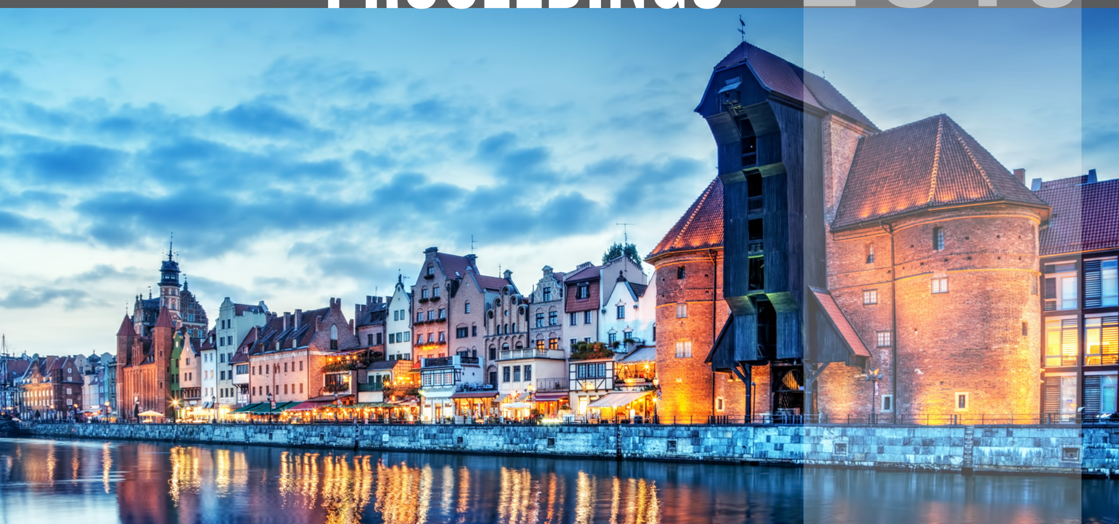
22nd European Symposium on Poultry Nutrition

10-13 JUNE 2019, GDAŃSK, POLAND

ESPN 2019

PROCEEDINGS



WWW.ESPN2019.COM

Proceedings of the 22nd European Symposium on Poultry Nutrition

Edited by:
Marta Kubiś, Marcin Hejdysz, Kamilia Dudek and Sebastian Kaczmarek

June 10-13, 2019
Gdańsk, Poland

Organized by:

The Working Group 2 Nutrition of the European Federation of Branches of the World's Poultry Science Association (WPSA)

And

The Polish Branch of the WPSA

THE WORLD'S POULTRY SCIENCE ASSOCIATION
education organization research

THE WORLD'S POULTRY SCIENCE ASSOCIATION

education organization research

Abstracts presented at ESPN2019 have been reviewed by members of the Scientific Committee and members of the Working Group. However, the contents in the abstracts and full papers are entirely the responsibility of the author or authors concerned and do not necessary represent the views of the organizers of the Symposium.

ISBN: 978-83-942760-6-5

Scientific Committee:

- Dr Pierre-Andre Geraert
- Prof. Dr Birger Svihus
- Dr Marcin Hejdysz
- Dr hab. Sebastian Kaczmarek
- Dr Rene Kwakkel
- Prof. Dr Markus Rodehutscord
- Dr hab. Andrzej Rosiński
- Dr Sanna Steenfeldt

The Organizers thank the following who reviewed abstracts submitted to this Symposium:

- Prof. dr Birger Svihus
- Dr Emily Burton
- Dr Joaquim Brufau
- Dr Ir. Evelyne Delezie
- Dr Karoly Dublecz
- Dr hab Damian Józefiak
- Dr Paweł Konieczka
- Dr Paweł Kołodziejski
- Dr hab. Krzysztof Kozłowski
- Dr Bartosz Kierończyk
- Dr Agnes Narcy
- Dr László Pál
- Dr Vasil Pirgozliev
- Prof. Dr Markus Rodehutscord
- Dr Anna Rogiewicz
- Dr Sanna Steenfeldt

Local Organizing Committee:

- Prof. Dr hab. Andrzej Rutkowski – President of the Organizing Committee
- Dr hab. Sebastian Kaczmarek – Vice President of the Organizing Committee
- Dr Marcin Hejdysz (UPP)
- Marta Kubiś (UPP)
- Kamilia Dudek (Symposium Cracoviense)

DIAMOND SPONSORS:

BIOMIN
DSM
NOVUS
PATENT CO.

PLATINUM SPONSORS:

CHR. HANSEN
EVONIK
J. RETTENMAIER & SÖHNE
ZOETIS

GOLD SPONSORS:

ABVISTA
ADISSEO
BASF
CJ BIO
DUPONT
ELANCO
EW NUTRITION
HAMLET PROTEIN
PERSTORP
PHYTOBIOTICS

SILVER SPONSORS:

AGROMED
CCPA GROUP
DELACON
DIAMOND V
EASTMAN
IMPEXTRACO
KEMIN
LALLEMAND
MIXSCIENCE
NUSCIENCE
OLMIX
PHILEO
SILVATEAM
TROUW NUTRITION

Table of Contents

Papers of invited speakers

Content

Session 1: Future protein sources - focus on the European market

European soya – possibilities and limitations

Volker Hahn

University of Hohenheim, Waldhof 2, 77731 Willstaett, Germany, Email: volker.hahn@uni-hohenheim.de

HISTORY OF SOYA IN EUROPE

The history section is taken from http://www.soyinfocenter.com/HSS/europe1.php. In this article, you will find many more details and references.

Starting in the 1850s soybeans and soyfoods began to take root in Europe, mainly thanks to the pioneering work of a group in France, the Society for Acclimatization. From 1854, when it received the first samples of soybeans from a French consul in China, the Society began distributing numerous packages of soybeans to its members throughout France, promoting the acclimatization and dissemination of this plant, and publishing the results of these culture trials in its widely read Bulletin of the Society for Acclimatization. After these first extensive efforts to spread the soybean in Europe, many successes were reported, but the Society did not succeed in establishing a permanent culture of the plant.

One of the great pioneers of soybeans and soyfoods in Europe was Dr. Friedrich Haberlandt from Vienna. Between 1873 and 1878, he initiated hundreds of successful soybean culture tests throughout southern central Europe. His 118-page classic Die Sojabohne, published in 1878, reported in detail on his work with soybean culture in Europe and discussed many European applications of soyfoods. Before Haberlandt, the soybean had largely been regarded as a food legume intended for human consumption in the form of a variety of soyfoods. Haberlandt pointed out that it could also be used for animal feed, which soon began to predominate, leading to a major reevaluation of the soybean's potential in Europe. Haberlandt had hoped to establish the soybean as a major commercial crop in Europe, but his death in 1878 prevented the realization of his vision.

In 1907 Japanese traders sent the first large trial shipment of Manchurian soybeans to England, where they were crushed in English oil mills at Liverpool and Hull to produce oil and flour. The results of this experimental crushing were favorable and the price of the imported soybeans was highly competitive. In 1908 the United Kingdom imported and crushed 40,600 tonnes (metric tonnes) of soybeans from Manchuria. Thereafter imports skyrocketed, reaching a peak of 449,000 tonnes imported and crushed in 1910. The vast majority of soybeans were imported from the United Kingdom up until 1912, followed by Germany, Denmark, and the Netherlands. Then in 1913, Germany and Russia became the major importers.

From 1945, after the war, imports of soybeans, soy oil, and soy flour increased dramatically, but now the USA and no longer East Asia were the main sources of these imports. European soybean imports from the US increased very rapidly, and total soybean imports rose from 105,000 tonnes in 1945 to 2,800,000 tonnes in 1960, a 26-fold increase in only 15 years, or an average annual growth rate of 25%. In 1973, a predicted shortage of soybeans in the US prompted the US government to impose a partial embargo on exports. This showed European livestock farmers their strong dependence on US soybeans and prompted the EEC to set itself the target of reducing its dependence, including by growing more soybeans in Europe.

Dr. Sven Holmberg from Sweden did pioneering work in Northern Europe. As a result, soybean could be grown (albeit in small quantities) at northern latitudes than had previously been the case. However, basic problems of latitude, climate, and population density, leading to low yields and high prices, limited expansion of acreage. The main increase of acreage took place in South-Eastern Europe, starting in the mid-1960s (with Romania) and greatly accelerating after the 1973 embargo, with Bulgaria, Yugoslavia, and Hungary. Total European soybean production increased from only 12,000 tonnes in 1960 to 108,000 tonnes in 1970 and 660,000 tonnes in 1980. From 1979 France became the only major soybean producer in Western Europe. Soybean production in the USSR was static during these two decades, but still larger than in any European country.

GLOBAL SITUATION TODAY

The world soybean production was about 361 million tonnes in 2018 (www.sopa.org). The USA, Brazil and Argentina dominated the production with 124, 117 and 55 million tonnes, respectively, followed by China, India, Paraguay and Canada. In total, more than 123 million hectares of soybeans were cultivated worldwide (Figure 1).

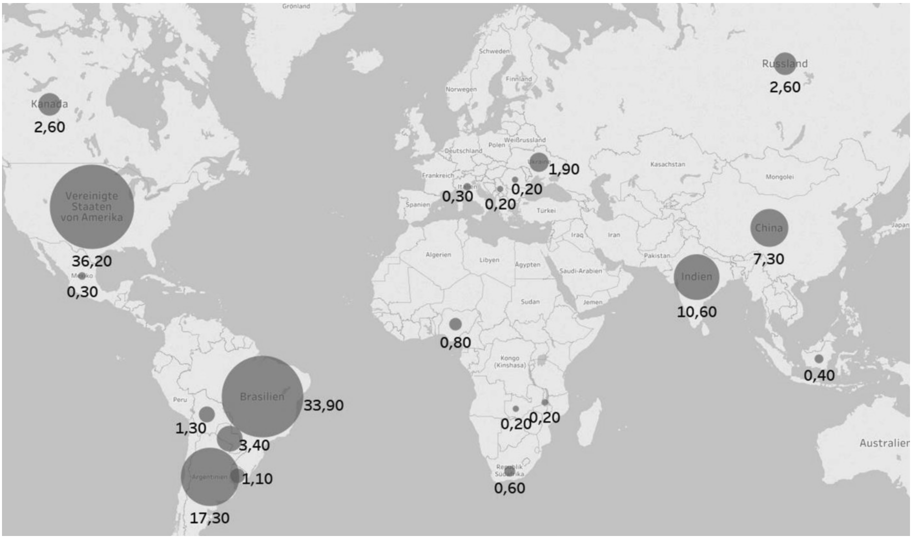


Figure 1: Soybean acreage 2017 in million hectares (source: www.fao.org/faostat)

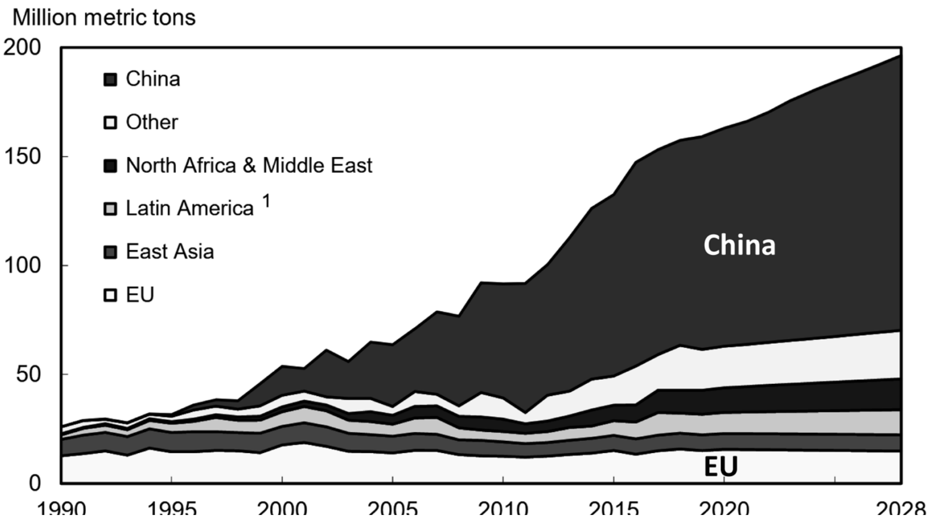


Figure 2: Soya imports (source: USDA agricultural projections)

China's soybean imports have risen steadily since the late 1990's. In 2017/18, China accounted for about 62 percent of world soybean trade (figure 2). EU soybean imports have been stable near 14.6 million tonnes over the past five years due to decreases in internal EU grain prices and increases in grain and rapeseed meal feeding (USDA agricultural projections).

SOYA ACREAGE IN EUROPE TODAY

In 2018, the acreage of soybean in total Europe was 4.3 million hectares, of which 1 million hectares were in the EU28. This means that the acreage has doubled since 2011. Production also doubled with now 10 million tonnes (2.8 million tonnes in EU28) (figure 3) and will expand to 15 million tonnes by 2025, according to Donau Soja's forecast. The most important countries in Europa are Ukraine, Russia and Italy (figure 4).

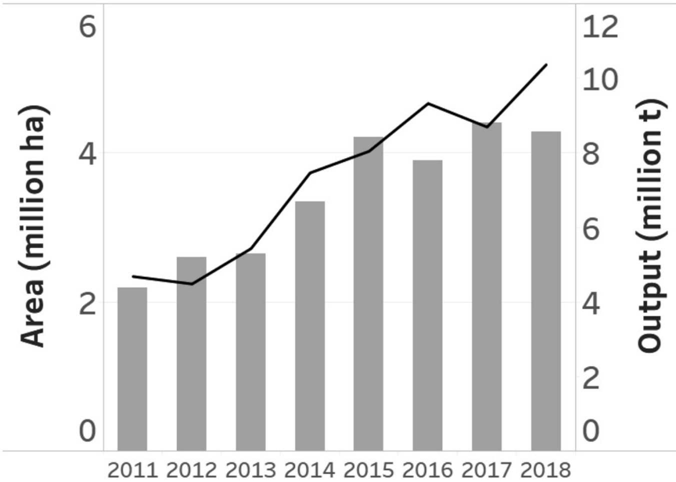


Figure 3: Soya area (bars) and output (line) in total Europe (source: Donau Soja)

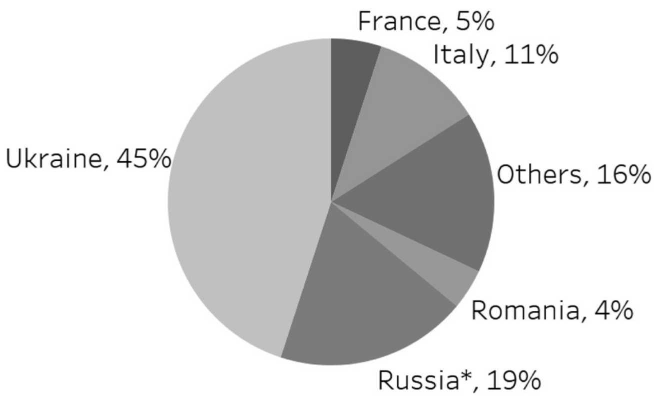


Figure 4: Soya output in percent by country in Europe (2017), * only European Russia, (source: Donau Soja)

YIELD

The mean yield in Europe between 2011 and 2017 was 2.05 t/ha with a maximum of 2.39 t/ha in 2016 and a minimum of 1.72 t/ha in 2012. The highest soya yield in recent years has mostly been achieved in Italy with often more than 3.0 t/ha. On the other hand, the average yield in the European part of Russia never exceeded 2.0 t/ha (source: Donau Soja).

QUALITY

Soybean meal is commonly used as feed source of animals due to its high protein concentration and excellent amino acid profile. However, experiments have shown that the concentration of standardized ileal digestible amino acids in soybean meal varies according to the country in which the soybeans were grown (LAGOS and STEIN, 2017). In addition, significant differences in the composition of soy meal in soybeans were found in different states of the United States (MOURTZINIS et al., 2018). For this reason, differences can be expected between European soybean meal and meal from other continents, but also within Europe.

SOYA BREEDING IN EUROPE

With the return of soya to European fields, breeding activities also increased. There are now several breeding companies working on soybean breeding like EURALIS Semences, France, RAGT, France, NS SEME, Serbia, S.I.S., Italy, SAATZUCHT DONAU, Austria, DANKO, Poland; Agesoya, Poland; GDM Seeds, Argentina, ARDS Turda, Romania, and several others breeding soybeans for South and Central Europe. In the plant variety database of the European Union, about 500 varieties are listed and this will increase considerably in the future. The breeding goals are mainly yield, disease resistances and protein content.

PROCESSING FACILITIES

Many soybean processing facilities are located in Europe. Most of them crush soybeans from overseas but some are also beginning to crash European soybeans. In 2018, around 3.5 million tonnes of European soybeans were crushed. However, some 15 million tonnes were imported into the EU28. Nevertheless, large companies are planning or have just begun to build soybean crushing plants for European soybeans such as ADM in Germany or the agricultural holding Cherkizovo in the European part of Russia.

REFERENCES

LAGOS, L. V., H.H. STEIN, 2017: Chemical composition and amino acid digestibility of soybean meal produced in the United States, China, Argentina, Brazil, or India. Journal of Animal Science 95, 1626-1636. DOI: 10.2527/jas2017.1440.

MOURTZINIS, S., B.S. BORG, S.L. NAEVE, J. OSTHUS, S.P. CONLEY, 2018: Characterizing soybean meal value variation across the United States: A swine case study. Agronomy Journal 110, 2343-2349. DOI: 10.2134/agronj2017.11.0624.

Low-glucosinolate rapeseed meal as a valuable source of protein for poultry

Anna Rogiewicz and Bogdan A. Slominski

Department of Animal Science, University of Manitoba, Winnipeg, Canada R3T 2N2

Canola meal (CM) or low-glucosinolate rapeseed meal (RSM) is a widely used protein supplement in poultry diets. This article presents the chemical composition and nutritive value of meals derived from canola-quality seeds and addresses the factors affecting digestibility of amino acids (AA) and availability of energy for poultry.

Chemical composition of CM and RSM is similar with crude protein, available sugars, total dietary fibre, and fat (ether extract) accounting for 40-42, 8-9, 38-42, and 3-4% DM, respectively. Dietary inclusion levels of CM have traditionally been limited to 5-10% due to concerns related to the high content of fiber and the presence of anti-nutritional factors, including glucosinolates (GLS). Over the years, the content of GLS in CM has been declining steadily, and it is now around 4.0 μmol/g. This value is similar to the levels of GLS in low-glucosinolate RSM produced in Australia. However, it is slightly lower than that in many European countries. Conventional plant breeding towards improving the quality of CM led to the development of low-fiber, yellow-seeded canola or the production of a new high-protein CM. Studies have been conducted to determine the standardised ileal digestibility (SID) of AA and AME_n contents with broiler chickens and turkeys. The SID for total AA and AME_n values of CM for broilers and turkeys averaged 82.4 and 78%, and 1,886 and 2,088 kcal/kg, respectively. It was documented in studies with broilers and turkeys that AME_n of CM and RSM could increase by around 200 kcal/kg following multi-carbohydrase supplementation. This review also presents the effects of high dietary levels of CM on growth performance and nutrient utilization in broilers, turkeys, and laying hens. In one broiler study, the effect of graded levels of CM from 0 to 30% was investigated, and the result showed that although fibre content of diets differed substantially with increased levels of CM, bird performance was not significantly affected. In another study, 15% of dietary inclusion of CM for young broilers (1 to 10 d of age) did not affect their growth performance. In the turkey studies (1-56d of age), dietary inclusion of 18 or 20% of RSM resulted in similar growth performance as that for the soybean meal (SBM)-based control diet. For laying hens, there were no significant differences in hen-day production, feed intake, feed efficiency, mortality, and egg quality between diets containing graded levels of CM from 0 to 20%. The egg mass was not affected as a result of the same or better hen-day egg production in hens consuming CM diets. It could be concluded that CM could be used effectively at 15-20% of broiler chicken, turkey, and laying hen diets, providing the diets are formulated based on digestible amino acids and available energy contents. It could also be concluded that due to the low GLS content, high inclusion levels of CM would not adversely affect animal health and growth.

Corresponding author: anna.rogiewicz@umanitoba.ca

INTRODUCTION

Low-glucosinolate rapeseed or canola is an offspring of rapeseed *Brassica napus* and *Brassica rapa* obtained by traditional selection programs used to reduce the content of erucic acid and GLS, making the oil and meal more valuable for human and animal consumption. Canola is the internationally recognized term for varieties containing no more than 2% erucic acid in the oil and less than 30 μmol/g of GLS in the meal. In Europe the term "double-zero rapeseed" (low-erucic acid, low-GLS) is used to identify canola quality seeds, oil and meal, although the term canola is used worldwide for all double-zero rapeseed varieties, regardless of their origin.

Canola is considered one of the greatest agricultural success making one of the world's healthiest vegetable oils, high-protein meal and biofuel feedstock. New varieties of canola are continuously being developed to improve yield, disease and insect resistance, and oil and meal quality. The production of canola has grown significantly since 1970s, due to the high popularity of the canola oil for human consumption, the increasing demand for the production of biodiesel industry, and growing needs for protein supplements in animal nutrition.

In 2018, the global canola production reached over 65 million metric tonnes. In Canada, the production of canola has been steadily increasing with 21 million metric tonnes being currently produced. However, the declining trend in canola production has been observed in many countries. It is partly attributed to the severe weather condition, particularly in Europe. Low precipitation combined with high temperature in central Europe in 2018 season have reduced the yields of rapeseed. According to the report from market analysis firm Oil World (www.oilworld.de), the EU produced 19.6 million tonnes of rapeseed last year (2018), down from the previous five-year average of 22.8 million tonnes. It is predicted that the EU crop will come in below 19.5 million tonnes, which would be the smallest crop in more than a decade. Australia harvested 2.8 million tonnes, well below the previous five-year average of 3.7 million. European crushers process about 20 million tonnes of canola a year, three million tonnes of which is imported. In Europe about 60 percent of the oil produced goes to the biodiesel sector, to fulfill the demand and to meet the requirement set by the Renewable Energy Directive 2009/28/EC, which mandates the use of renewable energy within the EU.

Canola seed is traditionally processed using pre-press solvent extraction to separate the oil from the meal. Methods involving the mechanical pressing have gained some popularity with the development of on-farm oil production, organic farming and in a response to the increasing demand for biodiesel. Expeller extraction methods are less efficient in extracting oil and produce meal with a greater (8.0 to 15.0%) residual oil content when compared to the pre-press solvent extracted CM. Canola meal has been widely used as a protein source for livestock. In recent years, breeding attempts to increase oil in the seed and to reduce the fibre content in the meal have led to the production of yellow-seeded *B. napus* canola and canola-quality *B. juncea*. As well, the development of a new high-protein CM has been achieved in North America under the trade name ProPound™ (DOW AgroSciences LLC, Indianapolis, USA.).

Canola meal is commonly used in poultry nutrition as an economically viable alternative to SBM. However, it still can't fully replace SBM due to the presence of antinutritive factors, lower metabolizable energy value, and lower and less consistent AA digestibility. Improved processing conditions, development of high-protein canola, or application of feed enzymes may mitigate such limitations. Research has shown that the nutritive potential of CM for poultry can be fully realized when diets are formulated based on the digestible AA and available energy contents.

CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF CANOLA/ LOW-GLUCOSINOLATE RAPESEED MEAL

A comprehensive Canadian CM quality survey was conducted between 2011 and 2017 with the partnership of Canola Council of Canada, Winnipeg, MB, Canada. A total of 249 samples of CM obtained from the large-scale, conventional pre-press solvent extraction processing were collected from all 13 canola processing facilities in Canada, and 22 samples of expeller-pressed CM were collected from form one processing facility to determine their chemical composition. In addition, samples of new yellow-seeded *B. napus* canola, conventional low-glucosinolate RSM from Poland and Australia were collected and analyzed. The meals were subjected to crude protein (Nx6.25) analysis using nitrogen analyzer TruSpec N (Leco, USA). Standard AOAC (2005) procedures were used for dry matter (900.15), fat (2003.06), ash (942.05), total phosphorus (965.17), starch (996.11), neutral detergent fiber (NDF) (2002.04), acid detergent fiber (ADF) (973.1) and AA (994.12) determination. Phytate phosphorus was determined using procedure described by HAUG and LANTZSCH (1983). Glucosinolates were determined by gas-liquid chromatography, as described by SLOMINSKI and CAMPBELL (1987). Sucrose, oligosaccharides (raffinose and stachyose) and fiber components were determined according to the procedure described by SLOMINSKI et al., (1994). Total dietary fiber (TDF) was determined by combination of NDF and detergent-soluble non-starch polysaccharides (NSP) measurements and was calculated as the sum of NDF and detergent soluble NSP. Total NSP were determined by gas-liquid chromatography (component sugars) and colorimetry (uronic acids) using the procedure described by ENGLYST and CUMMINGS (1984, 1988) with some modifications (SLOMINSKI and CAMPBELL, 1990). The results are presented in Table.1.

Protein and amino acids

The main components of CM/RSM include protein, carbohydrates (simple sugars, sucrose, oligosaccharides and starch), dietary fiber (NSP, lignin with polyphenols and glycoproteins) fat and ash. In general, the chemical compositions of CM and RSM were comparable. The crude protein levels of CM and RSM from Europe and Australia were similar and averaged 41.6, 40.1 and 40.4% DM, respectively. The profile of AA (content of AA as a percentage of CP) of CM and RSM was also not different. In comparison with the conventional meal, meals derived from yellow-seeded *B. napus* contained, on DM basis, more crude protein (43.4 vs. 41.6%). High-protein CM with 49% of CP, which was higher than that of the conventional CM and similar to that of SBM. Amino acids content of high-protein CM was higher than that of the conventional CM but of similar profile.

Table 1. Chemical composition of conventional CM/RSM, high-protein CM, meal derived from yellow-seeded canola, expeller/cold pressed canola and soybean meal (% DM)[1]

Component	Conventional CM/RSM			High-protein CM	Yellow-seeded canola	Expeller-pressed canola	Soybean meal
	Canada	Europe	Australia				
Dry matter	91.06	90.97	92.18	89.0	92.51	95.00	90.00
Crude protein	41.64	40.05	40.41	49.4	43.40	37.87	50.16
Ether extract	3.32	2.39	2.13	3.37	3.46	12.17	2.51
Ash	7.61	7.54	7.59	8.42	7.25	7.54	7.04
Essential amino acids							
Arginine	2.26	2.36	2.34	2.85	2.72	2.08	3.76
Histidine	1.21	1.08	1.16	1.53	1.28	1.07	1.36
Isoleucine	1.29	1.51	1.25	1.98	1.45	1.20	2.73
Leucine	2.61	2.71	2.71	3.46	2.89	2.42	3.94
Lysine	2.13	2.12	1.93	2.76	2.54	1.74	3.10
Methionine	0.71	0.77	0.75	0.99	0.71	0.63	0.67
Cystine	0.88	0.97	0.93	1.19	0.98	0.77	0.70
Phenylalanine	1.47	1.59	1.62	1.88	1.68	1.35	2.66
Tyrosine	0.97	1.20	1.07	1.44	1.12	0.87	1.88
Threonine	1.58	1.69	1.61	2.02	1.44	1.41	2.01
Valine	1.76	1.98	1.64	2.57	1.93	1.64	2.49
Carbohydrates							
Simple sugars [2]	0.28	0.49	0.44	0.90	0.22	0.10	0.66
Sucrose	6.28	6.84	6.73	6.18	10.05	5.53	6.82
Oligosaccharides [3]	2.98	2.86	3.21	3.26	2.81	2.88	6.16
Starch	0.83	0.61	1.22	0.33	0.47	0.52	2.53
Acid Detergent Fiber	20.07	21.00	20.26	15.73	9.34	16.44	9.24
Neutral Detergent Fiber	29.58	29.61	32.50	21.34	18.98	27.22	14.37
Total Dietary Fiber	38.44	39.56	42.92	27.10	29.81	34.35	23.98
Dietary Fiber fractions							
Non-starch polysaccharides	22.46	24.12	24.95	19.10	22.81	19.44	19.58
Lignin and polyphenols	10.65	11.04	10.90	4.49	3.09	10.11	2.86
Glycoproteins	5.33	4.40	7.05	3.51	3.91	4.80	1.54
Total P	1.12	1.21	1.10	1.40	1.23	1.04	0.73
Phytate P	0.70	0.87	0.74	1.01	0.95	0.66	0.42
Non-phytate P	0.41	0.34	0.36	0.39	0.28	0.38	0.31
Glucosinolates (μmol/g) [4]	3.60	6.25	1.79	8.99	14.57	9.23	NA[5]
AME_n for broilers (kcal/kg)	1,886	1,800	-	2,470	2,027	2,506	2,453
AME_n for turkeys (kcal/kg)	2,088	2,142	-		2,170		

1 Average values calculated from JIA et al., 2012; KHAJALI and SLOMINSKI, 2012; HALLE and SCHÖNE, 2013; ADEWOLE et al., 2016; GORSKI et al., 2017; KOZLOWSKI et al., 2018; RAD-SPICE et al., 2018; 2 Includes glucose and fructose; 3Includes raffinose and stachyose; 4Includes gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin and 4-hydroxyglucobrassicin; 5 not applicable

Protein with a well-balanced AA composition is the most valuable nutrient of CM. When compared to SBM, CM has less lysine and arginine but more methionine and cystine, thus it is advisable to use them complementarily in diet formulation. Amino acid contents are not the best indicators of CM quality. Emphasis should rather be placed on their digestibility as it might be affected by the processing conditions of oil extraction. Excessive heat treatment during the pre-press solvent extraction of canola seeds might reduce digestibility of AA and contribute to variation in the nutritive value of CM. Pre-press solvent extraction includes pre-conditioning, cooking, pressing, solvent extraction and desolventizing/toasting, most of which involve heat treatment. All these conditions contribute to variation in the chemical composition and nutritive value of the meal but the damaging effect of heat is most significant during the desolventizing/toasting where hexane from de-oiled meal is removed at high temperature for a prolonged time. The amount and duration of heat applied during processing can lead to destruction of heat sensitive AA. Some AA, especially lysine, can be turned to biologically unavailable derivatives during heat treatment due to Maillard reaction which occurs between AA and reducing sugars. Losses in available lysine, arginine, threonine, aspartic acid, or glutamic acid due to early and advanced Maillard reactions are quite common. The Maillard reaction products are indicatives of protein damage and their formation would lead to the increase in dietary fiber, NDF and lignin contents. Earlier research from our laboratory (SLOMINSKI, 1997) demonstrated a profound effect of moist heat treatment on CM quality. Application of temperatures higher than 105°C significantly increased the highly indigestible fraction of protein often referred to as neutral detergent insoluble crude protein (NDICP). This fraction contributes to the increase in TDF content and is indicative of protein damage caused by overheating. NEWKIRK et al. (2000) documented that NDICP values below 10% of total CP indicate CM with a greater than 85% lysine availability for poultry. In our recent studies (ADEWOLE et al., 2016) on the chemical composition of CM from Canadian canola crushing plants, differences between processing facilities in the contents of NDF and TDF were observed with NDICP, lignin and polyphenols showing the highest variation. There were positive correlations between NDF and TDF (R^2=0.97), and TDF and NDICP contents (R^2=0.79). In addition, the prediction equations have been developed to estimate the degree of protein damage. Moreover, the of NDF and CP have been used to predict the amount of available lysine in CM. In the same study, lysine damage due to heat treatment during the desolventizing/toasting has been well supported by a negative relationship between lysine and NDF (R^2=0.53), lysine and TDF (R^2=0.64), and lysine and NDICP (R^2=0.48), as well as by positive relationship between lysine and heat-sensitive GLS contents (R^2=0.54).

Glucosinolates

It has been widely recognized that GLS level in CM does not concern the meal users as it was in case of the high-glucosinolate RSM, because the content of GLS in CM has been declining steadily over the years and it is now below 4.0 μmol/g in Canadian and Australian CM, but slightly higher in RSM produced in European countries (i.e., 7.0 μmol/g). Such levels are not only the result of the intensive plant breeding but also result from GLS decomposition during seed processing. In the aforementioned study by ADEWOLE et al. (2016), GLS contents of CM from different crushing plants across Canada was reported to range from 2.0 to 10.1 μmol/g DM, indicating the effect of processing on GLS content. It has been observed that the GLS contents correlated well with the TDF contents indicating that the increase in the fibre content was directly related to overheating of CM, which, at the same time, was responsible for GLS decomposition.

Expeller/cold-pressed canola is not subject to desolventizing/toasting but is still subject to the potential effects of heat due to the friction generated during the expelling process. The meal temperature may be as high as 160°C but due to the low moisture content and the short duration, protein quality is generally preserved. As well, higher GLS content in expeller-pressed cake than that of the pre-press solvent extracted meal (9.23 vs. 3.60 μmol/g DM) would be expected due to the lack of any severe heat during expelling.

Carbohydrates and dietary fiber

The carbohydrate components of CM are composed of simple sugars, sucrose, oligosaccharides and NSP. As a consequence of small size and high oil content of the seed, the meal contains relatively high proportion of dietary fiber with the TDF and NDF contents being higher than those of SBM. As it was mentioned above, the processing conditions and temperature used in the desolventizing-toaster might increase the dietary fiber content due to formation of Maillard advanced glycation end-products, which resemble lignin. In addition, the dietary fiber content of CM can be increased further in the pre-press solvent extraction process since screenings and some other dockage are sometimes added back into the meal. Based on the survey conducted in Canada, the content of NDF and TDF of CM averaged 29.6 and 38.4% DM, respectively.

The high-protein type of CM contains less dietary fiber (27.1%, DM) compared to the conventional CM, with lignin and polyphenols levels being the main compositional changes. When compared with the conventional *B. napus* CM, the meal derived from yellow-seeded *B. napus* contain more sucrose (10.1 vs. 6.6 % DM) and less TDF (29.8 vs. 40.4 % DM) due to the low content of lignin with associated polyphenols, and thus thinner seed coat. Despite the improvements to the quality of yellow-seeded canola (i.e., more oil, more protein, less fibre), when the diets were formulated based on the digestible AA and metabolizable energy contents, similar growth performance parameters in broiler chickens and turkeys to those fed diets containing conventional CM were observed (SLOMINSKI et al., 2012; KOZLOWSKI et al., 2018; RAD-SPICE et al., 2018). It would appear that breeding for the yellow-seeded canola resulted in quantitative changes, such increased oil, protein and sucrose contents rather the qualitative changes due to decreased fiber content. MEJICANOS et al. (2017) demonstrated that canola fibre had a minimal effect on growth performance of broiler chickens fed diets containing meals from the tail-end dehulling process using sieving technology. Fractionated meals contained significantly more protein and less TDF than the parent meal and has been used at 15% from 1 to 10 days of age. Chicken performance was similar regardless of the fraction of CM used indicating that poultry can efficiently utilize diets containing high amount of canola fiber right at the early stages of growth. It has been concluded, that canola fiber would have a minimal effect on nutrient digestion and absorption due to the fact that most of the fiber in CM is water-insoluble.

Fat

Canola meal contains more fat than SBM, however fat content might vary between crushing plants as a result of the presence of by-products of oil-refining (i.e., gums, soapstocks, phospholipids), which are added back into the meal to reduce the dustiness and increase the energy content of the meal. It becomes a common practice in Canada, and in some European countries. However, soapstocks and gums are poorly digested and contribute less energy to the AME_n content of CM than oil (RAHMANI et al., 2017). Expeller-extracted meals contain more residual oil due to incomplete oil removal, thus resulting in the higher metabolizable energy content than the conventional pre-press solvent extracted meal (2,506 vs. 1,991 kcal/kg DM). For expeller-extracted CM, the residual oil content varies between 8.0 to 15.0%, depending on whether the seeds are passed through the system once or twice.

Phosphorus

Canola meal has relatively high amounts of phosphorus (P) (1.12% DM) in the form of phytate P (0.7% DM) and non-phytate (available) P (0.41% DM). Although high in phytate, CM is one of the richest sources of available P when compared to other feed ingredients.

NUTRITIVE VALUE OF CANOLA MEAL: AMINO ACIDS DIGESTIBILITY AND ENERGY AVAILABILITY FOR POULTRY

Several studies have been carried out in our laboratory to determine the SID of AA and AME_n value of CM for broiler chickens and turkeys. Standardized ileal amino acids digestibility (%) of canola meals for poultry is presented in Table 2.

An experiment was conducted to determine the SID of AA for broiler chickens using CM sourced from 13 canola processing facilities across Canada (ARIYIBI et al., 2018a). One-day-old broiler chickens were fed a pre-experimental starter diet from 1 to 14 d of age followed by the test diets from 15 to 21d. Test diets were formulated to contain 22% of CP with CM as a sole source of AA. One group received a casein-maize starch diet for the determination of endogenous AA losses. All diets contained Cr_2O_3 (0.3%) as an indigestible marker. On d 21, birds were euthanized and the ileal digesta samples were collected, freeze-dried and analyzed for AA and chromium contents. The total SID of AA of the all CM for broiler chicken averaged 82.4%. The variations

(P<0.05) in SID of indispensable AA was observed. Lysine, being the first limiting AA, was the most variable (86.3 vs. 74.2%) followed by tryptophan (94.5 vs. 76.7%) and threonine (87.3 vs. 71.6%). The SID of methionine (91.8 vs. 87.4%) and cystine (81.8 vs. 73.7%) were not significantly (P>0.05) affected, however SID of arginine which is known as a conditionally essential AA, varied significantly (92.0 vs. 83.8%) between different canola processing facilities. Variations in values of SID of AA of CM could result from differences in processing conditions. Similar results were obtained in the earlier study (ADEWOLE et al., 2017), using samples of Canadian CM from different processing facilities. In this study, standardized ileal digestibility for lysine, arginine, methionine and threonine averaged 79.1, 88.0, 89.9, and 74.9% respectively.

Standardized ileal digestibility of AA of CM has also been determined with turkeys. One-day-old poults were fed a pre-experimental starter diet from 1 to 21 d of age followed by the test diets from 22 to 28d. Test diets were formulated to contain 28% of CP with CM as a sole source of AA. The endogenous AA losses were determined. The total amino acids of CM were 78.0% digestible, with SID of lysine, methionine, cystine, threonine and arginine averaging 76.2, 79.1, 71.0, 72.5 and 85.8%e, respectively (KOZLOWSKI et al., 2018).

Table 2. Standardized ileal amino acids digestibility (%) of canola meals for poultry[1]

Amino acid	Conventional CM/RSM	High-protein CM	Yellow-seeded canola	Expeller-pressed canola	Soybean meal
Arginine	86	93	88	86	94
Histidine	64	90	64	81	93
Isoleucine	78	87	77	83	91
Leucine	82	88	81	82	90
Lysine	78	83	77	77	86
Methionine	85	91	88	88	91
Cystine	72	87	73	78	84
Phenylalanine	82	90	81	83	90
Tyrosine	83	NA	83	79	87
Threonine	75	83	74	79	87
Valine	76	84	74	82	86

1 Average values for broiler chickens and turkeys calculated from WOYENGO et al., 2010; ADEWOLE et al., 2016; ADEWOLE et al., 2017; GORSKI et al., 2017; ARIYIBI et al., 2018a; KOZLOWSKI et al., 2018; RAD-SPICE et al., 2018

The AME_n of CM and expeller/cold pressed canola has been determined in several studies (WOYENGO et al., 2010; JIA et al., 2012; HALLE and SCHÖNE, 2013; ADEWOLE et al., 2017; KOZLOWSKI et al., 2018; RADFAR et al., 2017; RAD-SPICE et al., 2018). The AME_n value of CM for broilers and turkeys averaged 1987 kcal/kg DM while for the expeller/cold pressed meal containing 11.5% of oil the AME_n averaged 2506 kcal/kg DM. The high-protein CM and meal from yellow-seeded canola contained more AME_n when compared to the conventional CM (GORSKI et al., 2017; JIA et al., 2012; KOZLOWSKI et al., 2018; RADFAR et al, 2017; RAD-SPICE et al., 2018).

IMPROVEMENT TO THE NUTRITIVE VALUE OF CM BY ENZYME APPLICATION

Supplementation of poultry diets containing CM with enzymes is an effective means of improving the quality of CM (KHAJALI and SLOMINSKI, 2012). Most of studies investigated the supplementation of carbohydrase enzymes to target NSP in attempt improve carbohydrates digestibility and to remove the potential oil encapsulating effect of cell wall polysaccharides. An *in vitro* study carried out in our laboratory demonstrated a significant depolymerization of NSP of canola seed when using a blend of cell wall-degrading carbohydrases, which allowed for more complete oil utilization by poultry by mitigating the insufficient rupture of oil-containing cells within the seed (MENG et al., 2005). This concept, however, would apply to canola seeds rather than CM where nutrients are more available for digestion due to the effective seed rapture during the oil extraction process. The enzymatic degradation of NSP of diets containing CM will, however, contribute to the production of NSP hydrolysis products which may have a prebiotic effect in improving health and development. The enzymatic hydrolysis of NSP would also generate some energy, and thus increase the available energy content of CM for poultry. In the most recent studies, the AME_n values increased from 1,886 and 2,088 to 1,955 and 2,196 kcal/kg for broilers and turkeys, respectively, following enzyme (multi-carbohydrase) supplementation (JIA et al., 2012; KOZLOWSKI et al., 2018; RAD-SPICE et al., 2018).

HIGH INCLUSION LEVELS OF CANOLA MEAL FOR POULTRY DIETS

Several studies on the effect of high dietary levels of CM on growth performance and nutrient utilization in broiler chickens, turkeys and laying hens has been conducted. The summary of such trials is presented in Table 3.

Table. 3 Summary of feeding trials with poultry fed diets containing CM

Species	Trial length (d)	CM level (% diet)	BW gain (kg/bird)	FCR (feed/gain)	Reference
Broilers	35	0	2.32	1.53	RAD-SPICE et al., 2018
		15	2.30	1.51	
Broilers	10	0	0.29	1.24	MEJICANOS et al., 2017
		15	0.29	1.19	
Broilers	28	0	1.32	1.45	ARIYIBI et al., 2018 b
		6	1.37	1.45	
		18	1.40	1.45	
		30	1.36	1.49	
Turkeys	56	0	3.90	1.71	KOZLOWSKI et al., 2018
		20	3.89	1.73	
Turkeys	56	0	3.67	2.16	ZDUNCZYK et al., 2013
		6	3.69	2.11	
		12	3.69	2.08	
		18	3.69	2.11	
			Egg production (%)	**Feed efficiency (g feed/g egg)**	
Laying hens	168	0	96.09	1.92	ROGIEWICZ et al., 2015
		12	96.53	1.95	
		20	96.33	1.96	

Broiler chickens

The determined SID values of amino acids and the AME_n contents of CM were used for the diet formulation in the recent growth performance study (RAD-SPICE et al., 2018). In this 35-day study broiler chickens were fed the control wheat/maize/SBM diet and the diet containing 15% of CM. Body weight gain (BWG) averaged 2.32 for the control, and 2.30 kg for CM. The benefit of formulating diet on available energy and digestible AA content has been observed showing no significant difference in feed conversion ratio (FCR) between the control and the diet containing CM, indicating that CM could be used effectively and replace SBM in broiler chicken rations. Similarly, no difference in the growth performance of broiler chickens fed a maize/SBM control diet and diet containing 15% of CM fed for 10 days in the pre-starter phase was observed in a study by MEJICANOS et al. (2017) indicating that CM can be used effectively at high inclusion levels at the early stage of chicken growth.

The effect of different inclusion levels of CM on the growth performance of broiler chickens has also been determined in another study by ARIYIBI et al. (2018b). The experiment had four phases of six treatment groups as follows: pre-starter (0, 3, 6, 9, 12, 15 % of CM), starter (0, 4, 8, 12, 14, 18 % of CM), grower 1 (0, 5, 10, 15, 20, 25 % of CM) and grower 2 (0, 6, 12, 18, 24, 30 % of CM), each lasting for one week. Diets were balanced for SID AA contents by replacing SBM in a maize-SBM basal diet with graded levels of CM. One-day-old broiler chickens (housed 5 birds/cage) were allotted into the six treatment groups with ten replicates/cages per treatment. Overall, the result showed that although dietary fibre content of diets differed substantially with increased levels of CM, growth performance was not significantly ($P>0.05$) affected by CM inclusion levels. Irrespective of the phase and CM inclusion levels, feed intake (FI), BWG, and FCR averaged 2.02 kg/bird/28 d, 1.39 kg/bird/28 d, and 1.46 kg/feed/kg gain and were similar to 1.92 kg/bird/28 d, 1.33 kg/bird/28 d, and 1.45 kg feed/kg gain for the control treatment, respectively. The results of the experiment are consistent with those reported by GORSKI et al. (2017) where no effect on growth performance of graded levels of 8, 16 and 24% of CM were observed. Study by GOPINGER et al. (2014) showed that CM inclusions up to 16.4% (7-14 d of age) and 22.9% (14-21 d of age) resulted in a statistically significant increase in daily BWG and FI, respectively, but the response to other performance parameters did not differ considerably. The same study reported a significant improvement in FCR and increased daily BWG for diets up to 20% and 30% CM inclusion in the grower and finisher diets fed from 22 to 28 d.

Turkeys

The nutritive value of CM was also investigated in a study with turkeys from 1 to 56 d of age fed wheat/SBM-based diets containing 20% of CM (KOZLOWSKI et al., 2018). All diets were balanced for digestible AA and available energy contents. There were no significant differences ($P>0.05$) in BWG and FCR between the control SBM-based and CM containing diets. These results were in agreement with those from the earlier study by ZDUNCZYK et al. (2013), in which inclusion of canola type RSM up to 18% did not affect the growth performance of turkeys from 1-56 d of age.

Laying hens

Canola meal inclusion levels in laying hen diets have traditionally been limited to maximum 10% due to the presence of antinutritional factors, including GLS and sinapine. KHAJALI and SLOMINSKI (2012) indicated that a dietary level of glucosinolates of 1.5 μmol/g would have no negative effect on laying hen performance, thus due to the relatively low GLS (less than 4.0 μmol/g DM), diets for laying hens could now contain significantly more CM without causing any adverse effect on egg production or mortality due to hemorrhagic liver syndrome. Therefore, the effect of different dietary levels of CM on egg production and egg quality parameters in laying hens has been investigated (ROGIEWICZ et al., 2015). A wheat/maize/SBM-based control diet, and diets containing 4, 8, 12, 16 or 20% of CM were fed to 6 replicate cage units of 18 Lohmann LSL laying hens each per treatment throughout the 24-week study consisting of 2 phases and three 28-d periods of data collection in each phase. Diets were formulated to contain 17.0 and 16.4% of CP and 2,800 and 2,700 kcal/kg of metabolizable energy in Phase 1 and 2, respectively. Dietary GLS content averaged 0.28, 0.52, 0.97, 1.30, and 1.49 μmol/g for the diets containing 4, 8, 12, 16 or 20% of CM, respectively. Hen-day production, egg mass, feed intake, and feed efficiency were determined three times in each phase at the end of a 28-d period. All eggs were weighed for 3 consecutive days in the middle of each period and 36 eggs per treatment were selected for egg quality measurements, including albumen height, Haugh units, specific gravity, yolk color, and egg shell elasticity and thickness. There were no significant differences in hen-day production, feed intake, egg mass, feed efficiency, and mortality between dietary treatments ($P>0.05$). When compared with the control diet, different dietary levels of CM had no effect on egg and egg shell quality parameters. Therefore, it would appear evident that CM could replace SBM and used effectively in laying hen diets at the dietary level of 15-20%. Similar study by HALLE and SCHÖNE (2013) with laying hens fed diets containing 5, 10 and 15% of expeller- pressed RSM showed no negative effect on the laying hen performance, even with the higher dietary GLS content than in our study (i.e., 1.05, 2.1 and 3.15 μmol/g, respectively). Moreover, the potential for the enrichment of the yolk fat with n-3 type of polyunsaturated fatty acids was documented.

Some egg producers have been reluctant of using CM because of its association with the fishy flavor of brown-shell eggs produced by certain strains of laying hens. It has to be emphasized that this genetic defect is no longer an issue, because since 2005 the breeder company Lohmann Tierzucht has been producing birds free of the defective gene. As a result, brown-shell eggs laying hens are not accumulating trimethylamine (TMA) in the yolk anymore, and despite the use of feed containing TMA precursors, the eggs will be free of the fishy odour.

Conclusions

It could be concluded that CM could be used effectively at 15-20% in broiler, turkey, and laying hen diets, providing the diets are formulated based on digestible AA and available energy contents. It could also be concluded that high inclusion levels of CM would not adversely affect animal health and growth.

REFERENCES

ADEWOLE, D., A. ROGIEWICZ, B. DYCK, B.A. SLOMINSKI, 2016: Chemical and nutritive characteristics of canola meal from Canadian processing facilities. Anim. Feed Sci. Technol. 222, 17-30.

ADEWOLE, D., A. ROGIEWICZ, B. DYCK, B.A. SLOMINSKI, 2017: Effects of canola meal source on the standardized ileal digestible amino acids and apparent metabolizable energy contents for broiler chickens. Poult. Sci. 96(12), 4298-4306.

ARIYIBI, S.A., A. ROGIEWICZ, B.A. SLOMINSKI, 2018a: Standardized ileal amino acid digestibility of canola meal in broiler chickens in relation to its source. Proceedings of the 2018 Animal Nutrition Conference of Canada, Edmonton, May 2018.

ARIYIBI, S.A., A. ROGIEWICZ, B.A. SLOMINSKI, 2018b: The effect of incremental dietary levels of canola (low-glucosinolate rapeseed) meal on growth performance of broiler chickens. Proceedings of the 15th European Poultry Conference, Dubrovnik, September 2018.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 2005: Official Methods of Analysis of AOAC International, 18th ed.; AOAC International: Gaithersburg, MD

DIRECTIVE 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Brussels, Belgium: European Council. 5 June 2009.

ENGLYST, H.N., J.H. CUMMINGS, 1984: Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 109, 937-942.

ENGLYST, H.N., J.H. CUMMINGS, 1988: Improved method for the determination of dietary fiber as non-starch polysaccharides in plant foods. J. Assoc. Off. Anal. Chem. 71, 808-814.

GOPINGER, E., E. XAVIER, M. ELIAS, A. CATALAN, M. CASTRO, A. NUNES, V. ROLL, 2014: The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens. Poult. Sci. 93(5), 1130-1136.

GORSKI, M., C. FORAN, P. UTTERBACK, C. PARSONS, 2017: Nutritional evaluation of conventional and increased-protein, reduced-fiber canola meal fed to broiler chickens. Poult. Sci. 96(7), 2159-2167.

HALLE, I., F. SCHÖNE, 2013: Influence of rapeseed cake, linseed cake and hemp seed cake on laying performance of hens and fatty acid composition of egg yolk. J. Verbr. Lebensm. 8,185-193.

HAUG, W., H.J. LANTZSCH, 1983: Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34, 1423-1426.

JIA, W., D. MIKULSKI, A. ROGIEWICZ, Z. ZDUŃCZYK, J. JANKOWSKI, B.A. SLOMINSKI, 2012: Low-fiber canola. Part 2. Nutritive value of the meal. J. Agric. Food Chem. 60 (50), 12231-122237.

KHAJALI, F, B.A. SLOMINSKI, 2012: Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91(10), 2564-2575.

KOZLOWSKI, K., D. MIKULSKI, A. ROGIEWICZ, Z. ZDUNCZYK, M. RAD-SPICE, H. JEROCH, J. JANKOWSKI, B.A. SLOMINSKI, 2018: Yellow-seeded Canola. Part 2. Nutritive value of the meal for turkeys. Anim. Feed Sci. Technol. 240, 102-116.

MEJICANOS, G.A., A. ROGIEWICZ, C.M. NYACHOTI, B.A. SLOMINSKI, 2017: Fractionation of canola meal using sieving technology. Can. J. Anim. Sci. 97, 613–621

MENG, X., B. A. SLOMINSKI, C. M. NYACHOTI, L. D. CAMPBELL, W. GUENTER, 2005: Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poult. Sci. 84, 37-47.

NEWKIRK, R.W., H.L. CLASSEN, T.A. SCOTT, M.J. EDNEY, D.R. DECLERCQ, A.J. SIEMENS, 2000: Prediction of lysine availability in canola meal. World's Poult. Sci. J. 56, 60

RADFAR, M., A. ROGIEWICZ, B.A. SLOMINSKI, 2017: Chemical composition and nutritive value of canola-quality *Brassica juncea* meal for poultry and the effect of enzyme supplementation. Anim. Feed Sci. Technol. 225, 97-108.

RAD-SPICE, M, A. ROGIEWICZ, J. JANKOWSKI, B.A. SLOMINSKI, 2018: Yellow-seeded Canola. Part 1. Nutritive value of the meal for broiler chickens. Anim. Feed Sci. Technol. 240, 66-77.

RAHMANI, M., A. ROGIEWICZ, B.A. SLOMINSKI, 2017: Chemical composition and available energy contents of canola meal from Canadian crushing plants. Proceedings of the Animal Nutrition Conference of Canada, Quebec City, May 10-11, 224.

ROGIEWICZ, A., B. DYCK, B.A. SLOMINSKI, 2015: High inclusion levels of canola meal in laying hen diets. Abstracts of the 14th International Rapeseed Congress, Saskatoon, Canada.139.

SLOMINSKI, B.A., 1997: Developments in the breeding of low fibre rapeseed/canola. J. Anim. Feed Sci. 6, 303-317.

SLOMINSKI, B.A., L.D. CAMPBELL, 1987: Gas chromatographic determination of indole glucosinolates- a re-examination. J. Sci. Food Agric. 40. 131-143.

SLOMINSKI, B.A., L.D. CAMPBELL, 1990: Nonstarch polysaccharides of canola meal: quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53, 175-184.

SLOMINSKI, B.A., L.D. CAMPBELL, W. GUENTER, 1994: Carbohydrates and dietary fiber components of yellow- and brown-seeded canola. J. Agric. Food Chem.42, 704–707.

SLOMINSKI, B.A., W. JIA, A. ROGIEWICZ, C.M. NYACHOTI, D. HICKLING, 2012: Low-fiber canola. Part 1. Chemical and nutritive composition of the meal. J. Agric. Food Chem. 60 (50), 12225-12230.

WOYENGO, T., E. KIARIE, C.M. NYACHOTI, 2010: Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poult. Sci., 89(6),1182-1189.

Zdunczyk, Z., J. JANKOWSKI, J. JUSKIEWICZ, D. MIKULSKI, B.A. SLOMINSKI, 2013: Effect of dietary levels of low-glucosinolate rapeseed (canola) meal and non-starch polysaccharide-degrading enzymes on growth performance and gut physiology of growing turkeys. Can. J. Anim. Sci. 93, 353-362.

The role of bioethanol production in producing environmentally sustainable feed materials for the poultry sector

Dawn Scholey and Emily Burton

Department of Animal, Rural and Environmental Sciences, Nottingham Trent University, Nottingham, UK NG25 0QF; Emily.burton@ntu.ac.uk

ABSTRACT

There is substantial potential to produce co-products for the animal feed industry from grain fuel production. Utilizing the high protein content remaining after fuel production is a complimentary part of production, optimizing value and sustainability of the process. Traditional bioethanol co-products provide a major route for converting excess fiber into food via animal production, but the use of these products is limited in monogastrics. There is a growing drive towards the production of sustainable protein for livestock and therefore, fuel bio-products may have high value as a protein source for both livestock and fish. There is the potential for yeast to be separated from the co-product stream to be marketed as feed protein and as a feed supplement, with the latter having potential to enhance immunity. This paper assesses the potential for yeast and other products from ethanol production as environmentally sustainable ingredients for the poultry sector.

INTRODUCTION

As fuel ethanol production increases worldwide, there will be substantial opportunity for production of co-products suitable for the animal feed industry. Traditional co-products provide a route for utilising excess fibre but complementary technological innovations now allow additional streams to utilise other portions of the stillage, optimising value of the process and improving the sustainability of production. These additional co-products will also support the demand for production of sustainable protein for livestock, particularly monogastrics, without some of the environmental challenges of protein sources such as soya and fishmeal. Traditional bioethanol and potable alcohol production generates a co-product Distiller's Dried Grains with Solubles (DDGS) which is used as a feed material mainly for ruminants due to its high fibre content. This paper will briefly draw together the current use of bioethanol co-products in poultry production, then investigate methods of producing extra product streams from bioethanol production, while evaluating the suitability of these additional co-products for feeding poultry.

The current global approach to sustainable agriculture hinges on balancing supply of the 4Fs: feed, fuel, food and fibre. The incorporation of biorefinery co-products into animal feeds provides a major conduit for finding balance; excess fibre and feed from production of fuel may be converted into food via animal production. Holistic biorefinery production, can produce a range of products including fuel and feed products to increase the sustainability of first-generation bioethanol, to bridge the period until later versions of bioethanol production become viable.

BIEOTHANOL PRODUCTION

Recent legislation globally continues to support growth in bioethanol production, led by commitments to 10% inclusion levels (E-10 blends) in countries such as China, Mexico and the UK. First generation bioethanol is defined as utilising starch and sugar crops to produce bioethanol, with the co-product of DDGS as a feed material. Global production uses maize and sugarcane, with wheat, potato and sugar beet more commonly used feedstocks in Europe (Havlik et al., 2011), and the majority of production (67%) being from maize (Rulli et al., 2016).. However, the feed versus fuel conundrum is a major limitation of this process since feed grade materials are used. Utilisation of crops for fuel can increase both food and feed prices and reduce availability, unless co-products from the bioethanol process can be utilised effectively for animal feed. The value of the resulting ethanol is also dependent on the price of mineral oil, and when the price of the latter falls, this places greater emphasis on the value of the co-product of the process in order than the refinery as whole remains profitable

In the future, there is potential for bioethanol production from second-generation bioethanol involving the processing of lignocellulosic feedstocks and waste residues from forestry or agriculture (Arifin et al., 2014). However, this technology has several hurdles before it can become widely available (Tao et al, 2011), including the poor ethanol yield of lignin containing material, high cost and advanced facilities required to support the conversion process (Nigam and Singh, 2011). Therefore, in the intervening decades while these advances technologies are developed, it is vital to improve the sustainability of first generation bioethanol production by increasing and diversifying co-product use. Maintaining several co-product streams from bioethanol can provide feed materials suitable for different farm species and improve both economic and environmental sustainability of the refineries.

DDGS USE IN POULTRY FEEDING

DDGS is mainly fed to ruminants, but there has been substantial research around feeding to poultry. DDGS has been widely incorporated into poultry diets over the last 15 years, with much research summarised in review papers (Swiatkiewicz and Koreleski, 2008; Salim et al., 2010). In practice dietary inclusion levels are kept relatively low to manage the nutrient variability, and conservative matrix values are applied by formulators, though this may diminish the value of DDGS.

Within the last few years, oil is being commonly extracted from stillage prior to drying DDGS, with the resultant DDGS being lower fat and therefore lower energy (Shurson, 2017). This lower oil DDGS is reported to be more variable in nutritional content and therefore requires further examination.

There are several processing issues with DDGS which need to be considered alongside it's nutritional value, including batch to batch and interplant variability (Lui, 2011), and the high energy costs (around 40% of the total energy required for production) required to dry the product so it can be transported and stored (Wheals et al., 1999). Transport can be an issue, with water adsorption causing caking and poor flow (Saragoni et al., 2007), and particle separation during transport can increase the variation in physical characteristics (Ileleji et al., 2007; Rosentrater, 2006). Pellet durability has also been negatively correlated with DDGS inclusion (Shim et al., 2011), whilst the high viscosity of DDGS mash also increases energy use through the condenser (Loar et al., 2010).

FRACTIONATION OF DDGS

Fractionation at the back end of the bioethanol process can produce several products, which make best use of the available nutrients in DDGS. These types of fractionation processes have the benefit of being a lower capital investment, with little effect on the overall bioethanol process. For example, the Elusieve process uses a mixture of sieving and an elutriation column to separate out a high fibre fraction and a higher protein "enhanced DDGS" fraction (Srinivasan et al., 2009), with a protein content of 41% and slightly increased fat content of 14% (Srinivasan et al., 2005). This process produces three product streams, including a small particle size DDGS and a larger particle size fibre fraction with up to 37% increased crude fibre in addition to traditional DDGS. This process is reported to be economically viable, producing modified DDGS at a cost of 84c/t (Srinivasan, 2016) and payback time for costs of installation of less than 5 years. The enhanced DDGS has been used successfully in broilers resulting in higher metabolizable energy and increased bodyweights (Kim et al., 2010; Loar et al., 2009) and also improved FCR and breast meat yield (Srinivasan et al., 2013).

SEPARATION OF OIL FROM BIOETHANOL PROCESS

Distiller's corn oil is a relatively new product, recovered from the liquid fraction post separation of the stillage, which has seen major uptake in bioethanol plants in the last five years. In 2018, the U.S. ethanol industry produced nearly 4 billion pounds of corn distillers' oil (RFA 2018), with over 85% of plants new extracting oil during the process. This corn oil is used by both the biodiesel industry (as a feedstock) and as a raw material for the animal feed industry, with a similar fatty acid profile to refined corn oil but is unable to be used for food purposes as it is structurally degraded. However, the content of unsaturated fatty acids is desirable for poultry feed, especially since it contains over 50% linoleic acid (Kerr et al., 2016). Distiller's corn oil appears to be relatively consistent in terms of fatty acid profiles and digestibility (Kerr et al., 2016) and inclusion in broiler diets did not adversely affect performance or yield (Kim et al., 2013), with an improvement in pellet quality reported by the same authors. DDGS produced after removal of the corn oil has a reduced fat content of between 4 and 8% compared with 8 to 12% seen in unmodified DDGS (Reis et al., 2017).

SEPARATION OF HIGH PROTEIN PRODUCTS

During the bioethanol fermentation, yeast multiplies, until it represents 8-10% of the dry matter of DDGS, with 0.071g yeast produced for every gram of starch (Spencer-Martins and Van Uden, 1977). A 400 million litre bioethanol plant could potentially produce 48 thousand tonnes of yeast per annum, if it can be isolated in an economically viable way. Yeast is a valuable protein, rich in minerals and vitamins, and its cell wall contains mannanoligosaccharides which have been shown to have positive effects on performance in poultry (Hooge et al., 2003). Yeast cell walls are often suggested as a prebiotic, with studies showing effects on immune factors, gut microbiota and improvements in gut morphology and mucin production (Santin et al., 2001; Moralez-Lopez et al., 2009).

Yeast separation

Yeast has commonly been removed from potable distilleries to reduce the concentration in the fermentation, by usually by a mechanical process such as membrane filtration or centrifugation. The filtration process has high energy and water usage, using a stack of Teflon membranes with high vibration to encourage filtration. Centrifugal procedures can include a decanter, to rapidly separate a fibre fraction, or a sedicanter (combined decanter and separator) which can clarify the suspension. The producers of the latter claim a lower power se and increased alcohol yield with this process (Gertsman, 2016). The yeast cream produced by these processes can be de-watered (for example by a disc separator), fed as cream, or dried to a powder product.

Yeast protein concentrate

A yeast protein concentrate has been produced using a decanter, with a nozzle centrifuge to produce a yeast cream, which is then dried to a power, with a DDGS product with lowered protein content (around 5% reduction in protein). This process can be added to first generation bioethanol plants with few engineering changes, and with a payback for the process in approximately two years (Williams, 2019). Interestingly this process has been reported to reduce DDGS variability, suggesting some of the nutrient variability seen in DDGS may be due to differences in yeast content.

Yeast protein from bioethanol has been fed successfully to several species, including fish, with up to 20% inclusion giving optimal performance in carp and bass (Gause and Trushenski, 2011; Omar et al, 2012). Knott and Shurson (2004) fed a yeast cream from corn bioethanol to pigs with improvements in gain feed ratio. In poultry, a study showed that feeding yeast can reduce serum cholesterol in broilers while increasing performance (Ahmed et al., 2015).

Bioethanol yeast protein concentrate has been shown to have a digestible amino acid content comparable with soya for broiler chicks (Burton et al., 2013) although this is influenced by the drying process, with severe drying reducing digestibility (Scholey et al., 2016). Bioethanol production may produce a toughened cell wall due to the high ethanol environment, which is resistant to proteolysis (Caballero-Cordoba and Sgarbieri, 2000), and disrupted cell walls improve nutrient utilisation when compared to whole yeast (Rumsey et al., 1991). Processing which produces high shear forces is therefore preferable as this provides access enzymes to the nutrients within the yeast.

Studies on broilers with up to 17.5% YPC improved broiler performance (Scholey et al, 2014) Digesta viscosity was also noted to increase with content of yeats protein from bioethanol, potentially as non-starch polysaccharides are also being seperated alongside the yeast (Scholey et al., 2011). However, as enzyme use of NSP-ases is now ubiquitous in the poultry industry, any antinutritional effects of increased visocisty should be simple to mitigate. The 17.5% inclusion did not have any detrimental effect on pellet quality (Burton et al., 2013), as previously mentioned, can sometimes be seen with DDGS inclusion.

SEPARATION OF OTHER HIGH VALUE PRODUCTS

It may be possible to harvest high value components such as amino acids, phytosterols and organic acids from bioethanol stillage, which allows further applications and further enhances the economic sustainability of the process.

Phosphorus

Cereal contains considerable phytate phosphorus and during ethanol production this is concentrated around three times in the DDGS (Almeida and Stein 2010). However, around half of the phosphorus present remains as phytate due to the conditions used during fermentation (Lui and Han, 2011), though there is an increase in the ratio of phytate to phosphorus of almost 50%, suggesting some phytate is degraded. Recent development of ion exchange technologies have been shown to be both efficient and specific in recovering phosphorus and phytate

(He et al., 2017), without effecting the other components of stillage, but whether the recovery of phosphorus is economically viable when exogenous p[phytase is so widely used in animal feed is debatable. Phytase can be added during the ethanol fermentation to release phosphorus, which is reported to have the additional benefit of improving production efficiency (Shetty et al., 2008)

Carotenoids

Carotenoid content is concentrated in DDGS and distiller's corn oil (as they are fat soluble) and are reported as being as bioavailable as commercial products sold for pigmentation purposes (Viguie et al., 2016). In countries where a yellow pigmentation of the skin is desirable, inclusion of distillery co-products can be utilised to improve skin colour, in addition to serving as an energy source (Cortes-Cuevas et al., 2015).

It is notable that distiller's corn oil is amber in colour due to high lutein and zeaxanthin levels (Moreau et al., 2007), which are important xanthophylls for treatment of macular degeneration. There may be potential benefits for addition of these products to poultry feed to improve colour of yolks and poultry meat, while potentially adding value in terms of enrichment for human health. A patent has been disclosed for extraction of these products and beta-carotene (Englert and Milos, 2014), but it may be more cost effective to feed the oil direct for the benefits of these compounds.

Phytosterols

There is potential for phytosterol production from distiller's corn oil with them making up around 5% on a dry matter basis (Moreau et al. 2010,) Phytosterols have potentially high value in reduction of low density lipo-proteins for human health. These have been widely characterised in DDGS but it can be assumed these have decreased since oil extraction has become common. The presence of tocopherols and tocotrienols may also have positive benefits in terms of the oxidative stability of corn oil and therefore, potentially, feed (Winkler-Moser and Breyer, 2011).

CONCLUSION

As the production of bioethanol increases globally, there is great potential for the utilisation of high nutrient value co-products in poultry feed. In addition to the use of relatively low levels of traditional DDGS, there are options for back end processing to add economic value and increase sustainability of production. Production of protein streams, oil streams, enhanced DDGS and specific high value products can reduce the issues around feed versus fuel seen with first generation bioethanol and help bridge the gap until later generations of bioethanol are viable.

REFERENCES

AHMED, M.E., ABBAS, T.E., ABDLHAG, M.A. AND MUKHTAR, D.A. (2015) Effect of dietary yeast (saccharomyces cerevisiae) supplementation on performance, carcass characteristics and some metabolic responses of broilers. Animal and Veterinary Sciences, 3: 5-10

ALMEIDA, F., STEIN, H. (2010) Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. Journal of Animal Science, 88: 2968–2977

ARIFIN, Y., TANUDJAJA, E., DIMYATI, A, PINONTOAN, R. (2014) A second generation biofuel from cellulosic agricultural by product fermentation using Clostridium species for electricity generation. Energy Procedia, 47: 310–5.

BURTON, E.J., SCHOLEY, D.V. AND WILLIAMS, P.E.V. (2013) Use of cereal crops for food and fuel - Characterization of a novel bioethanol co-product for use in meat poultry diets. Food and Energy Security, 2: 197-206

CABALLERO-CORDOBA, G.M. AND SGARBIERI, V.C. (2000) Nutritional and toxicological evaluation of yeast (Saccharomyces cerevisiae) biomass and a yeast protein concentrate. Journal of the Science of food and Agriculture, 80: 341-351

CORTES-CUEVAS, A., RAMIREZ-ESTRADA, S., ARCE-MENOCAL, J., AVILA-GONZALEZ, E., LOPEZ-COELLO, C. (2015) Effect of feeding low-oil DDGS to laying hens and broiler chickens on performance and egg yolk and skin pigmentation. Brazilian Journal of Poultry Science, 17: 247–254

ENGLERT, J., MILOS, K. (2014) Methods for producing carotenoids from fermentation by-products. US Patent App. 14/891,004, 201

GAUSE, B. AND TRUSHENSKI, J. (2011) Production performance and stress tolerance of sunshine bass raised on reduced fish meal feeds containing ethanol yeast. North American Journal of Aquaculture, 73: 168-175

GERTSMAN, A. (2016) Management of surplus yeast in modern breweries. Alexander, World Brewing Congress, Aug 13-17 2016

HAVLIK, P., SCHNEIDER, O.A., SCHMID, E., BOTTCHER, H., FRITZ, S., SKALSKY, R., et al. (2011) Global land-use implications of first and second-generation biofuel targets. Energy Policy, 39: 5690–702.

HE, Q., RODRIGUES, REIS, C.E., WANG, F., HU, B. (2017) Phytate extraction from coproducts of the dry-grind corn ethanol process. RSC Advances, 7(9): 5466–547

HOOGE, D.M., SIMS, M.D., SEFTON, A.E., CONNOLLY, A. AND SPRING, P.S. (2003) Effect of dietary mannanoligosaccharide, with and without bacitracin or virginiamycin, on live performance of broiler chickens at relatively high stocking density on new litter. Journal of applied poultry research, 12: 461-467

ILELEJI, K.E., PRAKASH, K.S., STROSHINE, R.L. AND CLEMENTSON, C.L. (2007) An investigation of particle segregation in corn processed dried distiller's grains with solubles (DDGS) induced by three handling scenarios. Bulk Solids Powder Science Technology, 2: 84–94.

KERR, B.J., DOZIER, W.A., SHURSON, G.C. (2016) Lipid digestibility and energy content of distillers' corn oil in swine and poultry. Journal of Animal Science, 94: 2900–2908.

KIM, E.J., PARSONS, C.M., SRINIVASAN, R., SINGH, V. (2010) Nutritional composition, nitrogen-corrected true metabolizable energy, and amino acid digestibilities of new corn distillers dried grains with solubles produced by new fractionation processes. *Poultry Science*, 89 (1): 44-51

KIM, E.J., PURSWELL, J.L., DAVIS, J.D., LOAR, R.E., KARGES, K. (2013) Live production and carcass characteristics of broilers fed a blend of poultry fat and corn oil derived from distillers dried grains with solubles. Poultry Science, 92: 2732–2736.

KNOTT, J.S. AND SHURSON, G.C. (2004) Nutritional value of yeast by-products from Ethanol Production for young pigs. Proceedings of the 65th Minnesota Nutrition Conference, St. Paul, MN. 65: 142-159

LIU, K., HAN, J. (2011) Changes in mineral concentrations and phosphorus profile during dry-grind processing of corn into ethanol. Bioresource Technology, 102: 3110–3118

LOAR, R.E., SRINIVASAN, R., KIDD, M.T., DOZIER, W.A. AND CORZO, A. (2009) Effects of elutriation and sieving processing (Elusieve) of distillers dried grains with solubles on the performance and carcass characteristics of male broilers. Journal of Applied Poultry Research, 18: 494-500

LOAR, R.E., MORITZ, J.S., DONALDSON, J.R. AND CORZO, A. (2010) Effects of feeding distillers dried grains with solubles to broilers from 0 to 28 days' post hatch on broiler performance, feed manufacturing efficiency and selected intestinal characteristics. Poultry Science, 89: 2242-2250

LIU, K. (2011) Chemical composition of distiller's grains, a review. Journal of Agricultural and Food Chemistry, 59: 1508–1526.

MORALES-LOPEZ, R., AUCLAIR, E., GARCIA, F., ESTEVE-GARCIA, E. AND BRUFAU, J. (2009) Use of Yeast cell walls: Beta-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poultry Science, 88: 601-607

MOREAU, R.A., JOHNSTON, D.B., HICKS, K.B. (2007) A comparison of the levels of lutein and zeaxanthin in corn germ oil, corn fiber oil and corn kernel oil. Journal of the American Oil Chemists Society, 84: 1039–1044

MOREAU, R.A., HICKS, K.B., JOHNSTON, D.B., LAUN, N.P. (2010) The composition of crude corn oil recovered after fermentation via centrifugation from a commercial dry grind ethanol process. Journal of the American Oil Chemists Society, 87: 895–902

NIGAM, P.S., SINGH, A. (2011) Production of liquid biofuels from renewable resources. Progress in Energy and Combustion Science, 37: 52–68.

OMAR, S.S., MERRIFIELD, D.L., KUHLWEIN, H., WILLIAMS, P.E.V. AND DAVIES, S.J. (2012) Biofuel derived yeast protein concentrate (YPC) as a novel feed ingredient in carp diets. Aquaculture, 330-333: 54-62

REIS, C.E.R., RAJENDRAN, A., HU, B. (2007) New technologies in value addition to the thin stillage form corn-to-ethanol process. Reviews in Environmental Science and Bio-technology, 16: 175-206

RENEWABLE FUELS ASSOCIATION (RFA) (2018) Ethanol co-products. https://ethanolrfa.org/resources/industry/co-products/ Accessed May 2019

ROSENTRATER, K.A. (2006) Some physical properties of distillers dried grains with solubles (DDGS). Applied Engineering in Agriculture, 22 (4): 589–595.

RULLI, M.C., BELLOMI, D., CAZZOLI, A., CAROLIS, G.D., ODORICOA, P.D. (2016) The water-land-food nexus of first-generation biofuels. Scientific.Reports, 6: 22521. doi: 10.1038/srep22521.

RUMSEY, G.L., KINSELLA, J.E., SHETTY, K.J. AND HUGHES, S.G. (1991) Effect of high dietary concentrations of brewer's dried yeast on growth performance and liver uricase in rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology, 33: 177-183

SALIM, H.M., KRUK, Z.A., LEE, B.D. (2010) Nutritive value of corn distillers dried grains with solubles as an ingredient of poultry diets: A review. Worlds Poultry Science Journal, 66: 411–432.

SANTIN, E., MAIORKA, A. AND MACARI, M. (2001) Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. Journal of Applied Poultry Research, 10: 236-244

SARAGONI, P., AGUILERA, J. M., AND BOUCHON, P. (2007) Changes in particles of coffee powder and extensions to cake. Food Chemistry, 104: 122–126.

SCHOLEY, D.V., MORGAN, N., WILLIAMS, P. AND BURTON, E.J. (2011) Effects of incorporating a novel bio-ethanol co-product in poultry diets on bird performance. British Poultry Abstracts, 7: 57

SCHOLEY, D., WILLIAMS, P. AND BURTON, E.J. (2014) Effect of alcohol derived yeast protein concentrate in broiler chick diets on pellet quality, bird performance and bone mineralisation. Proceedings of the 14th European Poultry Conference, P96

SCHOLEY, D., WILLIAMS, P., BURTON, E. (2016) Effect of drying process on amino acid digestibility of yeast protein separated from potable alcohol distilleries. *In*: Sustainable Poultry Production in Europe (Eds, Burton et al.): 285-286

SHETTY, J.K., PAULSON, B., PEPSIN, M., CHOTANI, G., DEAN, B., HRUBY, M. (2008) Phytase in fuel ethanol production offers economical and environmental benefits. International Sugar Journal, 110:160

SHIM, M.Y., PESTI, G.M., BAKALLI, R.I. (2011) Evaluation of corn distillers dried grains with solubles as an alternative ingredient for broilers. Poultry Science, 90: 369-376

SHURSON, G.C. (2017) The Role of Biofuels Coproducts in feeding the world sustainably. Annual Review of Animal Biosciences, 5: 229–254.

SPENCER-MARTINS, I. AND VAN UDEN, N. (1977) Yields of Yeast growth on starch. European Journal of Applied Microbiology, 4: 29-35

SRINIVASAN, R., MOREAU, R.A., RAUSCH, K.D., BELYEA, R.L., TUMBLESON, M.E., SINGH, V. (2005) Separation of fiber from distillers dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chemistry, 82: 528-533

SRINIVASAN, R., MOREAU, R.A., PARSONS, C., LANE, J.D. AND SINGH, V. (2008) Separation of fiber from distillers dried grains (DDG) using sieving and elutriation. Biomass and Bioenergy, 32: 468-172

SRINIVASAN, R., TO, F. AND COLUMBUS, E. (2009) Pilot scale fiber separation from distillers dried grains with solubles (DDGS) using sieving and air classification. Bioresource Technology, 100: 3548-3555

SRINIVASAN R., LUMPKINS, B., KIM, E., FULLER, L., JORDAN, J. (2013) Effect of fiber removal from ground corn distillers dried grains with solubles and soybean meal using the elusieve process on broiler performance and processing yield. Journal of Applied Poultry Research, 22: 177-189

SRINIVASAN, R. (2016) Fractionation of DDGS using sieving and air classification. In: Distiller's grains: Production, Properties and Utilization (Eds. K. Li, K. Rosentrator)

SWIATKIEWICZ, S., KORELESKI, J. (2008) The use of distillers dried grains with solubles (DDGS) in poultry nutrition. ´ Worlds Poultry Science Journal, 64: 257–266.

TAO, J., YU, S., WU, T. (2011) Review of China's bioethanol development and a case study of fuel supply, demand and distribution of bioethanol expansion by national application of E10. Biomass Bioenergy, 35: 3810–29.

VIGUIE, M., LIVINGSTON, K.A., MALHEIROS, R., FERKET, P.R. (2016) Effect of corn distillers solubles oil on broiler chicken skin pigmentation and plasma carotenoid content. Poultry Science, 95 (E-Suppl. 1): 13.

WHEALS, A.E., BASSO, L.C., ALVES, M.G. AND AMORIM, H.V. (1999) Fuel ethanol after 25 years. Trends in Biotechnology, 17: (12) 482-487

WILLIAMS, P. (2019) Long in energy - short in protein, a role for the bioethanol industry. British Poultry Abstracts, 15

WINKLER-MOSER, J.K., BREYER, L. (2011) Composition and oxidative stability of crude oil extracts of corn germ and distillers' grains. Industrial Crops and Products, 33: 572–578

Session 2: Nutrition of layers

'Focus on extended laying cycles'
Marathon versus 100 metres – what are the hallmarks of persistency in late lay?

Cormac O'Shea

University of Nottingham

Extending the length of the lay cycle in laying hen flocks is of interest to the industry in terms of greater utilisation of resources and profitability. The concept of 500 eggs at 100 weeks of age is now a key target of breeding companies, producers and associated disciplines. However the obstacles to longer time in lay are a general decline and greater variability in bird productivity, health and welfare, and egg quality as flocks progress from the mid to late lay cycle. Applied research in terms of nutrition and management has justifiably focused on addressing the problems which are manifest in late lay including bone status, egg quality and persistency in lay. While the genetic potential of the contemporary laying hybrid is remarkable, myriad factors prior to and post-hatch, during rearing and early lay may have implications for actual field outcomes for both individual animals and more generally for flocks. There is great need and potential to join up various scientific disciplines to produce and support resilient animals capable of optimal performance up to and exceeding 100 weeks. An area where greater knowledge is required is to understand the extent of variation for multiple traits between individual hens and how these change within individuals over time. Under experimental conditions, marked and persistent variation between individuals has been observed where some laying hens capable of producing in excess of 500 eggs have been recorded. Greater profiling of the general attributes of such animals and likewise individual animals with poor persistency will contribute to strategies for improvement and foster readiness for the advances in genetic potential that are underway.

EXTENDING THE LAY CYCLE TO 100+ WEEKS- CHALLENGES FOR THE HEN

A typical modern brown commercial laying hen is currently capable of an average of 86% hen day production from 20 until 90 weeks of age. From around 22-23 weeks of age egg production rates exceed 90% until approximately 50 weeks of age and decline steadily to approximately 74% as the flock approach 90 weeks of age. Across the laying phase, this rate of lay results in an egg mass of approximately 25kg and a feed conversion ratio of 2:1. (Hyline Brown, Management Guide UK). This tremendous output from a fairly static mature bodyweight of approximately 2kg reflects the culmination of successful breeding programs, supported by a greater understanding of the husbandry, veterinary and nutritional needs of hens throughout the rearing and specific laying phases.

The rate of improvement in egg output due to genetic selection has now slowed as the biological limits of egg formation rate are reached, at least in the early and mid-lay phases. Increasing the total output per hen are now focused on extending the lay cycle in terms of maintaining egg output to 100 weeks and beyond. Importantly, this goal is planned in the absence of leveraging the moulting process to rejuvenate the reproductive tract. This approach is feasible but beset with challenges that extend from the health and productivity of the hen to the quality and safety of the egg. Variation in production type from cage through to organic systems add complexity to identifying the best approach to managing flocks in extended laying cycles. The rate of lay undoubtedly has consequences for the physiology of the hen. From mid-lay onwards, some housing-specific conditions impact; such as fatty liver haemorrhagic syndrome in cage birds (SHINI et al., 2019), keel bone damage in multi-tier systems, infectious disease in outdoor birds (STOCKHOLM et al., 2010) or more general conditions such as osteoporosis (RICZU et al., 2004) and gout (FULTON et al., 2017). Whether such disease is a consequence of the aging process or a consequence of increasing risk due to longer times in lay or both, there are clear welfare concerns of increased morbidity and mortality associated with longer lay cycles. Interestingly, quite significant pathology such as fatty liver haemorrhagic syndrome may be present from at least 45 weeks of age in cage birds and associated with reduced productivity (unpublished data) but not necessarily manifest as increased morbidity and mortality until later in lay (SHINI et al., 2019).

DIMINISHING EGG QUALITY AND SAFETY IN AGED HENS

The egg is arguably less vulnerable to the passage of time as the animal, due to the evolutionary prioritisation of ensuring the survival of offspring (SAUVANT, 1994; LOESTSCHER et al., 2014). Thus the composition of the egg is essentially maintained throughout the lay cycle. Regardless, the late lay phase (70 weeks) is characterised by a gradual decline in external and internal egg quality. For the former, eggshell integrity is a challenge throughout the production chain as shell integrity weakens, while the latter results in lower Haugh unit scores and hence a greater proportion of lower grade eggs. SAMIULLAH et al. (2016) reported an increase in lighter coloured brown eggs with increasing flock age. There is not enough information from the literature on whether these visual and structural attributes progressively worsen past 75 weeks and therefore is an area that merits further investigation and intervention.

Egg safety is also a consideration in a lengthened lay cycle. Horizontal transmission is considered the principle route to egg contamination which arguably may occur at a greater rate when the egg shell quality declines. ROBERTS et al. (2013) showed an increase in translucency scores in late lay (>65 weeks), although not a reduction in cuticle coverage. However a change in cuticle composition associated with a decline in polysaccharides and lipids has been reported in aged Hyline hens and may have implications for egg contamination (RODRÍGUEZ-NAVARRO et al., 2013). Declining albumen quality is a feature of aged hens (Table 2). As an important source of defence against pathogens due an unfavourable pH and the presence of various antimicrobial peptides, it merits further investigation to assess whether albumen from aged hens can continue to effectively arrest microbial growth.

It is not fully understood if age increases the risk of egg contamination through vertical transmission. As mentioned earlier, late lay is characterised by an increased vulnerability to various infectious and metabolic diseases. If a greater pathogen load and diminished immune status were a feature of aged hens, it follows that the relative safety of the egg may be compromised. ZHENG and YOSHIMURA (1999) reported that aged hens (~90 wks) retained or had greater number of macrophages localised to the oviduct when compared with early lay hens. However a decrease in egg yolk IgY and an increase in plasma IgG was later observed in aged hens (BARUA et al., 2000). YOSHIMURA (2004) commented on the general decline in immunocompetent cells in aged hens. There is a paucity of data on the resilience of eggs from aged hens to microbial contamination via both vertical and horizontal transmission in the literature and is an area which merits further consideration in the context of a 100 week lay cycle.

OPTIMISING EGGSHELL AND SKELETAL INTEGRITY IN LATE LAY

Applied research has focused on manipulating nutrition (e.g. calcium, various supplements, feed strategies), husbandry (early life; bodyweight rate of gain in rearing, lighting) and health (e.g. vaccinations) to support the considerable demands placed on the hen throughout lay. Generally there is reasonable amount of information from the literature on the various management strategies in early life and throughout lay that may beneficially impact productivity and egg quality up to conventional end of lay (~70 weeks). There is a considerable dearth of information beyond this time period with some exceptions. Extrapolation of the productivity of hens and changes in general health, skeletal and egg characteristics from 70 weeks to 100+ weeks may predict either a continued decline in these variables or a levelling off. This is an area where more information is required to guide adjustment of current strategies and inform on new approaches to support the hen in late lay.

Improvement in eggshell and skeletal integrity in late lay has stimulated focus on optimising the macromineral content of the diet. The literature is considerable in this area up to approximately 70 weeks of age and will not be discussed here in detail. Generally, the literature reports a benefit of dietary Ca to 4% to 4.5% in terms of egg output and eggshell integrity, with less success in maintaining skeletal integrity (SAFAA et al., 2008). The challenge with Ca that is rather unique to layers is the high dietary inclusion rate and hence the high ratio of Ca to phosphorus relative to other non-ruminants, which may impact the availability of phytate-bound phosphorus (Grynspan and Cheryan 1983), and negatively impact digestion generally as a consequence of the high acid buffering capacity of calcium. Calcium particle size has shown promise as a means to meter the availability of luminal Ca in line with eggshell synthesis demands in aged hens (MOLNAR et al., 2016; ZHANG et al., 2017) and may be a more practical strategy than providing an alternative source of Ca. The response of eggshell and skeletal integrity to phytase has not been as evaluated to the same extent as with broilers but studies are promising where suboptimal levels of Ca and/or P are fed (Lim et al., 2003; Silverside et al., 2006). Factors that may affect phytase efficacy include the digestive maturity of the hen and whether feed is offered as mash or pellets. Marounek et al.

(2008) reported an age-associated increase in endogenous phytase activity in hens. Endogenous plant-derived phytase activity may be greatest when feed is not steam-pelleted. The role of phytase in hens greater than 70 weeks of age has not been thoroughly evaluated and it seems likely that there is potential to achieve greater utility in a scenario where eggshell quality is suboptimal.

The biochemical flux of microminerals such as Na, K and Cl changes sharply during the shell deposition process and it follows that this is a subject which merits further examination in the context of declining eggshell integrity in late lay. Yoruk et al. (2004) demonstrated improvements in a range of production variables such as mortality and feed efficiency in response to increased dietary sodium bicarbonate. The paradigm shift for many producers in this context is the impact of grass consumption under outdoor systems and the implications of a disrupted dietary electrolyte balance as a consequence of greater potassium intake. This has been discussed in detail by Singh and Cowieson (2013). Consideration of the adjustments required for dietary microminerals in late lay in both caged and free range systems is another area which will benefit from further attention.

Lessons from studying the individual hen; what are the hallmarks of high performance?

Understanding variation between animals for some production traits such as bodyweight is estimable from commercial systems; however the variation of, and relationships between important traits such as feed intake and bodyweight between animals and within animals over time are only possible under experimental conditions. Similarly, while an estimate of the variability in important egg characteristics may be achieved through random sampling, it is only possible to investigate associations between the egg and the hen using highly controlled experimental conditions. Characterising individual animals through the lens of animal breeding and genetics is an essential and well-established practice. Evaluating individual animals has a less obvious benefit from the perspective of nutritionists and technical disciplines partly based on the understanding that contemporary hybrid layers are relatively homogenous and rise or fall collectively based on their environmental conditions. The potential to capture the production parameters of individual animals has been markedly advanced through emerging technology and is an area where further advances can be enabled if prioritised. Some important examples of the application of various technologies to facilitate individual animal monitoring now exist for poultry (Puma et al., 2001; Tu et al., 2011; Aydin et al., 2014) including laying hens (Siegford et al., 2016). Using an individually caged experimental set-up, Akter et al. (2018) showed that mid-lay hens offered ad libitum access to a common diet exhibited considerable variation in voluntary feed intake, but comparatively less variability in rate of lay and egg mass, resulting in variation in feed to egg conversion ratio (FCR). This research showed that within a flock, some animals will exceed the breed target in terms of feed to egg efficiency and egg output while other animals will fail to achieve these targets. This inherent heterogeneity presents challenges for estimating the scale of response of nutritional and management intervention across all stages of lay and for the current focus of extending the lay cycle to 100+ weeks.

This work has been extended recently to understand the extent of variation in key production and egg traits in early, mid and late lay in a flock of individually caged, ad libitum fed ISA Brown hens (Tables 1 & 2). As expected, tracking a caged flock over time showed that production traits and egg quality decline gradually from early to late lay. It is worth noting, with the caveat that the data is from singly-caged hens under optimised experimental conditions, that the decline in persistency (92% rate of lay at 75 weeks) is better than suggested for the breed. This data also show that under highly controlled environmental conditions, tracking the same group of hens over time, that variation for production and egg quality traits is lowest in early lay and generally increases as those hens progress towards the late lay period (70+ weeks; Table 1 & 2). What this research has also suggested is that key production traits such as bodyweight, feed intake and estimates such as feed efficiency are remarkably stable for individual animals over time. For example, a hen exhibiting low feed intake relative to the average feed intake of the flock during early lay is likely to remain at a comparatively low feed intake level throughout mid and late lay. From this research, Akter et al. (2019) reported on the attributes of 45 week hens ranked on the basis of FCR throughout early lay. The characteristics of hens ranked as exhibiting low FCR (≤1.8) were a bodyweight close to the breed standard (1.9 kg; ISA Brown Management Guide, UK), while hens with a high FCR (≥2.3) were heavier (2.37 kg) and had greater liver and abdominal fat pad weight, and a greater incidence of FLHS at post mortem. Importantly, when retrospectively analysed, the divergence in bodyweight and appetite was evident (albeit to a far lesser extent) from prior to the onset of lay. This reinforces the concept that the period approaching onset of lay and early lay are important junctures at which the production profile of the entire cycle of individual birds may be strongly influenced. There is a lack of up-to-date research on the rearing conditions of brown layers on subsequent performance during the lay cycles. While it is reasonable to assume that pre- and early lay factors which promote persistency and other important traits to 70 weeks of age will be relevant in extending the lay cycle to 100+ weeks it is clear that more research is needed. Based on the preliminary findings reported here, it is unclear at the present time on the plasticity of production traits for individual hens once onset of lay has commenced. It is conceivable that inefficient hens will respond to dietary and management intervention but less apparent what the response would be of hens which are performing in excess of the breed target.

SUMMARY

Extending the lay cycle to 100+ weeks carries the risk of a general decline in productivity, welfare and egg quality and safety. Understanding the transitional physiology of individual hens which may be characterised as poor or exceptional performers in terms of persistency and other important traits will be greatly enabled by technology. Understanding the various factors that impact performance from preceding hatch, through rearing and during lay will allow fine tuning of nutritional and management strategies to achieve better uniformity across a range of important production and egg quality traits in late lay.

Table 1: Mean values and variability of production traits of ad libitum fed, individually caged ISA brown hens in early and late lay (n = 119)[1]

	Bodyweight (g)	ADFI (g)	BWC (g, 6 weeks)[2]	Rate of Lay (%)[3]	Egg weight (g)	Egg Mass (g)	FCR
25-30 weeks	2020	125	214	98	62	60	2.05
CV (%)	9.3	10	45	2.2	5.8	7.5	11
35-40 weeks	2078	117	57	97	64	62	1.92
CV (%)	10.5	10.3	132	3.8	6.5	8.8	8.5
70-75 weeks	2252	122	83	92	66	61	2.06
CV (%)	12	9.7	149	9.4	6.9	9.7	16
Range	(1297-3149)	(93-182)	(-670-266)	(62-100)	(49-78)	(45-73)	(1.60-4.58)

[1]All birds offered a wheat-soybean meal based diet in mash form

[2]Bodyweight change over 6 week period

[3]Hens not achieving 90% lay for the period 25-30 weeks were excluded from the data

Table 2: Average values and variability of egg traits of individually caged ISA brown hens in early and late lay (n = 27)

	Egg weight (g)	Albumen height (mm)	Haugh Unit	Albumen: yolk ratio	Shell weight g, (%)	Shell thickness[1]	Shell breaking strength	Yolk colour[2]
35-40 weeks	63	10	98	2.5	6.4 (10)	0.42	4813	11
CV (%)	4.2	10.5	4.8	7.5	4.3 (4.6)	2	11	-
70-75 weeks	65	8.2	89	2.2	6.2 (9.5)	0.47	4016	11
CV (%)	6	13	7.2	11	7	5.6	9.9	-
Range	(58-77)	(6-10)	(75-101)	(1.8-2.7)	(5-7; 7.5-10.6%)	(0.39-0.51)	(3209-4865)	(9-13)

[1]Average value calculated from thickness measured at poles and equator

[2]Measured with a DSM colour fan

ACKNOWLEDGEMENTS

The preliminary results presented here were supported by a grant awarded by Australian Eggs and a PhD Scholarship from the Schlumberger Foundation.

REFERENCES

AKTER, Y., S. GREENHALGH, M.R. ISLAM, C. HUTCHISON AND C.J. O'SHEA, 2018: Hens ranked as highly feed efficient have an improved albumen quality profile and increased polyunsaturated fatty acids in the yolk. Journal of Animal Science 96, 3482-3490.

AKTER, Y., P.J. GROVES, S.Y. LIU, A.F. MOSS, D. ANENE AND C.J. O'SHEA, 2019: Association of feed to egg efficiency with body weight and digestive organ characteristics in laying hens. Proceedings of the Australian Poultry Science Symposium, 30 (ISSN-1034-6260), 249-252

AYDIN, A., C. BAHR, S. VIAZZI, V. EXADAKTYLOS, J. BUYSE AND D BERCKMANS, 2014: A novel method to automatically measure the feed intake of broiler chickens by sound technology. Computers and Electronics in Agriculture 101, 17-23.

BARUA, A., S. FURUSAWA AND Y. YOSHIMURA, 2000: Influence of Aging and Estrogen Treatment on the IgY Concentration in the Egg Yolk of Chicken, *Gallus domesticus*. Japanese poultry science 37, 280-288.

FULTON, R.M., 2017: Causes of Normal Mortality in Commercial Egg-Laying Chickens, Avian Diseases 61 (3)

GRYNSPAN, F. AND M. CHERYAN, 1983: Calcium phytate: Effect of pH and molar ratio on in vitro solubility. Journal of the American Oil Chemists' Society 60, 1761-1764.

LIM, H.S., H. NAMKUNG AND I.K. PAIK, 2003: Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. Poultry Science 82, 92-99.

LOESTSCHER, Y., M. KREUZER AND R.E. MESSIKOMMER, 2014: Late laying hens deposit dietary antioxidants preferentially in the egg and not in the body, The Journal of Applied Poultry Research, 23, 647–660

MAROUNEK, M., M. SKŘIVAN, G. DLOUHÁ AND N. BŘEŇOVÁ, 2008: Availability of phytate phosphorus and endogenous phytase activity in the digestive tract of laying hens 20 and 47 weeks old. Animal Feed Science and Technology 146, 353-359.

MOLNÁR, A., L. MAERTENS, B. AMPE, J. BUYSE, J. ZOONS AND E. DELEZIE, 2016: Supplementation of fine and coarse limestone in different ratios in a split feeding system: Effects on performance, egg quality, and bone strength in old laying hens. Poultry Science 96, 1659-1671.

PUMA, M. C., H. XIN, R. S. GATES AND D. J. BURNHAM, 2001: An instrumentation system for studying feeding and drinking behaviour of individual poultry. Applied Engineering in Agriculture 17, 365-374.

RICZU, C.M., J. L. SAUNDERS-BLADES, HJK. YNGVESSON, F. E. ROBINSON AND D. R. KORVER, 2004: End-of-Cycle Bone Quality in White- and Brown-Egg Laying Hens, *Poultry Science*, 83, 375–383

ROBERTS, J. R., K. CHOUSALKAR AND SAMIULLAH, 2013: Egg quality and age of laying hens: implications for product safety. Animal Production Science 53, 1291-1297.

RODRÍGUEZ-NAVARRO, A. B., N. DOMÍNGUEZ-GASCA, A. MUÑOZ AND M. ORTEGA-HUERTAS, 2013: Change in the chicken eggshell cuticle with hen age and egg freshness. Poultry Science 92, 3026-3035.

SAFAA, H. M., M. P. SERRANO, D. G. VALENCIA, M. FRIKHA, E. JIMÉNEZ-MORENO AND G. G. MATEOS, 2008: Productive Performance and Egg Quality of Brown Egg-Laying Hens in the Late Phase of Production as Influenced by Level and Source of Calcium in the Diet. Poultry Science 87, 2043-2051.

SAUVANT, D., 1994: Modelling homeostatic and homeorhetic regulations in lactating animals. Livestock Production Science. 39, 105-113.

SHINI, A., S. SHINI AND W. L. BRYDEN, 2019: Fatty liver haemorrhagic syndrome occurrence in laying hens: impact of production system, Avian Pathology, 48, 25-34

SIEGFORD, J. M., J. BEREZOWSKI, S. K. BISWAS, C. L. DAIGLE, S. G. GEBHARDT-HENRICH, C. E. HERNANDEZ, S. THURNER AND M. J. TOSCANO, 2016: Assessing Activity and Location of Individual Laying Hens in Large Groups Using Modern Technology. Animals 6, 10.

SINGH, M. AND A. J. COWIESON, 2013: Range use and pasture consumption in free-range poultry production. Animal Production Science 53, 1202-1208.

SILVERSIDES, F. G., T. A. SCOTT, D. R. KORVER, M. AFSHARMANESH AND M. HRUBY, 2006: A Study on the Interaction of Xylanase and Phytase Enzymes in Wheat-Based Diets Fed to Commercial White and Brown Egg Laying Hens. Poultry Science 85, 297-305.

TU, X., S. DU, L. TANG, H. XIN AND B. WOOD, 2011: A real-time automated system for monitoring individual feed intake and body weight of group housed turkeys. Computers and Electronics in Agriculture 75, 313-320

ZHANG, B., JUSTINA V. CALDAS AND CRAIG N. COON, 2017: Effect of Dietary Calcium Intake and Limestone Solubility on Egg Shell Quality and Bone Parameters for Aged Laying Hens. International Journal of Poultry Science. 16, 132-138.

ZHENG, W. AND Y. YOSHIMURA, 1999: Localization of macrophages in the chicken oviduct: effects of age and gonadal steroids. Poultry Science 78, 1014-1018.

Nutritional approaches to reduce or prevent feather pecking in laying hens

A. J. W. Mens[12], M. M. van Krimpen[1] and R. P. Kwakkel[2]

[1]Wageningen Livestock Research, [2]Animal Nutrition Group, Wageningen University and Research, De Elst 1, 6708 WD Wageningen, The Netherlands; annemarie.mens@wur.nl

ABSTRACT

Feather pecking (FP) by laying hens is a significant welfare issue in the poultry industry. Pecking at and pulling out the feathers of conspecifics can seriously reduce the well-being of hens, and cause economic losses for the farmer. Records of the prevalence of FP in laying hen flocks from the last 20 years show an extent between 35-94%. Several research groups hypothesised about the causes of feather pecking. From a nutritional point of view, the re-directed behaviour and feather eating causes seem most plausible. The gut microbiome seems to be involved in FP by its influence on the hormonal pathways, and being influenced by the diet – which might include feathers because of FP – ingested by the birds. Experiences during the rearing period are related to FP in later life, by the possible effects on the physiological development the pullets endure. Most likely, pullets abide a sensitive period within the first weeks post hatch, during which FP can develop due to various factors such as hormonal influences, nutrition and environmental enrichment. Nutrition could influence FP in two ways. Specific nutrients, such as amino acids and dietary fibres, can have a direct effect on physiological mechanisms, by influencing deficiencies and imbalances. Furthermore, ingredients such as roughages, fibres and non-nutritive ingredients could have an effect on feeding behaviour, by increasing eating time, exploratory or foraging behaviour by occupation or increase the level of satiety. Using nutritional strategies during the sensitive period during rearing could prevent the development of FP altogether.

INTRODUCTION

Feather pecking (FP) is a serious problem in the laying hen sector. Feather pecking is an abnormal behaviour that has severe impact on the welfare of laying hens, as well as economical losses for the farmer. Feather pecking can result in plumage damage (PD), skin damage, increased disease susceptibility (Green *et al.*, 2000), productivity decrease, increase of food consumption and increased mortality (Rodenburg *et al.*, 2013). Feather pecking occurs in all housing systems (Bilcik and Keeling, 1999).

In practice, to prevent FP outbursts and to reduce the degree of damage, the beaks of the laying hens are trimmed (Nicol, 2018). Beak trimming itself does not influence pecking preferences or frequencies (Blokhuis and Van Der Haar, 1989), but simply decreases the impact of the pecking. Birds with intact beaks show substantial more FP than debeaked birds (Lambton *et al.*, 2010). On the other hand, the interference of trimming itself is quite painful, which can lead to short and long term pain or trauma. Beak trimming has also impact on the effectiveness of preening behaviour (Nicol, 2018) and can affect oral sensing (Cheng, 2007). When part of the beak is removed, the laying hens may lose these functions.

Ethical and societal concerns about the beak trimming procedure are increasing (Vanhonacker *et al.*, 2010; Heng *et al.*, 2013; Heleski *et al.*, 2015). As of January 2019, Dutch legislation banned beak trimming, since law forbids mutilation of an animal. In Denmark the poultry branch has voluntary agreed to stop beak trimming. Other parts of Europe have a legal prohibition on beak trimming (Sweden, Norway, Finland, Germany and Austria) (Jung and Knierim, 2017). In loose housing systems, FP has more impact since the large numbers of potential victims (Keeling, 1995). The aim of this paper is to give an overview of factors affecting FP, including the extent, ontogeny and nutritional solutions of the problem.

PECKING BEHAVIOUR IN POULTRY

Within bird pecking, five types of pecking are distinguished: 1. aggressive pecking (AP), 2. gentle feather pecking (GFP), 3. severe feather pecking (SFP), 4. tissue pecking (TP) and 5. vent pecking (VP) (Savory, 1995). Severe feather pecking, TP and VP are considered injurious pecking and can cause cannibalism. Aggressive pecking is a natural behaviour and has a clear purpose, which is not comparable to the other types. Directed at the conspecifics head and neck region, AP is used by hens to establish a stable dominance hierarchy (Savory, 1995), also known as the pecking order. Gentle feather pecking and SFP are non-aggressive pecking. Both are abnormal and destructive behaviours, which is only seen in birds in captivity (Bestman, 2002).

EXTENT OF FP

Most studies claim that FP is a big problem, without giving proper support. The prevalence of FP has not been recorded extensively in the last decade. Nevertheless, the laying hen sector still claims to have problems with FP outbreaks. Table 1 provides an overview of studies in the last two decades that focussed on the extent of FP. The use of plumage damage (PD) as an indicator for FP must be interpreted with caution. Whenever a FP outbreak occurs, the plumage is damaged and denuded areas both arise rapidly. However, it is possible a further increase of FP is not noted, since the plumage of the victims is already impaired, and the denuded areas might not be observed twice, or in increasing size or severity (Kjaer, 2000). Epidemiological studies to determine the prevalence of FP usually use questionnaires or interviews with farmers or by direct observations by researchers. When comparing studies, it is suggested by Nicol *et al.* (2013) that farmers may (i.e. because of lack of recognition or lack of systematic recording) underestimate the prevalence of FP in their barns.

Table 1: Prevalence of feather pecking (FP), severe feather pecking (SFP), gentle feather pecking (GFP) and plumage damage (PD) since 1999

Study	Country	Results	Reference
59 flocks	Sweden	PD: 62%	Gunnarsson *et al.* (1999)
32 aviary rearing flocks 34 deep litter rearing flocks	Switzerland	FP deep litter: 37.5% FP aviaries: 35.9%	Huber Eicher (1999)
214 beak trimmed flocks	United Kingdom	FP: 57%	Green *et al.* (2000)
25 flocks, starting at rearing	Switzerland	FP: 40% (wk 5) FP: 77.3% (wk 14) PD: 92.4% (wk 50)	Huber Eicher and Sebo (2001)
Beak trimmed flocks and organic flocks	The Netherlands	FP trimmed: 30-60% FP organic: 75%	Bestman (2002)
63 organic flocks	The Netherlands	PD moderate: 19% PD severe: 52%	Bestman and Wagenaar (2003)
Beak trimmed flocks	Switzerland	PD: 30% with more than 50% damaged	Häne *et al.* (2007)
26 flocks within 4 different housing systems	United Kingdom	PD: 15.5 – 26.9%	Sherwin *et al.* (2010)
61 free range and organic laying farms	United Kingdom	GFP: 82.9% SFP: 85.6%	Lambton *et al.* (2010)
34 rearing flocks, 12 beak trimmed	United Kingdom	GFP: 94% SFP: 27% (rearing) SFP: 65% (lay)	Gilani *et al.* (2013)
15 laying farms	Australia	PD: 93.3%	Au and Singh (2018)

Although FP is a common problem, not all birds in a flock show FP behaviour. Previous studies calculated that 9% (Keeling, 1994) or 12% (Wechsler *et al.*, 1998) of hens out of the group are severe peckers. Dutch poultry farmers estimate that between 5-20% of the hens within a group perform SFP (Bestman, 2002). According to Daigle *et al.* (2015) once the behaviour has developed, around 5% of the feather pecking hens remain consistent feather peckers, whereas around 30% are consistent victims throughout their life.

THEORIES OF FP

In the past 35-40 years, the causation of FP and the factors affecting FP have been extensively researched. However, no clear cause or one defining factor has been identified, while FP is a multifactorial problem. Several research groups postulated hypotheses about the ontogeny of FP, and the most plausible causations and the corresponding factors can be found in Figure 1. Some of the causes have influences or a relation with other causes. In the next two paragraphs, redirected behaviour and feather eating causes will be discussed extensively. These causes seem to be most related to nutrition (and could be counterbalanced by nutritional intervention).

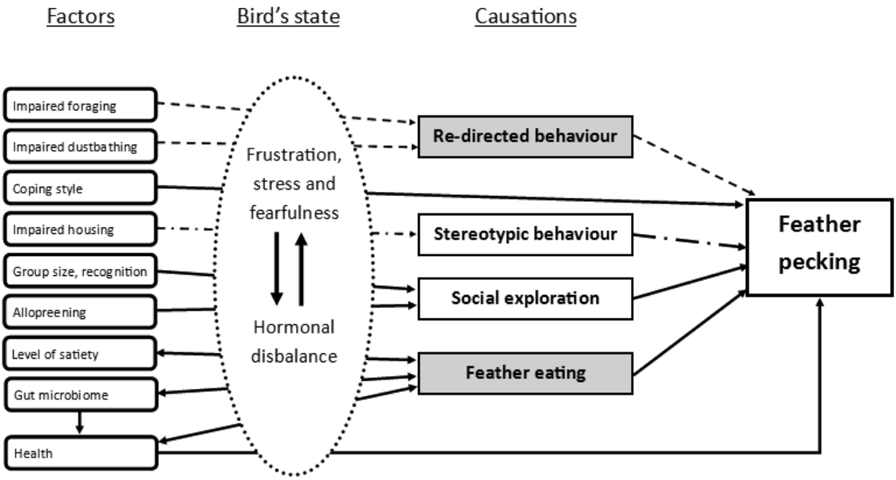


Figure 1: Overview of possible causations of feather pecking and the factors that influence the causations. Arrows indicate influences. Striped arrows and striped dotted arrow show connections within a hypotheses. Causations highlighted in grey are mostly related to nutrition.

Re-directed behaviour

The most common causations are the hypotheses of re-directed ground pecking behaviour (Savory, 1995), with the lack of foraging or dustbathing behaviour as the possible onset. Both theories share the same construction; something is missing in the environment (i.e. sufficient foraging or dustbathing substrate) with the same result: the bird has a strong motivation to express a certain behaviour, which however cannot be fulfilled, and therefore the behaviour is directed at something else. The birds get frustrated, since the motivated behaviour is blocked. Frustration can directly lead to FP, or indirectly by increasing stress in the birds.

From a nutritional point of view, the foraging theory is more interesting and more likely to play a role. As described by Hartcher *et al.* (2016) foraging consists out of food-searching and food consumption. In modern laying hen husbandry, feed is available in surplus and therefore the motivation to consume feed is quickly fulfilled. The motivation to search for feed , however, remains unfulfilled, especially when (appropriate) litter material is not provided. The motivation to forage is very high, birds would even use exclusively excreta or litter material with high amounts of excreta (Von Waldburg-Zeil *et al.*, 2018). In the absence of an appropriate foraging material feathers of conspecifics can be a good replacement. Numerous studies have shown that the absence of litter material affects pecking behaviour.

Feather eating

Even though the redirected behaviour theory has been largely accepted, it cannot explain the occurrence of FP in both experimental and practice settings in which foraging material is adequately provided. Another well-defined theory about the cause of FP includes feather eating. During SFP the feathers are usually ingested by the peckers. Although non-peckers also ingest feathers, feather peckers eat more feathers than non-peckers

(Harlander-Matauschek and Häusler, 2009). Those feathers clearly have an effect: food pellets that contained feathers decreased SFP and improved the plumage scores (Kriegseis *et al.*, 2012). The absence of loose feathers on the floor is in practice the first sign of a possible FP outbreak. Previous findings showed that the number of short feathers on the ground were negatively correlated with pecking and the number of droppings containing feathers was positively correlated with aggressive pecking. Those findings also showed that damaging pecking at young age was mostly directed at the tail and preen gland, were feathers are easily accessible and having the favoured eating size of 2-6 cm (Mckeegan and Savory, 1999). Feathers of that particular area are coated with preen oil, which might attract the peckers (Mckeegan and Savory, 2001).

It could also be suggested that the motivation for feather eating is regulated and enhanced by positive reinforcement. Harlander-Matauschek *et al.* (2008) showed that feather eating could

be increased when the birds had a positive experience, namely when ingesting feathers covered with a palatable substance. Furthermore they acquired an aversion of feathers when they were covered with an unpalatable substance. Hence, the ingestion of tasty feathers could be experienced as a reward, which will motivate the bird to eat more feathers.

Chicken feathers consist over 90% of protein, mainly beta-keratin (Saravanan and Dhurai, 2012). Since laying hens cannot break down keratin in the digestive tract, feathers do not have any nutritional value for the chicken (Mckeegan and Savory, 1999). However, some studies suggest that the ingestion of feathers might aid digestion, as it works as insoluble fibres. Harlander-Matauschek *et al.* (2006) showed that feathers increased the passage rate and thus could have similar effects as insoluble fibres (Figure 2). Nonetheless, it remains unclear which is the actual factor related to feather eating that makes the birds start FP. It could be that the lack of those components causes stress or frustration, and therefore induces redirected behaviour, or maybe the hens are not saturated, and are looking for gut filling.

Additionally, it is possible that something is missing in the diet (fibres, amino acid imbalance) which accelerates a physiological response, in terms of a hormonal disequilibrium or the production of metabolites in the gut by bacteria that influence gut health. Behaviour is in that situation the 'visible' response to an intrinsic physiological imbalance that we do not known yet.

Next to influences on digestion, the ingestion of feathers can influence the gut microbiota composition of the chicken due to bacterial feather degradation as well (Meyer *et al.*, 2012). The influence of microbiota on the gut-brain-axis is a rather unexplored field of study. Some studies have been focussing on the possible differences of the microbiome between feather peckers and non-feather peckers. Meyer *et al.* (2013) found differences in the microbial metabolites between high and low feather pecking birds, most pronounced in the ceca. Total short chain fatty acids (SCFA), propionate and n-butyrate were higher in laying hens from the HFP lines, that performed more FP as well. The authors suggest that especially SCFA and propionate are important, because of their direct and indirect effects on behaviour and the brain. It has been shown that HFP birds have lower amounts of Lactobacilli in their cecal microbiota (Birkl *et al.*, 2018). The authors suggest that Lactobacillus could have direct effects on the neurotransmission in the central nervous system, potentially reducing the sensitivity to stress-related behaviour. The exact pathways and roles of i.e. propionate and Lactobacillus still need to be determined. Furthermore, it is unclear if the microbiome is altered because of FP and/or feather eating behaviour, or whether a certain gut microbial composition provokes FP behaviour.

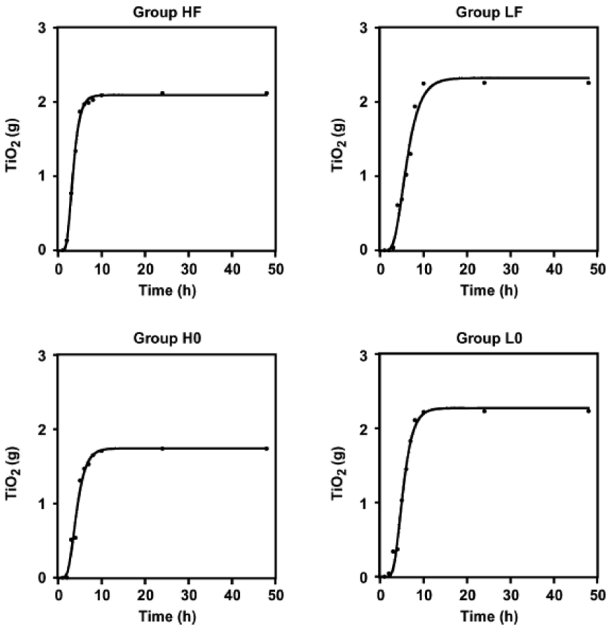


Figure 2: Cumulative excretion curves of TiO_2 (g) over a period of 48h in 4 groups of birds: H = high feather-pecking birds, L = low feather-pecking birds, F = access to feathers, and 0 = no access to feathers (from Harlander-Matauschek et al. 2006).

OTHER FACTORS AFFECTING FP

Next to a causation, many factors or combinations of factors may affect FP behaviour, mainly because those factors influence the birds' state (i.e. stress). An elevation in GFP and/or SFP has been observed in chickens fed pelleted diets, fed with a skip-a-day feeding management, housed in large groups, housed with lack of environmental enrichment or litter material, reared in suboptimal conditions, housed with high animal density, housed with high light intensity or excessive lights, with a different genetic background and being mixed (for an extensive review see Van Krimpen, 2008). Some factors have shown to decrease GFP and SFP, such as the quality and quantity of environmental enrichment, access to elevated perches, the use of warm-white lights or UV-lights. All these influencing factors however, contribute to the underlying motivational system, which is connected to the possible causations. Despite of this abundance of research reporting several factors influencing FP, practice is still struggling with FP.

HOW IMPORTANT IS THE REARING PERIOD?

Multiple studies focussing on FP during the laying period, suggest that experiences during early life are important in the development of FP. For example, rearing pullets on a wire floor resulted in more FP, less dust bathing behaviour and a higher mortality rate (Johnsen *et al.*, 1998; De Jong *et al.*, 2013). As reviewed by Campbell *et al.* (2018), rearing of laying hens should be enriched with physical, sensory and stimulatory aspects to aid the pullets potential of behavioural and (neuro)biological development. From a physiological point of view, the rearing period is a very important stage in development of the chick and experiences can have big influences on FP development (Gilani *et al.*, 2013). A study in rats showed a delay of brain maturation when the environment during the rearing period was poor (Narducci *et al.*, 2018). Post-hatching, the chicks brain continues to grow and develop until week 10 (Atkinson *et al.*, 2008). The synapse formation takes place during the first 3 weeks (De Haas, 2014), which sets the potential for the transmission of neurotransmitters. It is possible that within the first weeks of the rearing period the pullets go through a sensitive period in which FP can develop due to various factors such as imbalances in hormonal influences, nutrition and environmental enrichment. As reviewed by Hartcher *et al.* (2016), the first 10 days post-hatch seem to be critical for pullets to learn to interact with environmental enrichment. FP has already been observed within the first days post-hatch (Riedstra and Groothuis, 2002). However, since this involves mainly GFP and the birds will still moult, the onset of FP can be easily overlooked. Pinpointing the sensitive period to develop FP during the rearing period, and the physiological processes that coincide with the onset, have not been researched yet.

ROLE OF SEROTONIN IN THE DEVELOPMENT OF FP

Even though there are many hypotheses, speculations and studies about the causes and factors associated with FP, the underlying physiological mechanisms still remain unclear. Fundamental research has shown the involvement of the serotonergic system in FP (Van Hierden, 2003; Kops, 2014). Serotonin is a central (brain) neurotransmitter and peripheral signalling molecule. The serotonergic system can be influenced by gut microbiota (O'mahony *et al.*, 2015). Central serotonin has an influence on processes such as behaviour, mood, metabolic processes, sleep and growth (for review see: De Haas and van der Eijk, 2018). Mostly deficiencies in central serotonin during the rearing period have been associated with the onset of FP (Kops *et al.*, 2017). Amongst other organs, peripheral serotonin can be found in the gut, where it has numerous physiological functions (Spohn and Mawe, 2017). Lower peripheral serotonin levels were found in birds that were phenotyped as feather peckers, both in late rearing and laying (De Haas and Van Der Eijk, 2018). There are no data available about possible differences in peripheral serotonin around the onset of FP during early lay. In order to prevent the development of FP, it could be a strategy to influence serotonin towards an adequate level already in the rearing period.

POTENTIAL NUTRITIONAL STRATEGIES DURING REARING TO PREVENT OR REDUCE FP LATER IN LIFE

Nutrition can be considered as a rather promising way to influence animal behaviour. Effects can be observed quickly, and fast adjustments are feasible as well. Nutrition may influence FP in two ways. The first route is by the effects that specific nutrients directly could have on physiological mechanisms. This route is mostly driven by deficiencies and imbalances. Nutritional deficiencies or imbalances have been proven to enhance FP development (Van Krimpen *et al.*, 2005; Kjaer and Bessei, 2013). Thus, adequate nutrition, administrated at the right life stage of the bird could be a method to reduce FP or prevent a FP outbreak. Individual layers may have a specific appetite for certain nutrients (Roura and Cho, 2017). Such individual differences might be explained by a genetic variation in expression and/or sensitivity of nutrient sensors in these birds, and this may induce behavioural expressions, such as feather pecking (Roura *et al.*, 2013).

The second route is the effect that nutrition could have on feeding behaviour. This route focusses mostly on prolonging eating time, as shown by increasing exploratory or foraging behaviour by occupation or increasing the level of satiety, which reduces the drive to peck. Within this strategy, the pullets would be distracted from FP and simply have less time to perform FP. As mentioned before, the rearing period is important, since many believe that FP onsets during the developmental stages of a premature hen. Therefore, using nutritional strategies during rearing could prevent the development of FP altogether.

NUTRIENT SPECIFIC EFFECTS ON FP

Dietary protein and amino acids

Several studies have shown that low protein diets can increase FP (as reviewed by Kjaer and Bessei, 2013). Deficiencies in only dietary lysine have been associated with FP as well. Kumar et. al, 2018 fed several levels of dietary lysine to laying hens, and measured their plumage over time (Figure 3). In total 5 areas (neck, back, vent, wings and breast) were scored with each measurement a score from 1 (completely featherless) to 4 (fully feathered) at 27, 47 and 66 weeks of age. Over time, feather score decreased in all dietary treatments. However, the mean cumulative scores of all areas clearly show the biggest decrease in feather score with the lowest amount of dietary lysine. The authors found a positive correlation between dietary lysine level and feather score.

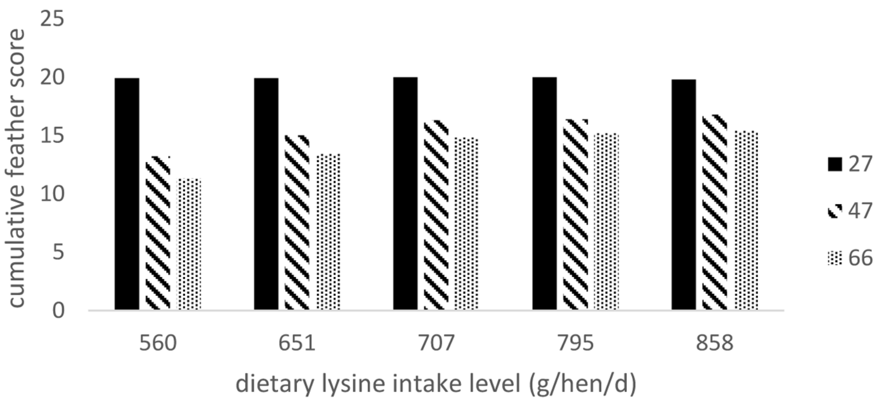


Figure 3: Effect of lysine intake level (g/hen/d) on mean cumulative feather score from 1 (completely featherless) to 4 (fully feathered) at 5 body areas: neck, back, vent, wings and breast, of laying hens fed different dietary lysine levels at 27 (black), 47 (striped) and 66 (dotted) weeks of age. (adapted from Kumar et al., 2018)

Furthermore, the levels of arginine (Sirén, 1963; Conson and Petersen, 1986; Bozakova and Yotova, 2009), methionine and cysteine (Elwinger *et al.*, 2002; Kjaer and Bessei, 2013) seem to influence FP as well. Unfortunately, these studies did not look into the physiological processes that might be altered because of imbalances of these amino acids. It seems that laying hens are sensitive for changes in the diet, and use their behaviour to deal with whatever is missing. Arginine, cystine, methionine and valine are important for the development of feathers (Emous and Krimpen, 2019), which could be a reason why pullets are trying to counteract deficiencies of these amino acids by eating to or pecking at feathers.

Tryptophan (TRP) is a pre-cursor for serotonin, which means that the amino acid is involved in the serotonin synthesis. Because TRP is an essential amino acid, serotonin production is limited by the availability of digestible TRP in the diet. Research in adult laying hens showed that higher supplementation of dietary TRP reduces FP (Savory *et al.*, 1999; Van Hierden *et al.*, 2004). Most likely additional TRP increases serotonin synthesis. The exact physiological mechanisms remain unclear. A study by Birkl *et al.* (2017) showed that a TRP depletion in the diet could seriously alter blood amino acid levels (aromatic amino acids as well as large neutral amino acids), which, in return, could have effects on all kind of physiological functions. Furthermore, the effects of depletion or supplementation of TRP on central and peripheral serotonin of laying hens are not studied yet.

If TRP could directly influence the synthesis of central serotonin, supplementation during rearing could be a method to prevent FP, since central serotonin levels during rearing are low (Kops *et al.*, 2017). A study focussing on the availability of TRP in plasma, found low levels of TRP in young laying hens (24 weeks) in which aggressive injurious pecking had onset the week before (Birkl *et al.*, 2017).

However, TRP has to compete with other large amino acids to pass the blood brain barrier to enter the brain (De Haas and Van Der Eijk, 2018). Therefore, only providing extra TRP might not work, and a more complete range of amino acids needs to be provided in the diet. Prescilla *et al.* (2018) studied the combination of TRP, methionine and glycine on plumage condition, and modelled the optimal inclusion rate for plumage score. Their model predicted that if these three amino acids were provided well above recommendations (relative increase of 23%, 10% and 49% of TRP, methionine and glycine respectively) the maximal likelihood of observing perfect plumage conditions was 61%. Furthermore, providing laying hens with a higher TRP:LYS ratio has decreased FP as well (Helmbrecht *et al.*, 2015). Since there isn't an abundance of studies focussing on combining TRP and the other mentioned amino acids (methionine, glycine and lysine), or on other combinations of amino acids that potentially influence FP and TRP, the area of AA profile is worthwhile to research in more detail.

Satiety by dietary fibres

High fibre contents in diets showed to have a consistent FP reducing effect (see for review Nicol et al., 2019). Furthermore, including high non-starch polysaccharides (NSP) concentrations in the diet may increase weights of the gizzard and its content, and aid digestion (Hetland *et al.*, 2003; Van Krimpen *et al.*, 2011). Conversely, low fibrous diets have been related to increased foraging behaviour. Most likely, low fibre content decreases satiety, which motivates the hens to perform more foraging behaviour, which might decrease FP (Nicol, 2019). These observations result in a bit of a dilemma, since reduction of FP and increasing foraging behaviour are both favourable as an aim for nutritional strategies.

By providing high coarse fibre content in the diet, birds might be more saturated, especially when the feed remains longer in the crop and gizzard. It is suggested that insoluble NSP sources accumulate in the gizzard and increase the mean retention time (MRT) in the foregut (Van Krimpen *et al.*, 2011). The MRT is often used as a measure for satiety. However, the exact physiological mechanisms involved in the regulation of satiety and feed intake in chickens are not yet completely understood. Satiety in humans is regulated by gastrointestinal mechanisms, in which gastric expansion is most important to stop eating and presence of nutrients in the small intestine is required to feel saturated (Read, 1992; Schwartz *et al.*, 2000). It is possible that expansion in the crop and gizzard of chicken might have a similar effect.

The increased foraging behaviour due to providing a low fibrous diet could have a negative effect as well. As mentioned before, when the hens are missing fibre in their diets, they might use feathers as a replacement, especially if litter material is absent or inadequate.

The gut microbiome

The diet consumed by an animal has influence on its gut microbiome. For example, feathers in a diet are proven to have effect on the gut microbiome composition: an increase of bacterial species that hydrolyze keratin in the ileum and cecum (*Enterococcu facium* and different strains of *Lactobacillus*) and a lower bacterial diversity in the cecum (Meyer *et al.*, 2012). Nevertheless, other nutrients might have an influence as well; for example providing probiotica, short-chain fatty acids and oxidized fatty acids (Zhang and Davies, 2016). A study in mice found that the ingestion of lactic acid bacteria regulates emotional behaviour (Bravo *et al.*, 2011), stressing the importance of gut bacteria. The gut microbiota affects the synthesis of serotonin, both central and peripheral. It may utilize or produce TRP, produce short chain fatty acids involved in the synthesis and release of neurotransmitter, and directly influence serotonin by the nervus vagus (see review de Haas and van der Eijk, 2018). Thus, changes in or influences on the gut microbiome might influence FP via several routes. However, causal relations between the gut microbiome and FP are studied at the moment, while results have not been published yet.

FEEDING RELATED BEHAVIOURAL EFFECTS ON FP

Occupation

Instead of prolonging the eating time, FP can also be prevented by occupying the hens with other pursuits. This could potentially increase foraging behaviour by providing environmental enrichment or special ingredients such as roughages. For example, providing laying hens with grass haylage as an enrichment material has improved hens welfare by lowering SFP and cannibalism (Albiker and Zweifel, 2017). A study done by Steenfeldt *et al.* (2007) tested high fibrous diet ingredients maize, i.e. silage, barley-pea silage and carrots, as a potential foraging enrichment. At 53 weeks of age, all three enrichments resulted in lower total, severe and gentle pecking bouts compared to the control group. Mortality was low in the groups receiving enrichment (0.5 - 2.5%) as compared to the control group (15%). In the control group, half of the mortality was due to cannibalism. Furthermore, the total plumage condition of the control group was worse than the groups that received maize silage or barley-pea silage. Although eating time was not observed, the authors suggested that the hens spent more time on feeding, which leaves less time to spent on FP.

Unfortunately, both studies did not look into the effect of the enrichment on the length of other behaviours, such as foraging or eating. It could be expected that providing extra nutrients next to the normal diet increases foraging behaviour. Additionally, also non-nutritive materials could occupy and distract the hens from pecking. Zepp *et al.* (2018) found a relation between FP and enrichment pecking (defined as direct pecking at enrichment), while testing enrichment in the form of a pecking block, pecking stone and lucerne bales. If enrichment pecking occurred, GFP, SFP and aggressive pecking was lower.

Prolonging eating time

Another strategy to prevent or reduce FP, is to aim for prolonging the time birds are eating. The time spent on eating, cannot be spent on other, unwanted behaviour such as FP.

In our lab, studies were performed on the effect of energy and nutrient dilution on eating time. The energy content demonstrated to have an effect on FP, where a high energy content resulted in FP and a low energy content decreased FP. When feeding a low energy level, the birds increased their feed intake to maintain their energy intake, consequently spending more time on eating. The increase in eating time can be achieved by nutrient dilution, i.e. providing sand or grit or high NSP raw materials. Even though studies have found effects from NSP on FP, not all results align. In a study providing high and normal NSP levels and normal and energy diluted diets to a feather pecking prone flock, only a delay in feather damage has been observed (Van Krimpen, 2008).

One of the studies in our lab included the rearing period, in which we found an increased feed intake during rearing in low NSP + energy dilution by sand and high NSP + energy dilution by oat hulls, in which a similar energy intake was ensured (Van Krimpen *et al.*, 2009). In the same study, energy dilution of the low NSP diets, however, did not affect eating time, while eating time was prolonged in the diluted high NSP diets, indicating that in particular the NSP-rich ingredients are responsible for prolonging eating time. In line with these findings, another study from the same group found that dietary dilution with insoluble NSP from common feed ingredients like barley and sunflower seed extract also affected the time-budget of pullets during rearing, resulting in increased eating time and decreased FP (Qaisrani *et al.*, 2013). Furthermore, when pullets received NSP-diluted diets during rearing and laying, FP has been reduced in the laying period (Van Krimpen *et al.*, 2010). Based on these results it can be concluded that dietary dilution with insoluble NSP indeed affects the time-budget of pullets, by training them during rearing towards more time spend on eating, which affects FP in later life.

The physiological processes that enable the influences of diluted diets on prolonged eating time, however, are not researched yet. Satiety and the processes that regulate satiety might play a role in this matter. Satiety could be explained by influences of energy dilution on physical thresholds for gut fill or by influencing the metabolic state of the bird (i.e. blood glucose levels). By diluting the diet, the birds might feel less saturated and therefore increase their eating time. On the contrary, the diluted diet could have a higher MRT in various part of the gastral-intestinal tract (Van Krimpen *et al.*, 2011), meaning the birds would feel more saturated. Future research should look into physiological parameters, such as hormones involved in satiety. When these mechanisms are fully understood, specialized diets could be formulated to influence satiety.

CONCLUSIONS

Feather pecking remains a welfare issue in the laying hen sector. The prevalence of SFP in laying hen flocks measured over the last 20 years remains above 50%. Changing legislation within Europe considering the ban on beak trimming might enlarge the severity of the problem. From a nutritional point of view, the re-directed foraging behaviour and feather eating theories seem the most plausible causes of FP. Furthermore, experiences during the rearing period seem to be linked to FP in later life. Nutrition can be used to reduce FP by having direct effects on physiological mechanisms such as the serotoninergic system, satiety and the gut microbiome. Moreover, nutrition can also be used to prolong eating time, to occupy the birds and to increase foraging behaviour; which indirectly decreases FP behaviour. Applying such nutritional strategies in the rearing period might alter the pullets physiology or train them in their time distribution, which both could result in the prevention of FP.

GAPS OF KNOWLEDGE

To prevent the development of FP, the ontogeny of FP needs to be studied more closely. The sensitive period in which pullets are most susceptible, as well as the role of the gut-brain axis should be unravelled. Future research should focus on the impact of nutritional strategies during early rearing on the development of FP and the gut microbiome. Thereby, the involved physiological and neuro-endocrine mechanisms of known and new nutritional strategies should be unravelled for developing more effective strategies to prevent FP. Emphasis is required to study the role of peripheral serotonin, the gut microbiome, energy metabolism and satiety and predisposing and increasing normal behaviours such as foraging and eating.

ACKNOWLEDGEMENTS

This research is co-financed by Lohmann Tierzucht GmbH, Ter Heerdt N.V., Agromix Broederij en Opfokintegratie B.V., De Heus Voeders B.V., Fonds voor Pluimveebelangen, Wageningen Livestock Research and Wageningen University, within the framework of the public private partnership "Feed4Foodure", partially funded by the Dutch Ministry of Economic Affairs.

REFERENCES

ATKINSON, R., MIGUES, P. V., CAMMAROTA, M., KAVANAGH, J. M., HUNTER, M. and ROSTAS, J. A. (2008) Biochemical, behavioural and electrophysiological investigations of brain maturation in chickens. *Brain Res Bull* **76**: 217-23.

BESTMAN, M. (2002) Kippen houden zonder verenpikken. De biologische legpluimveehouderij als uitgangspunt. Louis Bolk Instituut.

BESTMAN, M. W. P. and WAGENAAR, J. P. (2003) Farm level factors associated with feather pecking in organic laying hens. *Livestock Production Science. 2003;* **80**: 133-140.

BILCIK, B. and KEELING, L. J. (1999) Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *Br. Poult. Sci.* **40**: 444-451.

BIRKL, P., BHARWANI, A., KJAER, J. B., KUNZE, W., MCBRIDE, P., FORSYTHE, P. and HARLANDER-MATAUSCHEK, A. (2018) Differences in cecal microbiome of selected high and low feather-pecking laying hens. *Poultry Science* **97:** 3009-3014.

BIRKL, P., FRANKE, L., BAS RODENBURG, T., ELLEN, E. and HARLANDER- MATAUSCHEK, A. (2017) A role for plasma aromatic amino acids in injurious pecking behavior in laying hens. *Physiol Behav* **175**: 88-96.

BIRKL , P., KJAER, J. B., SZKOTNICKI, W., FORSYTHE, P. and HARLANDER- MATAUSCHEK, A. (2017) Testing a dietary acute tryptophan depletion mixture in feather pecking birds. *Xth European Symposium on Poultry Welfare*, Ploufragan, France.

BLOKHUIS, H. J. (1986) Feather-Pecking in Poultry - Its Relation with Ground-Pecking. *Applied Animal Behaviour Science* **16**: 63-67.

BLOKHUIS, H. J. (1989) The Effect of a Sudden Change in Floor Type on Pecking Behavior in Chicks. *Applied Animal Behaviour Science* **22**: 65-73.

BLOKHUIS, H. J. and VAN DER HAAR, J. W. (1989) Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Applied Animal Behaviour Science* **22**: 359-369.

BOZAKOVA, N. and YOTOVA, I. (2009) Ethological aspects of improving the welfare of turkey breeders in the hot summer period by dietary L-arginine supplementation. *Bulgarian Journal of Veterinary Medicine,* **12**: 185-191.

BRAVO, J. A., FORSYTHE, P., CHEW, M. V., ESCARAVAGE, E., SAVIGNAC, H. M., DINAN, T. G., BIENENSTOCK, J. and CRYAN, J. F. (2011) Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* **108**: 16050-5.

CAMPBELL, D. L. M., DE HAAS, E. N. and LEE, C. (2018) A review of environmental enrichment for laying hens during rearing in relation to their behavioral and physiological development. *Poultry Science* **98:** 9-28.

CHENG, H. (2007) Morphopathological changes and pain in beak trimmed laying hens. *World's Poultry Science Journal* **62**: 41-52.

CONSON, M. and PETERSEN, J. (1986) Feathering score of laying hybrids reared under different conditions. *Archiv Fur Geflugelkunde* **50**: 159-168.

DAIGLE, C. L., RODENBURG, T. B., BOLHUIS, J. E., SWANSON, J. C. and SIEGFORD, J. M. (2015) Individual Consistency of Feather Pecking Behavior in Laying Hens: Once a Feather Pecker Always a Feather Pecker? *Front Vet Sci* **2**: 6.

DE HAAS, E. N. (2014) *The fearful feather pecker*. PhD thesis, Wageningen University, Wageningen.

DE HAAS, E. N. and VAN DER EIJK, J. A. J. (2018) Where in the serotonergic system does it go wrong? Unravelling the route by which the serotonergic system affects feather pecking in chickens. *Neurosci Biobehav Rev* **95**: 170-188.

DE JONG, I. C., REUVEKAMP, B. F. J. and GUNNINK, H. (2013) Can substrate in early rearing prevent feather pecking in adult laying hens? *Animal Welfare* **22**: 305-314.

ELWINGER, K., TAUSON, R., TUFVESSON, M. and HARTMANN, C. (2002) Feeding of layers kept in an organic feed environment. *11th. European Poultry Conference*, Bremen.

EMOUS, R. A. V. and KRIMPEN, M. M. V. (2019) Effects of nutritional interventions on feathering of poultry - a review. (Poultry Science Symposium Series, Volume 32). In: 133-150 Poultry Feathers and Skin).

GILANI, A.-M., KNOWLES, T. G. and NICOL, C. J. (2013) The effect of rearing environment on feather pecking in young and adult laying hens. *Applied Animal Behaviour Science* **148**: 54- 63.

GREEN, L. E., LEWIS, K., KIMPTON, A. and NICOL, C. J. (2000) Cross-sectional study of the prevalence of feather pecking in laying hens in alternative systems and its associations with management and disease. *Vet Rec* **147**: 233-8.

GUNNARSSON, S., KEELING, L. J. and SVEDBERG, J. (1999) Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens. *Br Poult Sci* **40**: 12-8.

HÄNE, M., HUBER-EICHER, B. and FRÖHLICH, E. (2007) Survey of laying hen husbandry in Switzerland. *World's Poultry Science Journal* **56**: 21-31.

HARLANDER-MATAUSCHEK, A. and HÄUSLER, K. (2009) Understanding feather eating behaviour in laying hens. *Applied Animal Behaviour Science* **117**: 35-41.

HARLANDER-MATAUSCHEK, A., PIEPHO, H. P. and BESSEI, W. (2006) The effect of feather eating on feed passage in laying hens. *Poult Sci* **85**: 21-5.

HARLANDER-MATAUSCHEK, A., WASSERMANN, F., ZENTEK, J. and BESSEI, W. (2008) Laying hens learn to avoid feathers. *Poult Sci* **87**: 1720-4.

HARTCHER, K. M., WILKINSON, S. J., HEMSWORTH, P. H. and CRONIN, G. M. (2016) Severe feather-pecking in non-cage laying hens and some associated and predisposing factors: a review. *World's Poultry Science Journal* **72**: 103-114.

HELESKI, C. R., MERTIG, A. G. and ZANELLA, A. J. (2015) Stakeholder attitudes toward farm animal welfare. *Anthrozoös* **19**: 290-307.

HENG, Y., PETERSON, H. H. and LI, X. H. (2013) Consumer Attitudes toward Farm- Animal Welfare: The Case of Laying Hens. *Journal of Agricultural and Resource Economics* **38**: 418- 434.

HETLAND, H., SVIHUS, B., LERVIK, S. and MOE, R. (2003) Effect of Feed Structure on Performance and Welfare in Laying Hens Housed in Conventional and Furnished Cages. *Acta Agriculturae Scandinavica, Section A - Animal Science* **53**: 92-100.

HUBER EICHER, B. (1999) A survey of layer-type pullet rearing in Switzerland. *World's Poultry Science Journal* **55**: 83-91.

HUBER EICHER, B. and SEBO, F. (2001) The prevalence of feather pecking and development in commercial flocks of laying hens. *Applied Animal Behaviour Science* **74**: 223-231.

JOHNSEN, P. F., VESTERGAARD, K. S. and NORGAARD-NIELSEN, G. (1998) Influence of early rearing conditions on the development of feather pecking and cannibalism in domestic fowl. *Applied Animal Behaviour Science* **60**: 25-41.

JUNG, L. and KNIERIM, U. (2017) Are practice recommendations for the prevention of feather pecking in laying hens in non-cage systems in line with the results of experimental and epidemiological studies? *Applied Animal Behaviour Science* **200**: 1-12.

KEELING, L. J. (1994) Feather pecking - who in the group does it, how often and under what circumstances? *Proceedings of the 9th European Poultry Conference*, Glasgow, United Kingdom, pp. 288-289

KEELING, L. J. (1995) Feather pecking and cannibalism in layers. *Poultry International* 46–50.

KJAER, J. B. (2000) Diurnal rhythm of feather pecking behaviour and condition of integument in four strains of loose housed laying hens. *Applied Animal Behaviour Science* **65**: 331-347.

KJAER, J. B. and BESSEI, W. (2013) The interrelationships of nutrition and feather pecking in the domestic fowl - A review. *Archiv Fur Geflugelkunde* **77**: 1-9

KOPS, M. S., KJAER, J. B., GUNTURKUN, O., WESTPHAL, K. G. C., KORTE-BOUWS, G. A. H., OLIVIER, B., KORTE, S. M. and BOLHUIS, J. E. (2017) Brain monoamine levels and behaviour of young and adult chickens genetically selected on feather pecking. *Behav rain Res* **327**: 11-20.

KRIEGSEIS, I., BESSEI, W., MEYER, B., ZENTEK, J., WURBEL, H. and HARLANDER- MATAUSCHEK, A. (2012) Feather-pecking response of laying hens to feather and cellulose- based rations fed during rearing. *Poult Sci* **91**: 1514-21.

KUMAR, D., RAGINSKI, C., SCHWEAN-LARDNER, K., CLASSEN, H. L. and PLAIZIER, J. (2018) Assessing the response of hen weight, body composition, feather score, egg quality, and level of excreta nitrogen content to digestible balanced protein intake of laying hens. *Canadian Journal of Animal Science* **98**: 619-630.

LAMBTON, S. L., KNOWLES, T. G., YORKE, C. and NICOL, C. J. (2010) The risk factors affecting the development of gentle and severe feather pecking in loose housed laying hens. *Applied Animal Behaviour Science* **123**: 32-42.

MCKEEGAN, D. E. F. and SAVORY, C. J. (1999) Feather eating in layer pullets and its possible role in the aetiology of feather pecking damage. *Applied Animal Behaviour Science* **65**: 73-85.

MCKEEGAN, D. E. F. and SAVORY, C. J. (2001) Feather eating in individually caged hens which differ in their propensity to feather peck. *Applied Animal Behaviour Science* **73**: 131-140.

MEYER, B., BESSEI, W., VAHJEN, W., ZENTEK, J. and HARLANDER- MATAUSCHEK, A. (2012) Dietary inclusion of feathers affects intestinal microbiota and microbial metabolites in growing Leghorn-type chickens. *Poult Sci* **91**: 1506-13.

MEYER, B., ZENTEK, J. and HARLANDER-MATAUSCHEK, A. (2013) Differences in intestinal microbial metabolites in laying hens with high and low levels of repetitive feather- pecking behavior. *Physiol Behav* **110-111**: 96-101.

NARDUCCI, R., BARONCELLI, L., SANSEVERO, G., BEGENISIC, T., PRONTERA, C., SALE, A., CENNI, M. C., BERARDI, N. and MAFFEI, L. (2018) Early impoverished environment delays the maturation of cerebral cortex. *Sci Rep* **8**: 1187.

NICOL, C. (2018) Feather pecking and cannibalism. In: *Advances in Poultry Welfare*. 175-197.

NICOL, C. J. (2019) Feather pecking in laying hens: why they do it, and welfare implications. (Poultry Science Symposium Series, Volume 32). In: 31-46 Poultry Feathers and Skin.

NICOL, C. J., BESTMAN, M., GILANI, A. M., DE HAAS, E. N., DE JONG, I. C., LAMBTON, S., WAGENAAR, J. P., WEEKS, C. A. and RODENBURG, T. B. (2013) The prevention and control of feather pecking: application to commercial systems. *World's Poultry Science Journal* **69**: 775-788.

O'MAHONY, S. M., CLARKE, G., BORRE, Y. E., DINAN, T. G. and CRYAN, J. F. (2015) Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research* **277**: 32-48.

QAISRANI, S. N., VAN KRIMPEN, M. M. and KWAKKEL, R. P. (2013) Effects of dietary dilution source and dilution level on feather damage, performance, behavior, and litter condition in pullets. *Poult Sci* **92**: 591-602.

READ, N. W. (1992) Role of Gastrointestinal Factors in Hunger and Satiety in Man. *Proceedings of the Nutrition Society* **51**: 7-11.

RIEDSTRA, B. and GROOTHUIS, T. G. G. (2002) Early feather pecking as a form of social exploration: the effect of group stability on feather pecking and tonic immobility in domestic chicks. *Applied Animal Behaviour Science* **77**: 127-138.

RODENBURG, T. B., VAN KRIMPEN, M. M., DE JONG, I. C., DE HAAS, E. N., KOPS, M. S., RIEDSTRA, B. J., NORDQUIST, R. E., WAGENAAR, J. P., BESTMAN, M. and NICOL, C. J. (2013) The prevention and control of feather pecking in laying hens: identifying the underlying principles. *World's Poultry Science Journal* **69**: 361-374.

ROURA, E., BALDWIN, M. W. and KLASING, K. C. (2013) The avian taste system: Potential implications in poultry nutrition. *Animal Feed Science and Technology* **180**: 1-9.

SARAVANAN, K. and DHURAI, B. (2012) Exploration on Amino Acid Content and Morphological Structure in Chicken Feather Fiber. *Journal of Textile and Apparel, Technology and Management* **7**: 1-6.

SAVORY, C. J. (1995) Feather pecking and cannibalism. *World's Poultry Science Journal* **51**: 215- 219.

SAVORY, C. J., MANN, J. S. and MACLEOD, M. G. (1999) Incidence of pecking damage in growing bantams in relation to food form, group size, stocking density, dietary tryptophan concentration and dietary protein source. *British Poultry Science* **40**: 579-584.

SCHWARTZ, M. W., WOODS, S. C., PORTE, P. Y., SEELEY, R. J. and BAS KIN, D. G. (2000) Central nervous system control of food intake. *Nature* **404**: 661-671.

SHERWIN, C. M., RICHARDS, G. J., AND NICOL, C. J. (2010). Comparison of the welfare of layer hens in 4 housing systems in the UK. *British poultry science* **51:** 488-499.

SIRÉN, M. J. (1963) Cannibalism in cockerels and pheasants. *Acta Veterinaria Scandinavica* **4**: 1-47.

SPOHN, S. N. and MAWE, G. M. (2017) Non-conventional features of peripheral serotonin signalling - the gut and beyond. *Nat Rev Gastroenterol Hepatol* **14**: 412-420.

STEENFELDT, S., KJAER, J. B. and ENGBERG, R. M. (2007) Effect of feeding silages or carrots as supplements to laying hens on production performance, nutrient digestibility, gut structure, gut microflora and feather pecking behaviour. *Br Poult Sci* **48**: 454-68.

VAN HIERDEN, Y. M. (2003) *Behavioural neurobiology on feather pecking*. PhD thesis University of Groningen, Groningen.

VAN HIERDEN, Y. M., KOOLHAAS, J. M. and KORTE, S. M. (2004) Chronic increase of dietary l-tryptophan decreases gentle feather pecking behaviour. *Applied Animal Behaviour Science* **89**: 71-84.

VAN KRIMPEN, M. M. (2008) *Impact of nutritional factors on eating behaviour and feather damage of laying hens.* PhD thesis, Wageningen University, Wageningen.

VAN KRIMPEN, M. M., KWAKKEL, R. P., REUVEKAMP, B. F. J., VAN DER PEET- SCHWERING, C. M. C., DEN HARTOG, L. A. and VERSTEGEN, M. W. A. (2005) Reduction of feather pecking behaviour in laying hens by feeding management - a review. *Proceedings of 7th European Symposium of Poultry Welfare, 15-19 June 2005, Lublin, Poland*, Lublin, pp. 161-174

VAN KRIMPEN, M. M., KWAKKEL, R. P., VAN DER PEET-SCHWERING, C. M., DEN HARTOG, L. A. and VERSTEGEN, M. W. (2008) Low dietary energy concentration, high nonstarch polysaccharide concentration, and coarse particle sizes of nonstarch polysaccharides affect the behavior of feather-pecking-prone laying hens. *Poult Sci* **87**: 485-96.

VAN KRIMPEN, M. M., KWAKKEL, R. P., VAN DER PEET-SCHWERING, C. M., DEN HARTOG, L. A. and VERSTEGEN, M. W. (2011) Effects of dietary energy concentration, nonstarch polysaccharide concentration, and particle sizes of nonstarch polysaccharides on digesta mean retention time and gut development in laying hens. *Br Poult Sci* **52**: 730-41.

VAN KRIMPEN, M. M., KWAKKEL, R. P., VAN DER PEET-SCHWERING, C. M. C., DEN HARTOG, L. A. and VERSTEGEN, M. W. A. (2009) Effects of nutrient dilution and nonstarch polysaccharide concentration in rearing and laying diets on eating behavior and feather damage of rearing and laying hens. *Poultry Science* **88**: 759-773.

VANHONACKER, F., VAN POUCKE, E., TUYTTENS, F. and VERBEKE, W. (2010) Citizens' Views on Farm Animal Welfare and Related Information Provision: Exploratory Insights from Flanders, Belgium. *Journal of Agricultural and Environmental Ethics* **23**: 551-569.

VON WALDBURG-ZEIL, C. G., VAN STAAVEREN, N. and HARLANDER-MATAUSCHEK, A. (2018) Do laying hens eat and forage in excreta from other hens? *Animal* 1-7.

WECHSLER, B., HUBER EICHER, B. and NASH, D. R. (1998) Feather pecking in growers: a study with individually marked birds. *British Poultry Science* **39**: 178-185.

ZEPP, M., LOUTON, H., ERHARD, M., SCHMIDT, P., HELMER, F. and SCHWARZER, A. (2018) The influence of stocking density and enrichment on the occurrence of feather pecking and aggressive pecking behavior in laying hen chicks. *Journal of Veterinary Behavior* **24**: 9- 18.

ZHANG, L. S. and DAVIES, S. S. (2016) Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. *Genome Med* **8**: 46.

Feeding hens in a loose husbandry system - challenges and consequences on performance and welfare

I. Ruhnke, T. Z. Sibanda

Faculty of Science, Agriculture, Business and Law, University of New England, Armidale, 2351, NSW, Australia; iruhnke@une.edu.au

SUMMARY

Housing hens in loose husbandry systems such as barn, free-range, or organic systems is associated with many challenges that not only interfere with body weight uniformity, but also with adequate feed intake, as well as high inter-hen variation of nutrient and energy requirements. This brief review outlines recent findings about the characterisation of sub-populations within free-range flocks, and highlights their impact on flock performance and dietary considerations. Ongoing work with these subpopulations including determining of metabolic needs, clustering of various performance groups and hens of different health status within one flock may allow for distinct pattern recognition and targeted dietary management using different geographical locations within the hen house. Given the fact that subpopulations of layer flocks in experimental and commercial free-range facilities are commonly observed, the use of modern technology including routine Radio-Frequency Identification (RFID), location sensing and computed learning should be considered to improve current feeding strategies, allowing real-time flock management for a more economic, efficient and sustainable use of feed resources.

INTRODUCTION

Housing hens in loose husbandry systems such as barn, free-range, or organic systems is associated with many challenges that interfere with adequate feed and nutrient intake, resulting in suboptimal hen performance and inadequate egg quality (AERNI et al., 2005; EITS et al., 2005; FERRANTE et al., 2009; VAN HORNE, 1996). For example, the Feed Conversion Ratio (FCR) of hens housed in alternative housing systems is commonly observed to be higher than in hens housed in caged systems, and the additional energy requirement for maintenance has been estimated to be 10% (floor-housed) or 15% (free-range) higher compared to hens housed in cages (AERNI et al, 2005; TILLER, 2001). Similarly, other performance parameters including body weight were found to be less beneficial in hens kept in non-cage housing facilities. This has a direct consequence on feed formulation as heavier hens require lower protein:energy ratios. Adjusting diet formulation to the hen housing conditions is commonly performed throughout the industry. For example, in organic systems heavier hens can be economically more profitable if supplementation of synthetic amino acids is not an option, while for conventional cage, barn, or free range hens this would not be economically desired as the amino acid requirements can be balanced using synthetic amino acids. (LEENSTRA et al., 2014). The poultry industry is still one of the few food producing sectors where animals are fed on a group ration based on the average flock performance, rather than taking individual animal requirements based on performance or behaviour into account. Feeding individuals or performance groups has been shown to be beneficial to the pig and cattle industries and is widely adopted. The change in dairy production from herd management based on average performance values to individual feed concentrate rations on top of the basal ration depending on the milk production level of the cow has been so beneficial that this practice is widely spread throughout the world.

The objective of this brief review is to summarise recent findings that should be considered for future nutritional management of hens housed in loose-husbandry systems.

THE IMPACT OF FLOCK UNIFORMITY ON POULTRY NUTRITION

Pullet and hen uniformity is one of the key parameters to determine flock quality (HUDSON et al., 2001). However, housing flocks composed of 10,000 – 500,000 hens requires a significant amount of furnished space including feeders, drinkers, nest boxes and perches, sometimes arranged on different levels of aviary tiers. While adequate space per hen needs to be available at any given time, the use of these resources is usually continuous, preventing overcrowding of certain areas which can result in smothering, stress, misplaced eggs, reduced feather condition and increased flock mortalities (SIROVNIK et al., 2018; RODENBURG et al., 2013). When measuring the use of available feeder space in a commercial free-range system equipped with two 3-tier aviary systems, it was shown that hens spent on average most of their available time (7.92 h/hen/day) on the lower feeder tier, and 4.29 h/hen/day on the upper feeder tier (SIBANDA et al., 2019). The desired usage of the entire area space and the potentially available range area result in various subpopulations which may potentially impact body weight uniformity. For example, it has been shown that while some hens prefer to use the range area frequently, others choose to spend the majority of their life span in the sheltered shed (GEBHARDT-HEINRICH et al., 2014; HARTCHER et al., 2016).

THE IMPACT OF FLOCK SUB-POPULATIONS ON POULTRY NUTRITION

Ranging hens are subject to a variation of broad weather and temperature conditions (SOUSSIDOU et al. 2011). The metabolic energy of a hen changes especially at environmental temperatures above 21° C due to increased energy requirements for body temperature maintenance (DAGHIR, 1973). Therefore it can be hypothesised that hens on the range would have a higher metabolic energy requirement compared to hens that stay in the (temperature controlled) shed, and hens that stay in the shed would enjoy more frequent feed intake due to the physical closeness to feed and water resources, potentially leading to increased body weight associated with heavier eggs and/or increased hen-egg production. A first attempt to investigate the metabolic needs of hens that prefer to stay in the shed and hens that range frequently was performed by KOLAKSHYAPATI et al. (2019a). Selecting commercial laying hens based on their range usage during 18-74 weeks of age and measuring their metabolic energy in closed-circuit calorimetry chamber, it became evident that hens that prefer to stay in the shed had significantly higher metabolisable energy (ME) intake (P = 0.025), heat production (P = 0.005), and heat increment/ body weight$^{0.75}$ (P = 0.005) compared to hens that accessed the range frequently. This leads to the conclusion that hens that prefer to stay in the shed had significantly higher maintenance energy requirements and were less energy efficient compared to hens that used the range. Surprisingly, when investigating commercial hens reared within on flock, housed within the same shed and fed the same diet, hens of heavy body weight accessed the range significantly more frequently compared to lighter hens (Figure 1).

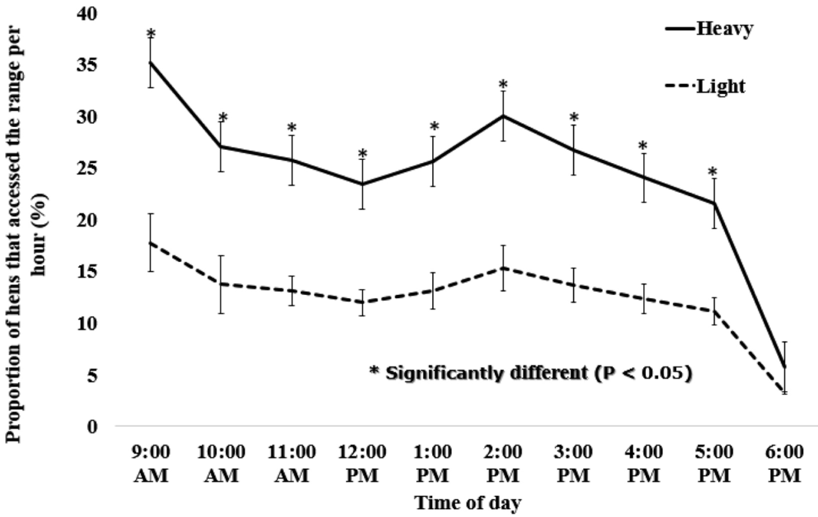


Figure 1: A significantly higher proportion of heavy hens (average body weight; mean ± SEM: 1.43 ± 0.001 kg; n = 1450 hens) used the range compared to lighter hens (average body weight 1.28 ± 0.002 kg; n = 1451 hens) throughout the day (P < 0.05). Range access was monitored for 7 consecutive days when hens were 17 weeks of age.

When investigating the impact of hen behaviour and fearfulness in that same flock, it was shown that fewer range visits during the first three weeks of range availability were associated with increased fearfulness at the end of lay (KOLASKHYAPATI et al., 2019b). It was also shown that range use was significantly negatively correlated (r = -0.30) to the time spent on the upper feeder tier and significantly positively correlated (r = 0.46) to use of the lower feeder tier (P = 0.001) (SIBANDA et al., 2019). As a consequence, we can conclude that the development of subpopulations is not only associated with range use, but extends further into the hen house and leads to the development of population clusters within the aviary system. If the more fearful hens that are not ranging prefer to stay on top of the aviary system, do we need to encourage hen confidence during rearing or address the different metabolic energy needs by offering a different diet on the feeder chains that run on the top tiers of the aviary system?

The consequences of these observations on hen performance are currently under investigation. Preliminary results investigating 5,625 hens within 3 replicate flocks demonstrate that significant differences of range usage can lead to the development of subpopulations that are associated with significantly different performance characteristics for each of the subpopulations including the hen house percentage of egg production as well as the onset and the duration of lay (RUHNKE and SIBANDA, 2018). In detail, Lohmann Brown hens that spent more time on the range, came into lay significantly earlier compared to hens that preferred to stay in the shed. At 22 weeks of age, rangers performed at 89.5%, while stayers reached 72.9% hen-day egg production. Hens that stayed in the shed did not achieve a comparable egg performance until 52 weeks of age. In this research, hens that stayed in the shed contributed 20% of the overall flock population, therefore representing a significant loss for the egg producer allowing for serious reconsideration of current feed practices. In contrast, the performance of hens that accessed the range frequently exceeded the expected performance of the breed standard. Further information regarding the total egg mass produced, as well as differences observed on internal and external egg quality is under current investigation.

CONSEQUENCES OF FLOCK SUBPOPULATIONS FOR FUTURE IMPLICATIONS POULTRY NUTRITION

Many investigations have previously demonstrated significant differences and challenges in production, physiological, behavioral and welfare parameters associated with different housing systems (AHAMMED et al., 2014; FERRANTE et al., 2009, YILMAZ DIKMEN et al., 2016)

While many researchers found significantly more disadvantages associated with loose husbandry systems, others have clearly demonstrated that hens housed in barn, free-range or organic systems can exceed caged production or breeder guidelines (CLERICI et al, 2006). Therefore, impact of hen management and stockmanship skills on hen performance, health and farm economics cannot be underestimated (BLOKHUIS et al., 2007). If the skillset and knowledge about flock management is extended to a substantial understanding of the differences and needs of various flock subpopulations, the use of loose husbandry systems will become more sustainable and profitable. Modern technology such as integrated RFID systems that allow for constant and automatic flock monitoring will help to determine the load on resources, detecting unusual movement pattern, and provide indicators about the localisation of different performance groups. The use of RFID systems in poultry breeder feeding systems, for example, has been used to increase flock uniformity to 100% (ZUIDHOF et al., 2017). Automated feeding stations that recognize individual birds and use body weight information may be part of future flock management, similar to the dairy industry where cows are fed with supplements based on the their daily milk yield. However, being able to determine the % of hens that are not using the nest boxes, not accessing certain feeder lines, or using the range in real time can allow for adequate changes in dietary manipulations. Furthermore, offering different diets or feed additives through different feeder lines may directly target the different requirements for hens that favor these specific locations. It is not only of highest interest to investigate how feed technology can be used to support under-performing subpopulations, but also how to ensure that over-performing hens are truly nurtured to their best long-term care.

Using big data and computer learning is a powerful tool to allow an in-depth understanding about hen usage of the aviary system and assist in decision making regarding the height, width, design and number of tiers to ensure desired performance and welfare outcomes. This is especially true when aiming for flock longevity and laying persistence beyond 100 weeks of age. Investigating reasons and key events that are associated with the development of flock subpopulations needs to be extended to the rearing facilities as well as the consequences of modified rearing strategies on the use of the hen house including aviary system and other resources.

CONCLUSION

The characterisation of subpopulations within one flock using modern technology allows for identification of hen clusters and classification into various groups according to their performance, health status, and metabolic energy requirements. Using this information routinely to manage flocks for commercial egg production will allow nutrient support to achieve outstanding performance of all flock subpopulations, while at the same time providing efficient and responsible use of feed resources. The use of modern technology including integrated Radio-Frequency Identification (RFID) systems, location sensing and computed learning to improve flock management needs further research and should be considered to improve current feeding strategies allowing real-time flock management for a more economic, efficient and sustainable use of feed resourced.

The author declares no conflicts of interest.

REFERENCES

AERNI, V., M.W.G. RINKHOF, B. WECHSLER, H. OESTER, E. FRÖHLICH, 2005: Productivity and mortality of laying hens in aviaries: a systematic review. World's Poultry Science Journal. **61**, 130-142.

AHAMMED, M., B. J. CHAE, J. LOHAKARE, B. KEOHAVONG, M. H. LEE, S. J. LEE, D. M. KIM, J. Y. LEE, S. J. OHH, 2014: Comparison of aviary, barn and conventional cage raising of chickens on laying performance and egg quality. Asian-Australasian Journal of Animal Sciences **27**(8), 1196-1203.

AHAMMED, M. and S. J. OHH, 2013: Effect of housing systems-barn vs cage on the first phase egg production and egg quality traits of laying pullet. Korean Journal of Poultry Science, **40**(1), 67-73.

BLOKHUIS, H. J., T. FIKS VAN NIEKERK, W. BESSEI, A. ELSON, D. GUÉMENÉ, J.B. KJAER, M. LEVRINO, C.J. NICOL, R. WEEKS, H.A. VAN DE WEERD, 2007: "The LayWel project: welfare implications of changes in production systems for laying hens. World's Poultry Science Journal **63**: 101-114.

CLERICI, F., E. CASIRAGHI, A. HIDALGO, AND M. ROSSI, 2006: Evaluation of eggshell quality characteristics in relation to the housing system of laying hens. Proceedings of the XII European Poultry Conference: 10-14.

DAGHIR, N.J., 1973. Energy requirements of laying hens in a semi-arid continental climate. British Poultry Science, **14**(5), 451-461.

EITS, R. M., R. P. KWAKKEL, B.G.E. REINDSEN, T. ZANDSTRA, AND A.A. MAATMAN. 2005: Effect of housing system on balanced protein requirements in laying hens. In 15th European symposium poultry nutrition, **15**, 133-135.

FERRANTE, V., S. LOLLI, G.VEZZOLI, L. G. CAVALCHINI, 2009: Effects of two different rearing systems (organic and barn) on production performance, animal welfare traits and egg quality characteristics in laying hens. Italian Journal of Animal Science. **8**(2), 165-174.

GEBHARDT-HENRICH, S.G., M.J. TOSCANO, E.K. FRÖHLICH, 2014: Use of outdoor ranges by laying hens in different sized flocks. Applied Animal Behaviour Science. **155**, 74-81.

HARTCHER, K.M., K.A. HICKEY, P.H. HEMSWORTH, G.M. CRONIN, S.J. WILKINSON, M. SINGH, 2016. Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens. Animal. **10**, 847-853.

HUDSON B. P., R. J. LIEN, J. B. HESS, 2001: Effects of body weight uniformity and pre-peak feeding programs on broiler breeder hen performance. Journal of Applied Poultry Research. **10**, 24-32.

KOLAKSHYAPATI M, S.B.WU, T.Z. SIBANDA, I. RUHNKE, 2019a: Body weight but not range usage is the main factor affecting net energy utilisation in commercial free-range laying hens. Animal Nutrition - submitted

KOLAKSHAYAPATI, M., P.S. TAYLOR, T.Z. SIBANDA, I. RUHNKE, 2019b: Association of fearfulness at the end of lay with range visits during 18-22 weeks of age in commercial laying hens. Proceedings of the Congress of the International Society for Applied Ethology - accepted.

LEENSTRA, F., V. MAURER, M. BESTMAN, F. VAN SAMBEEK, E. ZELTNER, B. REUVEKAMP, F. GALEA, AND T. VAN NIEKERK, 2012: Performance of commercial laying hen genotypes on free range and organic farms in Switzerland, France and The Netherlands. British Poultry Science. **53**(3), 282-290.

LEENSTRA, F., V. MAURER, F. GALEA, M. BESTMAN, Z. AMSLER-KEPALAITE, J. VISSCHER, I. VERMEIJ, M. VAN KRIMPEN, 2014: Laying hen performance in different production systems; why do they differ and how to close the gap? Results of discussions with groups of farmers in The Netherlands, Switzerland and France, benchmarking and model calculations. European Poultry Science. **78**,1-10.

RODENBURG, T.B., M.M. VAN KRIMPEN, I. C. DE JONG, E.N. DE HAAS, M.S. KOPS, B.J. RIEDSTRA, R.E. NORDQUIST, J.P. WAGENAAR, M. BESTMAN, C.J. NICOL, 2013: The prevention and control of feather pecking in laying hens: identifying the underlying principles. World's Poultry Science. **69**(2), 361-374.

RUHNKE I. and T.Z. SIBANDA, 2018: Nutritional management of free range laying hens. Proceedings of the Poultry Information Exchange.

SIBANDA T.Z., S.W. WALKDEN-BROWN, M. KOLAKSHYAPATI, B. DAWSON, D. SCHNEIDER, M. WELCH, Z. IQBAL, A. COHEN-BARNHOUSE, N.K. MORGAN, J. BOSHOFF, I. RUHNKE, 2019: Flock use of the range is associated with the use of different components of a multi-tier aviary system in commercial free-range laying hens. British Poultry Science - submitted.

SIROVNIK, J., H. WÜRBEL, M.J. TOSCANO, 2018: Feeder space affects access to the feeder, aggression, and feed conversion in laying hens in an aviary system. Applied Animal Behaviour Science. **198,** 75-82.

SOSSIDOU, E.N., A. DAL BOSCO, H.A. ELSON, and C.M.G.A. FONTES, 2011: Pasture-based systems for poultry production: implications and perspectives. World's Poultry Science Journal **67**(1) (2011): 47-58.

TILLER, H., 2001: Nutrition and animal welfare in egg production systems. 13th European Symposium Poultry Nutrition. **13**, 226-232.

VAN HORNE, P.L.M., 1996: Production and economic results of commercial flocks with white layers in aviary systems and battery cages. British Poultry Science. **37**(2), 55-261.

YILMAZ DIKMEN, B., A. İPEK, Ü. ŞAHAN, M. PETEK, A. SÖZCÜ, 2016: Egg production and welfare of laying hens kept in different housing systems (conventional, enriched cage, and free range). Poultry Science. **95**(7), 1564-1572.

ZUIDHOF M. J., M. V. FEDORAK, C. A. OUELLETTE, I. I. WENGER, 2017: Precision feeding: Innovative management of broiler breeder feed intake and flock uniformity, Poultry Science. 96(7), 2254-2263.

Is biological interaction about to supplant the notion of organism?

Marc-André Selosse

Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205 –CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005, Paris, France

&

Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

SUMMARY

Two revolution have emerged in biology at the beginning of the twenty-first century: microbes are everywhere, and, beyond diseases or decomposition, they weave vital relationships with mutual benefits with larger organisms. All the functions of plants and animals are affected by the microbiota that they contain and which influence their nutrition, health but also development (together with, in the case of animals, behavior). This text is a general presentation of the physical and functional presence of microbiota, and how it is acquired. It discusses the notion of the individual and organism, facing the emergence of the holobiont, and also the strength of interactions and networks of interactions in ecosystems.

INTRODUCTION

The dominant Western view of biology since the 19th century has been based on the concept of the organism, ie, a living entity that by and large is structurally and functionally independent (we speak of the biology of organisms). We generally approach this view through the description and naming of the species ("a rabbit") and functional analysis in the "physiology of the organism." The organism is imagined regardless of scale, but once we go below one millimeter we add the prefix micro, as if to distinguish at all costs two worlds, the visible and the invisible: on the microscopic scale, therefore, we speak of microorganism or microbe.

In recent years, albeit with remarkable epistemological slowness, the scientific community has belatedly and gradually come to see that the macroscopic functions of animals and plants are accomplished through interactions with microbes. One determinant in this has been the possibility, using the tools of molecular biology, of describing extremely varied microbial communities associated with animals and plants, without the need for culture or direct observation, which long delayed awareness of the taxonomic and functional diversity of these communities. The so-called next-generation sequencing techniques (Buermans et al., 2014), which simultaneously determine the primary structure of numerous mixed fragments of DNA, enable the description of the composition, even genetic, of highly diverse and complex microbial communities. This is doubtless one reason why there is now greater recognition of the microbial diversity present in animals and plants. But a functional approach has also emerged in parallel. In the last ten years, microbial communities and their roles have been described in all large organisms, not only in the digestive tract of mammals, but also of insects, or in plant roots and flowers, and even in algae. This is how the concept and the importance of cooperation with microbes, which is widely acknowledged among specialists in symbiosis (Margulis et al., [1991,] Selosse et al., 2004) but has long been marginalized, have finally spread to biological research as a whole.

COLONIZING MICROBES SHAPE THEIR HOST'S ECOLOGY

Microbes are ubiquitous and, although their presence is discreet, they may have a major impact everywhere, as witnessed by a terrible agronomic mistake made in North America. In 1931, the University of Kentucky began a program to improve tall fescue (a grass) to enrich the pastures of the American west. After selection of the most competitive plants, the cultivar Kentucky 31 was marketed in 1943 (Saikkonen, 2000). Widely sown, it quickly formed dense prairies. Alas, cattle feeding on it soon developed "fescue foot:" vasoconstriction of the extremities leading to gangrene and tail loss, hooves sloughed off, stress behavior, nocturnal activity, 30% drop in milk and meat production, and more frequent abortions (Bacon, 1995). Kentucky 31 today is an invasive plant that has cost agriculture dear (Clay et al., 1999).

What was the cause of fescue foot? The culprit was unmasked rather late in the day as an ascomycetes (fungus) of the genus *Neotyphodium* which lives within the plant and produces ergot alkaloids. Some of these fungi are toxic for insects, others for mammals: among their mycotoxins, ergovalin, a vasoconstrictor, explains the gangrene of the extremities in cattle; lysergic acid (from which the psychedelic drug LSD is derived) alters behavior. However, and this is what led to it being overlooked, this fungus is invisible as it never "leaves" the plant and so produces no sporulating lesion: it colonizes all the plant tissues and, in particular, the seeds. It reproduces by transmission to the offspring, and unwittingly this led to selection of the most toxic fungi, which improve plant growth because their repellent effect wards off herbivores (Tanaka et al., 2005)!

Major properties (economic and ecological in this case) of large organisms sometimes result from the microorganisms that live within them. Such microbial contributions are often decisive: if the fungus in cultivar Kentucky 31 is removed by antifungal treatment or by mild heating of the seeds, the plant becomes more palatable to herbivores and so does not become lastingly established in nature (Clay et al., 1999). It's the plant-fungus consortium that is competitive!

Plants and animals are therefore inhabited by microbes that shape their traits. This gave rise to the concept of holobiont (Rosenberg et al., 2011) (from the Greek *holo*, whole, and *bios*, life), which designates the biological unit composed of a host (plant or animal) and all its microbes. This concept replaces the vision of isolated organisms, because all organisms, as we shall see, are accompanied. The holobiont cumulates the properties of the host organism and of its passengers (like the alkaloids of *Neotyphodium*), all the genomes of which the host assembles. Emergent properties also occur. It is often asserted that the holobiont has an evolutionary dimension (ie, a new entity subject to natural selection), but while it is clear that the holobiont contributes to the success of its members as a target for selection, it is not itself fully reproduced (see below) and this evolutionary approach is debated (Douglas et al., 2016). The holobiont leads to the notion of hologenome, which extends the genome of the organism by adding those of its microbes. How are the associations that structure holobionts set up? How and via which functions do they modify the host? We shall see that biological interactions between the host and its microbes act powerfully on the evolution of both.

INHERITED MICROBES AND HITCHHIKING MICROBES

A holobiont teems with microbes. A recent reassessment of the quantity of microorganisms carried by humans suggests that the human body has as many microbial cells as human cells. Or, if we ignore the non-nucleated and abundant red blood cells, there are ten microbial cells for every human cell (Sender et al., 2016). And this inventory does not include mitochondria! Some of these microbes are inherited, while others are transient. Some, like *Neotyphodium*, are inherited and persist from generation to generation without leaving the host. Far from being the most frequent, these microbes are often interlinked with holobiontic functions so closely that they may be discovered late in the day, as with Kentucky 31. For example, mitochondria, which are responsible for respiration and numerous biosyntheses in eukaryotes, are bacteria that have lived in our cells for aeons. Although early observers guessed that this was the case, because mitochondria divide in two like bacteria, the idea took a long while to catch on. The elucidation, in the early 20th century, of the biochemistry of respiration revealed close ties between respiration and the rest of cellular metabolism and this functional unity tended to marginalize the idea that mitochondria are of bacterial origin (Selosse, 2012). The discovery that mitochondria contain DNA and a reduced bacterial genome firmly established their bacterial nature after the 1970s, driven by the overarching vision of Lynn Margulis (1970). Respiration and the eukaryotic cell are therefore holobiontic states that cannot in evolutionary terms be reduced to one organism with a single origin.

The same is true for photosynthesis: algae and plants photosynthesize using plastids, cellular organelles that also contain a reduced bacterial genome and which are, in fact, intracellular photosynthetic cyanobacteria! Some vital cellular properties therefore are those of microbes that long ago became heritable. These microbes remain because within the cell they acquired two properties: division in the cell and transmission to host daughter cells – notably, the reproductive cells, spores or gametes (often the ovule). So, plastids and mitochondria are intracellular bacteria, though their extreme dependence on the cell host and their status as an organelle lead some authors to consider that they are no longer bacteria. This is naturally a question of definition, but it also ignores that all organisms are dependent (so, dependence is not itself a criterion). Here, and for heuristic reasons (Pallen, 2011, Selosse, 2011) we shall consider the bacterial nature as being of phylogenetic origin (eubacterial) – the bacteria can be more or less free or dependent and become organelles when they import some or all of their proteins.

Insects contain bacteria in advanced states of dependency. Their hereditary interactions with microbes has enabled the diversification of ecological niches, through feeding on a wide variety of foods (Moran et al., 2008). The 4000 species of aphids, for instance, have become feeders on plant sap thanks to the heritable bacteria *Buchnera*, which complete the nutritional needs of aphids by synthesizing tryptophan, leucine, and vitamins absent from sap (Jiang et al., 2013). Other insects can feed on blood because although it is poor in vitamins of the B group (thiamine, folate, thiazole, etc.) these are produced by inherited bacteria: *Wigglesworthia* in tsetse flies, *Riesia* in fleas, and *Wolbachia* in bedbugs. Some insects that feed on dry, vitamin-poor foodstuffs benefit from food complements synthesized by yeasts. Examples are the coleoptera called drugstore or bread beetles (*Stegobium paniceum*) and cigarette beetles (*Lasioderma serricorne*). In the latter, yeasts detoxify nicotine, which is normally insecticidal (Dowd, 2007). The bacteria involved in these endosymbioses have extremely reduced genomes 5 to 30 times smaller than that of *Escherichia coli*. The genome of *Carsonella*, an endosymbiont of the sap-sucking hemiptera *Psylla*, is reduced to 160 kb (180 genes) (Moran, 2011). What with specialization in a metabolic function and regression of functions associated with living independently, these bacteria have lost many genes and have become closely dependent: like cellular organelles, certain endosymbiontic bacteria import some of their proteins (Nakabachi et al., 2014). Here we have reached the point where mitochondria and plastids have acquired the full status of organelles. Inherited bacteria have therefore contributed to the extensive evolutionary diversification of insects.

The heritable part of the holobiont, the inherited endosymbionts, are, however, less diverse than the symbionts that “climb aboard” at each generation. At germination or at birth, a microbial horde colonizes the organism, in particular on the surfaces where the organism feeds. There are oft-overlooked resemblances between roots and the gut, not only in nutritional function, but also through the great microbial diversity recruited at each generation (Mommer et al., 2016). The soil surrounding the root, the rhizosphere, is enriched in dead cells and secretions and attracts myriad fungi and bacteria – from 100 to 1000 million per gram of soil! The root takes up mineral resources and emits molecules that create conditions that filter a specific community from the soil (Müller et al., 2016). The gut also harbors an abundant microbial community: each human being harbors more than 1000 species of bacteria and yeasts (more than one kilogram!) and has as many bacteria as body cells (Sommer et al., 2013)! Nourished by ingested foodstuffs, the intestinal community is also filtered from the environment by feeding behaviors, the conditions (anaerobiosis and the presence of enzymes) and the immune system, which determines which microbes thrive.

VERTICAL AND HORIZONTAL TRANSMISSION HAVE THEIR ADVANTAGES

Microbial passengers acquired at each generation or ever-present heritable microbes: which most favor the host? All depends on the criteria used. Inherited microbes are less varied (no choice of partner in this case!) than those recruited at each generation, which are of such variety that they can adapt to different environments and new food. Our gut microbiota, for example, adapt us to our diets: Asians have bacteria that oxidize a toxin of soybeans (daidzein, a natural endocrine disruptor) (Gaya et al., 2016); the Japanese have others that digest the walls of the red algal cells that are characteristic of their cuisine, such as the edible seaweed nori (Hehemann et al., 2012). However, an inherited microbe is reliable, whereas a recruited microbe might be scarce in some places. The germination of orchids depends on soil fungi that feed the seedling and anyone who has tried to sow orchids in their natural milieu will have failed in many places because of a lack of suitable fungi (Selosse, 2014). This is doubtless why some orchid species are rare (McCormick et al., 2014).

Microbes present for the duration and microbes that hitch a ride also differ in the mechanisms that avoid unfavorable partners. Microbes inherited long ago tend to be more favorable to the host: their reproduction depends on that of the host, so those that favor their host are, of course, selected because they reproduce more (Sachs et al., 2006). Vertical transmission, if strict, favors the emergence of mutualism. This selects mitochondria, plastids, or the fungi of Kentucky 31 that are favorable for the host. The environment, on the other hand, can be a source of good and bad, such as a root parasite or an intestinal illness. Microbes that hitch a ride can take advantage of resources provided by the host, to which their future is not definitively tied. To counter this, the host deploys the “carrot and stick” strategy.

Carrot-wise, the host actively attracts favorable microbes. A plant root, for example, emits strigolactones and these are recognized by favorable soil fungi, which colonize the roots of 90% of plants thereby forming a mixed organ called the mycorrhiza (Gough et al., 2016). These soil fungi swap sugars for mineral salts and water collected by the root from the soil. Legumes have roots that emit flavonoids, which attract rhizobia, soil bacteria that colonize the roots by producing nodules where they transform atmospheric nitrogen into nitrogen resources for the legumes (Long, 2006). As some of this nitrogen is released, on the death of the plant for instance, or in exudates, this turns the legumes into "green manure," even if nitrogen fixation is, in fact,… holobiontic! In humans, maternal milk favors the colonization of the infant's gut by bacteria, which aid digestion and protect against diarrheal agents. Human milk contains oligosaccharides composed of three to five sugar molecules whose concentration (15 g/L) makes them the third constituent of breast milk, after lactose and lipids, but well ahead of proteins. As the infant does cannot digest them, these sugars were omitted from formula milk. They are, however, easily digestible by appropriate bacteria, like certain bifidobacteria, which are highly specialized in their use (Ward et al., 2006).

Stick-wise, immune reactions reject the pathogens that harm tissues while mechanisms sort the best partners. For example, on roots, not all fungi able to form mycorrhiza are also favorable to a given plant, which therefore risks nourishing partners that are of little or no use. After the plant has formed mycorrhizae with various partners, it selectively feeds those fungi that supply it with more mineral nutrients, like phosphorus and nitrogen, and feeds less those fungi that prove useless (Selosse et al., 2011). Hence, a long joint evolution with microbes has shaped, in animals and plants, mechanisms optimizing the use of microbes, which adapted to their hosts: the holobiont therefore is the outcome of reciprocally influenced evolution, otherwise called coevolution.

THE MICROBIOTE PLAYS VARIOUS ROLES IN THE HOST ORGANISM

Over the course of evolution, longstanding and temporary partners have ended up assuming multiple roles in the holobiont. In recent decades, plants grown in sterile soil and rodents raised without any exposure to microorganisms (ie, germ-free or axenic) have enabled eloquent comparisons that reveal varied roles which, once again, are similar in the animal gut and in plant roots.

Feeding is a holobiontic affair. Mycorrhizal fungi lower mineral salt levels in the soil around the root, bacteria solubilize iron and phosphate or, like rhizobia, mobilize atmospheric nitrogen… to the point that many plants, like pines, grow little or poorly in sterile soil (van der Heijden et al., 2015). In the intestine, bacteria help digestion via their enzymes and produce vitamins (K and B12) and other essential molecules: deprived of bacteria, germ-free mice require, for the same growth, 25% more food (Wostmann et al., 1983).

Microbes that offer protection to the host, which provides them with room and board, have been selected. They keep pathogens at bay through local competition and at the price of an antibiotic war that rages in the gut and around the root. For example, *Staphylococcus lugdunensis* of the human microbiote synthesizes a peptide antibiotic that destroys *Staphylococcus aureus* (Zipperer et al., 2016), which 10 to 30% of healthy human carry and which, when uncontrolled, can produce skin diseases, even septicemia. But the protective effect of microbes also involves modification of the host: germ-free mice have a less developed immune system, both in the gut (fewer lymphocytes, attenuated expression of immune system genes) and in the rest of the body (Thaiss et al., 2016). Likewise, plants devoid of root microbes react more slowly and less aggressively to parasitic attack and so suffer increased damage (Martinez-Medina et al., 2016).

This shows that microbes play a part in development. Their colonization induces maturation of the immune system (Selosse et al., 2014) – not just a one-off immune reaction, but a capacity to react more effectively that is not acquired without them (this phenomenon in plants is also termed defense priming). Immunity is not only activated, but is also completely modulated and reprogrammed: some of its components are repressed, such as lymphocytes involved in inflammation, the natural killer T cells in mice (Olszak et al., 2012). The current explosion in the Western world of allergies and auto-immune diseases, in which the immune system reacts disproportionately or inappropriately or both, may be the result of a life that has become overly sanitized, where microbial diversity arrives too late in a child's development to play this role of maturation (Fujimura et al., 2015). Allergies are less frequent in rural settings where exposure to microbes is commoner and can be prevented by the addition of targeted bacteria to infant foods. An example of such "virtuous" exposure is the effect of the presence in the home of a dog, which increases the diversity of the child's microbiota and correlatively reduces the risk of asthma by 13% (Gupta, 2017).

Lastly, germ-free mice show behavioral alterations: increased activity, reduced timidity and anxiety, poor memorization (Luczynski et al., 2016). Their nervous system differs from that of holobiontic mice, notably in the functioning of the synapses and in the expression of major genes. These traits return to normal after microbial colonization, but only when the animals are young and still developing (Diaz Heijtz et al, 2011). So, in a way yet to be elucidated, the presence of bacteria also induces the correct development of the nervous system! There is a complex dialogue between the nervous system and the microbes that persist in the adult, whose behavior can be affected by these microbes. For example, bacteria alter mood in rodents and humans. *Campylobacter* species seem to favor depressive states (Marshall, 2009); lactobacilli and bifidobacteria reduce depression and stress: they partially mimic the effects of anxiolytics on behavior and also, in rodents and humans, on brain activity (Bharwani et al., 2017)! One day, maybe, chosen bacteria will heal us. In the meantime, we wonder at so many intrusions in functions that we (wrongly) imagine are independent.

Microbes even have a say in reproduction. When we sterilize elderflower (a fragrant species used in various flavored drinks), it stops synthesizing certain compounds that attract pollinators, notably volatile terpenes, which decrease three-fold (Peñuelas et al., 2014). In animals, microbial interference even affects the choice of sexual partner. When fruit flies fed on molasses are mixed with others fed on starch, they prefer to mate with those fed the same food. After antibiotic treatment, this mating preference disappears… but returns when the flies are contaminated with excrement. The origin of the excrement, and of the microbes it contains, therefore determines the sexual choice of the flies (Sharon et al., 2010)! Lactobacilli favored by eating starch seem to modify sexual pheromones, which attract partners during mating.

THE EVOLUTION OF INTERDEPENDENCE

Why are there these microbial intrusions in so many processes that could be independent? We have proposed two simple mechanisms to explain this evolution towards dependence (Selosse et al., 2014). We should not forget that germ-free plants and animals are laboratory creations useful for investigating and understanding the role of microbes. In nature, microbes are always around, diverse and countless. On the one hand, evolutionary innovation, like a new floral fragrance or a supplementary food for an insect, can be based on one of these ever-present microbes. On the other hand, if microbes perform a function also performed by the host, they can supplant the host's function by, for instance, synthesizing a vitamin or replacing a stimulus in a developmental process. Such events are doubtless rare in evolution, but hundreds of millions of years of coevolution and tens if not hundreds of two-by-two interactions have increased the probability. What's more, when functions are lost in the host or microbe, the mutations that would restore these functions (reverse mutations) are unlikely. This drift towards dependence is therefore unlikely to be reversed and secondarily strengthens the interaction. The dependence works and increases symmetrically for the microbes, as shown, for example, by genome reduction in the endosymbiotic bacteria of insects referred to above, and by mitochondria and plastids. Loss of dependence in the holobiont is an authentic product of coevolution.

This is a harsh lesson for those who wanted to postpone the teaching of ecology until after learning about physiology, development, reproduction, and so on, the biology of organisms in effect. The interactions between microbes, or between microbes and their host, shape part of the biology of organisms into a complex ecology! Because, in the end, the holobiont is a stage for ecological interactions. Ecology is no longer subordinated to the biology of organisms: like the chicken and the egg, these disciplines mutually underpin each other.

THE LIMITS OF THE HOLOBIONT: TOWARDS NETWORKS OF INTERACTIONS

Now we come to the limits of the holobiontic vision. A modern concept in vogue, I feel it already has a whiff of the outdated about it. Interactions, particularly microbial, deny the relevance of an independent vision of the organism: this is appreciable for endosymbiotic microbes, for example, but germ-free mice and other animals show that the isolated organism is unable to function correctly in an independent fashion. The holobiont enables the survival of the organism as an enlarged unit, but in a world of interactions, are there still really discernible "units?" Consider for a moment an interaction that is life-sustaining for 90% of plants: the abovementioned mycorrhizal association, which is studied by my own research team.

Mycorrhizal fungi in the soil colonize the roots of various plants, sometimes of different species. Likewise, each plant associates with dozens of species of fungi. Mycorrhizal symbiosis therefore creates a network of plants connected by fungi, and vice versa (Selosse et al., 2006). Now, certain plants feed on this network: they capture from their fungi sugars that the fungi have acquired from neighboring plants. Species of the orchid and heather families have sometimes lost chlorophyll and are entirely fed by the network (Merckx, 2013). Other plants are still green and are sustained by both photosynthesis and the network (Selosse et al., 2009). Most of these plants live in undergrowth where the network provides supplementary carbon in low-light conditions that limit photosynthesis. What's more, the mycorrhizal network can send signals between plants: when a plant is attacked by a parasite, plants connected to the same mycorrhizal network react to this attack by, for example, triggering the synthesis of defense compounds (Barto et al., 2012). This is so, for instance, in tomatoes connected by a shared mycorrhizal fungus (Song et al., 2015): when one tomato plant is attacked by *Alternaria*, its neighbors elicit defense responses if, and only if, there is a connection to the same mycorrhizal fungus. Although experiments in "natural" conditions, far from the greenhouse models used hitherto, remain desirable, it seems clear that the plant functions within a mycorrhizal network (Roy et al., 2015).

In this example, how do we delimit the independent units? Where does the holobiont end? The plant is linked to fungi, but what these fungi supply to the plant—signals or nutrients—come from other plants, which themselves owe their functioning to other fungi, and so on. Other interaction networks exist: pollinating insects interact with several plants each of which interacts with several insects. Our pathogens also form networks, each of which interact with several hosts, which themselves are linked to several microbes. The network is, of course, materialized in the case of plants, less so for animals. But the example mentioned above of how the sexual behavior of fruit flies can be manipulated by a clone of bacteria that the flies share, or not, reveals that the sharing of clones between animals can alter their interactions, even their evolution. Another group of examples of manipulation of a host species by a clone is that of *Wolbachia*, which manipulate the sexuality of their host to maximize their own transmission by females (Werren et al., 2008) and can cause sterility in uninfected males. In *Nasonia*, a genus of wasps, interspecific hybrids survive poorly, except after antibiotic therapy (Trucker et al., 2013): the microbial communities inherited from the parents oppose crosses such that, albeit invisible, the network transcends the limits of the organism.

The holobiont, by actualizing the idea of organism, masks the importance of the interactions themselves and delays the shift to another vision of life, that of a network of interactions, which has already been modeled by ecologists (Bascompte et al., 2014). Modern science has transposed a Western philosophy based on the individual into a biology based on the organism. A genuine rupture would be—will be, I believe—to place interactions center stage (Selosse, 2017). A cobweb is not a collection of points where threads cross, but rather the links between these points! Similarly, life comprises organisms and above all the links between them. The notion of organism, in two centuries of research, has yielded a great deal in understanding and knowledge. Increasingly though, this notion seems to be an artefact of the macroscopic world, where the plant or animal is seen as an observable whole, concealing its microbes. Somewhat belatedly, but insistently, our understanding of organisms is now revealing their multiple functional contributions (McFall-Ngai et al., 2013).

Today, interactions are in the spotlight. Tomorrow, the interdependences and interrelations with the microbes we harbor will open new horizons for environmental management, food production, healthcare, and nutrition.

ACKNOWLEDGMENTS

A version of this article first appeared in *Pour la Science* (Selosse, 2016), which has kindly authorized the use of this modified and extended version translated into English.

REFERENCES

Bacon C.W., "Toxic endophyte-infected tall fescue and range grasses: historic perspectives", *Journal of Animal Science* 73, 1995, p. 861-870.

Barto E.K., J.D. Weidenhamer, D. Cipollini & M.C. Rillig, "Fungal superhighways: do common mycorrhizal networks enhance belowground communication?", *Trends in Plant Science* 17, 2012, p. 633-637.

Bascompte J. & P. Jordano, "Mutualistic networks", Princeton University Press, 2014.

Bharwani A., M.F. Mian, M.G. Surette, J. Bienenstock & P. Forsythe, "Oral treatment with *Lactobacillus rhamnosus* attenuates behavioural deficits and immune changes in chronic social stress", *BMC Medicine* 15, 2017, 7.

Buermans H.P.J. & J.T. den Dunnen, "Next generation sequencing technology: Advances and applications", *Biochimica and Biophysica Acta (BBA)* 1842, 2014, p. 1932-1941.

Clay K. & J. Holah, "Fungal endophyte symbiosis and plant diversity in successional fields", *Science* 258, 1999, p. 1742-1744.

Diaz Heijtz R., S. Wang, F. Anuar, Y. Qian, B. Björkholm, A. Samuelsson, M.L. Hibberd, H. Forstberg & S. Pettersson, “Normal gut microbiota modulates brain development and behavior”, *Proceedings of the National Academy of Sciences of the United States of America* 108, 2011, p. 3047–3052

Douglas A.E. & J.H. Werren JH, “Holes in the hologenome: why host-microbial symbioses are not holobionts”, *mBio* 7, 2016, e02099-15.

Dowd P.F., “Insect fungal symbionts - a promising source of detoxifying enzymes”, *Journal of Industrial Microbiology* 9, 2007, p. 149–161.

Fujimura K.E. & S.V. Lynch, “Microbiota in allergy and asthma and the emerging relationship with the gut microbiome”, *Cell Host Microbe* 13, 2015, p. 592-602.

Gaya P., M. Medina, A. Sánchez-Jiménez & J.M. Landete, “Phytoestrogen metabolism by adult human gut microbiota”, *Molecules* 21, 2016, 1034.

Gough C. & G. Bécard, “Strigolactones and lipo-chitooligosaccharides as molecular communication signals in the arbuscular mycorrhizal symbiosis”, *in* F. Martin (dir.), *Molecular mycorrhizal symbiosis*, John Wiley & Sons, 2016.

Gupta S., “Microbiome: Puppy power”, *Nature* 543, 2017, p. S48–S49.

Hehemann J.H., A.G. Kelly, N.A. Pudlo, E.C. Martens & A.B. Boraston, “Bacteria of the human gut microbiome catabolize red seaweed glycans with carbohydrate-active enzyme updates from extrinsic microbes”, *Proceedings of the National Academy of Sciences of the United States of America* 109, 2012, p. 19786-19791.

Jiang Z., D.H. Jones, S. Khuri, N.F. Tsinoremas, T. Wyss, G. Jander & A.C. Wilson, “Comparative analysis of genome sequences from four strains of the *Buchnera aphidicola* Mp endosymbiont of the green peach aphid, *Myzus persicae*”, *BMC Genomics* 24, 2013, 917.

Long S.R., “*Rhizobium* symbiosis: nod factors in perspective”, *The Plant Cell* 8, 1996, p. 1885-1898.

Luczynski P., K.-A. McVey Neufeld, C.S. Oriach, G. Clarke, T.G. Dinan & J.F. Cryan, “Growing up in a bubble: Using germ-free animals to assess the influence of the gut microbiota on brain and behavior. *International Journal of Neuropsychopharmacology* 19, 2016

Margulis L. & R. Fester, “Symbiosis as a source of evolutionary innovation. Speciation and morphogenesis”, MIT Press, 1991.

Margulis L., *Origin of Eukaryotic Cells*. Yale University Press, 1970.

Marshall J.K., “Post-infectious irritable bowel syndrome following water contamination”, *Kidney International* Suppl. 2009, p. S42-S43

Martinez-Medina A., V. Flors, M. Heil, B. Mauch-Mani, C.M.J. Pieterse, M.J. Pozo, J. Ton, N.M. van Dam & U. Conrath, “Recognizing plant defense priming”, *Trends in Plant Science* 21, 2016, p. 818-822.

McCormick M.K. & H. Jacquemyn, “What constrains the distribution of orchid populations?”, *New Phytologist* 202, 2014, p. 392-400.

McFall-Ngai M., M.G. Hadfield, T.C.G. Bosch, H.V. Carey, T. Domazet-Lošo, A.E. Douglas, N. Dubilier, G. Eberl, T. Fukami, S.F. Gilbert, U. Hentschel, N. King, S. Kjelleberg, A.H. Knoll, N. Kremer, S.K. Mazmanian, J.L. Metcalf, K. Nealson, N.E. Pierce, J.F. Rawls, A. Reid, E.G. Ruby, M. Rumpho, J.G. Sanders, D. Tautz & J.J. Wernegreen, “Animals in a bacterial world, a new imperative for the life sciences”, *Proceedings of the National Academy of Sciences of the United States of America* 110, 2013, p. 3229–3236.

Merckx V. (ed.), *Mycoheterotrophy: the biology of plants living on fungi*, Springer, 2013, .

Mommer L., J. Kirkegaard, & J. van Ruijven, “Root interactions: Towards a rhizosphere framework”, *Trends in Plant Sciences* 21, 2016, p. 209-217.

Moran N.A. & J.P. McCutcheon, “Extreme genome reduction in symbiotic bacteria”, *Nature Review Microbiology* 8, 2011, p. 13-26.

Moran N.A., J.P. McCutcheon & A. Nakabachi, “Genomics and evolution of heritable bacterial symbionts”, *Annual Review of Genetic* 42, 2008, p. 165-190.

Müller D.B., C. Vogel, Y. Bai & J.A. Vorholt, “The plant microbiota: systems-level insights and perspectives”, *Annual Review of Genetics* 50, 2016, p. 211-234.

Nakabachi A., K. Ishida, Y. Hongoh, M. Ohkuma & S.Y. Miyagishima, "Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont", *Current Biology* 24, 2014, p. R640-R641.

Olszak T., D. An, S. Zeissig, M.P. Vera, J. Richter, A. Franke, J.N. Glickman, R. Siebert, R.M. Baron, D.L. Kasper & R.S. Blumberg, "Microbial exposure during early life has persistent effects on natural killer T cell function", *Science* 336, 2012, p. 489–493.

Pallen M.J., "Time to recognise that mitochondria are bacteria?", *Trends in Microbiology* 19, 2011, p. 58-64

Peñuelas J., G. Farré-Armengol, J. Llusia, A. Gargallo-Garriga, L. Rico, J. Sardans, J. Terradas & I. Filella, "Removal of floral microbiota reduces floral terpene emissions", *Science Reports* 4, 2014, 6727.

Rosenberg E. & I. Zilber-Rosenberg, "Symbiosis and development: The hologenome concept". *Birth Defects Research C Embryo Today* 93, 2011, p. 56–66

Roy M. & M.-A. Selosse, "Les réseaux mycorhiziens, des réseaux mutualistes entre champignons et racines des plantes", *in* C. Sueur (ed.), *Analyse des réseaux sociaux appliquée à l'éthologie et l'écologie*, Éditions Matériologiques, 2015, p. 291-328

Sachs J.L. & T.P. Wilcox, "A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*", *Proceedings of the Royal Society London B* 273, 2006, p. 425-429.

Saikkonen K., "Kentucky 31, far from home", *Science* 287, 2000, p. 1887.

Selosse M.A. & F. Rousset, "The plant-fungal marketplace", *Science* 333, 2011, p. 828-829.

Selosse M.A. & M. Roy "Green plants eating fungi: facts and questions about mixotrophy", *Trends in Plant Sciences* 14, 2009, P. 64-70.

Selosse M.A., "Au-delà de l'organisme, l'holobionte", *Pour La Science* 469, 2016, p. 80-84

Selosse M.A., "Jamais seul : ces microbes qui construisent les plantes, les animaux et les civilisations", Actes Sud, 2017.

Selosse M.A., "Morts d'amour: mitochondria are bacteria that sometimes become extinct through symbiosis", *Trends in Microbiology* 19, 2011, p. 255-256.

Selosse M.A., "Symbiose et mutualisme *versus* évolution: de la guerre à la paix? ", *Atala* 15, 2012, p. 35-49.

Selosse M.A., "The latest news from biological interactions in orchids: in love, head to toe". *New Phytologist* 202, 2014, p. 337–340.

Selosse M.A., A. Bessis & M.-J. Pozo, "Microbial priming of plant and animal immunity: symbionts as developmental signals", *Trends in Microbiology* 22, 2014, p. 607-613.

Selosse M.A., E. Baudouin, P. Vandenkoornhuyse, "Symbiotic microorganisms, a key for ecological success and protection of plants", *Comptes-rendus Biologies*, 327, 2004, p. 639-648.

Selosse M.A., F. Richard, X. He, S. Simard, "Mycorrhizal networks: the liaisons dangereuses", *Trends in Ecology and Evolution* 11, 2006, p. 621-628.

Sender R., S. Fuchs & R. Milo, "Revised estimates for the number of human and bacteria cells in the body", *PLoS Biology* 14, 2016

Sharon G., D. Segal, J.M. Ringo, A. Hefetz, I. Zilber-Rosenberg & E. Rosenberg, "Commensal bacteria play a role in mating preference of *Drosophila melanogaster*". *Proceedings of the National Academy of Sciences of the United States of America* 107, 2010, p. 20051-20056.

Sommer F. & F. Bäckhed, "The gut microbiota - masters of host development and physiology", *Nature Reviews. Microbiology* 11, 2013, p. 227-238.

Song Y., D. Chen, K. Lu, Z. Sun & R. Zeng, "Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus", *Frontiers in Plant Science* 6, 2015, 786.

Tanaka A., B.A. Tapper, A. Popay, E.J. Parker & B. Scott, "A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory", *Molecular Microbiology* 57, 2005, p. 1036–1050.

Thaiss C.A., N. Zmora, M. Levy & E. Elinav, "The microbiome and innate immunity", *Nature* 7, 2016, p. 65-74.

Trucker R.M. & S.R. Bordenstein, "The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus *Nasonia*", *Science* 341, 2013, p. 667-669.

van der Heijden M.G.A., F. Martin, M.-A. Selosse & I. Sanders, "Mycorrhizal ecology and evolution: the past, the present and the future", *New Phytologist* 205, 2015, p. 1406–1423.

Ward R.E., M. Niñonuevo, D.A. Mills, C.B. Lebrilla & J.B. German, "In vitro fermentation of breast milk oligosaccharides by *Bifidobacterium infantis* and *Lactobacillus gasseri*", *Applied and Environmental Microbiology* 72, 2006, p. 4497-4499.

Werren J.H., L. Baldo & M.E. Clark, "*Wolbachia*: master manipulators of invertebrate biology", *Nature Reviews. Microbiology* 10, 2008, p. 741-751.

Wostmann B.S., C. Larkin, A. Moriarty, & E. Bruckner-Kardoss, "Dietary intake, energy metabolism, and excretory losses of adult male germfree Wistar rats", *Laboratory Animal Science*, 33, 1983, p. 46–50.

Zipperer A., M.C. Konnerth, C. Laux, A. Berscheid, D. Janek, C. Weidenmaier, M. Burian, N.A. Schilling, C. Slavetinsky, Marschal M., M. Willmann, H. Kalbacher, B. Schittek, H. Brötz-Oesterhelt, S. Grond, A. Peschel & B. Krismer, "Human commensals producing a novel antibiotic impair pathogen colonization", *Nature* 535, 2016, p. 511-516.

Improving gut health by modulating the digestive microbiota of chickens? How metagenomics can help.

Fanny Calenge

INRA - UMR GABI, Domaine de Vilvert, 78352 Jouy-en-Josas cedex, France, fanny.calenge@inra.fr

ABSTRACT

High throughput sequencing technologies led to a tremendous progress in the knowledge of gut microbiota in human as well as in livestock and model animal species. Now considered as a symbiont, this microbial ecosystem realizes essential functions for its host through a complex functional dialog. Gut health in particular depends on the composition and functions of the gut microbiota through the regulation of digestion and innate and adaptive immunity. Nutritional strategies making use of feed composition and additives have been used for a long time in poultry production to improve chicken gut health, but their mode of action is generally poorly understood beyond their effect on microbiota composition. Metagenomic approaches, by giving access to the functional potential of the gut microbiota, might become a decisive tool to decipher the complex functional interactions at stake in these effects on gut health. The access to the chicken intestinal metagenome will first allow us to perform in-depth diversity analyses to describe the microbes present in chicken production conditions in relation to the many factors influencing the intestinal microbiota ecosystem. Research is needed to understand what can be considered a healthy microbiota at the functional level and to understand how the different tools available to influence it interfere with the complex host-microbiota molecular crosstalk, in particular during the early intestinal colonisation by micro-organisms. Finally, we will have to relate individual microbiota characteristics with fine descriptors of gut health, related host phenotypes of interest, and host genotypes, to identify key functional elements and putative biomarkers predicting the ability of animals to cope with different kinds of health challenges. Only a multidisciplinary research relying on a multi-omics data integration approach will allow us to take up this challenge. This research will allow us to fine-tune prevention or healing nutritional strategies to optimize the gut microbiota and the related phenotypes, in particular gut health, by acting on the holobiont, i.e. both on the host and its gut microbiota.

Keywords: *Gallus gallus*, metagenome, gut health, functional metagenomics

1.INTRODUCTION

The impressive progress of sequencing technologies during the last decades has led to a huge progress in microbial ecology, by giving a rapid and much more exhaustive access to the many microbes of each microbial ecosystem. The intestinal microbiota in particular, comprised of a majority of anaerobic, uncultivable microorganisms, is now considered as a symbiotic partner for its host, realising essential functions that the host is unable to achieve and contributing to the regulation of its whole physiology and metabolism (Blottière *et al.* 2013, Sommer and Bäckhed 2013). It is now very clear that the host and its intestinal microbiota both contribute to the expression of phenotypic traits of interest (Hanning and Diaz-sanchez 2015). Health in particular, and especially intestinal health, is the result of complex functional interactions between intestinal microbes and host immunity (Broom and Kogut 2018) . Disruptions of the intestinal microbiota can lead to many kinds of non-infectious diseases by altering the host physiology and metabolism and triggering inflammation (Guchte *et al.* 2018). Conversely, alterations of the host immunity can disrupt the intestinal microbiota. Research led on the human intestinal microbiota is currently leading in therapeutic innovation in human medicine. It consists mainly in personalised nutrition or action of pre- or pro-biotics to direct the microbiota to a composition considered as more favourable to its host, development of diagnostic tools for complex diseases such as Crohn's disease or the response to cancer treatments, and fecal transplants to treat critical infections by the bacterium *Clostridium difficile* (Aziz *et al.* 2013).

These observations are true for animal species as well, although potential applications to heal or prevent diseases differ. In poultry production, many diseases cause gut dysfunction, or originate from the gut (Kogut and Arsenault 2016). Gut health is therefore at the centre of animal health. The adult gut microbiota in chicken was identified a long time ago for its protective effect toward the colonisation of intestines of young chicks by *Salmonella* sp. through a mechanism called competitive exclusion (Clavijo *et al.* 2018). It has since be proven effective toward other enteric pathogens, in chicken as well as in every livestock species and in human. The gut microbiota also largely contributes to digestion by the production of essential vitamins and metabolites such as short chain fatty acids (SCFA) and the digestion of complex molecules such as non-starch polysaccharides. It also contributes to host metabolism and physiology by the production of metabolites circulating in the entire organism, and it interacts with host immunity permanently through the intestinal epithelium. There is no universal definition of gut health (Kogut and Arsenault 2016). We will define it here as an optimal functioning of the gut, ensuring resistance to diseases and an efficient energy extraction from feed.

The composition of the gut microbiota in bacterial species in poultry is highly dynamic. In current production conditions, eggs are separated from laying hens and chicks hatch without contact with their mothers, so that the first microorganisms colonising the digestive tract are found in the immediate environment: eggshell, surfaces in the hatchery, air/ water supply and the first feed ingested. For this reason, chicken production is unique since, without the maternal influence, in theory the gut microbiota composition can be modulated very early, which offers the possibility to influence the development of host immunity and digestion with presumably long-lasting effects throughout the animal life. Beyond this early influence of the immediate environmental micro-organisms, gut microbiota varies according to the animal age, the intestinal segment, the genetic breed, and is also influenced by many environmental parameters (Kers *et al.* 2018).

The feed composition and the use of additives is probably the most important factor influencing gut microbiota composition in poultry, as the primary source of nutrients for microbes themselves (Oakley *et al.* 2014, Pan and Yu 2014). Many studies report the positive effect on mainly feed efficiency or resistance to intestinal colonisation by bacterial pathogens of a vast array of feed additives, sometimes in relation to variations in gut microbiota composition. Nevertheless, their mode of action is generally not understood, or only poorly, which limits the possibility to improve their efficiency. Taxonomic information gathered about the ecosystem composition through high-throughput sequencing of the 16S rRNA gene of bacterial DNA, although very informative, is not sufficient to understand the functions at stake in the interactions between gut microbiota and host phenotypes of interest, among which gut health. For this, studies of whole metagenomes are needed. After an overview of the existing knowledge about the effect of feed and additives in poultry production on gut microbiota and gut health, this paper reviews the whole metagenomics studies applied to chicken available. Finally, it will discuss the directions research should take to improve our knowledge of functional host-microbiota interactions based on metagenomics approaches and thus implement successful nutritional approaches to improve gut health in poultry production.

2. THE CHICKEN DIGESTIVE MICROBIOTA: MAIN FEATURES AND EFFECTS OF FEED AND ADDITIVES ON GUT HEALTH

a. Current knowledge of the digestive microbiota

The digestive microbiota of chicken has been studied for a long time, first using cultivation techniques and, like other microbial ecosystems using DNA-based approaches and eventually next-generation DNA sequencing approaches targeting the 16S rRNA bacterial gene as a phylogenetic marker.

We know that with current breeding practices, the maternal influence on the early colonisation of the chick's digestive tract is much reduced compared to Mammals. Recent results though suggest that the embryo is colonised by bacteria from the maternal microbiota (Ding *et al.* 2017), which means that there might be a maternal influence, and that host genetics might have an impact on the early crosstalk between the embryo and its microbiota. Chicks hatch with no, or very few bacteria in their gut, which is quickly colonised by micro-organisms from the immediate environment: surfaces, eggshell, water, litter, and of course, feed. Very few studies focus on the identity of these primo-colonising micro-organisms, probably because their low abundance makes their isolation difficult, even through DNA amplification. It is nevertheless worth of interest, because their functional importance might be fundamental, by priming the immune system and the first molecular cross-talk occurring between host immunity and microbiota, with long-lasting consequences over the entire lifetime of the animal.

After this primo-colonisation, bacterial populations grow rapidly, reaching 10^9 to 10^{11} bacteria per gram the third day after hatching to stay relatively stable until 30 days of age. Qualitative changes also occur in the different digestive compartments according to the animal's age, with an increase of diversity over time (Ocejo *et al.* 2019)"type":"article-journal"},"uris":["http://www.mendeley.com/documents/?uuid=33f0ab25-aa88-4087-a71b-fe0840724b73"]}],"mendeley":{"formattedCitation":"(Ocejo <i>et al.</i> 2019. Segments of the upper part of the digestive tract: crop, gizzard and ileum contain mostly *Lactobacillus* species. Caeca, at the distal part of the digestive tract, are the most favourable site for bacterial development and are actually the more diverse and the richest in bacteria, due to a longer retention time than in other parts of the GI tract. Major phylas in caeca are *Bacteroidetes*, *Protebacteria* and *Firmicutes*, with for the latter *Clostridiales* more represented. The long retention time favours fermentations and the production of short chain fatty acids (SCFA) from the degradation of polysaccharides and interactions of bacteria with their host across the epithelium, in particular with host immunity due to the presence of secondary immune tissues like caecal tonsils. It is also, for the same reasons, the segment where pathogens are the most abundant. For all of these reasons, it is certainly the most studied intestinal segment in relation with gut health.

Microbiota composition of luminal content and intestinal mucosa also differ (Awad *et al.* 2016). The mucosal microbiota is of great functional importance since it directly interacts with the host cells and immunity. Nevertheless, it is not often studied, probably for practical reasons, being less easily collected and difficult to dissociate from the luminal content. Research should certainly focus on this microbiota since its role is most probably essential in the crosstalk occurring with the host through the intestinal epithelium, thus contributing to the immune homeostasis allowing the control of pathogenic bacteria while maintaining beneficial microbes.

b. Effects of feed and additives on gut health through modifications of the gut microbiota: what do we know?

Micro-organisms living in the gut mainly depend on the feed ingested by animals to survive. Chicken feed consists in plants, mainly cereals, complemented with enzymes allowing an improved degradation of specific molecules. Many kinds of additives are also used: vitamins, specific amino acids, minerals, and prebiotics, probiotics and sometimes synbiotics or postbiotics supposed to stimulate the growth of beneficial bacteria with an expected improvement of productivity and health. Looking for new additives able to improve gut health has become even more important with the suppression of antibiotics as growth promoters. Many studies report the effect of the modification in each of these components on the taxonomic composition of the digestive microbiota. Their impact on the gut microbiota composition is huge. Some of these studies also document the correlated effect on host phenotypes of interest, mainly feed efficiency, and sometimes pathogen load or resistance to disease. This has been excellently reviewed (for instance (Oakley *et al.* 2014, Borda-molina *et al.* 2018)), and here is only a short survey of these studies. Given the amount of studies published, it is easy to get lost as to the most efficient additives and feeding strategies. This is further complicated by the lack of standard practices and the highly dynamic composition of the microbiota, which is influenced by many factors, among which environmental factors. Furthermore, the actual mechanisms by which these changes in composition occur, and how they improve (or degrade) host traits of interest are not well known, in general. Applying functional genomics approaches to know which bacterial genes change in abundance when using an additive would certainly improve our knowledge of the molecular mechanisms involved and improve our understanding of the host-microbiota crosstalk. At last, many of these studies focus on very specific host traits, whereas modifications of microbiota composition might have effects on multiple host phenotypes. For instance, increased fractions of NSP reduce nutrient digestibility (Choct *et al.* 1996), but also decrease the occurrence of necrotic enteritis and the colonisation by *Clostridium perfringens* (Pan and Yu 2014). We therefore advocate that integrated studies are needed, to document as completely as possible the effects of gut microbiota changes on its chicken host.

The cereal used as the main source of feed is known to affect the microbiota composition through the quantity of non-starch polysaccharides (NSP) available . Wheat thus comprises more NSP than maize, which is usually preferred as a source of cereal. NSP are not well digested by host enzymes; their digestion requires the intervention of bacteria equipped with enzymes such as glycosyl hydrolases. A higher proportion of NSP through a diet containing more wheat, for instance, is known to trigger an increased retention time of feed and a proliferation of slow-growing bacteria able to degrade NSP. More generally, the proportions of carbohydrates, proteins (Stanley *et al.* 2014)or lipids in the feed are known to affect gut microbiota composition. In addition, feed structure and technological processes applied such as the presence of whole grains also have an impact.

Several enzymes are used with success to improve feed digestibility. Their direct effects are well known since they target specific, well-known substrates. Furthermore, it is now clear that metabolites derived from the hydrolysis reactions they catalyse act as prebiotics, thus modifying the gut microbiota composition (Kiarie et al, 2013).

Prebiotics are molecules non-digestible by the host, which favour the growth of specific bacteria supposed to be beneficial to the host. The most used are oligo-saccharides such as fructo-oligosaccharides (FOS) or mannan-oligosaccharides (MOS), which both favour the growth of *Lactobacillus* species and reduce the quantity of

Clostridium species (Pourabedin and Zhao 2015). Some studies demonstrate that they reduce the colonisation by pathogens. The study of xylo-oligosaccharides is more recent and promising. They are found in raw materials such as inuline extracted from endive or yeast growth medium. Prebiotics act through the increase of beneficial bacteria in the gut (*Lactobacillus*), a decrease in bacteria considered as detrimental (*Clostridia*), or the decrease in the colonisation by enteric pathogens.

Probiotics are selected live bacteria ingested by the animal, which are able to reach the intestine where they grow and bring benefits to their host. Their benefits on host health are documented (Clavijo *et al.* 2018), but their effects on performances are less obvious and depend on the strain used as a probiotic. Commercialised strains belong to ten to twenty genera, mainly *Lactobacillus*, *Bacillus*, *Bifidobacterium* and *Enterococcus*. These probiotic strains increase the population of beneficial bacteria, in particular *Lactobacilles* and *Bifidobacteria*, which inhibit the growth of pathogenic bacteria through the production of bacteriocins or organic acids (Bajagai et al, 2016).

Synbiotics are the combination of specific probiotics and prebiotics chosen to favour their growth once they have reached the intestine. Currently, post-biotics are also used: they are specific metabolites produced by known probiotic bacteria (Kareem *et al.* 2016). The reasoning behind their development is that probiotic bacteria act on their host through the metabolites they produce, so that beneficial effects on gut health can be delivered by directly administering these metabolites to animals. Butyrate is one of them. This SCFA is a major source of energy for host cells, and it inhibits pathogen growth in the chicken gut (Gonzalez-Ortiz *et al.* 2017).

3. METAGENOMIC APPROACHES APPLIED TO CHICKEN INTESTINAL MICROBIOTA: WHERE ARE WE?

a. Definition and methods: metagenomics/ metaproteomics/ metatranscriptomics (and metabolomics?)

Most published studies of the chicken digestive microbiota made use of the targeted sequencing of the bacterial 16S rRNA gene to perform an inventory of the bacteria present. This gene is present with at least one copy in every bacterial genome and possesses highly conserved domains surrounding highly variable domains, which allows the definition of conserved primers for PCR amplifications of the variable regions. Amplicons are sequenced and assigned to known taxons through bio-informatic analyses. This allows to perform an inventory of the dominant bacteria present in any ecosystem, without the need to cultivate bacteria. Although very informative, this approach has many limitations, like a probable bias in the quantification of some types of bacteria due to the unequal number of 16S rRNA gene according to bacterial species. It also rarely allows the identification of bacteria at the species level, and furnishes a list of OTU (operational taxonomic units) identified generally at the family and sometimes at the genus level, while many OTUs remain without annotation.

True metagenomic approaches, i.e. whole metagenomics approaches relying on the full sequencing of the metagenome are both more difficult and much more expensive. They are also many times more informative, because they give access not only to one gene used as a phylogenetic marker, but to all the genes of the bacteria of a given ecosystem. These approaches rely on the massive sequencing of fragments of bacterial DNA from the ecosystem, followed by bioinformatics analyses to assemble the reads produced into genes. It leads to lists of hundreds of thousands of genes for every sample sequenced. This allows not only a taxonomic inventory of the bacteria present, but also a functional inventory of the genomic potential of the ecosystem. The building of reference metagenomes greatly facilitates such studies by allowing a lower sequencing depth in subsequent analyses and a much improved annotation of the genes. Such reference metagenomes have been first produced for human (Qin et al. 2010) and then for model and livestock species: in mice (Xiao et al. 2018), pig (Xiao et al. 2016), cows (Stewart et al. 2018), dog (Coelho et al. 2018), rat (Pan et al. 2018) and recently for chicken as well (Huang et al. 2018).

Most studies conducted until know looked at the impact of feed and additives on gut microbiota on the taxonomic composition. Nevertheless, taxonomic composition might not always be the more relevant criterion to follow: changes might occur at the functional level without visible changes at the taxonomical level, because bacterial genes could display differential expression levels, and because some slight and hence undetectable changes in abundances could nevertheless have important functional consequences detectable through a functional metagenomics approach. Furthermore, understanding the functional mechanisms at stake with the help of functional metagenomics approaches, could allow us to identify the pathways responsible for the multiple effects of gut microbiota changes on host phenotypes and hence to more efficiently improve the desirable host traits without degrading other traits.

b. Lessons learned from first metagenomics studies

In chicken, a few studies making use of true metagenomics approaches have been published. This has been reviewed recently (Borda-molina *et al.* 2018). The first study compared the microbiotas of one healthy animal, and one animal challenged with *Campylobacter jejuni*. The number of animals was too low to identify the functional elements associated with the increase in *Campylobacter* abundance. Nevertheless, this first study underlined the functional importance of the metavirulome, i.e. the virulence genes of a given microbiota, and identified a high percentage of mobile elements (tranposases) responsible for horizontal gene transfer among bacteria (Qu *et al.* 2008). The second study used a higher number of animals to understand how antibiotics treatment (chlortetracycline) modified the caecal microbiota. A whole metagenome approach applied to 16 pools of caecal microbiota differing according to animal age and antibiotic treatment received succeeded in identifying microbial genes potentially explaining the positive effect of antibiotic treatments on growth and health (Danzeisen *et al.* 2011); a modification of the availability of transport systems for a wide array of molecules was observed. A more recent study used the same antibiotics to compare the 16S rRNA gene based composition of the fecal microbiota with the abundance of antibiotic resistance genes identified through a whole-metagenome sequencing approach (Xiong *et al.* 2018). It concludes that chlortetracycline selected for resistant bacteria and inhibited the sensitive bacteria, thus causing a shift in bacterial abundances that in turn changed the the resistome structure. Another study identified a high number of enzyme involved in the degradation of poly-saccharides and in fermentation pathways leading to the production of beneficial SCFA, but also uptake hydrogenases. It hypothesizes that several of the most abundant genera present in the caecal microbiome (in this case *Megamonas*, *Helicobacter* and *Campylobacter*) act as hydrogen sinks, thus allowing a more productive fermentation to acetate and an increased production of SCFA benefiting the host (Sergeant *et al.* 2014). At last, other studies focused on the fecal microbiota in relation to feed efficiency or lipid metabolism (Singh *et al.* 2014, Hou *et al.* 2016)which may in part be due to variation in their gut microbiota. In this paper we analyse the fecal microbiota of low and high feed conversion ratio (FCR. The more recent study, which also produced the first reference intestinal metagenome in chicken, tested the effects of a plant derived growth promoter compared to the effects of an antibiotic on the microbial genes composition. It confirmed the predominant role of *Lactobacillus* in the observed favorable effect on growth of the plant growth promoter used, and the effect of the antibiotic on the growth of antibiotic-producing bacteria and on the abundance of antibiotic resistance genes, thus emphasizing the need for safe alternatives to antibiotics in poultry production (Huang *et al.* 2018).

These studies illustrate the interest of whole metagenomics approaches and emphasize the importance of the chicken gut metagenome for gut health. These research areas will soon all benefit from the existence of chicken reference metagenomes, which extraordinarily extend the identification of microbial genes and will allow much more precise studies of the functional potential of the gut microbiota. The first reference metagenome built used an Illumina HiSeq technology to produce a saturated catalog of 9.04 million genes and included all compartments of the digestive tract, with samples collected at different timepoints on mainly broiler chickens (Huang *et al.* 2018). Another initiative coordinated by INRA (the MetaChick consortium) is currently focusing on the comparison of chicken microbiotas in the many production systems encountered in chicken production in France, to perform an extensive assessment of the existing biodiversity of the caecal microbiota and to gain insight into the actual differences between farms and production systems. This knowledge will be for applied perspectives of nutritional modulations of the gut microbiota in chicken, since different strategies might be necessary in different production systems according to the microbiota encountered. It will soon lead to the publication of an extensive caecal reference metagenome for both laying hens and broilers, whereas most other studies conducted focus on broiler chickens.

c. Other –Omics approaches: metabolomics/ metaproteomics

A few published studies refer to other –omics approaches to study the chicken gut metagenome. No metatranscriptomics approach has been used in chicken, to our knowledge, which is understandable given the absence of reference metagenome until now and the technical difficulties of this approach with rapidly degraded mRNA in fecal or caecal contents. Its development would be of high interest, since metatranscriptomics gives access to the actually expressed genes of the metagenome in a given condition, whereas metagenomics furnishes the functional potential of the studied ecosystem. Just like host transcriptomics though, it only gives a transient image of the gene expression and several time points and conditions should be considered in carefully designed experimentations.

Metabolomics and metaproteomics furnish the metabolites and proteins actually produced and are thus highly interesting. These molecules are those that supposedly interact with the host and mediate the impact of gut microbiota on host traits of interest. A few studies made use of a metaproteomics approach to study the chicken gut microbiota. The first one made use of two pools of feces from 18 weeks old hens and identified abundant stress proteins as well as proteins involved in metabolic processes of carbohydrates, alcohol and protein (Tang *et al.* 2014). Another study used this approach on pools of three chickens at different time points as a way to identify bacteria species eligible as potential probiotics based on their proteic profiles (Polansky *et al.* 2016), in particular for enzymes leading to the production of the SCFAs acetate, propionate and butyrate. Eligible bacteria had also to be able to colonize newly hatched chicks from donor microbiotas and to form spores, which finally led to the identification of *Anaerostipes*, *Anaerotruncus* and *Subdoligranulum* suitable probiotic candidates. The latest study reports the effects of a supplementation in phosphorus and microbial phytase on the microbial proteins using 24 pools of 2 animals (Tilocca *et al.* 2016). They showed the benefits of this supplementation on the gut microbiota, which thrives under supplementation and is stressed without it.

At last, metabolomics approaches are complementary to metaproteomic approaches, by identifying the metabolites produced through the expressed enzymes. One difficulty though is the impossibility to distinguish metabolites from the host from metabolites resulting from the activity of the gut microbiota. On study made use of this approach and showed than modifications in metabolite profiles in birds fed with rice bran were associated with a reduction in the colonization of caeca by *Salmonella Typhimurium* (Rubinelli *et al.* 2017).

4. DISCUSSION – PERSPECTIVES : HOW METAGENOMICS WILL ASSIST MICROBIOTA MODULATION STRATEGIES

Metagenomics furnishes a detailed vision of the array of genes present in an ecosystem at the time of the collect and of the dominant bacterial genomes present, which allows both taxonomic and functional analyses of the studied ecosystem. Attempts at whole metagenomics approaches are more and more frequent, although they remain too complex and expensive to be used routinely by most laboratories. The 16S approach remains the reference method. Nevertheless, the publication of reference metagenomes should pave the way toward more studies making use of whole metagenomes. Many research domains can benefit from this approach, and we will only focus on those that can improve nutritional modulation strategies. In chicken production, nutritional strategies in relation to gut health mainly aim at preventing health challenges. In addition to the use of different cereals or additives, strategies such as early nutrition or *in-ovo* application of probiotics aim at accelerating the colonisation of the gut by a favourable microbiota and at efficiently priming the host immune system. The early days post-hatch, and even before hatch to some extent, are certainly a window of opportunity to apply nutritional strategies in chicken with long-term favourable consequences for the animal.

Whatever the strategy considered, knowing the bacterial genes potentially involved can improve our understanding of the molecular mechanisms at stake. Nevertheless, knowing the genes involved does not necessarily gives insight into the effects they cause in the host, and we advocate that integrative studies detailing with the same precision both the gut microbiota and the host phenotypes should be conducted. Host genetic variations should also be depicted, since microbiome and host contribute together to the expression of traits of interest such as feed efficiency and gut health. Fine descriptors of the host immunity should be described through transcriptomics in intestinal tissues or even systemic organs, genomics, observation of histological modifications and isolation of specific molecules such as for instance IgA. This will allow the identification of correlated phenotypes and changes in microbial gene abundances and give a comprehensive view of the modifications occurring in the holobiont. This will improve our knowledge of the early molecular interactions occurring in the perinatal period between host immunity and microbiota. In addition, such integrative strategies should allow the identification of early biomarkers of different kinds for gut health dysfunctions, which are highly needed in current poultry production (Ducatelle *et al.* 2018). Furthermore, all –omics approaches are complementary and their simultaneous analysis using adapted bio-informatics tools could really improve our global understanding of how interactions between host and microbiota shape gut health in poultry. Although not adapted to every experiment due to their cost and complexity, such strategies applied to well designed experimental studies applied to very large cohorts to ensure statistical robustness should lead to a huge progress in the coming years. Such studies should help in the definition of a "healthy" microbiota, or rather of a "healthy" microbiota in a given environment. They might also lead to the identification of microbial genes of interest such as specific enzymes (Al-Darkazali et al, 2017) and help researchers to define sets of probiotics strains based on their functions (Brugiroux et al, 2016). The development of standard practices, from sample collect and DNA extraction to bio-informatic analyses, as in human research on the intestinal metagenome (Costea *et al.* 2017), will also be essential to compare results from distinct studies.

Finally, the field application of the knowledge acquired experimentally, be it diagnostic tools based on signature markers (genes, bacterial species, metabolites, enzymes, etc) or the application of an additive, might be difficult due to the highly dynamic nature of the gut microbiota and the many factors modifying its functioning. It is advisable to conduct studies in conditions very close to the field conditions, or even in field conditions when applicable. The key functional molecules at the heart of the host-gut microbiota interactions are probably less influenced by endogenous and environmental parameters than taxonomic compositions alone, which should be a help for applied perspectives.

REFERENCES

AWAD, W. A., MANN, E., DZIECIOL, M. and HESS, C. 2016. Age-Related Differences in the Luminal and Mucosa-Associated Gut Microbiome of Broiler Chickens and Shifts Associated with Campylobacter jejuni Infection. *Frontiers in Cellular and Infection Microbiology* **6**: 1–17.

AZIZ, Q., DORÉ, J., EMMANUEL, A., GUARNER, F. and EMM, Q. 2013. Gut microbiota and gastrointestinal health : current concepts and future directions. *Neurogastroenterol Motil* **25**: 4–15.

BLOTTIÈRE, H. M., VOS, W. M. De and EHRLICH, S. D. 2013. Human intestinal metagenomics : state of the art and future. *Current Opinion in Microbiology* **16**: 232–239.

BORDA-MOLINA, D., SEIFERT, J. and CAMARINHA-SILVA, A. 2018. Current Perspectives of the Chicken Gastrointestinal Tract and Its Microbiome. *Computational and Structural Biotechnology Journal* **16**: 131–139.

BROOM, L. J. and KOGUT, M. H. 2018. Veterinary Immunology and Immunopathology The role of the gut microbiome in shaping the immune system of chickens. *Veterinary Immunology and Immunopathology* **204**: 44–51.

CHOCT, M., HUGHES, R. J., WANG, J., BEDFORD, M. R., MORGAN, J. and ANNISON, G. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* **37**: 609–621.

CLAVIJO, V., JOSEFINA, M. and FL, V. 2018. Non-Invited Review The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production : A review. *Poultry Science* **97**: 1006–1021.

COELHO, L. P., KULTIMA, J. R., COSTEA, P. I., FOURNIER, C., PAN, Y., CZARNECKI-MAULDEN, G., HAYWARD, M. R., FORSLUND, S. K., SEBASTIAN, T., SCHMIDT, B., DESCOMBES, P., JACKSON, J. R., LI, Q. and BORK, P. 2018. Similarity of the dog and human gut microbiomes in gene content and response to diet: 1–11.

COSTEA, P. I., ZELLER, G., SUNAGAWA, S., PELLETIER, E., ALBERTI, A., LEVENEZ, F., TRAMONTANO, M., DRIESSEN, M., HERCOG, R., JUNG, F., KULTIMA, J. R., HAYWARD, M. R., COELHO, L. P., ALLEN-VERCOE, E., BERTRAND, L., BLAUT, M., BROWN, J. R. M., CARTON, T., COOLS-PORTIER, S., DAIGNEAULT, M., DERRIEN, M., DRUESNE, A., VOS, W. M. De, FINLAY, B. B., FLINT, H. J., GUARNER, F., HATTORI, M., HEILIG, H., LUNA, R. A., VLIEG, J. V. H., JUNICK, J., KLYMIUK, I., LANGELLA, P., CHATELIER, E. Le, MAI, V., MANICHANH, C., PATIL, K. R., PENDERS, J., PERSSON, S., PONS, N., POPOVA, M. and SALONEN, A. 2017. A n a ly s i s Towards standards for human fecal sample processing in metagenomic studies. *Nature Biotechnology* **35**: 1069–1076.

DANZEISEN, J. L., KIM, H. B., ISAACSON, R. E., TU, Z. J. and JOHNSON, T. J. 2011. Modulations of the Chicken Cecal Microbiome and Metagenome in Response to Anticoccidial and Growth Promoter Treatment. *PLoS ONE* **6**.

DING, J., DAI, R., YANG, L., HE, C., XU, K. and LIU, S. 2017. Inheritance and Establishment of Gut Microbiota in Chickens. *Frontiers in Microbiology* **8**: 1–11.

DUCATELLE, R., GOOSSENS, E., MEYER, F. De, EECKHAUT, V., ANTONISSEN, G., HAESEBROUCK, F. and IMMERSEEL, F. Van. 2018. Biomarkers for monitoring intestinal health in poultry : present status and future perspectives: 1–9.

GONZALEZ-ORTIZ, G., SANTOS, T. T., VIENOLA, K., VARTIAINEN, S., APAJALAHTI, J. and BEDFORD, M. R. 2017. Response of broiler chickens to xylanase and butyrate supplementation. *Poultry Science* **0**: 1–12.

GUCHTE, M. Van De, BLOTTIÈRE, H. M. and DORÉ, J. 2018. Humans as holobionts : implications for prevention and therapy. *Microbiome* **6**: 4–9.

HANNING, I. and DIAZ-SANCHEZ, S. 2015. The functionality of the gastrointestinal microbiome in non-human animals. *Microbiome*: 1–11.

HOU, Q., KWOK, L., ZHENG, Y., WANG, L., GUO, Z., ZHANG, J., HUNAG, W., WANG, Y., LENG, L., LI, H. and ZHANG, H. 2016. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. *Scientific Repo*: 1–13.

HUANG, P., ZHANG, Y., XIAO, K., JIANG, F., WANG, H., TANG, D., LIU, D., LIU, B., LIU, Y., HE, X., LIU, H., LIU, X., QING, Z., LIU, C., HUANG, J., REN, Y., YUN, L., YIN, L., LIN, Q., ZENG, C., SU, X., YUAN, J., LIN, L., HU, N. and CAO, H. 2018. The chicken gut metagenome and the modulatory effects of plant-derived benzylisoquinoline alkaloids: 1–17.

KAREEM, K. Y., LOH, T. C., FOO, H. L., AKIT, H. and SAMSUDIN, A. A. 2016. Effects of dietary postbiotic and inulin on growth performance , IGF1 and GHR mRNA expression , faecal microbiota and volatile fatty acids in broilers. *BMC Veterinary Research*: 8–17.

KERS, J. G., VELKERS, F. C., FISCHER, E. A. J., HERMES, G. D. A., STEGEMAN, J. A., SMIDT, H. and MOORE, R. J. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. *Frontiers in Microbiology* **9**: 1–14.

KOGUT, M. H. and ARSENAULT, R. J. 2016. Editorial : Gut Health : the New Paradigm in Food animal Production. *Frontiers in Veterinary Science* **3**: 10–13.

OAKLEY, B. B., LILLEHOJ, H. S., KOGUT, M. H., KIM, W. K., MAURER, J. J., LEE, M. D., COLLETT, S. R., JOHNSON, T. J. and COX, N. A. 2014. The chicken gastrointestinal microbiome. *FEMS Microbiology Letters* **360**: 100–112.

OCEJO, M., OPORTO, B. and HURTADO, A. 2019. 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow- growing chickens throughout their productive lifespan. *Scientific Reports*: 1–14.

PAN, D. and YU, Z. 2014. Intestinal microbiome of poultry and its interaction with host and diet © 2014 Landes Bioscience . Do not distribute © 2014 Landes Bioscience . Do not distribute. *Gut Microbes* **5**: 108–119.

PAN, H., GUO, R., ZHU, J., WANG, Q., JU, Y., XIE, Y., WANG, Z., LI, T., LIU, Z., LU, L., LI, F., TONG, B., XU, X., LI, R., YUAN, Z., YANG, H. and WANG, J. 2018. A gene catalogue of the Sprague-Dawley rat gut metagenome.

POLANSKY, O., SEKELOVA, Z., FALDYNOVA, M., SEBKOVA, A., SISAK, F. and RYCHLIK, I. 2016. Chicken Cecal Microbiota. *Applied and Environmental Microbiology* **82**: 1569–1576.

POURABEDIN, M. and ZHAO, X. 2015. Prebiotics and gut microbiota in chickens. *FEMS Microbiology Letters* **362**: 1–8.

QIN, J., LI, R., RAES, J., ARUMUGAM, M., BURGDORF, K. S., MANICHANH, C., NIELSEN, T., PONS, N., LEVENEZ, F., YAMADA, T., MENDE, D. R., LI, J., XU, J., LI, S., LI, D., CAO, J., WANG, B., LIANG, H., ZHENG, H., XIE, Y., TAP, J., LEPAGE, P., BERTALAN, M., BATTO, J., HANSEN, T., LE, D., LINNEBERG, A., NIELSEN, H. B., PELLETIER, E., RENAULT, P., SICHERITZ-PONTEN, T., TURNER, K., ZHU, H., YU, C., LI, S., JIAN, M., ZHOU, Y., LI, Y., ZHANG, X., GUARNER, F., LI, S., QIN, N., YANG, H., WANG, J., BRUNAK, S. and DORE, J. 2010. ARTICLES A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**.

QU, A., BRULC, J. M., WILSON, M. K., LAW, B. F., THEORET, J. R., JOENS, L. A., KONKEL, M. E., ANGLY, F., DINSDALE, E. A., EDWARDS, R. A., NELSON, K. E. and WHITE, B. A. 2008. Comparative Metagenomics Reveals Host Specific Metavirulomes and Horizontal Gene Transfer Elements in the Chicken Cecum Microbiome **3**.

RUBINELLI, P. M., KIM, S. A., PARK, S. H., ROTO, S. M., NEALON, J., RYAN, E. P. and RICKE, S. C. 2017. Differential effects of rice bran cultivars to limit Salmonella Typhimurium in chicken cecal in vitro incubations and impact on the cecal microbiome and metabolome. *PLoS ONE* **12**: 1–20.

SERGEANT, M. J., CONSTANTINIDOU, C., COGAN, T. A., BEDFORD, M. R., PENN, C. W. and PALLEN, M. J. 2014. Extensive microbial and functional diversity within the chicken cecal microbiome. *PLoS ONE* **9**: e91941.

SINGH, K. M., SHAH, T. M., REDDY, B., DESHPANDE, S., RANK, D. N. and JOSHI, C. G. 2014. Taxonomic and gene-centric metagenomics of the fecal microbiome of low and high feed conversion ratio (FCR) broilers Title. *Journal of Applied Genetics* **55**: 145–154.

SOMMER, F. and BÄCKHED, F. 2013. The gut microbiota - masters of host development and physiology. *Nature Reviews in Microbiology* **11**: 227–238.

STANLEY, D., HUGHES, R. J. and MOORE, R. J. 2014. Microbiota of the chicken gastrointestinal tract : influence on health , productivity and disease. *Applied and Environmental Microbiology* **98**: 4301–4310.

STEWART, R. D., AUFFRET, M. D., WARR, A., WISER, A. H., PRESS, M. O., LANGFORD, K. W., LIACHKO, I., SNELLING, T. J., DEWHURST, R. J., WALKER, A. W., ROEHE, R. and WATSON, M. 2018. metagenomic sequencing of the cow rumen. *Nature Communications* **9**: 1–11.

TANG, Y., UNDERWOOD, A., GIELBERT, A., WOODWARD, M. J. and PETROVSKA, L. 2014. Metaproteomics Analysis Reveals the Adaptation Process for the **80**: 478–485.

TILOCCA, B., WITZIG, M., RODEHUTSCORD, M. and SEIFERT, J. 2016. Variations of Phosphorous Accessibility Causing Changes in Microbiome Functions in the Gastrointestinal Tract of Chickens: 1–20.

XIAO, L., ESTELLÉ, J., KIILERICH, P., RAMAYO-CALDAS, Y., XIA, Z., FENG, Q., LIANG, S., PEDERSEN, A. Ø., KJELDSEN, N. J., LIU, C., MAGUIN, E., DORÉ, J., PONS, N., CHATELIER, E. Le, PRIFTI, E., LI, J., JIA, H., LIU, X., XU, X., EHRLICH, S. D., MADSEN, L., KRISTIANSEN, K., ROGEL-GAILLARD, C. and WANG, J. 2016. A reference gene catalogue of the pig gut microbiome. *Nature Microbiology* **1**: 1–6.

XIAO, L., FENG, Q., LIANG, S., SONNE, S. B., XIA, Z., QIU, X., LI, X., LONG, H., ZHANG, J., ZHANG, D., LIU, C., FANG, Z., CHOU, J., GLANVILLE, J., HAO, Q., KOTOWSKA, D., COLDING, C., LICHT, T. R., WU, D., YU, J., JAO, J., SUNG, Y., LIANG, Q., LI, J., JIA, H., LAN, Z., TREMAROLI, V., DWORZYNSKI, P., NIELSEN, H. B., BÄCKHED, F., DORÉ, J., CHATELIER, E. Le, EHRLICH, S. D. and LIN, J. C. 2018. resource A catalog of the mouse gut metagenome. *Nature Biotechnology* **33**.

XIONG, W., WANG, Y., SUN, Y., MA, L., ZENG, Q., JIANG, X. and LI, A. 2018. Antibiotic-mediated changes in the fecal microbiome of broiler chickens define the incidence of antibiotic resistance genes: 1–11.

Interaction between Nutrition, Metabolism and the Gut Microbiome Influence Poultry Health and Immunity

Michael H. Kogut

USDA-ARS, SPARC, College Station, TX 77845, Mike.kogut@ars.usda.gov

ABSTRACT.

Nutrition and the immune system are decidedly interconnected, where metabolism and nutrition undoubtedly impact the immune system, and immune responses, in turn, influence metabolism. The established perception that host defense and nutritional status impact each other must now be expanded to include a third component of this network: the gut microbiota. This biological multi-faceted relationship can no longer be considered exclusively dependent on the host, but is also dependent on a second genomic component within the host, the microbiome. The microbiome programs host immunity and drives a metabolome that impacts micronutrients and energy balance. In turn, the host immunity shapes the microbiome and host nutritional status influences elements of host defenses and make-up of commensal microbial community. Lastly, perturbation of microbiota composition can trigger intestinal inflammation by driving inappropriate immune responses.

Immediate and sustained metabolic alterations modulate host defensive responses during infection through metabolic regulations governed by nutrient-sensing machineries. These regulatory mechanisms affecting immune cells are known as immunometabolic regulations. Characterizing these mechanisms reveal how these immunometabolic regulators fine-tune host immunity. In addition, metabolites generated by gut microbiota and metabolic tissues instruct tissue immune responses and contribute to immune system functionality. Thus, the interplay between immune cells and metabolic homeostasis lead to effective and detrimental immune responses. Harnessing immunometabolic regulations is a promising strategy to boost antimicrobial immunity as an alternative to antibiotics. This delicate immunometabolic regulation provides a new leverage for immunologists to unleash and restraint immunity.

INTRODUCTION

In the broiler and turkey industries, antibiotics have been widely used as feed additives to improve growth and performance [1, 2]. However, the spread of antibiotic-resistant pathogens in medical and veterinary environments has resulted in the ban of antibiotics in animal feeds in the EU and the dramatic reduction in their use in the US [3, 4]. With poultry producers under increasing pressure to reduce their use of antibiotics to control disease and enhance production, the development of cost-effective alternatives to antibiotics to reduce these microbial pathogens in poultry products would be of great value to the food industry and to the consumer.

OVERVIEW OF THE IMMUNE SYSTEM

Innate immunity is the first line of defense against pathogenic organisms and the interface of the interactions between the host and the microbiota [5]. At the cellular level, the innate immune response is mediated by epithelial cells in mucosal surfaces and phagocytic cells that reside in the tissues or are recruited from the blood, including granulocytes, monocytes, and macrophages. At the molecular level, innate immune cells sense microbes through pattern recognition receptors (PRRs), which recognize molecular signatures (also known as pathogen-associated molecular patterns) from microbial cells [5, 6] including proteins, lipids and nucleic acids. PRRs also recognize host signature molecules that are indicative of disease and cellular damage. Pathogen recognition through these receptors results in the activation of cellular defense mechanisms and the production of secreted pro-inflammatory cytokines, which alert other host cells to the presence of infection, drive further recruitment of immune cells from the blood to the site of infection, and induce systemic responses to the disease, such as fever. Pathogen recognition through PRRs also stimulates the microbicidal mechanisms of innate immunity, such as the production of reactive oxygen species and antimicrobial peptides, in part through the activation of phagocytic cells.

The defense mechanisms of acquired immunity are based on the recognition of 'foreign' molecular shapes termed antigens. Adaptive immunity is activated more slowly (on the time scale of 3 days to a couple of weeks) by a combination of signals from the innate immune system and by antigens, and is largely mediated by B cells and T cells. These cells carry receptors that recognize foreign molecular patterns but have no intrinsic bias towards pathogen recognition. Adaptive immunity is therefore dependent on the innate immune system for initial pathogen recognition. Signals from the innate immune response drive the selective expansion and activation of the B cell and T cell populations with specificity for the ongoing infectious challenge. The main effector mechanisms of adaptive immunity include the production of antibodies by B cells, the killing of infected host cells by cytotoxic T cells, and various helper T cell-mediated actions. Crucially, activation of adaptive immunity results in the production of memory B cells and T cells which can provide life-long specific protection against subsequent infections with a pathogen bearing the same antigens.

MODULATING HOST IMMUNITY AS AN ALTERNATIVE TO ANTIBIOTICS

Despite efforts to halt the increase and spread of antimicrobial resistance, bacteria continue to become less susceptible to antimicrobial drugs over time, and rates of discovery for new antibiotics are declining. Thus, it is essential to explore new paradigms for anti-infective therapy in animal agriculture. An effective host immune response to pathogens in the earliest stages of infection is a critical determinant of disease resistance and susceptibility. One promising approach involves host-directed immunomodulatory therapies, whereby natural mechanisms in the host are exploited to enhance therapeutic benefit. The objective is to initiate or enhance protective antimicrobial immunity while limiting inflammation-induced tissue injury. What is evident is that stimulation of the innate immune response has the greatest potential for protection against foodborne pathogens, regardless of whether they are viral, bacterial, or protozoal in nature. The advantages of modulating the innate response are threefold: (1) induction is rapid, (2) non-specificity of the response allows for cross-protection against unrelated pathogens, and (3) different levels of therapeutic potential; i.e., prophylactic affects, adjuvant effects, systemic and local protection, and multiple immune cellular targets.

Immune modulation can be defined as the manipulation of immune system to control infections and other adverse health effects with precise regulation to avoid complications while suppressive or potentiating efforts are made to benefit the animal and human health. Immune modulation is one approach for new anti-infective therapies, whereby natural mechanisms in the host can be exploited to strengthen therapeutic benefit. The stimulation of innate immunity has considerable potential to induce a profound and rapid cross-protection against multiple pathogens.

There has been extensive research into developing and evaluating contemporary alternatives to antibiotic growth promoters that are at least partially immunomodulatory in their function in poultry including: prebiotics/probiotics/direct fed microbials [7-12], phytochemicals [13, 14] antimicrobial peptides (host defense peptides) [15-18], essential oils [19], butyrate and organic acids [15, 20], feed enzymes [21, 22], and egg yolk antibodies [23, 24].

NUTRITIONAL IMMUNE MODULATION

The interaction between poultry nutrition and nutrients and the bird's immune response has long been known and has been reviewed extensively [25-31]. Studies on feed restriction have brought further insights into the effect of nutrition on the immune competency of poultry [reviewed in 32]. Klasing's [25] seminal paper classifies nutritional modulation of immune function via seven broad categories: (1) immune system development, (2) immune cell substrates, (3) leukocyte regulatory activity, (4) reduction of immune pathology, (5) regulation of hormonal activity, (6) physiological actions on the intestinal tract, and (7) limiting nutrient availability to pathogens during infections. The field of nutritional immunology has become a specialty within avian immunology over the past decade. There is a large body of evidence that the impact of nutritional components on the avian gut and systemic immunity has been increasingly recognized. Furthermore, the implications of nutrition and nutritional intervention on prevention of disease and even corrections of pathophysiological conditions have become established concepts [28, 29, 33].

Despite the number of papers demonstrating the ability of nutrients to influence the avian immune response, relatively few studies have actually been successful using nutritional modulation of immunity to induce protection against infectious diseases [34-36]. Recent studies [30, 37] have argued that part of the problem is that most research has only used a single immune measurement as a marker for immune effectiveness. On the other hand, despite recent advances in the field, the interplay between nutritional processes and immune system is incompletely understood. Particularly, specific cellular and molecular immune responses invoked by feed components and the role of the gut barrier and microbiota on the interaction between the immune system and nutrition remains to be fully elucidated. The rest of this review will point out some of the areas have been either neglected when studying the effects of nutrition and nutrients on immune function (the effect of the gut microbiome) or the non-intentional effects of over-feeding various nutrients on the avian immune response (feed-induced inflammation and meta-inflammation).

IMMUNOMETABOLISM

The interface of the immune system and metabolism is an emerging field of study. Relatively recently, immunity and metabolism were treated as distinct processes carried out by an organism. Immunity was focused on the recognition and resistance to a pathogen and involved its own set of cells and tissue activities. Metabolism was solely the chemical processes that provided the energy to carry out the various functions of the organism; this included immune functions, but metabolism was simply the source of energy for the immune system.

Within animal agriculture, a consideration of immunometabolism in animal production has been ongoing, though not coined as such. It has been clear to poultry producers that a focus solely on maximizing animal growth can be detrimental to immune potential, while an innate immune response has negative consequences on growth (38). Integrating metabolism and immunity provides a research avenue for the ultimate goal of maximizing growth and animal production without having a negative impact on animal health and immunity. Our own research has shown the nearly innumerable links between cellular signaling proteins classically characterized as members of either the immune or metabolic functional groups (39). Due to these links, we feel that an integrated immunometabolic approach is worth considering for anyone researching animal production from either a nutrition/metabolism or immunity/disease perspective.

GROWTH/IMMUNITY BALANCE

A significant avenue of research combining immunity and metabolism in animal production was how mounting an immune response affected energy levels and the transfer of energy from growth to immunity (38). Research into the energy consequences of immunity is relatively advanced in animal science. It has been well understood for many decades that an animal that initiates an innate immune/inflammatory response will likely grow slower and have worse feed conversion (40, 41). It is thought that one mode of action of growth-promoting antibiotics given to food animals is a general reduction in inflammation. Indeed, it has been argued that the anti-inflammatory effects of growth-promoting antibiotics are even more important than the reduction/elimination of disease-causing pathogens (42).

INFLAMMATION, IMMUNITY, AND METABOLISM

In the animal science field, feed-induced inflammation has been a concern. Certain feeding ingredients can lead to an inflammatory gut response; examples include non-digestible components of wheat and rye in chicken (43) and soybean meal in fish (44, 45). Even an excess of feed can lead to changes in immune response (46). One current animal feed strategy involves adding exogenous enzymes to the diet to breakdown certain indigestible and/or inflammatory feed components in the gut, with the aim to reduce immune response and redirect this energy to growth (47). A current feed trend involves trying to find natural additives that enhance the animal's resistance to disease, either by influencing the host immune response or the gut microbiota. Caution must be exercised when evaluating these feed additives; robust scientific methodology must be used to determine efficacy and understand the mechanism of action.

In poultry, there has been a significant amount of research into nutrition's effects on immunity (48) and the use of pre- and probiotic feeding ingredients to improve growth and disease resistance (49-51). However, the literature is limited on the immunometabolism link between stress or disease and production issues. The links between disease and production issues are certainly there, and poultry production problems ranging from lameness (52) to muscle fat deposition (53) have been explored.

INTRACELLULAR IMMUNOMETABOLISM INTERACTIONS

The recent expansion of the immunometabolism field involves characterizing the direct intracellular pathway links between metabolism and immunity (38, 54). Research is focusing on signaling molecules that integrate both metabolic energy sensing and immune response signals. The protein synthesis pathway is regulated by mTOR and is also involved in T-cell fate (55), determining whether the cell becomes an effector T-cell or a regulatory T-cell

(40). AMPK is an energy sensor that monitors the ratio of AMP:ATP, altering an anabolic and catabolic process; it is also involved in innate immune response and has a direct link to mTOR (56). Evidence also points to metabolic-induced epigenetic reprograming of immune pathways via the sirtuins (57). The past perspective of separating immunity and metabolism meant a focus on targeting immune pathways in infectious disease and metabolism pathways for growth/metabolic disorders. With an integrated approach, we can broaden our potential targets for disease intervention and our understanding of how metabolic processes can influence health.

REFERENCES

1. GASKINS, H.R, C.T. COLLIER, D.B. ANDERSON, 2002:. Antibiotics as growth promotants: mode of action. Ani. Biotechnol. 13,29–42.
2. DIBNER, J.J., J.D. RICHARDS, 2005: Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 84,634–643.
3. SINGER, R.S., C.L. Hofacre, 2006: Potential impacts of antibiotic uses in poultry production. Avian Dis. 50,161-172.
4. SEAL, B.S., H.S. LILLEHOJ, D.M. DONOVAN, C.G. GAY, 2013: Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Ani. Health Res. Rev. 14,78-87.
5. MEDZHITOV, R, 2017: Recognition of microorganisms and activation of the immune response. Nature 449,819-826.
6. KUMAR, H., T. KAWAI, S. AKIRA, 2011: Pathogen recognition by the innate immune system. Int. Rev. Immunol. 30,16-34.
7. LOWRY, V.K., M.B. FARNELL, P.J. FERRO, C.L. SWAGGERTY, A. BAHL, M.H. KOGUT, 2005: Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar Enteritidis. Int. J. Food Microbiol. 98,309-318.
8. REZNAEI, S., M. F.. JAHROMI, J. B. LIANG,1 I. ZULKIFLI, A.S. FAMARJAM, V. LAUDADIO, V. TUFARELLI, 2015: Effect of oligosaccharides extract from palm kernel expeller on growth performance, gut microbiota and immune response in broiler chickens. Poult. Sci. 94,2414-2420.
9. FILHO, R.A.C.P., S.J.A. DIAZ, F. S. FERNANDO, Y.-F. CHANG, R.L.A. FILHO, A.B. JUNIOR, 2015: Immunomodulatory activity and control of *Salmonella* Enteritidis colonization in the intestinal tract of chickens by *Lactobacillus* based probiotic. Vet. Immunol. Immunopathol. 167,:64-69.
10. LEE, K-W, D. K. KIM, H.S. LILLEHOJ, S.I. JANG, S.-H. LEE, 2015: Immune modulation by *Bacillus subtilis*-based direct-fed microbials in commercial broiler chickens. Ani. Feed Sci. Technol. 200,76-85.
11. BRAUKMANN, M., P.A. BARROW, A. BERNDT, U. METHNER, 2016: Combination of competitive exclusion and immunization with a live Salmonella vaccine in newly hatched chickens: Immunological and microbiological effects. Res. Vet. Sci. 107,34-41.
12. ASGARI, F., Z. MADID, R. FALAK, M.A. BAHAR, M. HEYDARI NASRABADI, M. RAIANI, M. SHEKARABI,. 2016. Probiotic feeding affects T cell populations in blood and lymphoid organs in chickens. Beneficial Microbes 7,669-675.
13. WLODARSKA, M., B. P. WILLING, D.M. BRAVO, B. B. FINALY, 2016: Phytonutrient diet supplementation promotes beneficial Clostridia species and intestinal mucus secretion resulting in protection against enteric infection. Sci. Reports 5,9253.
14. LIU, X., M. S. DIARRA, Y. ZHANG, Q. WANG, H. YU, S.-P. NIE, M.-Y. XIE, J. GONG, 2016: Effect of encapsulated carvacrol on the incidence of necrotic enteritis in broiler chickens. Av. Pathol. 45,357-364.
15. SUNKARA, L.T., M. ACHANTAa, N.B. SCHREIBERr, Y. R. BOMMIENI, G. DAI, W. JIANG, S. LAMONT, H.S. LILLEHOJ, A. BEKER, R. G. TEETER, G. ZHANG. 2011: Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. Plos One 7,e49558.
16. KOGUT, M.H., K.J. GENOVESE, H. HE, C. L. SWAGGERTY, Y. W. JIANG, 2012: BT cationic peptides: Small peptides that modulate innate immune responses of chicken heterophils and monocytes. Vet. Immunol. Immunopathol. 145,151-158.

17. KOGUT, M.H., K.J. GENOVESE, H. HE, C. L. SWAGERTY, Y. W. JIANG, 2013: Modulation of chicken intestinal immune gene expression by small cationic peptides as feed additives during the first week post-hatch. Clin. Vaccine Immunol. 20,1440-1448.
18. ZHANG, G., L.T. SUNKARA, 2014: Avian antimicrobial host defense peptides: from biology to therapeutic applications. Pharmaceuticals 7,220-247.
19. DU, E., W. WANG, L. GAN, Z. LI, S. GUO, Y. GUO, 2016: Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. J. An. Sci. Biotechnol. 7,19.
20. SUNKARA, L.T., X. ZENG, A.R. CURTIS, G. ZHANG, 2014: Modulation of antimicrobial host defense peptide gene expression by free fatty acids. Mol. Immunol. 57,171-180.
21. MARTINEZ-CUMMER, M.A, 2015: Immunogenic ingredients in poultry: applications of innovative concepts leading to sustainable solutions for improved productivity. Aust. Poult Sci. Proceedings 2015,209-212.
22. FERRIERA, H.C. Jr, M. I. HANNAS, L. F. T. ALBINO, H. S. ROSTAGNO, R. NEME, B. D. FARIA, M. L. XAVIER, Jr,, L. N. RENN, 2016: Effect of the addition of $\boldsymbol{\beta}$-mannanase on the performance, metabolizable energy, amino acid digestibility coefficients, and immune functions of broilers fed different nutritional levels. Poult Sci. 95,1848-1857.
23. WITCOMBE, D.M., N.C. Smith, 2014: Strategies for anti-coccidial prophylaxis. Parasitol. 141,1379-1389.
24. SAND, J.M., M.K. ARENDT, A. REPASY, G. DENIZ, M. E. COOK, 2016: Oral antibody to interleukin-10 reduces growth rate depression due to *Eimeria* spp. infection in broiler chickens. Poult. Sci. 95,439-446.
25. KLASING, K.C., 1998: Nutritional modulation of resistance to infectious diseases. Poult. Sci. 77,119-1125.
26. KLASING, K.C., 2007: Nutrition and the immune system. Br. Poult. Sci. 48:525-537.
27. KIDD, M.T., 2004: Nutritional modulation of immune function in broilers. Poult. Sci. 83:650-657.
28. KOGUT, M.H., 2009: Impact of nutrition on the immune response to infection in poultry. J. Appl. Poult. Res. 18,111-124.
29. KOGUT, M.H., K.C Klasing, 2009: An immunologist's perspective on nutrition, immunity, and intestinal diseases: introduction and overview. J. Appl. Poult. Res. 18,103-110.
30. KORVER, D.R., 2012: Implications of changing immune function through nutrition in poultry. An. Feed Sci. Technol. 173,54-64.
31. LILLEHOJ, H.S., K.W. LEE, 2012: Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. Poult. Sci. 91,1286-1291.
32. KOUTSOS, E.A., K.C. KLASING, 2014: Factors modulating the avian immune system. In: K.A Shat, B. Kaspers, and P. Kaiser (eds.) Avian Immunology (2nd edition), Academic Press, Boston. pp. 299-313.
33. KOGUT, M.H., 2013: The gut microbiome and host innate immunity: Regulators of host metabolism and metabolic diseases in poultry? J. Appl. Poult. Res. 22,637-646.
34. REDMOND, S.B., R. M. TELL, D. COBLE, C. MUELLER, D. PALIC, C. B. ANDREASEN, S. J. LAMONT, 2010: Differential splenic cytokine responses to dietary immune modulation by diverse chicken lines. Poult. Sci. 89,1635-1641.
35. WILS-PLOTZ, E.L., R.N. DILGER, 2013: Combined dietary effects of supplemental threonine and purified fiber on growth performance and intestinal health of young chicks. Poult. Sci. 92,726-734.
36. ECHEVERRY, H., A. YITBAREK, P. MUNYAKA, M. ALIZADEH, A. CLEVER, G. CAMELO-JAIMES, P. WANG, K. O J. C. RODRIGUEZ-LECOMPTE, 2016: Organic trace mineral supplementation enhances local and systemic innate immune responses and modulates oxidative stress in broiler chickens. Poult. Sci. 95,518-527.
37. SELVARAJ, R.K., R. SHANMUGASINDARAM, R. RENGASAMY, 2016: The use and abuse of immune indices in nutritional immunology studies. J. Appl. Poult. Res. 25,284-291.
38. KLASING, K.C., 2002: Nutrition and the immune system. Br. Poult.Sci. 48,525–37.

39. ARESENAULT, R.J., B. TROST, M.H. KOGUT., 2014: A comparison of the chicken and turkey proteomes and phosphoproteomes in the development of poultry specific immunometabolism kinome peptide arrays. Vet. Infect.Dis. 1,22.
40. KLASING, K C., 1988: Nutritional aspects of leukocytic cytokines. J. Nutr. 118,1436–46.
41. KLASING, K.C., D.R. KORVER, 1997: Leukocytic cytokines regulate growth rate and composition following activation of the immune system. J. Anim.Sci. 75,58–67.
42. NIEWOLD, TA., 2007: The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci. 86,605–9.
43. TEIRLYNCK, E., L. BJERRUM, V. EECKHAUT, G. HUYGEBAER, G, F. PASMANS, F. HAESEBROUCK, (2009: The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. Br. J.Nutr. 102,1453–61.
44. URAN, P.A., A.A. GONCALVES, J.J. TAVERNE-THIELE, J.W. SCHRAMA, A.J. VERRETH, J.H.W.M. ROMBOUT, 2008: Soybean meal induces intestinal inflammation in common carp (Cyprinus carpio L.). Fish Shellfish Immunol. 25,751–60.
45. BAKKE-McKELLEP, A.M., C.M. PRESS, G. BAEYERFJORD, A. KROGDAHL, T. LANDSVERK, 2000: Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, Salmo salar L.,with soybean meal- induced enteritis. J. Fish Dis. 23,115–27.
46. KLASING, K.C., 1988: Influence of acute feed deprivation or excess feed intake on immunocompetence of broiler chicks. Poult.Sci. 67,626–34.
47. CHOCT, M., 2006, Enzymes.for the feed industry: past, present and future. Worlds Poult. Sci. J. 62,5–16.
48. KORVER, D.R., 2012: Implications of changing immune function through nutrition in poultry. Anim. Feed Sci. Technol. 173,54–64.
49. PATTERSON, J.A., K.M. BURKHOLDER, 2003: Application of prebiotics and probiotics in poultry production. Poult. Sci. 82,627–31.
50. RIGGS, J.P., J.P. JACOB, 2005: Alternatives to antibiotics for organic poultry production. J. Appl. Poult. Res. 14,750–6.
51. HUME M.E., 2011: Historic perspective: prebiotics, probiotics, and other alternatives to antibiotics. Poult. Sci. 90,2663–2669.
52. KOGUT, M.H., 2013: The gut microbiota and host innate immunity: regulators of host metabolism and metabolic diseases in poultry? J. Appl. Poult. Res. 22,637–46.
53. ARSENAULT, R.J., S. NAPPER, M.H. KOGUT, 2013: Salmonella enterica Typhimurium infection causes metabolic changes in chicken muscle involving AMPK, fatty acid and insulin/mTOR signaling. Vet. Res. 44,35.
54. KELLY, B., L.A. O'NEILL, 2015: Metabolic reprogramming in macrophages and dendritic cells ininnate immunity. Cell Res. 25,771–84.
55. POLLIZZI, K.N., J.D. POWELL, 2015: Regulation of T cells by mTOR: the known and the unknowns. Trends Immunol. 36,13–20.
56. O'NEILL, L.A.J., D.G. HARDIE, 2013: Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature 493,346–55.
57. PREYAT, N., O. LEO, 2013: Sirtuin deacylases: a molecular link between metabolism and immunity. J. Leukoc. Biol. 93.669–80.

Sustainability goals and approaches of the feed industry

Yves Tohermes

DVT – German Feed Association / FEFAC

According to the UN World Population Prospects 2017, the world population will grow to more than 9.7 billion people by 2050. The economic development, and consequently rising salaries, in emerging economies worldwide will enable more and more people to raise their standard of living. As a result of increased income, peoples' daily diets change and their living patterns shift. The increasing consumption of meat is just one of the many aspects that is observed to go hand in hand with an improvement in family income and prosperity.

Germany provides a good example of the linkage between meat consumption and economic prosperity. At the beginning of the reconstruction of Germany after the Second World War in 1950/51, national meat consumption was at a low. In the following 30 years, German meat consumption grew continuously and reached its peak in the early 1980s (100 kg/capita/year), mainly driven by the increase in pork consumption. After the peak of consumption, the German meat and feed industry developed further and professionalised its value chain in order to sustain its international competitiveness, which it holds until today.

Over the past decades, consumer diets and perceptions starting changing, while public debates on environmental issues such as deforestation, monocultures and the use of GM crops moved along the agricultural value chain, became louder and eventually turned into political agendas. The European food value chain nowadays finds itself in a stress field where the majority of costumers are still very price sensitive, the number of national and European regulations/laws keep on increasing, and the public as well as the political focus on sustainability grows constantly. Consequently, the European feed industry – as part of the food value chain – has to face these challenges and accommodate current customers' demands, while operating in a highly competitive environment.

Sustainability within the European feed value chain means balancing social, ecological and economic interests. This balance is highly critical for the existence of feed manufacturers, as it defines the economic necessities, ecological boundaries, and the social claim of a good. An impact on either of these points has to be followed by countermeasures in order to sustain a stable environment for feed production. The feed industry often has to find practical compromises, for instance between the current scientific truth and the customer's will, or between pressure from NGOs and the market reality.

WORKING THE MARKET FROM DIFFERENT ANGLES

Soy certification

The advances of modern breeding technologies have enabled famers to produce more efficiently and to promote soy as an essential protein source for modern animal husbandry. The cultivation of South American soy is publicly often linked to issues like deforestation, soil degradation, excessive monocultures, and violated labour and land rights, for instance. In 2006 the European Feed Manufacturers' Federation (FEFAC) started to engage on the topic as part of taking responsibility.

In order to anticipate increased market, the FEFAC Soy Sourcing Guidelines (SSGs) were created to ensure transparency and to provide an interpretation to what "responsible soy" should minimally comply with.

Existing soy certification standards can apply to be benchmarked against the FEFAC SSGs (performed by ITC) to verify whether they meet the feed industry's requirements for good environmental, social and agricultural practices of soy production. There currently are 18 standards listed, all of which comply with the FEFAC guidelines. These standards originate from NGOs, farmer associations, and companies involved in the feed value chain. FEFAC will soon start the reviewing process of its guidelines in order to ensure that they serve political and future market demand for "deforestation-free" products.

Although certification – as a tool – can contribute to more sustainable production, its overall effect is always limited. Consumers' willingness to pay a premium for certified commodities, as well as the implementation of environmental standards in cultivating countries, still promises the biggest potential of sustainable development.

VLOG – GMO-FREE FEED

In Europe, and particular in Germany, the use of genetic engineering is a highly controversial and often publicly discussed issue. Although there is no scientific proof, so far, of any hazardous effect from the consumption of GMO food, the use of GMO technology and GMO commodities in the EU is highly restricted.

Meanwhile, a lot of customers in Germany equate the consumption of GMO-free products to the promotion of sustainability. This misinterpretation, however, does not hinder the success of GMO-free products on the German market. In 2013, the German VLOG (Verband Lebensmittel ohne Gentechnik e. V.; English: Association Food without Genetic Engineering) introduced its seal, "no genetic engineering". Since then, more and more food products of animal origin in Germany are produced GMO-free. One of the main drivers of the VLOG seal was the dairy industry, which widely implemented GMO-free inputs as a new standard. As a consequence, around 40 percent of German dairy feed is GMO-free, as is 60 percent of the poultry feed.

The German feed industry thereby currently operates in a field of ambivalence in which it has to balance its interests. The VLOG standard allows the industry to find another channel to market its feed, as it is the answer to a certain market demand. Meanwhile, the same standard negates the positive effect of resource efficiency by using the latest breeding and cultivation technology.

FEFAC VISION FOR SUSTAINABILITY

The European Feed Manufacturers' Federation (FEFAC) published its "FEFAC Vision 2030" in 2016 in which sustainability represents one of the three main pillars.

Sustainability should always be regarded in the context of globalised markets and value chains. Creating single-spot markets with high-end sustainability segments in a fragmented market will not bring solutions to the issue of global resource depletion, and may set the level of ambition too high for many agricultural producers to be an appealing incentive. The overall integration and gradual improvement of farm-level production in exporting countries will maintain natural capital and the long-term capacity of production.

Sustainability is a dynamic process that moves along societal definitions and perceptions over time as more and more insight is gained. Because of this, societal demands, for example, can cause trade-offs in the light of feed efficiency, since the consumer demand for slower growing animals has increased. Moreover, sustainability has its limits. The push to reduce waste accumulation in other processing industries should never come at the expense of feed and food safety. Besides for the legal principles, it has to be accepted by a sustainable livestock sector that only feed ingredients with high safety standards qualify and are eligible for valorisation.

Improving environmental sustainability of poultry production using innovative feeding strategies

B. Méda[1], P. Belloir[2], A. Narcy[1] and A. Wilfart[3]*

[1]BOA, INRA, Université de Tours, 37380, Nouzilly, France, [2]Ecole d'Ingénieurs de Purpan, INP Toulouse, 31000 Toulouse, France, [3]SAS, INRA, Agrocampus Ouest, 35000, Rennes, France; bertrand.meda@inra.fr

SUMMARY

Poultry production is facing many sustainability challenges, among which the reduction of environmental impacts such as climate change, eutrophication or acidification. In that context, nutrition is a powerful tool to improve the environmental sustainability of poultry production. First, nutrient utilization (in particular nitrogen and phosphorus) should be improved with a better fit between supply and requirements, and by using feed additives (amino acids, enzymes).

Secondly, using life cycle assessment (LCA) methodology, the environmental impacts associated to the production of feed ingredients should be considered in feed formulation. Finally, the LCA environmental impacts of poultry production "at farm gate", taking into account feed production and on-farm emissions, should also be assessed in order to design "environmental friendly" feeding strategies. To do so, modelling tools are still required to simultaneously evaluate these new strategies at different scales (animal performance, manure emission, feed production), both on economic and environmental criteria.

INTRODUCTION

Because world population should reach almost 10 billion in 2050, the demand for animal products will strongly increase in the coming decades. In particular, poultry products are expected to have the highest growth rate with 120 and 65% for poultry meat and eggs respectively (MOTTET and TEMPIO, 2017). Simultaneously to the increase of production, poultry supply chains are facing many sustainability challenges (VAARST *et al.,* 2015). Besides consumers' expectations regarding animal welfare, they should tackled several environmental issues, among which climate change, water eutrophication or soil acidification (GERBER *et al.*, 2007). To do so, nutrition is one of the most effective lever as feed is the main driver of animal performance (growth or egg production) and nutrient excretion (nitrogen, phosphorus). Moreover, feed production (*i.e.* the production, transport and processing of feed ingredients) has also been shown to be a source of pollution associated to livestock production (STEINFELD *et al.*, 2006). Consequently, in order to improve its environmental sustainability, poultry production should design new feeding strategies. This review therefore focuses on the environmental consequences of different feeding strategies both at animal scale (feed efficiency and nutrient utilization), at farm scale (manure emissions) and at a production system global scale, including the impacts associated to feed production (life cycle approach).

DECREASING NUTRIENT EXCRETION AND EMISSIONS FROM MANURE

Adjustment of nutrient supply to requirements and improvement of feed utilization

In poultry, ammonia (NH_3) in the main nitrogen compound emitted by manure. It is responsible for several environmental impacts such as water pollution (eutrophication) and soil acidification (GERBER *et al.*, 2007). The most efficient way to reduce NH_3 emissions is to reduce nitrogen (N) excretion of poultry, by adjusting crude protein (CP) supply to the requirements of the animals (MÉDA *et al.*, 2011). The first step is the reduction of dietary CP content over time with phase-feeding strategies. In the past two decades, many studies focused on the reduction of dietary CP content during the growing-finishing period. These studies generally succeeded in reducing nitrogen excretion, with an average value of -10% for a reduction of one CP point in the diet (MÉDA *et al.*, 2011). Nevertheless, several authors also reported a negative impact on growth and/or feed efficiency. For instance, when decreasing CP from 23.4 to 19.2% in broiler chicks between 7 and 21d, BREGENDAHL *et al.* (2002) observed an increase of feed conversion ratio by 8% (1.35 and 1.46 respectively) and a 4% decrease of body weight gain (654 and 631 g respectively). AFTAB *et al.* (2006) suggested that essential/non-essential amino acids (AA) or net energy/metabolizable energy ratios could explained these negative effects.

Defining the optimal amino acids profile (*i.e.* all amino acids being expressed according to lysine level) is a therefore challenge for poultry production, and the use of crystalline AA is often required to reach this optimal profile during feed formulation. The profile proposed by MACK *et al.* (1999) for broilers is one of the most famous. Recently, this profile was updated for arginine and threonine, and successfully used in finishing broilers by BELLOIR *et al.* (2017). Using a large range of crystalline AA (lysine, methionine, threonine, arginine, valine, isoleucine and tryptophan), the decrease of dietary CP content by 2 percentage points (from 19 to 17%) had no effect on growth or feed efficiency (Table 1). Concerning N excretion, results were consistent with the literature, with a decrease by about 10% per CP point, even when feed efficiency was negatively impacted below 17% (Table 1). In the future, to achieve an even greater decrease in dietary CP (*i.e.* below 17%), the requirements for the next limiting AA (*e.g.* valine, isoleucine) and non-essential AA (*e.g.* glycine) should be assessed. Finally, the use of exogenous proteases could also improve feed efficiency but contrasted results were reported in the literature. Further research is thus required to better understand the interactions between the animal (genetics, age), dietary characteristics (proteins, fibre) and other exogenous enzymes (ADEOLA and COWIESON, 2001).

Table 1 Effect of dietary crude protein content in finishing broilers on animal performances and nitrogen balance according to BELLOIR *et al.* (2017). Diets were formulated to respect an ideal protein profile (digestible amino acids) and crystalline amino acids were used to balance the diets*. Nitrogen balance was recalculated using data and equations from the study.

	19%	18%	17%	16%	15%
Animal performances					
Body weight (g)	2460	2470	2466	2451	2461
Body weight gain (g)	1479	1496	1494	1446	1478
Feed intake (g)	2430	2477	2472	2459	2528
Feed conversion ratio†	1.64[b]	1.65[b]	1.65[b]	1.69[a]	1.71[a]
Nitrogen balance					
N intake (g)	73.9	71.3	67.2	63.0	60.7
N retention (g)	42.9	43.4	43.3	41.9	42.9
N excretion (g)‡	31.0	28.0 (-10%)	23.9 (-23%)	21.0 (-32%)	17.8 (-43%)
N volatilization (g)‡	8.5	6.6 (-23%)	4.7 (-45%)	3.3 (-61%)	2.1 (-75%)

*DL-Methionine, L-Lysine-HCl and L-Threonine in all diets + L-Arginine, L-Valine, L-Isoleucine and L-Tryptophan in 18% to 15% diets. †$p<0.01$; ‡In brackets: reductions compared to the 19% diet.

Phosphorus (P) supply in poultry should also be considered, because of its impact on the environment (water eutrophication) and because phosphate resources are non-renewable (GERBER *et al.*, 2007; CORDELL *et al.*, 2009). Similarly, to nitrogen, significant reductions in P content were achieved in the past. For instance, POWERS and ANGEL (2008) reported that in the middle of the 2000's, the average dietary content for non-phytate P (NPP) in broilers up to 21 days of age was about 3.6 g/kg *vs* 4.5 g/kg according to the last NRC recommendations (1994). The use of exogenous phytase also add a significant role in the decrease of P supply in poultry diets, as this enzyme can substantially improve P availability in the digestive tract (POWERS and ANGEL, 2008). For example, RIBEIRO JR. *et al.* (2016) estimated that the addition of 500 and 1000 phytase units of *Escherichia coli* is equivalent to the respective addition of 1.3 and 2.4 g of inorganic phosphorus (dicalcium phosphate) per kg in broiler diets between 1 and 21 days. More recently, new feeding strategies based on the adaptive response of birds under P (and calcium) restriction have shown promising results to decrease even further dietary P in poultry. For instance, VALABLE *et al.* (2018) showed that a 25% decrease of NPP contents in growing broilers, followed by "classical" finisher diet did not impaired performance nor bone mineralization. In particular, the adaptive response could involve an improvement of P digestibility and absorption (ROUSSEAU *et al.*, 2016), and consequently an increase in body P retention. NARCY *et al.* (2015) showed indeed that in growing broilers, a simultaneous decrease in dietary NPP and calcium contents (-1.5 and -4 g/kg respectively) improved P body retention by 12%. Using this strategy in growing period and by lowering NPP and calcium contents in finishing period (-1.1 and -4.2 g/kg respectively), ROUSSEAU *et al.* (2016) showed that P manure content could be substantially reduced (about -40%).

In the future, the development of precision feeding (PF) strategies, such as those successfully developed in pigs (POMAR *et al.*, 2010; ANDRETTA *et al.*, 2016), could be a new step for adjusting nutrient supply to the requirements of the animals. DUSART *et al.* (2019) evaluated *in silico* the economic and environmental potential gains when applying a precision feeding strategy in broilers between 10 and 47 days of age. This PF strategy was based on a daily mix of two different pre-diets in different proportions according to the requirements of the birds (d10-d24: pre-diets A/B; d25-d42: pre-diets B/C; d43-d47: pre-diets C/D), and was compared to a classical phase-feeding strategy (3 phases). The authors showed that feeding cost, N and P excretion could be decreased by 6, 11 and 4%, respectively. Yet, PF strategies in poultry are not currently used in commercial farms, as many issues still have to be tackled (modelling of daily requirements, processing of on-farm collected data such as body weight, implementation of decision rules into farm equipment…).

Reduction of ammonia emissions from manure

As reported by MÉDA *et al.* (2011), many factors control ammonia emission from poultry manure. Besides the amount of ammoniacal N available in manure, manure moisture is a key factor in the ammonification process, *i.e.* the aerobic decomposition by microorganisms of excreted uric acid into NH_3. According to GROOT KOERKAMP (1994), the optimal rate of ammonification is observed when manure moisture is between 40 and 60%. Manure moisture is essentially controlled by ventilation rate in the poultry house, bedding material characteristics and direct water inputs (water spillage), but feeding can also influence this parameter (MÉDA *et al.*, 2011). First, feed viscosity directly affects water excretion in poultry as reported by FRANCESCH and BRUFAU (2004). Yet, the practical characterization of viscosity (*e.g.* by feed producers) is somehow difficult. Secondly, as reported by BORGES *et al.* (2004), the increase in dietary electrolytic balance (DEB) linearly increases water consumption (+30% for an increase of DEB from 40 to 340 mEq/kg of feed), and consequently, manure moisture. As reported by QUINIOU and NARCY (2019), the optimal DEB associated to the lowest litter moisture content should be close to 150 mEq/kg of feed (quadratic response). So far, in conventional diets, DEB is generally above this value, mostly because of the use of K^+-rich feed ingredients, such as soybean meal.

The decrease of soybean use in low-CP diets can thus help reducing excreta or manure moisture, as shown by several authors in the literature (HERNÁNDEZ *et al.*, 2012; FERGUSON *et al.*, 1998). Such a decrease in manure moisture could have indeed a positive effect on ammonia volatilization as shown by BELLOIR *et al.* (2017). By reducing dietary CP (and soybean meal use) in the finisher diet of broilers, the authors showed a synergic effect on total N volatilization associated both to the decrease of N excretion and to the lower volatilization rate of the excreted N (-4 percentage point per CP point). For a CP level of 15% (*vs* a control diet at 19%), this means a decrease by 75% of volatilized N for a reduction by "only" 43% of N excretion (Table 1). Finally, another way of reducing NH_3 volatilization is to decrease manure pH, the chemical equilibrium between NH_4^+ and NH_3 being shifted towards the non-volatile form NH_4+ (MÉDA *et al.*, 2011) by using acidifying compounds in feed (LI *et al.*, 2008; WU-HAAN *et al.*, 2007).

TAKING INTO ACCOUNT THE ENVIRONMENTAL IMPACTS OF FEED INGREDIENTS

Evaluation of the environmental impacts of feed ingredient using Life Cycle Assessment (LCA)

Because of its normalized methodological framework (ISO 14040 and 14044, 2006) and its large set of environmental impact categories, Life Cycle Assessment (LCA) has become one of the most popular method to assess, using a holistic approach, the environmental impact of a product or a system throughout its life cycle.

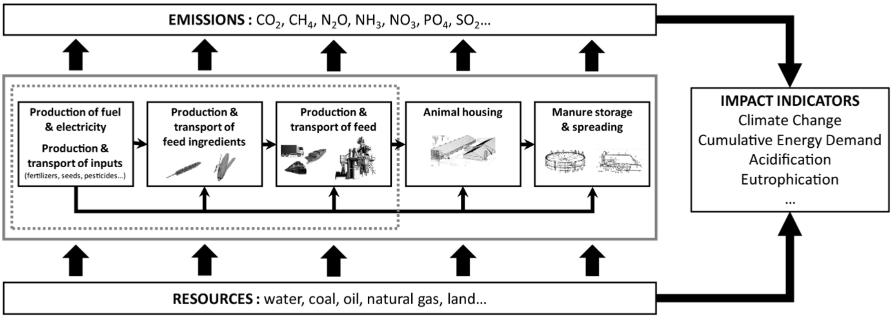


Figure 1 System boundaries for the environmental assessment of feed and poultry production (dotted and full grey lines respectively) using life cycle assessment. For each stage of the production process, all the resources consumed and the compounds emitted into the environment are considered in order to calculate environmental impact indicators.

To perform a LCA, the first step is to define the goal and scope of the studied system by determining its boundaries and the function of the product/system. In the case of feed production, all the stages, from the production of inputs (fuel, electricity, seeds, fertilizers…) for the production of feed ingredients until their processing in the feed mill and the transport of concentrated feed to the poultry farm, have to be considered (Figure 1). When focusing on the environmental impacts of poultry production, the rearing of animals in the farm and manure emissions will also be taken into account in the LCA (Figure 1). The second step is the inventory analysis, during which, all the resources consumptions and the emissions to the environment are listed (using enquiries, database, models, expertise…). The last step is the aggregation of the resources use and emissions into impact categories for environmental impacts such as climate change or eutrophication (Figure 1). Depending on the function of the product/system considered in step 1, the results will be expressed using a specific functional unit. In the case of poultry production, the functional units to be considered would be the ton of feed and the ton of live weight (or eggs) for concentrated feed and poultry productions, respectively.

WILFART *et al.* (2016) proposed a consistent database for LCA impacts of feed ingredients used in animal nutrition in France (ECOALIM database). According to the authors, LCA impacts vary greatly according to the category of feed ingredients considered. For instance, vegetal fats or crystalline AA have the largest climate change (CC) impacts per ton of ingredient whereas cereals grains, oil seeds and proteins crops have the lowest impacts (Table 2). The variability observed among and within feed ingredient categories is mostly due to the production process of the feed ingredient (for crops: fertilizers, pesticides; for industrial products such as vitamins or AA: energy input) and/or to the economic allocation of impacts such as in cereals coproducts (*i.e.* coproducts are generally cheaper that the primary products, and produced in smaller volumes). For instance, WILFART *et al.* (2016) showed that acidification (AC) and eutrophication (EU) impacts for conventional wheat grain (*i.e.* with mineral fertilization) are about 15% higher than wheat grain produced with a systematic organic fertilization (data not shown), due to lower ammonia emission and nitrate leaching in the field.

Table 2 Variability of environmental impacts (per ton of feed ingredient) assessed using life cycle assessment for different feed ingredient categories. CC: Climate Change; CED: Cumulative Energy Demand; AC: Acidification; EU: Eutrophication.

Feed ingredient category	CC (kg CO_2-eq)	CED (GJ)	AC (kg SO_2-eq)	EU (kg PO_4-eq)
Cereals*	493 ± 207	24 ± 5	1818 ± 628	42 ± 14
Cereals coproducts*	583 ± 533	25 ± 22	954 ± 709	46 ± 45
Oil seeds and protein crops*	484 ± 438	26 ± 6	3103 ± 1177	24 ± 27

Oil meals*	769 ± 832	21 ± 8	2283 ± 1058	24 ± 8
Vegetal fats*	2334 ± 1727	52 ± 20	4972 ± 2343	78 ± 56
Animal coproducts*	557 ± 641	13 ± 13	800 ± 1331	46 ± 63
Insects (fresh and meals) †, ‡	2205 ± 1317	52 ± 60	17 ± 13	10 ± 7
Algae and duckweed†	1810 ± 683			
Yeast and bacteria meals†	1285 ± 290			
Minerals*	967 ± 900	14 ± 15	175 ± 205	23 ± 41
Crystalline amino acids*	11021 ± 6101	284 ± 144	2510 ± 1758	311 ± 210

* ECOALIM French database (WILFART *et al.*, 2016); † TALLENTIRE *et al.*, 2018; ‡ OONINCX and DE BOER, 2012; THÉVENOT *et al.*, 2018; VAN ZANTEN *et al.*, 2015.

The LCA impacts of new feed ingredients such as insects (fresh or meal), aquatic biomass (algae, duckweed) and single cell protein sources (yeast and bacteria meals) have also been studied, although few data are available in the literature (Table 2). In Europe, such feed ingredients are considered as alternatives to replace imported soybean meal, but further work is still required to conclude on their environmental benefits as environmental impacts are found to be either lower (*e.g.* TALLENTIRE *et al.*, 2018) or higher (THÉVENOT *et al.*, 2018) than those of soybean meal. Furthermore, as stressed out for insect production by THÉVENOT *et al.* (2018) and VAN ZANTEN *et al.* (2015), production processes are still in development. Large improvements are thus possible to decrease the impacts of these new ingredients. In particular, in the European Union, authorizing the use of bio-waste (manure or household waste) should be considered to produce insects in a more sustainable way.

Finally, the incorporation rate of the feed ingredient in the diet must also be considered. By doing so, the LCA impacts per ton of ingredient can be put into perspective. For instance, in the study by TALLENTIRE *et al.* (2017), crystalline AA impacts are generally 10 times higher (or more) than the impacts of most of the other feed ingredients (cereal grains and coproducts, oil meals…). However, as the total incorporation rate of synthetic AA in broilers feeds is very low (<1%), they only contribute to less than 10% of the final impacts of the feed, whereas cereals + soybean meal contribute to 10 to 70%. Similar results were found in the studies of GARCIA-LAUNAY *et al.* (2018) and WILFART *et al.* (2018).

Feed formulation with environmental criteria

LCA impacts of feed ingredients can be easily integrated as new criteria in formulation matrices to calculated environmental impacts of the formulated diet. Maximal constraints on impacts can be set to limit the environmental impacts (*e.g.* defining a maximal CC impact per ton of feed). If the objective is to reduce substantially one impact, linear programming is adapted to perform a "least-impact" formulation rather than the classical "least-cost" one. Yet, this approach has be shown by several authors to increase feed price, sometimes up to +30%, and to cause pollution swapping among impacts (NGUYEN *et al.*, 2012; TALLENTIRE *et al.*, 2017). For instance, when formulating a UK broiler diet using a "least-CC" approach, TALLENTIRE *et al.* (2017) managed to decrease CC impact per ton of feed by about 35% but with an increase by about 15 and 30% in price and the use of non-renewable energy, respectively.

In order to avoid (or limit) pollution swapping among impacts while controlling the increase in feed price, GARCIA-LAUNAY *et al.* (2018) proposed a method called "multi-objective formulation" (MOF) that takes simultaneously into account feed price and several environmental impacts. MOF is based on linear programming as "least-cost" formulation (LCF). The objective function to be minimized (*min MO*) includes here both a price and an environmental component, with a weighting factor a (0 £ a £ 1) to set the weight of the environmental component. When a = 0, MOF is similar to LCF, while when a = 1, feed price is no longer considered in formulation. Four LCA impacts are considered in the environmental component of the objective function: climate change (CC), non-renewable energy use (NREU), land occupation (LO) and phosphorus demand (PD). Impacts are normalized by the impacts of feed formulated with LCF, and the relative weight of each LCA impact can be set using the b coefficients. For instance, GARCIA-LAUNAY *et al.* (2018) chose to give more weight to climate change ($b_{CC} = 0.4$) compared to the other βs (0.2). Finally, acidification and eutrophication (AC and EU) impacts are indirectly considered by setting a maximal constraint so that these impacts cannot exceed 105% of LCF feed impacts. The best compromise between environmental impact reduction and price increase can be identified when the marginal decrease in the environmental impact becomes lower than the marginal increase in the price ($a = \alpha_{lim}$).

$$\min MO = (1-\alpha) \times \left(\frac{Cost_{MOF}}{Cost_{LCF}}\right) +$$
$$\alpha \times \left(\beta_{CC}\frac{CC_{MOF}}{CC_{LCF}} + \beta_{NREU}\frac{NREU_{MOF}}{NREU_{LCF}} + \beta_{LO}\frac{LO_{MOF}}{LO_{LCF}} + \beta_{PD}\frac{PD_{MOF}}{PD_{LCF}}\right)$$

with $\beta_{CC} + \beta_{NREU} + \beta_{LO} + \beta_{PD} = 1.$

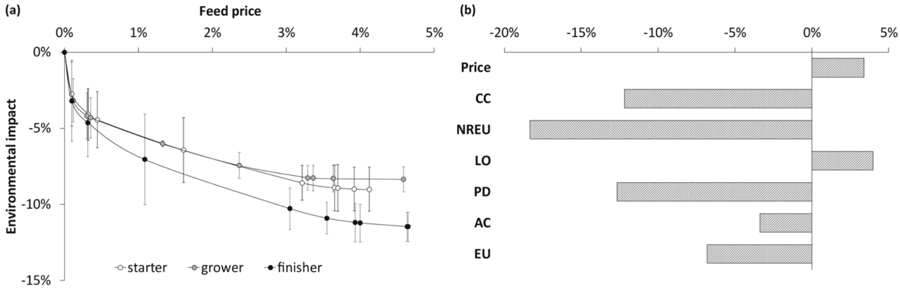


Figure 2 a) Variations of price and environmental impacts for broiler feeds using multiobjective formulation (a from 0 to 1 with a step of 0.1), compared to least-cost formulation. b) Variations of price and environmental impacts for an average broiler feed (starter 6%; grower 20%; finisher 74%) using multiobjective formulation ($a = \alpha_{lim}$) compared to least-cost formulation (CC: climate change; NREU: non-renewable energy use; LO: land occupation; PD: phosphorus demand; AC: acidification; EU; eutrophication). (adapted from GARCIA-LAUNAY *et al.*, 2018)

Using this approach, GARCIA-LAUNAY *et al.* (2018) showed that, in broilers, a substantial reduction in environmental impact of feed is achievable for an increase in feed price below 5% (Figure 2a). In particular, at $a = \alpha_{lim}$, five of the six considered LCA environmental impacts could be decreased: NREU (-18%), PD (-17%), CC (-12%), EU (-7%) and AC (-3%), while LO and feed price were increased by 4 and 3% respectively per ton of average feed (Figure 2b). This improvement in the environmental impact of broiler feed was possible with several changes in the feed composition. For instance, in finishing broiler, maize coproducts and protein seeds (8 and 4% in LCF feed) were totally removed in MOF feed, while wheat coproducts were increased up to 12% (0% in LCF feed). In the same time, cereals were decreased by 11 percentage points while oil meals were increased by 6 percentage points (GARCIA-LAUNAY *et al.*, 2018; data not shown).

Finally, it is essential to remind that the actual reduction in the environmental impacts of feed will greatly depend on the price, the availability and the origin of feed ingredients. For instance, TALLENTIRE *et al.* (2017) showed that the CC impact of broiler feed produced in the UK could be three times higher in the USA. This can be explained by the use, in the UK diet, of soybean from South America with a CC impact almost 8 times higher than the US one (3.1 and 0.4 kg CO_2-eq/kg respectively), in relation with the recent land use change for the cultivation of soybean in South America (mainly deforestation releasing carbon from large carbon sinks).

GETTING THE BIG PICTURE: ASSESSING THE CONSEQUENCES OF FEEDING STRATEGIES ON ENVIRONMENTAL SUSTAINABILITY OF POULTRY PRODUCTION

As previously mentioned, LCA is one of the most adequate method to assess the environmental sustainability of poultry production “at farm gate”, and many studies can be found in the literature, mostly focusing on broiler and egg productions. In these studies, regardless of the species or the production system (conventional, organic…), feed production stage is described as the main contributor to several environmental impacts expressed per kg of product. In particular, the contribution of feed production to climate change and energy use represents about 60-75% of the total impact (Figure 3). Feed production also significantly contributes to eutrophication (35-60%; Figure 3) and in a lesser extent to acidification (<30%; Figure 3), because of the major contribution of NH_3 emissions from manure to this impact.

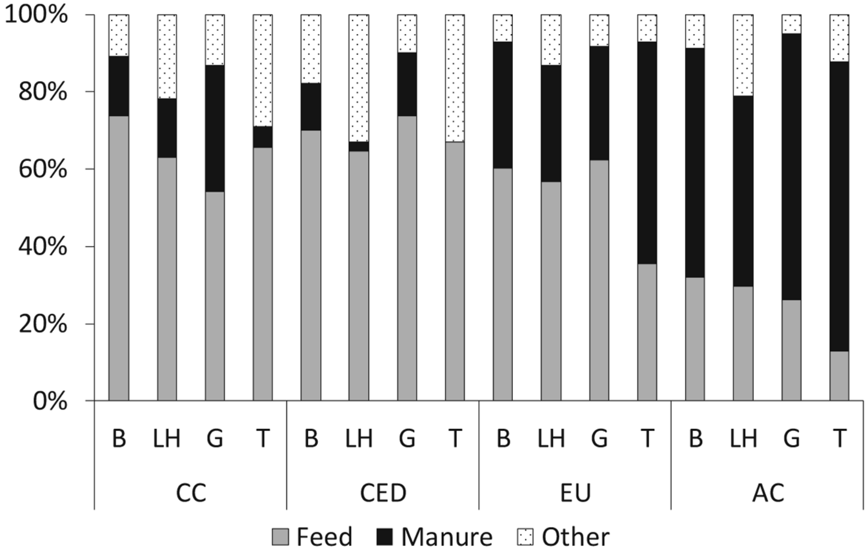


Figure 3 Average contribution of feed production and manure emissions to environmental impacts per kg of product at farm gate for different poultry productions (CC: Climate Change; CED: Cumulative Energy Demand; EU: Eutrophication; AC: Acidification). Broiler (B): PRUDÊNCIO DA SILVA *et al.* (2014); LEINONEN *et al.* (2012a); PELLETIER (2008). Laying hen (LH): LEINONEN *et al.* (2012b); DEKKER *et al.* (2011); MOLLENHORST *et al.* (2006). Goose “foie gras” (G): ARROYO *et al.* (2013). Turkey (T): LEINONEN *et al.* (2014).

Given the significant contribution of feed production to environmental impacts of poultry production, different feeding strategies in poultry have been assessed in the literature using LCA at farm gate (*i.e* impacts per kg of live weight, kg of edible carcass, kg of egg…), especially in fast-growing broiler (Table 3). The study of KEBREAB *et al.* (2016) perfectly illustrates the environmental benefits that were obtained in Europe in the past with the use of crystalline AA in broiler diets. This led to an important decrease in dietary CP content (-80 g/kg on average from a diet without any AA supplementation to a diet using lysine, methionine and threonine + phytase) and an improvement of feed conversion ratio (1.85 vs 2.01 in the “reference” scenario). This strategy reduced significantly N intake (-38% per kg of body weight gain) and consequently, N excretion and N emissions (about -55% per kg of body weight gain; recalculated using data available in the study). Therefore, EU and AC impacts were decreased by about 50% (Table 3). In the diets supplemented with crystalline AA, soybean meal use was decreased by about -50%, leading to a reduction of 44% of CC impact (Table 3), because of soybean meal was considered to be imported from South America. The studies of LEINONEN and WILLIAMS (2015) and MÉDA *et al.* (2017) showed that in « actual » diets, CP reduction is still a relevant tool to reduce the environmental impacts in poultry, especially CC, EU and AC impacts but to a lesser extent compared to the "historical" initial situation described by KEBREAB *et al.* (2016) with very high dietary CP contents.

Other European studies focused on the partial or total substitution of one or several feed ingredients in the diet. For instance, DEKKER *et al.* (2013) showed that in the Netherlands, replacing several imported feed ingredients (wheat, sunflower and soybean meals…) by Dutch ones, the CC impact and energy use of organic egg production could be decreased by 9% and 21%, respectively. Similarly, the substitution of maize and soybean meal, by sorghum and other protein sources respectively, was found to be very efficient to decrease environmental impact in geese "fois gras" production and slow-growing chicken, respectively (ARROYO *et al.*, 2013; MÉDA *et al.*, 2017). Concerning the use of insects, TALLENTIRE *et al.* (2018) concluded that using insect meal could be promising to significantly decrease CC impact in broilers, because of a lower CC impact per ton of meal compared to soybean meal. Yet, further research on insects is required, as other authors found opposite results (*e.g.* THÉVENOT *et al.*, 2018).

Finally, the environmental benefits of using multiobjective formulation taking into account feed price and impacts (MOF; GARCIA-LAUNAY *et al.*, 2018) are confirmed when the final environmental impacts of broiler production at farm gate are considered. WILFART *et al.* (2018) indeed found that, in conventional broiler, MOF could decrease CC impact and energy use per ton of broiler by 12 and 14% respectively (Table 3).

Table 3 Potential reductions in the environmental impacts at farm gate for different poultry production systems in Europe using various feeding strategies. Reductions are expressed comparatively to the "reference" system considered in each study (CC: Climate Change; CED: Cumulative Energy Demand; EU: Eutrophication; AC: Acidification).

Production	Feeding strategy	CC	CED	EU	AC	Reference
Standard broiler	Inclusion of crystalline amino acids (+ phytase)	-44%	-6%	-50%	-50%	KEBREAB *et al.* (2016)
Standard broiler	Crude protein reduction (-5 to -20 g/kg) + protease	-2%		-3%	-5%	LEINONEN and WILLIAMS (2015)
Standard broiler	Crude protein reduction in finisher period (-30 g/kg)	-8%	-1%*	-7%	-5%	MÉDA *et al.* (2017)
Standard broiler	Multiobjective formulation accounting for cost and impacts	-12%	-14%*	-5%	-2%	WILFART *et al.* (2018)
Free range broiler	Soy-free diets	-41%	+5%	0%		MÉDA *et al.* (2015)
Goose "foie gras"	Total substitution of maize by sorghum	-17%	-23%	0%	-12%	ARROYO *et al.* (2013)
Organic egg	Substitution of imported feed ingredients by Dutch ones	-9%	-21%		-1%	DEKKER *et al.* (2013)

* Non-renewable energy only

CONCLUSIONS

Designing new feeding strategies to improve the environmental sustainability of poultry production is crucial as poultry products are expected to be more and more consumed in the next decades. In particular, nutritionists should continue their efforts in adjusting nutrient supply to the requirements of the animals, through a better knowledge of these requirements, the use of feed additives (amino acids, enzymes), and the development of new formulation systems (*e.g.* to consider interactions between nutrients or diet components). The environmental impacts associated to the production, transport and processing of feed ingredients should also be taken into account using life cycle assessment. To consider all these aspects, modelling tools are required to design and evaluate feeding strategies at different scales (animal, farm, life cycle) both on economic and environmental criteria.

REFERENCES

ADEOLA, O., A.J. COWIESON, 2011: Opportunities and challenges in using exogenous enzymes to improve non-ruminant animal production. J. Anim. Sci., 89, 3189-3218.

AFTAB, U., M. ASHRAF, Z. JIANG, 2006: Low protein diets for broilers. World Poultry Sci. J., 62, 688-701.

ANDRETTA, I., C. POMAR, J. RIVEST, J. POMAR, J. RADÜNZ, 2016: Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. Animal, 10, 1137-1147.

ARROYO, J., L. FORTUN-LAMOTHE, A. AUVERGNE, J.P. DUBOIS, F. LAVIGNE, M. BIJJA, J. AUBIN, 2013: Environmental influence of maize substitution by sorghum and diet presentation on goose foie gras production. J. Clean. Prod, 59, 51-62.

BELLOIR, P., B. MÉDA, W. LAMBERT, E. CORRENT, H. JUIN, M. LESSIRE, S. TESSERAUD, 2017: Reducing the CP content in broiler feeds: impact on animal performance, meat quality and nitrogen utilization. Animal, 11, 1881-1889.

BREGENDAHL, K., J.L. SELL, D.R. ZIMMERMAN, 2002: Effect of low-protein diets on growth performance and body composition of broiler chicks. Poultry Sci. 81, 1156-1167.

CORDELL, D., J.O. DRANGERT, S. WHITE, 2009: The story of phosphorus: Global food security and food for thought. Global Environ. Chang., 19, 292-305.

DEKKER, S.E.M., I.J.M. DE BOER, I. VERMEIJ, A.J.A. AARNINK, P.W.G. GROOT KOERKAMP, 2011: Ecological and economic evaluation of Dutch egg production systems. Livest. Sci., 139, 109-121.

DEKKER, S.E.M., I.J.M. DE BOER, M. VAN KRIMPEN, A.J.A. AARNINK, P.W.G. GROOT KOERKAMP, 2013: Effect of origin and composition of diet on ecological impact of the organic egg production chain. Livest. Sci., 151, 271-283.

DUSART, L., Y. RHOULAM, B. MÉDA, 2019: La formulation bilinéaire permet de minimiser le coût alimentaire et les rejets en alimentation de précision. Proceedings of the 13th Journées de la Recherche Avicole et Palmipèdes à Foie Gras, Tours, France, 298-302.

FERGUSON, N.S., R.S. GATES, J.L. TARABA, A.H. CANTOR, A.J. PESCATORE, M.J. FORD, D.J. BURNHAM, 1998: The effect of dietary crude protein on growth, ammonia concentration and litter composition in broilers. Poultry Sci., 77, 1481-1487.

FRANCESCH, M., J. BRUFAU, 2004. Nutritional factors affecting excreta/litter moisture and quality. World Poultry Sci. J., 60, 64-75.

GARCIA-LAUNAY, F., L. DUSART, S. ESPAGNOL, S. LAISSE-REDOUX, D. GAUDRÉ, B. MÉDA, A. WILFART, 2018: Multiobjective formulation is an effective method to reduce environmental impacts of livestock feeds. Brit. J. Nutr., 120, 1298-1309.

GERBER, P., C. OPIO, H. STEINFELD, 2007: Poultry production and the environment – a review. Proceedings of the International Poultry Conference, Bangkok, Thailand, 379-405.

GROOT KOERKAMP, P.W.G., 1994: Review of emissions of ammonia from housing systems for laying hens in relation to sources, processes, building design and manure handling. J. Agr. Eng. Res., 59, 73-87.

HERNÁNDEZ, F., M. LÓPEZ, S. MARTÍNEZ, M.D. MEGÍAS, CATALÁ P., MADRID J., 2012. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1- to 48-day-old broilers. Poultry Sci., 91, 683-692.

ISO 14040, 2006: Environmental management-Life cycle assessment-Principles and framework. International Organization for Standardization, Geneva, Switzerland.

ISO 14044, 2006: Environmental management-Life cycle assessment-Requirements and guidelines. International Organization for Standardization, Geneva, Switzerland.

KEBREAB, E., A. LIEDKE, D. CARO, S. DEIMLING, M. BINDER, M. FINKBEINER, 2016: Environmental impact of using specialty feed ingredients in swine and poultry production: A life cycle assessment. J. Anim. Sci., 94, 2664-2681.

LEINONEN, I., A.G. WILLIAMS, J. WISEMAN, J. GUY, I. KYRIAZAKIS, 2012a: Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: Broiler production systems. Poultry Sci., 91, 8-25.

LEINONEN, I., A.G. WILLIAMS, J. WISEMAN, J. GUY, I. KYRIAZAKIS, 2012b: Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: Egg production systems. Poultry Sci., 91, 26-40.

LEINONEN, I., A.G. WILLIAMS, I. KYRIAZAKIS, 2014: Comparing UK turkey production systems using analytical error propagation in uncertainty analysis. Proceeding of the 9th International Conference LCA of Food, San Francisco, USA, 702-710.

LEINONEN, I., WILLIAMS A.G., 2015: Effects of dietary protease on nitrogen emissions from broiler production: a holistic comparison using Life Cycle Assessment. J. Sci. Food Agr., 95, 3041-3046.

MÉDA, B., M. HASSOUNA, C. AUBERT, P. ROBIN, J.Y. DOURMAD, 2011: Influence of rearing conditions and manure management practices on ammonia and greenhouse gas emissions from poultry houses. World Poultry Sci. J., 67, 441-456.

MÉDA, B., L. DUSART, M. LESSIRE, J.M. HALLOUIS, T. BORDEAU, C. SOUCHET, F. MERCERAND, C. HATTÉ, I. BOUVAREL, C. BERRI, 2015: Remplacement du tourteau de soja par des matières premières riches en protéines : Évaluation multicritère chez le poulet de chair de type Label Rouge. Proceedings of the 11th Journées de la Recherche Avicole et Palmipèdes à Foie Gras, Tours, France, 1082-1087.

MÉDA, B., P. BELLOIR, M. LESSIRE, H. JUIN ; E. CORRENT, W. LAMBERT, S. TESSERAUD, 2017: Réduction du taux protéique dans l'aliment finition chez le poulet de chair : Conséquences environnementales. Proceedings of the 12th Journées de la Recherche Avicole et Palmipèdes à Foie Gras, Tours, France, 356-360.

MOLLENHORST, H., P.B.M. BERENTSEN, DE BOER I.J.M, 2006: On-farm quantification of sustainability indicators: an application to egg production systems. Brit. Poultry Sci., 47, 405-417.

MOTTET, A., G. TEMPIO, 2017: Global poultry production: current state and future outlook and challenges. World Poultry Sci., 73, 245-256.

NGUYEN, T.T.H., I. BOUVAREL, P. PONCHANT, H.M.G. VAN DER WERF, 2012: Using environmental constraints to formulate low-impact poultry feeds. J. Clean. Prod., 28, 215-224.

NATIONAL RESEARCH COUNCIL (NRC), 1994. Nutrient requirement of poultry. 9th rev. ed. National Academy Press, Washington, DC, USA, ISBN 978-0-309-04892-7, 176 p.

OONINCX, D.G.A.B, I.J.M. DE BOER, 2012: Environmental Impact of the Production of Mealworms as a Protein Source for Humans – A Life Cycle Assessment. Plos One, 7(12), e51145.

PELLETIER, N., 2008: Environmental performance in the US broiler poultry sector: Life cycle energy use and greenhouse gas, ozone depleting, acidifying and eutrophying emissions. Agr. Syst., 98, 67-73.

POMAR, C., L. HAUSCHILD, G.H. ZHANG, J. POMAR, P.A. LOVATTO P.A., 2010: Precision feeding can significantly reduce feeding cost and nutrient excretion in growing animals. In: SAUVANT, D., J. VAN MILGEN, P. FAVERDIN, N. FRIGGENS: Modelling nutrient digestion and utilisation in farm animals. Wageningen Academic Publishers, Wageningen, The Netherlands, ISBN 978-90-8686-156-9, 327-334.

POWERS, W., R. ANGEL, 2008: A review of the capacity for nutritional strategies to address environmental challenges in poultry production. Poultry Sci., 87, 1929-1938.

PRUDÊNCIO DA SILVA, V., H.MG. VAN DER WERF, S.R. SOARES, M.S. CORSON, 2014: Environmental impacts of French and Brazilian broiler chicken production scenarios: An LCA approach, J. Environ. Manage., 133, 222-231.

QUINIOU, N., A. NARCY, 2019: Pourquoi et comment intégrer la balance électrolytique dans les critères de formulation des aliments. Proceedings of the 13th Journées de la Recherche Avicole et Palmipèdes à Foie Gras, Tours, France, 145-156.

RIBEIRO JR., V., S.C. SALGUERO, G. GOMES, V.R.S.M BARROS, D.L. SILVA, S.L.T. BARRETO, H.S. ROSTAGNO, M.I. HANNAS, L.F.T. ALBINO, 2016: Efficacy and phosphorus equivalency values of two bacterial phytases (*Escherichia coli* and *Citrobacter braakii*) allow the partial reduction of dicalcium phosphate added to the diets of broiler chickens from 1 to 21 days of age. Anim. Feed Sci. Tech., 221, 226-233.

ROUSSEAU, X., A.S. VALABLE, M.P. LÉTOURNEAU-MONTMINY, N. MÊME, E. GODET, M. MAGNIN, Y. NYS, M.J. DUCLOS, A. NARCY, 2016: Adaptive response of broilers to dietary phosphorus and calcium restrictions. Poultry Sci., 95, 2849-2860.

STEINFELD, H., P. GERBER, T. WASSENAAR, V. CASTEL., M. ROSALES, C. DE HAAN, 2006: Livestock's long shadow. Environmental issues and options. Food and Agriculture Organisation (FAO), Rome, Italy. 390 p.

TALLENTIRE, C.W., S.G. MACKENZIE, I. KYRIAZAKIS, 2017: Environmental impact trade-offs in diet formulation for broiler production systems in the UK and USA. Agri. Syst., 154, 145-156.

TALLENTIRE, C.W., S.G. MACKENZIE, I. KYRIAZAKIS, 2018: Can novel ingredients replace soybeans and reduce the environmental burdens of European livestock systems in the future? J. Clean. Prod., 187, 338-347.

THÉVENOT, A., J.L. RIVERA, A. WILFART, F. MAILLARD, M. HASSOUNA, T. SENGA-KIESS, S. LE FÉON, J. AUBIN, 2018. Mealworm meal for animal feed: Environmental assessment and sensitivity analysis to guide future prospects. J. Clean. Prod., 170, 1260-1267.

VAARST, M., S. STEENFELDT, K. HORSTED, 2015: Sustainable development perspectives of poultry production. World Poultry Sci., 71, 609-620.

VALABLE, A.S., A. NARCY, M.J. DUCLOS, C. POMAR, G. PAGE, Z. NASIR, M. MAGNIN, M.P. LÉTOURNEAU-MONTMINY, 2018: Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. Animal, 12, 1555-1563.

VAN ZANTEN, H.E., H. MOLLENHORST, D.G.A.B. OONINCX, P. BIKKER, B.G. MEERBURG, I.J.M. DE BOER, 2015: From environmental nuisance to environmental opportunity: housefly larvae convert waste to livestock feed. J. Clean. Prod., 102, 362-369.

WILFART, A., S. ESPAGNOL, S. DAUGUET, A. TAILLEUR, A. GAC, F. GARCIA-LAUNAY, 2016: ECOALIM: A dataset of environmental impacts of feed ingredients used in French animal production. Plos One, 11(12), e0167343.

WILFART, A., L. DUSART, B. MÉDA, A. GAC, S. ESPAGNOL, L. MORIN, Y. DRONNE, F. CARCIA-LAUNAY, 2018: Réduire les impacts environnementaux des aliments pour les animaux d'élevage. INRA Prod. Anim., 31, 289-306.

Phytate destruction and efficiency of phosphorus use: problems solved?

Markus Rodehutscord[*] *and Vera Sommerfeld*

Institute of Animal Science, University of Hohenheim, 70599, Stuttgart, Germany

[*]Correspondence: inst450@uni-hohenheim.de

SUMMARY

Phosphorus (P) nutrition is a key element to making poultry production more sustainable. Much progress has been made in understanding the mode of action of phytases and the utilisation of phytate-bound P, especially in broiler chickens. This allows for reduction in the use of feed phosphates produced from finite rock phosphate resources. A further increase in utilisation of phytate-bound P seems to be possible mainly through more degradation of intermediate products of the phytate destruction chain. However, more important to the management of P resources is the precise knowledge of digestible P requirements and the avoidance of P oversupply. Progress has been made in modelling the digestible P requirement of broiler chickens, but more work is needed in order to make systems more flexible and adjustable to the diversity of conditions found in poultry production systems. Other growing poultry such as turkeys and waterfowl have different phytate destruction and P digestibility than broilers. More research is needed with these species and also with layers in regard to P digestibility and P requirement.

INTRODUCTION

Phosphorus (P) is a non-renewable resource and the rock phosphate reserves are unequally distributed on earth. All living organisms depend on the continuous supply of available P sources for many metabolic processes. Saving P resources for future generations is an important objective for participants along the entire food chain and likely one of the greatest challenges for agricultural production systems to become more sustainable (GROSS, 2010, NESET and CORDELL, 2012). For the animal industries including poultry this is related to a need for further improvement in the efficiency of P utilisation.

In poultry feeds, a large proportion of P originates from plant seeds (such as cereal and leguminous grains) and by-products of the food and energy sector such as oilseed meals and press cakes, brans, and dried distillers' grains. In many of these plant feeds, more than half of the P is contained in the form of phytic acid and its salts called phytate ($InsP_6$). Birds are restricted in their digestive capacity to release P from $InsP_6$. Hence, mineral feed phosphates are often added to the feed of poultry to meet the requirements. This is of special relevance in regions such as the EU, where animal proteins (and P contained therein) are not allowed to be used. This is not sustainable, in particular when feed phosphates are manufactured from rock phosphates. Different approaches are of interest and need to be combined in an attempt to further increase the efficiency of P utilisation in the poultry sector. They are related to an increase of digestibility of P bound in the form $InsP_6$ ($InsP_6$-P), revision and diversification of dietary P allowances, and attempts to keep P cycles closed (again) on different levels of production. This contribution, though not intended to be a comprehensive review, will summarise the progress achieved and research gaps to be filled.

PHOSPHORUS EVALUATION SYSTEM

Traditionally, different P evaluation systems were suggested and are in use in different regions of the world. Systems consider aspects of P utilisation by the animal to a different extent and are based on total P, non-phytate P, available P, bioavailable P, retainable P, and digestible P (SHASTAK and RODEHUTSCORD, 2013). These expressions do not mean the same, which causes confusion and does not support improvements of evaluation systems. According to the consensus opinion of the Working Group No 2 –Nutrition– of the European Federation of Branches of WPSA, P evaluation for poultry should be done using precaecal digestible P to overcome limitations (WPSA, 2013). This includes that P evaluation of feedstuffs, evaluation of phytase, and P requirements are based on digestible P.

RELEASE OF P FROM INSP$_6$ IN THE DIGESTIVE TRACT

Broiler chickens

For a long time, it has been assumed that endogenous InsP$_6$ degradation in the digestive tract of poultry plays little role or even does not exist. However, several studies conducted in recent years showed something different. When the broiler chicken is challenged by a low supply of Calcium (Ca) and P, for instance by complete removal of feed phosphates from the diet, up to two third of the InsP$_6$ contained in the diet is degraded and the released phosphate absorbed until the end of the small intestine, even when the diet does not contain the enzyme phytase. This degradation is catalysed by phytases and other phosphatases provided by epithelial cells or microorganisms of the digestive tract. When mineral P sources are added to the diet, which is very common in the poultry industry, endogenous InsP$_6$ degradation of the broiler is strongly reduced, an effect even more pronounced when Ca is included in the supplement and especially when Ca is supplemented in excess of the requirement. When phytase is added to the feed, it can compensate for these diminishing effects of mineral P and Ca on InsP$_6$ degradation. The literature in this field has recently been reviewed elsewhere (RODEHUTSCORD, 2017) and the reader is referred to this reference for more detailed information.

Progress has been made in the development and application of phytase as a feed additive and this strongly contributes to making the poultry sector more sustainable. Many recent studies have shown that phytase supplementation to broiler diets can increase gastrointestinal InsP$_6$ degradation to a very large extent. In studies of our group using maize-soybean meal-based diets, InsP$_6$ degradation up to the terminal ileum reached a level of 80-90 % (ZELLER et al., 2015a, ZELLER et al., 2015b, SOMMERFELD et al., 2018a, SOMMERFELD et al., 2018b, INGELMANN et al., 2019) and was even higher than 90 % when phytase supplementation exceeded 1500 FTU per kg of feed. Accordingly, possibilities to achieve further improvements in P digestibility by increasing InsP$_6$ accessibility above what currently is possible are not big.

Of note, measurement of InsP$_6$ disappearance in the digestive process does not mean more than release of one (or more) phosphate group from the *myo*-inositol ring. Although the concentration of *myo*-inositol in the digesta of the ileum and in blood of broilers are considerably higher when InsP$_6$ degradation is increased upon phytase supplementation (GAUTIER et al., 2018, SOMMERFELD et al., 2018a, b), this does not mean dephosphorylation is complete once the initial phosphate group is released by phytase. Phytases are known for not being able to catalyse entire dephosphorylation and other phosphatases of endogenous origin are assumed to continue degradation on InsP$_4$ and InsP$_3$ isomers. Wherever the involved enzymes may come from, lower InsP isomers occur at the end of the ileum, demonstrating that the degradation process is not complete (Figure 1). Even when phytase is supplemented at a very high level (3000 or 12,500 FTU/kg) in order to maximise initial dephosphorylation in the anterior section of the digestive tract, some lower InsP remain undigested in the content of the ileum (ZELLER et al., 2015b, SOMMERFELD et al., 2018a). Should it be possible to overcome these limitations in the degradation cascade, further improvements in the utilisation of InsP$_6$-P are possible.

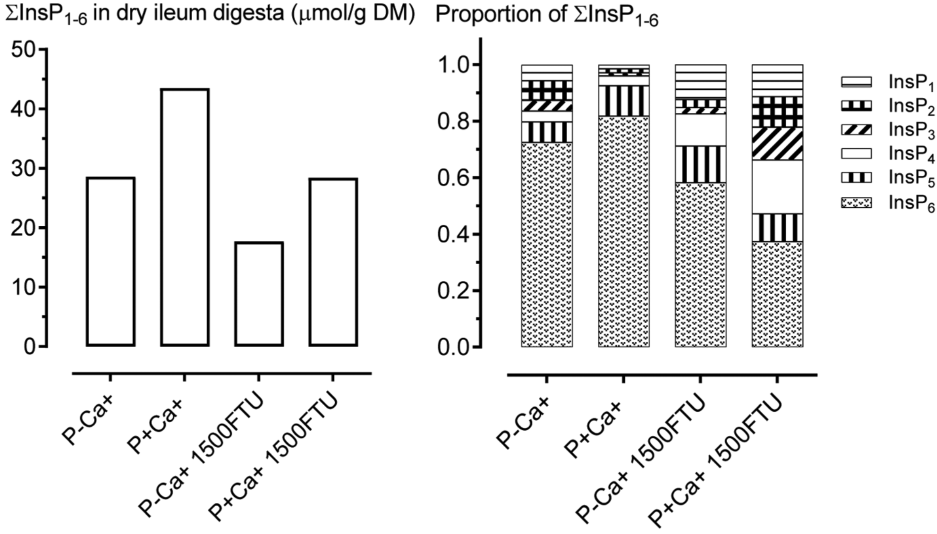


Figure 1. Concentrations of inositol phosphates measured in the ileum digesta of broilers that were fed diets with different combinations of P, Ca, and phytase supplements (SOMMERFELD et al., 2018b)

Several studies measured P digestibility together with $InsP_6$ disappearance. Analysing data from such studies showed that with each increment of $InsP_6$-P disappearance at the end of the ileum, P digestibility increased by a factor of 0.78 (RODEHUTSCORD, 2017). The difference of this factor from one (0.22) is reflective of the amount of P that is indigestible because bound to lower InsP. This seems to be an untapped potential of P digestibility that could be activated by enzymes that help avoiding accumulation of lower InsP in the $InsP_6$ degradation cascade.

Supplements of Ca sources, especially limestone, are well known to reduce the solubility and precaecal degradation of $InsP_6$ with corresponding effects on P digestibility (TAMIM and ANGEL, 2003). This effect seems to be specifically relevant when diets do not contain a phytase supplement while negative effects of monocalcium phosphate supplementation on $InsP_6$ degradation disappeared at very high levels of phytase supplementation (ZELLER et al., 2015b). However, when 1500 FTU/kg were used in diets adequate in Ca concentration, precaecal $InsP_6$ degradation remained below the value achieved at a reduced Ca concentration (SOMMERFELD et al., 2018b). Therefore, concepts that target a maximum in P digestibility in broilers by using phytase must consider a meaningful dosage of Ca and avoid Ca oversupply. Evaluation of Ca sources on a digestible basis and factors influencing Ca digestibility have close linkages with P digestibility but are a separate matter (ANWAR et al., 2018, KIM et al., 2018) and will not be further addressed herein.

LAYING HENS

Degradation of $InsP_6$ in laying hens has been investigated less intensively than in broiler chickens and results were not as consistent. In the early work of VAN DER KLIS et al. (1997), principal responses of laying hens were similar to those characterised above for broiler chickens: When maize-soybean meal-based diets were used, precaecal $InsP_6$ degradation and P digestibility were significantly lower at 4 % Ca in the diet compared to 3 % Ca in the diet, but monocalcium phosphate did not affect $InsP_6$ degradation. Phytase supplementation (500 FTU/kg) increased precaecal $InsP_6$ degradation up to 72 %, but the negative effect of Ca remained to exist in the diets containing phytase. The response in precaecal $InsP_6$ degradation was not reflected in performance and bone data of hens, which was considered as an indicator of the P requirement of laying hens being overestimated (VAN DER KLIS et al., 1997). In contrast, precaecal phytate ($InsP_6$ – $InsP_2$) degradation was not significantly affected or even reduced upon phytase supplementation to maize-based diets in the study of HUGHES et al. (2009). Reduction of dicalcium phosphate concentration in this study did not significantly affect precaecal phytate degradation. Of note, the Ca concentration of the diets in this study was very high (4.4 %). In another study, phytase supplementation effects were investigated using single ingredients as the only source of P in the diet (LESKE and COON, 1999). The diets were provided without or with 300 FTU/kg phytase. Excreta $InsP_6$ degradation was increased from 26 % to 62 % in soybean meal, 23 % to 52 % in maize, and 4 % to 51 % in rice bran.

A recent work showed that in content of gizzard and ileum of hens, concentrations of *myo*-inositol were significantly higher and those of $InsP_6$ and $InsP_5$ lower when wheat-based diets were supplemented with 1500 FTU/kg phytase (TAYLOR et al., 2018). The authors did not report precaecal $InsP_6$ degradation; however, precaecal P digestibility was increased from 39 % to 70 % by 1500 FTU/kg phytase. Again, the diets had a very high Ca concentration (> 4.4 %).

MAROUNEK et al. (2008) used hens of different age and found that excreta $InsP_6$ degradation was more than twice as high in 47-weeks old hens (53 %) than in 20-weeks old hens (24 %). This corresponded to significantly higher estimates of endogenous phytase activity in the digestive tract, especially in the caeca, but also precaecal. It is not clear whether age effects can partially explain the inconsistencies of results from the studies mentioned above. Experimental protocols, site of sampling and phytate assays were all also different among the studies. Compared to broilers, the process and extent of $InsP_6$ degradation and factors of major impact on $InsP_6$ degradation are not well understood in laying hens.

YOUNG TURKEYS

When using a low-P basal diet and further diets containing graded supplements of monobasic calcium phosphate, P utilisation of the basal diet was higher in broiler chickens than turkeys, but utilisation of the supplemented mineral P was higher in turkeys (RODEHUTSCORD and DIECKMANN, 2005). ADEBIYI and OLUKOSI (2015) studied precaecal P digestibility and P retention of wheat DDGS using the regression approach. Estimated digestibility and retention values were very high in broiler chickens (94 and 92 %) and lower in turkeys (76 and 71 %). Likely, differences in $InsP_6$ degradation in the digestive tract and release of phosphate have contributed to the differences between species. When feeding low-P wheat-soybean meal-based diets to turkeys, $InsP_6$ degradation until the end of the ileum was 29 % in the absence of a phytase supplement and 45 % when 500 FTU/kg phytase was added (INGELMANN et al., 2018). This level of $InsP_6$ degradation was substantially lower than that reported in similar studies with broiler chickens mentioned above. When using different genotypes of maize in studies with broiler chickens and turkeys with and without a phytase supplement, precaecal $InsP_6$ degradation was much lower in the turkey study than in the broiler study, irrespective of phytase addition (INGELMANN et al., 2019). Endogenous mucosal phytase activity was detected in the small intestine of broiler chickens (MAENZ and CLASSEN, 1998, HUBER et al., 2015) and it cannot be ruled out that this is different in young turkeys, thus leading to differences in phosphate release from $InsP_6$. Other variables such as passage rate and pH in different sections of the digestive tract can also contribute to the differences between species. More studies are necessary to understand the factors that limit $InsP_6$ degradation and phytase action in the digestive tract of turkeys.

Phosphorus requirement and dietary allowances

A plethora of papers targeted the P requirement of **broiler chickens** and factors influencing the requirement. Summarizing this research goes beyond the scope of this presentation. Perhaps a summary is not possible because of the differences in main feed ingredients, P sources, interactions between mineral P, Ca, and phytate, response criteria, and ways of expression. Age of the broilers is important, and perhaps strain also plays a role. Nonetheless, some of the literature has been extracted following defined criteria and used for meta-analysis, for instance by LÉTOURNEAU-MONTMINY et al. (2010). These authors made calculations for 3 weeks-old broilers using non-phytate P, and calculated how variation in Ca concentration and phytase supplementation affect the concentration of non-phytate P needed in the diet for growth, gain to feed ratio, and tibia ash concentration. Calculations were consistent with the Ca effects on $InsP_6$ degradation mentioned in the previous chapter by showing that more non-phytate P was needed in the diet when Ca concentration was increased (LÉTOURNEAU-MONTMINY et al., 2010, FARIDI et al., 2015). Of note, both meta-analyses showed that more P is needed for high bone mineralisation than growth or gain to feed ratio. Such meta-analyses are very useful in generating data for optimising diets, but have the disadvantage that they are related to predefined classes of growth or age.

Following guidance of WPSA (2013), requirement data for precaecal digestible P are needed. In line with this and as an alternative to meta-analysis, factorial approaches were suggested to be used in poultry (RODEHUTSCORD, 2006, KHAKSAR et al., 2017). This implies that single factors such as endogenous P loss and P accretion during growth can be determined separately and used in flexible models. Such models can consider variables such as broiler strain and its P content of the body and its change with age, growth rate, and feed conversion ratio. Such a model of digestible P requirement has recently been suggested by KHAKSAR et al. (2017). When entry variables are chosen that represent broiler standards, the required digestible P concentration of the diet continuously decreases with increasing age (Figure 2). The graph also shows how total P concentration is affected in dependence of the P digestibility of the diet, irrespective of whether this is caused by the choice of feed raw materials or phytase supplementation, or both. It is clear that there is considerable possibility to reduce the P concentration in the diet with increasing age. The older broilers grow, the higher the possibility for saving P resources is. This is a combined effect of what is shown in Figure 2 and the fact that the feed to gain ratio is the higher the older broilers are.

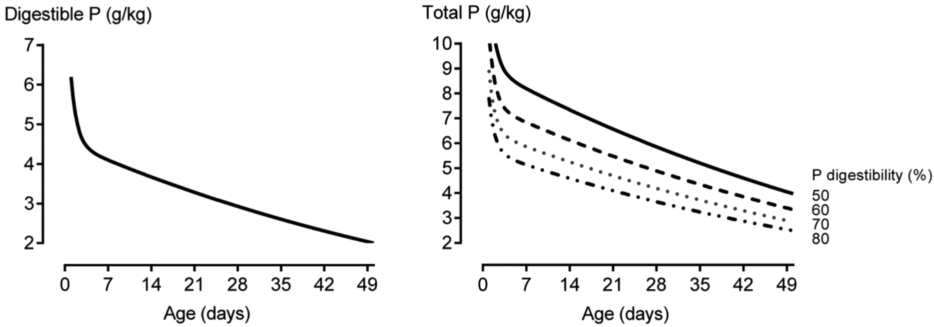


Figure 2. Course of digestible P and total P concentration needed in diets for broilers during growth. The left panel is based on the model of KHAKSAR et al. (2017) and feed intake curves of commercial broilers. The right panel shows resulting concentrations of total P in dependence of P digestibility of the diet.

In a meta-analysis of data from 14 experiments with **laying hens** using maize-based diets, estimated non-phytate P requirements ranged from 1.5 to 2.2 g/kg feed, depending on what response trait was chosen and whether 300 FTU/kg phytase were supplemented or not (AHMADI and RODEHUTSCORD, 2012). Compared to recommendations of different committees, this range is much lower. More recent studies confirmed that traditional recommendations overestimate the requirements. In a study of 12-week duration and using several response criteria, authors concluded that their data indicate a reduction to 1.5 g 'available' P is possible without affecting health and performance of laying hens (JING et al., 2018). Other authors even suggested that an oversupply with P can reduce the digestibility of other nutrients (HUGHES et al., 2009). Thus, the potential to save feed phosphates in laying hen feeding is high.

The P concentration that is needed in diets of **growing turkeys** can be modelled in the same way as mentioned before for broiler chickens by using the factors specific for turkeys. Dietary P levels in the diet are much higher in turkeys than broilers in the starter phase. However, the possibilities to reduce the P concentration in turkeys with increase in age are much bigger, especially in those production systems that grow males for more than 20 weeks (RODEHUTSCORD et al., 2003).

CONCLUSIONS

Phosphorus nutrition is a key element to making poultry production more sustainable. This includes application of a P evaluation system based on digestible P, refinement of digestible P requirements, increasing digestibility of phytate-bound P, and avoidance of oversupply with P and Ca. Optimizing P supply in poultry feeding by using these tools helps saving finite resources for future generations, avoiding undesired environmental effects, and saving feeding cost. Much progress has been made in increasing phytate-P digestibility by the application of enzymes and there is not much room left for further increase. The greatest potential and challenge is the avoidance of oversupply with P. To support transition to more sustainable P use, models of P requirement need to be improved and validated. Finally, extension is needed for getting the knowledge gain transferred to the industry.

REFERENCES

ADEBIYI, A. and OLUKOSI, O. (2015) Determination in broilers and turkeys of true phosphorus digestibility and retention in wheat distillers dried grains with solubles without or with phytase supplementation. *Animal Feed Science and Technology* 207: 112-119.

AHMADI, H. and RODEHUTSCORD, M. (2012) A meta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. *Poultry Science* 91: 2072-2078.

ANWAR, M. N., RAVINDRAN, V., MOREL, P. C. H., RAVINDRAN, G. and COWIESON, A. J. (2018) Measurement of the true ileal calcium digestibility of some feed ingredients for broiler chickens. *Animal Feed Science and Technology* 237: 118-128.

FARIDI, A., GITOEE, A. and FRANCE, J. (2015) A meta-analysis of the effects of nonphytate phosphorus on broiler performance and tibia ash concentration. *Poultry Science* 94: 2753-2762.

GAUTIER, A. E., WALK, C. L. and DILGER, R. N. (2018) Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poultry Science* 97: 211-218.

GROSS, M. (2010): Fears over phosphorus supplies. *Current Biolog*y 20: R386.

HUBER, K., ZELLER, E. and RODEHUTSCORD, M. (2015) Modulation of small intestinal phosphate transporter by dietary supplements of mineral phosphorus and phytase in broilers. *Poultry Science* 94: 1009-1017.

HUGHES, A. L., DAHIYA, J. P., WYATT, C. L. and CLASSEN, H. L. (2009) Effect of quantum phytase on nutrient digestibility and bone ash in white leghorn laying hens fed corn-soybean meal-based diets. *Poultry Science* 88: 1191-1198.

INGELMANN, C.-J., WITZIG, M., MÖHRING, J., SCHOLLENBERGER, M., KÜHN, I. and RODEHUTSCORD, M. (2019) Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with or without added phytase. *Poultry Science* 98: 912-922.

INGELMANN, C. J., WITZIG, M., MÖHRING, J., SCHOLLENBERGER, M., KÜHN, I. and RODEHUTSCORD, M. (2018) Effect of supplemental phytase and xylanase in wheat-based diets on prececal phosphorus digestibility and phytate degradation in young turkeys. *Poultry Science* 97: 2011-2020.

JING, M., ZHAO, S., ROGIEWICZ, A., SLOMINSKI, B. A. and HOUSE, J. D. (2018) Assessment of the minimal available phosphorus needs of laying hens: Implications for phosphorus management strategies. *Poultry Science* 97: 2400-2410.

KHAKSAR, V., MEDA, B. and NARCY, A. (2017) Updating the available P requirements of broilers. In: Proceedings of the 21st European Symposium on Poultry Nutrition, Salou/Vila-seca, Spain, 124-129.

KIM, S. W., LI, W., ANGEL, R. and PROSZKOWIEC-WEGLARZ, M. (2018) Effects of limestone particle size and dietary ca concentration on apparent P and Ca digestibility in the presence or absence of phytase. *Poultry Science* 97: 4306-4314.

LESKE, K. L. and COON, C. N. (1999) A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poultry Science* 78: 1151-1157.

LÉTOURNEAU-MONTMINY, M. P., NARCY, A., LESCOAT, P., BERNIER, J. F., MAGNIN, M., POMAR, C., NYS, Y., SAUVANT, D. and JONDREVILLE, C. (2010) Meta-analysis of phosphorus utilisation by broilers receiving corn-soyabean meal diets: Influence of dietary calcium and microbial phytase. *Animal* 4: 1844-1853.

MAENZ, D. D. and CLASSEN, H. L. (1998) Phytase activity in the small intestinal brush border membrane of the chicken. *Poultry Science* 77: 557-563.

MAROUNEK, M., SKŘIVAN, M., DLOUHÁ, G. and BŘEŇOVÁ, N. (2008) Availability of phytate phosphorus and endogenous phytase activity in the digestive tract of laying hens 20 and 47 weeks old. *Animal Feed Science and Technology* 146: 353-359.

NESET, T.-S. S. and CORDELL, D. (2012): Global phosphorus scarcity: identifying synergies for a sustainable future. *Journal of the Science of Food and Agriculture* 92: 2-6.

RODEHUTSCORD, M. (2006) Optimising the use of phosphorus sources in growing meat ducks. *World's Poultry Science Journal* 62: 513-523.

RODEHUTSCORD, M. (2017) Advances in understanding the role of phytate in phosphorus and calcium nutrition of poultry. In: APPLEGATE, T. (Ed.) Achieving sustainable production of poultry meat. Volume 2: Breeding and nutrition, pp. 165-180 (Cambridge, Burleigh Dodds).

RODEHUTSCORD, M. and DIECKMANN, A. (2005) Comparative studies with three-week-old chickens, turkeys, ducks, and quails on the response in phosphorus utilization to a supplementation of monobasic calcium phosphate. *Poultry Science* 84: 1252-1260.

RODEHUTSCORD, M., WENDT, P. and STROBEL, E. (2003) Reducing the phosphorus concentration in diets for turkeys between 10 and 22 weeks of age. *British Poultry Science* 44: 591-597.

SHASTAK, Y. and RODEHUTSCORD, M. (2013) Determination and estimation of phosphorus availability in growing poultry and their historical development. *World's Poultry Science Journal* 69: 569-585.

SOMMERFELD, V., KÜNZEL, S., SCHOLLENBERGER, M., KÜHN, I. and RODEHUTSCORD, M. (2018a) Influence of phytase or *myo*-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. *Poultry Science* 97: 920-929.

SOMMERFELD, V., SCHOLLENBERGER, M., KÜHN, I. and RODEHUTSCORD, M. (2018b) Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. *Poultry Science* 97: 1177-1188.

TAMIM, N. M. and ANGEL, R. (2003) Phytate phosphorus hydrolysis as influenced by dietary calcium and micro-mineral source in broiler diets. *Journal of Agricultural and Food Chemistry* 51: 4687-4693.

TAYLOR, A. E., BEDFORD, M. R., PACE, S. C. and MILLER, H. M. (2018) The effects of phytase and xylanase supplementation on performance and egg quality in laying hens. *British Poultry Science* 59: 554-561.

VAN DER KLIS, J. D., VERSTEEGH, H. A. J., SIMONS, P. C. M. and KIES, A. K. (1997) The efficacy of phytase in corn-soybean meal-based diets for laying hens. *Poultry Science* 76: 1535-1542.

WPSA [Working Group Nutrition of the European Federation of Branches of WPSA]. (2013) Determination of phosphorus availability in poultry. *World's Poultry Science Journal* 69: 687-698.

ZELLER, E., SCHOLLENBERGER, M., KÜHN, I. and RODEHUTSCORD, M. (2015a) Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. *Journal of Nutritional Science* 4: e1.

ZELLER, E., SCHOLLENBERGER, M., WITZIG, M., SHASTAK, Y., KÜHN, I., HOELZLE, L. E. and RODEHUTSCORD, M. (2015b) Interactions between supplemented mineral phosphorus and phytase on phytate hydrolysis and inositol phosphates in the small intestine of broilers. *Poultry Science* 94: 1018-1029.

Advances of amino acid requirements of ducks

Shuisheng Hou

State Key Laboratory of Animal Nutrition, Key Laboratory of Animal (Poultry) Genetics Breeding and Reproduction, Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100193, China

* Corresponding author: Shuisheng Hou. E-mail address: houss@263.net. Tel: +86 10 62815832. Fax: +86 10 62815832.

Address: Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan West Road, Haidian, Beijing, 100193, China

ABSTRACT

Over past decades, tremendous improvements have been made in Pekin ducks. The continued increase in duck performance dictates the need for continual reevaluation of dietary nutrient specification. Amino acids, especially essential amino acids, play vital roles in protein synthesis and metabolism in ducks. Therefore, this paper reviews the latest advances of amino acid requirements and roles on growth of ducks.

Key words: duck, amino acid, requirement, growth

INTRODUCTION

Requirement is defined as the lowest recommendation for animals to maintain the normal growth and development at a certain period. Nutrients in diet below the recommendation would affect the growth and development of animal, and above the dietary recommendation is a waste of natural resources and increases economic costs in diet. Amino acids, especially essential amino acids, play vital roles in protein synthesis and metabolism in ducks. Although the most amino acids recommendation of starter or growing Pekin ducks were provided by NRC (1994), tremendous improvements have been made in Pekin ducks over past decades. Therefore, the new data of amino acids (especially essential amino acids) requirements of Pekin ducks should be reevaluated to adapt to modern strain ducks.

METHIONINE AND DUCK GROWTH

Methionine on growth performance and carcass traits of ducks

Methionine (Met) is the first limiting amino acid in conventional corn-soybean meal-based diets for poultry (Elkin et al., 1986; Matsushita et al., 2007), and plays vital roles in protein synthesis and metabolism. Optimal Met supplementation to diet could increase the growth performance, carcass yield and egg production of poultry (Xie et al., 2006; Ruan et al., 2018). The studies of the roles of Met on growth of broiler chickens and laying hens have been done (Kalinowski et al., 2003; Zhai et al., 2012; Liu et al., 2017). Like other poultry species, Met requirement of ducks and its roles on growth were also investigated by several studies in our group. Dietary Met deficiency resulted in poor growth performance and carcass yield of ducks (Xie et al., 2004; 2006). The final body weight (BW), average daily weight gain (ADG) and average daily feed intake (ADFI) were increased, and feed/gain (F/G) decreased when optimal Met levels were supplemented in diets. Our previous studies showed that ADG was increased and then decreased as dietary Met level increased in corn-peanut meal diets of ducks from hatch to 21 d or from 21 to 49 d of age (Xie et al., 2004; 2006). Moreover, optimal Met supplementation in diets could increase breast and leg meat yield, and decrease abdominal fat deposition. For ducklings from 1 to 21 d, the breast and leg meat yield were both quadratically increased (Xie, 2003), but only the breast meat yield was increased for the growing ducks from 21 to 49 d of age with Met supplementation (Xie et al., 2006). Meanwhile, we observed the abdominal fat of growing ducks was decreased as dietary Met level increased (Xie et al., 2006).

Methionine requirements of ducks

The Met requirement of NRC (1994) recommendation is prehistoric, and the data was taken from only one study in Muscovy ducks (Leclerq and de Carvile, 1977). Hence, Met requirement of Pekin ducks should be reevaluated to adapt to modern strain duck. The studies of Met requirement of Pekin ducks in recent years were listed in Table 1. Xie et al. (2006) reported that Met requirement of male Pekin ducks from 21 to 49 d of age for maximum weight gain and breast meat yield were 0.377% and 0.379%, respectively. Recently, however, the greater Met requirement (Table 1) of growing Pekin ducks was reported by Zeng et al (2015), who also found that Met requirement for optimal feather coverage was greater than the values for BW, F/G and breast meat yield. The different results are probably ascribed to differences in experimental setup, such as the basal diets, strain. The energy level of diet formulation was regarded to be a major factor, which influence the feed intake. The higher dietary energy level often requires greater amino acids to compensate for lower feed intake for ducks. Hence, we designed a study to investigate dietary energy level on Met requirement of growing Pekin ducks fed a conventional corn-soymeal diet from 15 to 42 d of age. We observed an interaction effect on F/G between dietary metabolizable energy (ME) and supplemental Met level. Met requirements of Pekin ducks from 15 to 42 d of age for optimal F/G at 10.82 and 12.95 MJ/kg of ME were different (0.381% vs 0.475%) when expressed as percentage of diet, but had no difference (0.352 vs 0.367 g/MJ) when expressed as g/MJ ME of diet. It indicated that dietary ME level affected the Met requirements, but the Met/ME ratio was relatively constant of Pekin ducks from 15 to 42 d of age in corn-soymeal basal diet (unpublished data).

Table 1. The Met requirement of Pekin ducks.

Growth stage	Basal diet type	Energy level (MJ/kg)	Response criterion[1]	Model[2]	Requirement (%)	Reference source
1~21 d	Corn-Peanut meal	12.32	ADG	Quadratic regression	0.481	Xie et al. (2003)
21~49 d	Corn-Peanut meal-Soymeal	12.18	ADG, BMY	Quadratic regression	0.377, 0.379	Xie et al. (2006)
1~14 d	Corn-Peanut meal-Soymeal	12.45	BW	-	0.46	Wang et al. (2006)
15~35 d	Corn-Peanut meal-Soymeal	12.75	ADG	Quadratic broken line	0.37	Xue (2018)
15~42 d	Corn-soymeal	10.82	F/G	Linear broken line	0.381	unpublished data
		12.95			0.475	
15~28 d	Corn-Soymeal-mixed meal	13.37	BW, BMY, FPWFL	Quadratic broken line	0.510, 0.445, 0.404	Zeng et al. (2015)
				Quadratic regression	0.606, 0.576, 0.559	
15~35 d			BW, BMY, FC	Quadratic broken line	0.468, 0.408, 0.484	
				Quadratic regression	0.605, 0.564, 0.612	

1. ADG, average daily gain; BMY, breast meat yield; BW, final body weight; F/G, feed/gain; FPWFL, fourth primary feather length; FC, feather coverage.

2. "-" means no model used.

The bioefficacy of new methionine sources of ducks

Met deficiency resulted in growth depression and reduced carcass yield of ducks, which could be counteracted via addition of synthesized DL-methionine (DLM) or its hydroxy analogue (2-hydroxy-4-methylthiobutanoic acid; DL-HMTBA) in diets (Xie et al., 2007). Xie (2007) reported that ADG was increased and F/G was decreased with supplementation of DLM and DL-HMTBA in diet, and the bio-efficacies of DL-HMTBA relative to DLM were 78% and 67% based on ADG, and 66% and 44% based on F/G, respectively, for Pekin ducks from 7 to 21 d and 7 to 42 d of age. Recently, however, two studies showed that DLM and DL-HMTBA have the same bio-efficacy of Pekin ducks from 1 to 42 d (relative bio-efficacy was 99%; Zhao et al., 2018), and from 1 to 21 d (relative bio-efficacy was 101%; Kluge et al., 2016). The explanation of this discrepancy was probably due to the difference of DL-HMTBA sources and processing technology.

Usually, chemically synthesized DL-Met supplemented in diets is a racemic (50:50) mixture of D- and L-Met. L-Met could be incorporated directly into body with 100% efficacy, but D-Met must be converted to L-Met before it is incorporated into protein, which might influence its absorption and utilization. At present, L-Met, a new Met source, has been commercially available for feed formulation. Hence, we evaluated the bio-efficacy of L-Met relative to DL-Met, and found that the bio-efficacies of L-Met relative to DL-Met were 134% of Pekin ducks from 1 to 21 d, and 125% of Pekin ducks from 15 to 35 d for ADG by the linear ratio-slope regression (Xue, 2018).

Methionine toxicity of ducks

Regardless of which Met source, optimal Met supplementation to diet had positive effects on duck growth, but excess Met supplementation was toxic and resulted in growth depression (Xie et al., 2007; Xue et al., 2018a). Xie et al. (2007) reported that excess DLM or DL-HMTBA had adverse effect on growth of Pekin duck from 21 to 42 d, and DL-HMTBA was less growth-depressing than equimolar DLM. In addition, Xue et al. (2018a) showed that excess DL- and L-Met were toxic and both sources induced growth depressing equally for starter Pekin ducks. The total tolerable upper limit of Met was 0.87% for Pekin ducks from 7 to 28 d (Xue et al., 2018b). In two aforementioned previous studies, the higher plasma homocysteine concentration in excess Met groups was also observed (Xie et al., 2007; Xue et al., 2018b). In addition, the concentrations of neuropeptide Y, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), maleic dialdehyde (MDA) in plasma were decreased with supplementation of 2% DLM or DL-HMTBA (Xie, 2007). In liver, MDA was increased, and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) were decreased with supplementation of 2% DLM or 2.26% DL-HMTBA (Xie, 2007). Moreover, supplementation of 2% DLM or 2.26% DL-HMTBA caused DNA hypermethylation (Xie, 2007). The aforementioned results might explain the growth depression of ducks by causing liver damage, oxidative damage and genomic DNA hypermethylation when excess Met was supplemented to diet.

LYSINE AND DUCK GROWTH

Lysine biological roles of ducks

Lysine (Lys) plays an important role in protein structure, and participates in the synthesis of muscle, enzymes, serum protein, and polypeptide hormones (Hall and Soares, 2018). The most important role for Lys is protein synthesis. Optimal Lys supplementation could promote protein deposition and further promote the growth of ducks. Many studies showed that the adequate Lys level in diet could increase the breast meat yield and weight gain, and decrease F/G of ducks (Attia, 2003; Xie et al., 2009; Wen et al., 2017). Furthermore, Lys participates in energy metabolism (Luo and Xiong, 2006) and could promote the absorption of minerals such as calcium and iron by chelating with minerals to form soluble small molecular chelates (Civitelli et al., 1992).

Lysine requirements of ducks

As the second limiting amino acid in ducks, the Lys requirements for ducks have been evaluated extensively (Table 2). The Lys requirement of NRC (1994) recommendation for Pekin ducks from hatch to 14 d of age was 0.90%, which came just from few early studies. However, according to the modified Gauss-newton iterative algorithm, the Lys requirements of for ADG and F/G were 1.17% and 1.06% for Pekin ducks from hatch to 21 d of age, and 0.87% and 0.82% for Pekin ducks from 22 to 49 d of age (Bons et al., 2002). Wang et al (2006) reported that the requirement of Lys for Pekin ducklings from hatch to 14 d of age was 1.10%. Xie et al (2009) estimated that Lys requirements of Pekin ducks from 7 to 21 d of age for ADG, F/G, breast meat weight, and breast meat yield were

0.84%, 0.90%, 0.97%, and 0.98% respectively. Zhou et al. (2016) showed that the Lys requirements for ADG, F/G and breast meat yield were 0.948%, 0.986% and 0.961%, respectively, for ducks from 1 to 14 d of age, and 0.758%, 0.792% and 0.761%, respectively, for ducks from 15 to 35 d of age. The aforementioned data of Lys requirements were greater than NRC (1994) recommendation and lower than Ministry of Agriculture of China (2012) for meat-type ducks. The Lys recommendations of ducks are various, which might be due to the response criterion influenced easily by environmental conditions, other nutrient limitations, genetic potential (Garcia et al., 2006; Ghahri et al., 2010; Wen et al., 2017). Zhang et al (2009) reported the Lys requirement of Pekin ducks from 7 to 21 d of age for F/G increased from 0.80% to 0.90% as increased energy and protein level in diets. Wen et al (2017) found that Lys requirement of Pekin ducks for ADG at 12.76 MJ/kg (0.98%) was greater than at 11.51 MJ/kg (0.94%), which might be to compensate for a lower feed intake since dietary energy levels affected feed intake of ducks.

Table 2 The Lys requirements for Pekin ducks

Growth stage	Energy (MJ/Kg)	Protein (%)	Response criterion[1]	Model[2]	Requirement (%)	Reference
1~14 d	12.43	20.26	BW	-	1.10	Wang et al, 2006
1~21 d	12.68	22.40	ADG	Modified Gauss-Newton iterative algorithm	1.17	Bons et al, 2002
			F/G		1.06	
7~21 d	12.13	20.00	F/G	Broken-line model	0.90	Zhang et al, 2009
	10.92	18.00			0.80	
7~21 d	12.13	20.30	ADG	Broken-line model	0.84	Xie et al, 2009
			F/G		0.90	
			BMW		0.97	
			BMY		0.98	
1~21 d	11.51	19.65	ADG	Broken-line model	0.94	Wen et al, 2017
	12.76	19.58			0.98	
1~14 d	12.18	20.10	ADG	Quadratic regression model	0.948	Zhou et al, 2016
			F/G		0.986	
			BMY		0.961	
15~35 d	12.30	16.50	ADG	Quadratic regression model	0.758	
			F/G		0.792	
			BMY		0.761	

[1.] ADG, average daily gain; BMY, breast meat yield; BMW, breast meat weight; BW, final body weight; F/G, feed/gain.

[2.] "-" means no model used.

THREONINE AND DUCK GROWTH

Threonine (Thr) is the third-limiting amino acid behind Met and Lys in poultry diets (Kidd & Kerr 1996; Malinovsky 2018) and increasing dietary Thr could improve growth performance of starter Pekin ducks (Xie et al. 2014b). Thr supply is critical for maintaining intestinal structure and promoting intestinal mucin secretion of birds (Horn et al. 2009; 2010; Zhang et al. 2014), and plays an important role in lipid metabolism (Jiang et al. 2017; 2018).

Threonine requirements of Pekin ducks

Thr is the third-limiting essential amino acid for poultry, and it plays key roles in growth for poultry. However, the Thr requirement for Pekin ducks was not provided by NRC (1994). Therefore, a series of experiments were conducted to evaluate the dietary Thr requirements for Pekin ducks in our laboratory or others (Table 3). Three experiments were conducted to estimate the Thr requirements of Pekin ducks from 1 to 14 d (Jiang et al., 2018) or 1 to 21d of age (Jiang et al., 2016; 2017). Jiang et al. (2018) showed that the Pekin ducks from 1 to 14 d of age required 0.56%, 0.61%, 0.60%, 0.63%, 0.67% Thr, respectively, to achieve maximal ADG in diets containing 16.0%, 17.5%, 19.0%, 20.5%, 22.0% crude protein. Based on quadratic broken-line model, Thr requirements were 0.66% and 0.70% for optimal ADG, and 0.67% and 0.73% for breast muscle percentage of Pekin ducks from 1 to 21 d of age fed diets containing 19% and 22% crude protein, respectively (Jiang et al., 2017). In addition, Xie et al. (2014b) reported that the Thr requirement of Pekin ducks from 1 to 21 d for maximum ADG was 0.67%. Dietary Thr requirements were estimated to be 0.81 to 1.00% by quadratic broken-line regression, and 0.90 to 0.98% by quadratic regression, for Pekin ducks from hatch to 14 d of age (Zhang et al., 2016), and to be 0.70 to 0.80% by quadratic regression, and 0.62 to 0.72% when using quadratic broken-line regression, for Pekin ducks from 15 to 35 d of age (Zhang et al., 2014).

Obviously, the dietary requirements of Pekin ducks are affected by several factors, such as dietary protein levels, phases, fitting models and response criterion. Dietary Thr requirements of Pekin ducks are increased as increased CP levels in diets (Jiang et al., 2016; 2017). Furthermore, the requirements fitted by quadratic regression are greater than those fitted by quadratic broken-line regression (Zhang et al., 2014; 2016). Besides, the Thr requirements evaluated for optimal breast muscle percentage were greater than that for weight gain (Jiang et al., 2017).

Table 3 The threonine requirements of Pekin ducks

Growth stage	Protein level (%)	Response criterion[1]	Model	Requirement (%)	Reference
1~21 d	20.89	ADG	quadratic broken-line	0.61	Jiang et al., 2016
	17.78			0.56	
1~21 d	19.0	ADG	quadratic broken-line	0.66	Jiang et al., 2017
	22.0			0.70	
	19.0	BMY		0.67	
	22.0			0.72	
1~21 d	18.98	ADG	quadratic broken-line	0.67	Xie et al., 2014b
1~14 d	16.0	ADG	linear broken line	0.56	Jiang et al., 2018
	17.5			0.61	
	19.0			0.60	
	20.5			0.63	
	22.0			0.67	

1~14 d	20.65~20.98	BW	quadratic broken-line	0.87	Zhang et al., 2016
		ADG		0.86	
		F/G		0.92	
		BMY		0.95	
		BW	quadratic regression	0.94	
		ADG		0.94	
		F/G		0.98	
		BMY		0.96	
15~35 d	16.39~18.46	BW	quadratic broken-line	0.70	Zhang et al., 2014
		ADG		0.71	
		F/G		0.69	
		BMY		0.69	
		BW	quadratic regression	0.76	
		ADG		0.76	
		F/G		0.74	
		BMY		0.73	

[1.] ADG, average daily gain; BMY, breast meat yield; BW, final body weight.

Threonine regulates lipid metabolism of Pekin ducks

Thr supplementation reduced the fat contents in body (Rangel-Lugo et al., 1994) and breast muscle (Ciftci & Ceylan, 2004) in broilers. Study from our laboratory showed that Thr plays an important role in hepatic lipids metabolism, and dietary Thr and protein levels had an interaction on hepatic lipids metabolism of Pekin ducks (Jiang et al., 2017; 2018).

Previous studies showed that low protein in the diet increased the percentage of abdominal fat in ducks (Zeng et al., 2015; Xie et al., 2016; Jiang et al., 2017; 2018). Dietary Thr supplementation reduced the lipids deposition in liver of Pekin ducks, indicating that Thr supplementation decreased the catabolism of amino acid and prevented the conversion from amino acids to lipids by balancing the dietary amino acids at low protein diet (Jiang et al., 2017). A study conducted in Pekin ducks from 15 to 35 d of age, showed that dietary excess Thr supplementation reduced sebum and abdominal fat percentage, and increased breast meat percentage (unpublished data). Further study showed that dietary Thr supplementation improved hepatic lipid metabolism of Pekin ducks though regulating the genes expression related to lipid synthesis, transport, and oxidation (Jiang et al., 2018). In order to explore the potential pathway that dietary Thr deficiency increases hepatic triglyceride accumulation of Pekin ducks, we determined the hepatic gene expression profiles. The results showed that dietary Thr deficiency increased hepatic triglyceride and fatty acid accumulation via increasing fatty acid and triglyceride synthesis, and reducing fatty acid oxidation and triglyceride transport (unpublished data).

TRYPTOPHAN AND DUCK GROWTH

Tryptophan (Trp) is an indispensable amino acid for poultry, and plays an important role in growth development of poultry. NRC (1994) recommended the Trp requirements (0.23%) for ducks. However, over past decades, tremendous improvements have been made in Pekin ducks, and the continued increase in duck performance dictates the need for reevaluation of dietary Trp. Therefore, a study was conducted to evaluate the Trp requirement for Pekin duck from 1 to 21 d of age (Xie et al., 2014a). As we know, Trp has niacin-sparing activity because of its conversion to niacin (Heidelberger et al., 1948). Thus, we evaluated the effects of dietary nicotinamide levels on Trp requirements of ducks from 1 to 21 d of age. The results showed that the Trp requirements of ducks fed nicotinamide-unsupplemental diet (0.24%) was higher than those fed nicotinamide-supplemental diet (0.17%,

Table 4). The Trp requirement (0.24%) of ducks fed nicotinamide-unsupplemental diet was similar with the NRC (1994) recommendation (0.23%), but the Trp requirement (0.17%) of ducks fed nicotinamide-supplemental diet was lower than the NRC (1994) recommendation (0.23%). In summary, the optimal dietary Trp level is 0.17% for Pekin ducks from 1 to 21 d of age when the diet supplemented with adequate nicotinamide.

Table 4 The tryptophan requirements of Pekin ducks

Growth stage	Response criterion	Nicotinamide supplemental level (mg/kg)	Requirement (%)	Reference
1-14 d			0.23	NRC (1994)
15-49 d			0.17	
1~21 d	ADG	0	0.24	Xie et al., (2014)
		40	0.17	
		80	0.17	
		120	0.17	

ARGININE AND DUCK GROWTH

Arginine requirement of ducks

Arginine (Arg) is an essential amino acid in poultry and plays important physiological roles in poultry growth. So far, limited literature on Arg requirement is available for Pekin duck. Arg requirement of NRC (1994) for Pekin duck from 0 to 2 and 2 to 7 wk of age is 1.1% and 1.0%, respectively. However, the value is derived from Mule duck (Chen and Shen, 1979). Pekin duck grows faster than Mule duck, and the growth performance of modern Pekin ducks has been greatly increased in past 30 years. Therefore,we estimated Arg requirement for modern Pekin ducks from 1 to 21 d of age(Wang et al.,2013a). The results showed that dietary Arg supplementation significantly improved ADG, ADFI and breast meat yield, and decreased F/G of Pekin ducks. Based on quadratic broken-line regression analysis, Arg requirements of male Pekin ducks from 1 to 21 d of age were 0.95, 1.16 and 0.99% for ADG, F/G and breast meat yield, respectively.

Arginine supplementation stimulates muscle development, and reduces fat deposition in duck

Arginine was reported to elevate breast muscle yield in broilers (Labadan et al., 2001) and growing ducks (Wu et al., 2011), and stimulate protein synthesis of longissimus muscle by mTOR pathway in neonatal pigs (Yao et al.,2008). In our study, dietary Arg affects feed intake and carcass yield, and breast muscle yield were increased with each increment of dietary Arg level (0.60, 0.85 or 1.70% Arg) in Pekin duck from 15 to 35 days of age, and birds fed the diet containing 1.70% Arg had greatest leg muscle yield and total muscle yield (Wang et al., 2013b). Dietary Arg supplementation could reduce the fat deposition and abdominal adipose cell size (diameter and volume) (Wu et al., 2011). Under daily cyclic hot temperature environment, the addition of Arg improves feed conversion ratio, and increases hepatic weight relative to body weight while decreases the hepatic water content (Zhu et al.,2015).

Arginine regulates appetite via nitrate oxide pathway

Dietary Arg deficiency impairs appetite and thus inhibits growth and development in broiler chickens (Kidd et al., 2001; Labadan et al., 2001). Our reports have also shown that low dietary Arg decreases feed intake, especially from 1 to 21 d of age (up to 39%) in ducks (Wang et al.,2013a; 2013b). However, the reason why feed intake is depressed by Arg deficiency is not completely clear in ducks. Arg is the nitrogenous precursor for the endogenous synthesis of nitric oxide by nitric oxide synthase, the orexigenic effect of ghrelin, orexin A, and morphine in rats was attenuated by inhibition of endogenous nitric oxide synthesis via peripheral or central administration of nitric oxide synthase inhibitor L-NG-nitro-arginine methyl ester HCl (L-NAME)(Calignano et al., 1993; Gaskin et al., 2003; Farr et al., 2005). We studied the relationship among feed intake and dietary Arg provision and plasma nitric oxide concentration (Wang et al.,2014a), ducks fed the diet containing 0.65% Arg had lower feed intake and plasma nitric oxide level than those in 0.95 and 1.45% Arg groups. Therefore, we concluded that Arg modifies feeding behaviour possibly through controlling endogenous synthesis of nitric oxide in Pekin ducks.

Relationship between arginine and lysine

The specific relationship between dietary Arg and Lys for modern poultry has been reviewed (Balnave and Brakez, 2002). Inappropriate Arg: Lys ratio have adverse effects on poultry growth, feed utilization, plasma and muscle amino acid concentration, and the effects are more evident with an excess of Lys (low Arg: Lys ratio) than that with an excess of Arg (high Arg: Lys ratio). The calculation from our previous experiment shown that Arg: Lys ratio ranging from 0.86 to 1.55 doesn't influence growth performance and muscle yield in duck from 1 to 21 d of age (Wang et al., 2013a).

REFERENCES

Attia Y.A. 2003. Performance, carcass characteristics, meat quality and plasma constituents of meat type drakes fed diets containing different levels of lysine with or without a microbial phytase. Archives of Anim Nutr 57: 39-48.

Balnave D., and Brakez J. 2002. Re-evaluation of the classical dietary arginine:lysine interaction for modern poultry diets: a review. World's Poult Sci J. 58:275-289.

Bons A., Timmler R., and Jeroch H. 2002. Lysine requirement of growing male Pekin ducks. Brit Poult Sci. 43: 677-686.

Calignano A., Persico P., Mancuso F., and Sorrentino L. 1993. Endogenous nitric oxide modulates morphine-induced changes in locomotion and food intake in mice. Eur. J. Pharmacol. 231: 415-419.

Chen B., and Shen T.F. 1979. Studies on duck nutrition. III. Arginine and lysine requirements of mule ducklings. Poult Sci. 58: 1316-1320.

Ciftci I., and Ceylan N. 2004. Effects of dietary threonine and crude protein on growth performance, carcase and meat composition of broiler chickens. Brit Poult Sci 45: 280-2899.

Elkin R. G., Stewart T. S., and Rogler J. C. 1986. Methionine requirement of male White Pekin ducklings. Poult Sci 65:1771-1776.

Farr S.A., Banks W.A., Kumar V.B., and Morley J.E. 2005. Orexin-A-induced feeding is dependent on nitric oxide. Peptides. 26: 759-765.

Garcia A.R., Batal A.B., and Baker D.H. 2006. Variations in the digestible lysine requirement of broiler chickens due to sex, performance parameters, rearing environment, and processing yield characteristics. Poult Sci. 85: 498-504.

Gaskin F.S., Farr S.A., Banks W.A., Kumar V.B., and Morley J.E. 2003. Ghrelin-induced feeding is dependent on nitric oxide. Peptides. 24: 913-918.

Ghahri H., Gaykani R., and Toloie T. 2010. Effect of dietary crude protein level on performance and lysine requirements of male broiler chickens. Afr J Agric Res. 5: 1228-1234.

Hall C, and Soares da Costa T. 2018. Lysine: biosynthesis, catabolism and roles. Wiki J Sci. doi:10.15347/wjs/2018.004.

Heidelberger C., Gullberg M.E., Morgan A.F., and Lepkovsky S. 1948. Tryptophan metabolism. 1. Concerning the mechanism of the mammalian conversion of tryptophan into kynurenine, kynurenic acid, and nicotinic acid. J Bio Chem 179:151-155.

Horn N., Radcliffe J., Applegate T., and Adeola O. 2010. Gut morphology and nutrient retention responses of broiler chicks and White Pekin ducklings to dietary threonine deficiency. Can. J. Anim. Sci. 90: 513-520.

Horn N.L., Donkin S.S., Applegate T.J., and Adeola O. 2009. Intestinal mucin dynamics: response of broiler chicks and White Pekin ducklings to dietary threonine. Poult Sci 88: 1906-1914.

Jiang, Y., Zhu, Y. W., Xie, M., Tang, J., Wen, Z. G., Qiao, S. Y., and Hou S. S. 2018. Interactions of dietary protein and threonine on growth performance in pekin ducklings from 1 to 14 days of age. Poult Sci 97: 262-266.

Jiang, Y., Uzma, M., Tang, J., Wen, Z. G., Hou, S. S., Huang, W., and Hou S. S. 2016. Effects of dietary protein on threonine requirements of Pekin ducks from hatch to 21 days of age. Anim Feed Sci Tech, 217: 95-99.

Jiang Y., Liao X.D., Xie M., Tang J., Qiao S.Y., Wen Z.G., and Hou S.S. 2018. Dietary threonine supplementation improves hepatic lipid metabolism of Pekin ducks. Anim Prod Sci AN17633.

Jiang Y., Tang J., Xie M., Wen Z.G., Qiao S.Y. and Hou S.S. 2017. Threonine supplementation reduces dietary protein and improves lipid metabolism in Pekin ducks. Brit Poult Sci 58: 687-693.

Kalinowski A., Jr M. E., and Wyatt C. L. 2003. Methionine and cystine requirements of slow- and fast-feathering broiler males from three to six weeks of age. Poult Sci 82:1428-1437.

Kidd M.T., and Kerr B.J. 1996. L-threonine for poultry: A review. J Applied Poult Res 5:358-367.

Kidd M.T., Peebles E.D., Whitmarsh S.K., Yeatman J.B., Wideman R.F. 2001. Growth and immunity of broiler chicks as affected by dietary arginine. Poult Sci. 80: 1535-1542.

Kluge H, Gessner D. K., Herzog E., and Eder K. 2016. Efficacy of DL-methionine hydroxy analogue-free acid in comparison to DL-methionine in growing male white Pekin ducks. Poult Sci 95:590-594.

Labadan J.M.C., Hsu K.N., and Austic R.E. 2001. Lysine and arginine requirements of broiler chickens at two- to three-week intervals to eight weeks of age. Poult Sci. 80: 599-606.

Leclerq B., and de Carvile H. 1977. On the sulfur amino acid requirement of Muscovy ducklings. Arch. Geflgelk. 41:270-272.

Liu Y., Lin X., Zhou X., Wan D., Wang Z., Wu X., and Yin Y. 2017. Effects of dynamic feeding low and high methionine diets on egg quality traits in laying hens. Poult Sci 96: 1459-1465.

Luo M.R., and Xiong Z.Y. 2006. The relationship between lysine and exercise capacity. Sichuan Sports Sci 3:43-46. (In Chinese)

Malinovsky A.V. 2018. Why threonine is an essential amino acid in mammals and birds: studies at the enzyme level. Biochemistry (Moscow) 83: 795-799.

Matsushita K., Takahashi K., and Akiba Y. 2007. Effects of adequate or marginal excess of dietary methionine hydroxyl analogue free acid on growth performance, edible meat yields and inflammatory response in female broiler chickens. J Poult Sci 44:265-272.

Ministry of Agriculture of the People's Republic of China. 2012. Nutrient requirements of meat-type ducks of China. China Agriculture Press, Beijing. (In Chinese)

Rangel-Lugo M., Su C.L., and Austic R.E. 1994. Threonine requirement and threonine imbalance in broiler chickens. Poult Sci 73:670-681.

Ruan D., Fouad A. M., Fan Q., Xia W., Wang S., Chen W., Lin C., Wang Y., Yang L. and Zheng C. 2018. Effects of dietary methionine on productivity, reproductive performance, antioxidant capacity, ovalbumin and antioxidant-related gene expression in laying duck breeders. Brit J Nutr 119, 121–130.

Wang C., Xie M., Huang W., Xie J.J., Tang J., and Hou S.S. 2013a. Arginine requirements of White Pekin ducks from 1 to 21 days of age. Poult Sci. 92:1007-1010.

Wang C., Xie M., Huang W., Yu J.Y., Feng Y.L., and Hou S.S. 2013b. Dietary arginine from 15 to 35 days of age affects feed intake and carcass yield in White Pekin ducks. Anim Prod Sci. 53:1041-1045.

Wang C., Hou S.S., Huang W., Xu T.S., Rong G.H., and Xie M. 2014. Arginine affects appetite via nitric oxide in ducks. Poult Sci. 93:2048-2053.

Wang Y., Hou S., Huang W., Zhao L., Fan H., and Xie M. 2006. Lysine, methionine and tryptophan requirements of Beijing ducklings of 0-2 weeks of age. Agr Sci in China 5: 228-233.

Wen Z.G., Rasolofomanana T.J., Tang J., Jiang Y., Xie M., Yang P.L., and Hou S.S. 2017. Effects of dietary energy and lysine levels on growth performance and carcass yields of Pekin ducks from hatch to 21 days of age. Poult Sci. 96: 3361-3366.

Wu L.Y., Fang Y.J., and Guo X.Y. 2011. Dietary L-arginine supplementation beneficially regulates body fat deposition of meat-type ducks. Brit Poult Sci. 52: 221-226.

Xie M., Hou S. S., Huang W., Zhao L., and Yu J. Y. 2003. Interrelationship between methionine and cystine of early Peking ducklings. Poult Sci 83: 1703-1708.

Xie M., Han X.F., Huang W., and Hou S.S. 2014a. Effects of niacin status on tryptophan requirements of starter white Pekin ducks. Livestock Sci 159: 75-78.

Xie M., Zhang L., Wen Z.G., Tang J., Huang W., and Hou S.S. 2014b. Threonine requirement of White Pekin ducks from hatch to 21 d of age. Brit Poult Sci 55: 553-557.

Xie M., Jiang Y., Tang J., Wen Z.G., Zhang Q., Huang W., and Hou S.S. 2017. Effects of low-protein diets on growth performance and carcass yield of growing White Pekin ducks. Poult Sci 96:1370–1375.

Xie M., Hou S.S., and Huang W. 2006. Methionine requirements of male white Peking ducks from twenty-one to forty-nine days of age. Poult Sci 85:743-746.

Xie M., Hou S.S., and Huang W. and Fan H.P. 2007. Effect of excess methionine and methionine hydroxy analogue on growth performance and plasma homocysteine of growing Pekin ducks. Poult Sci 86:1995-1999.

Xie M. 2003. A Study on the interaction between methionine and cystine of early Pekin ducklings. MS. Thesis, Chinese Academy of Agricultural Science. (In Chinese)

Xie M. 2007. Requirement, bioefficacy, and toxicity of Methionine in Peking ducks. Ph.D. Thesis, Chinese Academy of Agricultural Science. (In Chinese)

Xie M., Guo Y., Zhang T., Hou S., and Huang W. 2009. Lysine requirement of male White Pekin ducklings from seven to twenty-one days of age. Asian-australas. J. Anim. Sci. 22: 1386-1390.

Xue J. J. 2018. Bioefficacy and toxicity of methionine with different optical activity in Pekin ducks. MS. Thesis, Chinese Academy of Agricultural Science. (In Chinese)

Xue J. J., Xie M., Tang J., Huang W., Zhang Q., and Hou S. S. 2018a. Effects of excess DL- and L-methionine on growth performance of starter Pekin ducks. Poult Sci 97:946-950.

Xue J., Xie M., Tang J., Huang W., Zhang Q., and Hou S. 2018b. Effects of excess methionine on growth performance and blood parameters of Peking ducks during 7 to 28 days of age. Chinese J. Anim Nutr 30 :2979-2986. (In Chinese)

Yao K., Yin Y.L., Chu W.Y., Liu Z.Q., Deng D., Li T.J., Huang R.L., Zhang J.H., Tan B.,Wang W.C., and Wu G.Y. 2008. Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. J Nutr 138:867-872.

Zeng Q.F., Cherry P., Doster A., Murdoch R., Adeola O., and Applegate T.J. 2015. Effect of dietary energy and protein content on growth and carcass traits of Pekin ducks. Poult Sci 94: 384-394.

Zeng Q. F., Zhang Q., Chen X., Doster A., Murdoch R., Makagon M., Gardner A. and Applegate T. J. 2015. Effect of dietary methionine content on growth performance, carcass traits, and feather growth of Pekin duck from 15 to 35 days of age. Poult Sci 94: 1592-1599.

Zhang Q., Xu L., Doster A., Murdoch R., Cotter P., Gardner A., and Applegate T.J. 2014. Dietary threonine requirement of Pekin ducks from 15 to 35 days of age based on performance, yield, serum natural antibodies, and intestinal mucin secretion. Poult Sci 93: 1972-1980.

Zhang, Q., Zeng, Q. F., Cotter, P., and Applegate, T. J. 2016. Dietary threonine response of pekin ducks from hatch to 14 d of age based on performance, serology, and intestinal mucin secretion. Poult Sci, 95:1348–1355.

Zhang T., Hou S.S., Xie M., Huang W., Yu J.Y., and Zhao L. 2009. Effects of dietary energy and protein levels on lysine requirement of Beijing ducks from 7 to 21 days of age. Chinese J Anim Nutr. 21(3): 294-300. (In Chinese)

Zhao L, Zhang N. Y., Pan Y. X., Zhu L. Y., Batonon-Alavo D. I., Ma L. B., Khalil M. M., Qi D. S., and Sun L. H. 2018. Efficacy of 2-hydroxy-4-methylthiobutanoic acid compared to DL-Methionine on growth performance, carcass traits, feather growth, and redox status of Cherry Valley ducks. Poult Sci 97:3166-3175.

Zhou Y.F., Liu Y.Q., Wei H.K., and Peng J. 2016. Estimation of the optimum digestible lysine level for Cherry Valley ducks. Poult Sci. 96: 838-843.

Zhu W., Jiang W., and Wu L.Y. 2015. Dietary L-arginine supplement alleviates hepatic heat stress and improves feed conversion ratio of Pekin ducks exposed to high environmental temperature. J Anim Physiol Anim Nutr. 98:1124-1131.

Recent Developments in Practical Aspects of Duck Feeding

P. Garland[1] and N. Lynn[2]

[1]Premier Nutrition, Rugeley, Staffordshire, WS15 1RD, United Kingdom

[2]Cherry Valley Farms Ltd, Laceby, Lincolnshire, DN37 7DP, United Kingdom

Corresponding author: patrick.garland@premiernutrition.co.uk

Abbreviated Title: Practical duck feeding

INTRODUCTION

The global duck market is expanding and there are now some 5 billion ducks grown annually for meat production of which 3.8 billion are Pekin type. The remaining 1.2 billion are either Muscovy or mule birds primarily produced for liver, egg production strains and local breeds for both meat and eggs (Rae, A. personal communication). Despite the growth in the production of ducks it is an industry that is dwarfed by the other poultry sectors and makes up only 8% of poultry meat production globally. This figure is distorted by the fact that in Asia, and China in particular, duck has a higher proportion of meat sales. In China it is estimated that duck is 31% of total poultry consumption, so for the rest of the world duck meat is just 3% of total poultry meat consumption.

The dominance of chicken in the world poultry meat market is a challenge for those involved in practical duck nutrition because there is less published work concerning duck feeding and management. Nutritionists working with ducks have to adapt chicken focussed research and take into account the differences in growth profiles, understand environmental and nutritional responses to achieve the best results. It is clear that, "The duck is not a water-proof chicken" and different nutritional strategies are needed.

EARLY DEVELOPMENT

Genetic selection of Pekin ducks has resulted in impressive improvements in weight gain and feed conversion efficiency in the grower generation. Between 1920 and 2001 the 7 week weights for Pekin ducks strains increased from <2.0kg to >4.0kg and feed conversion ratio (FCR) improved from 3.0 to 2.5 (Cherry and Morris, 2008, p11-12.). The performance for Pekin ducks in terms of weight gain outstrips that of the current broiler chicken by a considerable margin, typical weight at 42 days is given as 3.3 to 3.55 kg (Cherry Valley, Grimaud Freres and Orvia). Early growth is rapid with 7 day weights of 260g (Orvia). The values for broiler chickens are 2.85kg and 185g respectively (Cobb-Vantress and Aviagen), but with greater feed conversion efficiency.

The rapid growth of the duck requires due consideration in the supply of nutrients to meet the requirements for the early development of the skeleton. Duggan *et al.,* (2015) demonstrated that leg bone growth of both an unselected duck strain and modern Pekin genotypes was initially faster than that shown by broiler and layer type chickens. This growth plateaued at about 5 weeks of age whilst the chicken leg bone continued to increase in length. It was postulated by Duggan that this is a reflection of the ancestral behavioural requirements of both in evading predators. The duckling runs to water whilst the chick has short burst of flight to gain access to cover in vegetation. Similarly, duck wing and breast muscle development is slower than in the chicken since neither would have been required for use until close to adulthood when migration takes place.

Mineral nutrition for the broiler chicken in the first diet fed is given as calcium 0.90 to 0.96% and available phosphorus 0.45 to 0.48% by the major breeders (Cobb-Vantress, Aviagen), by the time 35 days has been reached these values are, for calcium 0.76 to 0.81% and for available phosphorus 0.38 to 0.405%. These levels are not appropriate for Pekin duck strains.

Required dietary phosphorus concentrations for pekin ducks were modelled by Rodehutschord (2005) and estimates of 0.52% in the first week falling to 0.29% in the fifth week given along with a calcium:phosphorus ratio of 2:1 being advised. This higher level of available phosphorus is corroborated by Xie *et al* (2016) where growth performance of pekin ducklings was improved by using higher levels of non-phytate phosphorus (nPP), at 0.6% nPP growth was higher in the 0-4 day period and the authors concluded that there may even have been responses beyond this level. Practical experience has demonstrated that ensuring higher levels of available phosphorus are present in diets, without excess calcium, gives benefits in terms of early skeletal development. The increases in genetic potential for growth rate means that early mineral nutrition, during the first 10-14 days, needs to keep pace with this advance. A study by Van Wyhe *et al* (2012) compared ducks of the same strain that were selected for improved growth rates. There were differences in epiphysial ash values when the 1993 genotype was compared with that developed over 17 years and selected in 2010. The contemporary ducks had a significantly lower percentage of ash content at 10 and 14 days, but there were no differences by 18 days.

The application of exogenous phytase is common practice in commercial growing diets and this is supported by published work, for example Adeola (2010 and 2018).

ENVIRONMENT

Stocking density and temperature have marked effects on the growth rates of pekin ducks. This is relevant because, around the world, commercial duck production systems vary tremendously in the housing systems used and the environmental temperatures to which the birds are subjected. It is well established that in the cooler times of year in naturally ventilated or open sided housing growth rates and weights at a given age are increased, driven by higher feed intakes. Where high stocking densities are employed, even in controlled environment housing, there is a marked reduction in live weight. Both of these features are demonstrated by Cherry and Morris (2008). For stocking density a series of studies allowed the development of the equation:-

Live weight% = $97.253+2.123x-0.348x^2$ where x = number of birds placed per m^2 at 17 days

The result is expressed as a percentage of the weight achieved at 46 days when the 17 day stocking density is 4.1 birds per m^2. The equation gives a weight reduction of 8% for birds stocked at 8 per m^2 compared to those stocked at 4.1 per m^2.

For temperature the equation is:-

Live weight (kg) at 48 days = $3.281+0.0102x-0.0009x^2$ where x= temperature (°C ; mean of daily temperatures).

In the example given in the publication a move from 10°C to 28°C results in a loss of 0.4kg live weight at 48 days.

Both the stocking density and ambient temperature effects are driven by feed intake changes and therefore it becomes more challenging for nutritionists to set nutrient levels, particularly for diets fed after 15-20 days, without understanding the basic flock husbandry. It also makes it harder to interpret published trial results regarding responses to individual nutrients if neither temperature nor stocking density information is provided. Additionally, the impact of stocking density is greater in litter based systems than where slatted or wire floors are used, for the latter, slightly higher densities can be tolerated by the birds which will achieve similar weights to litter housed birds at lower density. The possibility of stress effects at high stocking densities is of interest and Liu *et al* (2015) showed that elevated dietary tryptophan at 0.48% could alleviate stress, improve physical performance and give better meat quality compared to 0.18% tryptophan. There were 4 levels of dietary tryptophan employed, 0.18, 0.48, 0.78 and 1.08% and it is possible that the beneficial effect could lie somewhere between the commercially typical 0.18% and the next level tested.

AMINO ACIDS

The impact of nutrient intake changes due to environmental factors influencing feed intake becomes important when considering amino acid nutrition where slight differences in the inclusion level in final feeds can result in significant diet cost changes. Valid observations made by Helmbrecht (2012) are that the amino acid requirement of the chicken cannot be applied to ducks and that there are strain differences in requirements for the different genetic sources of "White Pekin" ducks. It is not economically feasible to develop precise requirement values for each genetic strain, but it would help the commercial nutritionist if the breed source of birds under test is provided.

In the paper by Helmbrecht (2012) a methionine and methionine plus cystine (M+C) dose response study was reported. Under the conditions employed (wire floors) the birds demonstrated that the optimal levels of total methionine and M+C for body weight gain (BWG) during 1-14 days were 0.42% and 0.75% of diet. For the period 15-35 days this increased to 0.55% and 0.84% respectively (for M+C the optimum level was calculated to be > 1.0% but the highest level applied in the study was 0.84%), in the finisher phase the determined values were 0.35% and 0.58%. Zeng *et al* (2015) demonstrated higher methionine requirement for optimal feather growth than for either body weight or feed efficiency in 15 to 35 day old ducks.

This corresponds with the data given by Cherry and Morris (2008) where it is stated that breast feather development commences at about 20 days, primary wing feathers at 24 days and down continues until 28 days. They also report increased length of the sixth primary wing feather when dietary M+C levels were increased from 0.6% to 0.8% in the 14 day onwards period. Therefore, it seems reasonable that there is a higher total sulphur amino acid (TSAA) requirement in the mid growth period. Compared to standard broiler recommendations this increase in TSAA is another example of the marked differences between chickens and ducks.

Published amino acid requirement data is limited and there are, in some cases, marked differences in the values proposed as optimum. Optimum determined values given in Table 1. have been taken as the level at which maximum live weight, breast meat yield or FCR is recorded and is the upper reported figure. However, as outlined by Chen and Applegate (2016) the very flexible nature of the duck allows it to be grown for a multitude of markets dependant on the local consumer demands. These demands may vary in terms of the weight at slaughter, carcass conformation, lean meat and fat content and other carcass traits.

Studies frequently focus on either the starter phase, typically 0-21 days or the finisher phase, typically 21-40 days or more. Whilst the data generated is welcomed, the insight from Helmbrecht (2012) that the nature of growth and feather development is different as the duck ages, suggests a 3 stage approach to feeding in a commercial situation, this is perhaps difficult to replicate in research facilities. In work published by Zeng *et at* (2015b) there were clear responses to increased energy and protein supplied to ducks from 14 until 35 days. The data suggests that rather than using a single diet from 14 until 35 days there would be a commercial advantage to apply a higher plane of nutrition in the 14-28 day period. This would effectively be a 3 stage feeding programme. In the same report the trial diets were used in a digestibility study in Pekin ducks from 15 to 19 days. Valuably, duck amino acid digestibility and apparent metabolizable energy (AME) and nitrogen corrected AME (AMEn) were assessed. The authors concluded that the optimum performance was achieved using a 12.68 MJ/kg, 19% crude protein and 1.21% standardised ileal digestibility (SID) lysine regimen and it was also noted that the growth performance suggested that different diets for the 14-21, 21-28 and 28 to 35 day periods would probably be appropriate.

The work by Zeng *et al* (2015b) tends to be at odds with some of the published results for optimum amino acids levels shown in Table 1. The total lysine in the high energy diets was 1.31% which is considerably higher than any of the values given in Table 1. However the threonine at 0.66% was not dissimilar but at a lower percentage of lysine than reported by Jiang *et al* (2016)

Table 1. Published amino acid levels for optimum performance. All given as total amino acids except Zhou *et al* (2016)

Author	Amino acid	Age (days)	Optimum determined	Sex	Strain
Bons *et al* (2002)	Lysine	0-21	1.17	Male	Pekin
		22-49	1.0		
Evonik (2012)	Lysine	35-49	0.7 (>0.83 for breast yield)	Male	Pekin
Zeng *et al* (2015b)	Lysine (SID)	14-35	1.21	Unknown	Pekin
Zhou *et al* (2016)	Lysine (digestible)	1-14	0.96	Male	Cherry Valley
		15-35	0.76		
Wen *et al* (2017)	Lysine	1-21	0.94 at 11.5 MJ/kg 0.98 at 12.8 MJ/kg	Male	White Pekin
Helmbrecht (2012)	Methionine	1-14	0.42	Unknown	Pekin
		15-35	0.55		
	36-49	0.35			

Xie *et al* (2006)	Methionine	21-49	0.38	Male	White Pekin
Zeng *et al* (2015a)	Methionine	15-35	0.51-0.61	Unknown	Pekin
Jiang *et al* (2016)	Threonine	0-21	0.61 (53% of lysine)	Male	White Pekin
Xie *et al* (2014)	Threonine	0-21	0.67	Male	White Pekin
Jiang *et al* (2017)	Threonine	0-21	0.73	Unknown	Pekin
Timmler and Rodehutscord (2003)	Valine	0-21	0.8	Male	Stolle Steddin Vital
Wang *et al* (2013)	Arginine	0-21	1.16	Male	Z4 White Pekin

Valine was similar to that given by Timmler and Rodehutscord (2003) albeit their value is for 0-21 day birds. It is reasonable to assume from the joint author affiliations and acknowledgements that the birds used in the trial were from a genetic stable where rapid growth and low feed conversion have been selected.

In Cherry and Morris (2008) is it shown that feeding diets with high nutrient density does not give improved performance in unselected strains and therefore application of high energy and amino acid diets should be used only where there are going to be performance gains thereby avoiding nutrient wastage.

Nevertheless, the Zeng *et al* (2015b) report corroborates the commercial practice of using diets with higher amino acid profile in terms of lysine compared to the data reported in Table 1. when selected strains are being used.

Another paper cited in the table where digestible lysine is given (Zhou *et al*, 2016) relies on unpublished amino acid digestibility values determined in the author's own laboratory.

There is no means of assessing if environmental temperature, stocking density or genetics are confounding the differences between the studies given in Table 1. but it is quite possible that this is the reason for differences in reported optimum levels.

Cherry and Morris (2008) report a number of trials showing responses to dietary protein level. Increasing the crude protein in the feed, from 15% to 27%, from 14 days until slaughter at 42-48 days increased live weight and reduced feed conversion ratio in different genotypes and in both temperate and hot climates. It is also demonstrated that feeding a 15.7% compared to a 21% crude protein diet resulted in birds being 3 days behind at 14 days and 2.5 days behind at 42 days in terms of their weight gain. A more precise means of assessing protein quality is of course to consider amino acids. Cherry and Morris (2008) also provide insights into the response of Pekin ducks to available lysine, increasing available lysine daily intakes over 14 to 46 days of age at an average ambient temperature of 16°C increased daily live weight gain. The diets used were 12.6MJ/kg and available lysine levels were 0.7, 0.9 and 1.1%. The greatest gain in growth rate was achieved by increasing from 0.7 to 0.9% lysine, but further improvements in performance were made at the 1.1% lysine level.

There is a need to reduce crude protein levels in diets for all species of farmed livestock to meet environmental concerns whilst maintaining performance levels. The ability to reduce dietary crude protein in pekin ducks from 14 to 35 days through the use of crystalline amino acids has been demonstrated by Xie *et al* (2017). Diets were formulated to achieve digestible amino acid levels based on the work by Kong and Adeola (2013) and crude protein levels from 17.22% down to 13.54% were employed. Weight gain and feed intake were not affected but at the lower level both FCR and abdominal fat increased. To maintain performance and carcass characteristics the authors calculated that 15% protein supplemented with amino acids is the lower protein level required to maintain performance to the 17.22% protein diet level.

The concept of ideal amino acid profiles is well established for other poultry species but is less well applied in designing feeds for ducks.

The profile provided by Evonik (2016) shown in Table 2. gives a basis for working with an ideal amino acid balance.

Whilst this is based on total amino acids and as previously discussed responses to higher levels of individual amino acids, in selected genetics, have been reported it provides a useful guide to the relationships between amino acids and commercial practice has demonstrated its validity.

Table 2. Total amino acid recommendations and calculated ideal profile (Evonik, 2016)

	1-22 Days		23-49 Days	
	% of diet	As % Lysine	% of diet	As % Lysine
Lysine	1.16	100	0.90	100
Methionine	0.42	36	0.42	47
M+C	0.76	66	0.77	86
Threonine	0.84	72	0.66	73
Tryptophan	0.21	18	0.20	22
Arginine	0.94	81	0.76	84
Valine	0.77	66	0.59	66
Histidine	0.42	36	0.32	36

ENERGY

Ducks are adept at adjusting feed intake according to energy requirement and this is closely linked to environmental temperature. The thermal neutral zone for mature pekin ducks is between 8 and 23 °C but will vary according to degree of feather cover (Cherry and Morris, 2008). Feed conversion efficiency will be adversely affected by environmental temperatures outside the thermoneutral zone; at low temperatures the birds will eat more and use energy to keep warm, whereas when they are hot, they will cut their feed intake and need to use energy to increase heat loss. Experience tells us that for birds older than 18 days of age the ideal growing temperature is 15-18 °C.

Selecting an appropriate energy density for feeds will therefore be influenced by environmental issues. Zeng *et al* (2015b) noted performance improvements as a result of feeding a high energy diet of 13.75 MJ/kg and proposed an optimum value of 12.68MJ/kg for 15-35 day old Pekin ducks along with an SID lysine of 1.21%. Several studies reported in Cherry and Morris (2008) demonstrate lower feed intake, higher live weight gain and improved feed conversion ratio as diet energy increased from less than 10.5 MJ/kg to 12.5 MJ/kg. It is also shown that whilst feed intake may be reduced by higher energy there needs to be a corresponding increase in protein level to maintain growth. This is confirmed by the paper from Wen *et al* (2017) who showed that energy level determined feed intake in 1 to 21 day old Pekin ducks and that high energy diets require higher amino acid contents to compensate. Increasing the energy density of the feed can, however, result in the overconsumption of calories because the birds cannot precisely adapt their feed intake at high energy densities. This may improve carcass yield but there is an increase in carcass fat content which may not be desirable to some sectors of the market. Cherry and Morris (2008) analysed data from trials with both slow and rapidly growing ducks where energy and protein levels were increased. Although both energy and protein increases gave gains in weight and conversion efficiency there was no evidence of interaction between energy and protein. Therefore, it seems that local application of diet energies within the range of 12.0 to 13.0 MJ/kg are appropriate, depending on environmental temperature, carcass conformation targets and feed formulation cost constraints.

EVALUATION OF RAW MATERIALS

Kluth and Rodehutscord (2006) compared the digestibility of soya bean meal and rapeseed meal in broilers, turkeys and ducks. Protein digestibility in ducks was found to be lower than that for either broilers or turkeys and all amino acids exhibited lower values with lysine and methionine being significantly lower. The authors conclude that the use of broiler or turkey derived digestibility values are not applicable in the formulation of diets for ducks. Similarly, Kong and Adeola (2013) reached the same conclusion having studied the digestibility of soya bean meal and canola (rapeseed) meal in 3 week old broilers and ducks. Their research showed higher endogenous amino acid losses in ducks, when compared with broiler chickens, although ducks had higher ileal digestibility of cystine and proline.

In the paper by Zeng *et al* (2015b) we have access to the diet formulations used and the predicted digestible amino acid contents based on studies in their own laboratory. From a purely practical perspective this gives an opportunity to compare the values with those used for diet formulation based on our chicken knowledge. Taking two of the Zeng *et al* (2015b) diets and calculating the digestible amino acid content from the results of the amino acid analysis and the digestibility coefficients given, an estimate of the digestible amino acid profile is calculated as shown in Table 3. The determined AMEn values are also included. These are compared to values calculated by applying a standard raw material matrix containing values for amino acids and AMEn for chickens (Premier Atlas 2014, internal document). Whilst the flaws in this approach, including a lack of knowledge of the proximate analysis of the raw materials, should be highlighted, the degree of agreement between the two systems is remarkable for most amino acids. Table 3. shows a clear discrepancy in the values for threonine, with the standard matrix (Premier Atlas 2014, internal document) overestimating the digestible threonine value. This could be addressed on a practical level by increasing the values used for the diets during the formulation process. Energy values show a greater degree of variation and warrant further investigation.

Table 3. After Zeng *et al* (2015b), trial diet digestible amino acids values and determined energy compared to predicted chicken values taken from practical raw material data base using predicted raw material chicken values.

	Zeng et al 12.8MJ/kg, 19.0% protein diet		Zeng et al 13.8MJ/kg, 19.0% protein diet	
	Zeng *et al*	Premier	Zeng *et al*	Premier
Dig Lysine	1.21	1.18	1.19	1.18
Dig Threonine	0.56	0.64	0.54	0.63
Dig Tryptophan	0.19	0.19	0.21	0.20
Dig Isoleucine	0.73	0.70	0.73	0.72
Dig Valine	0.80	0.79	0.79	0.81
Dig Leucine	1.48	1.45	1.49	1.44
Dig Arginine	1.16	1.14	1.17	1.16
AMEn MJ/kg	12.68	12.44	13.75	13.31

The differences in amino acid digestibility between chickens and ducks are also highlighted by Rodehutscord (2015), although this author did state that no clear conclusion could be made on species differences due to inconsistencies in the literature reviewed and that magnitudes of difference depend very much on the raw material evaluated. The available data bases arising from comparable methods is very limited. Herein lies another issue for the practical nutritionist. Mateos (2015) has clearly demonstrated the large differences between recognised raw material databases for energy values which are calculated from analysed parameters for individual ingredients. Until there is greater consistency between data sources it is essential to apply values from single sources rather than select information from multiple providers.

Bearing in mind that the information for digestible amino acids, energy and available phosphorus is reasonably robust for chickens we need to rely on these at present until species specific sources covering a wide range of materials become available. This is where the commercial nutritionist needs to adapt diet specifications for ducks to minimise the possible effects on performance or adverse cost implications.

FEED FORM AND SUPPLY

Feed intake is key to attaining good growth rates in selected strains of ducks and good access to feed and water is, therefore, important. Ducks do not possess a crop and are less able to consume large meals which chickens and turkeys can manage. This is particularly important in high stocking density situations where bird number may inhibit free movement between feed and water sources. Preferably feed should be offered as a pellet with the minimum of dust and fines. Dusty feed will depress feed intake and hence growth rates, lead to higher feed conversion rates due to wastage and increases demand for water to allow washing of fines from the beak.

The authors are not aware of work reporting the grist profile of feed before pelleting for ducks, as has been examined for chickens. However, the feeding of whole grains alongside pelleted feed is not uncommon and is successful, providing that the overall diet matches the nutrient requirements for the particular stage of growth.

SUMMARY

Pekin ducks were originally developed from wild mallards and to this day retain many of the growth and behavioural traits associated with their ancestors. These traits are a significant influence on the nutrition of modern Pekin ducks, making it different to common practice for chickens.

Environmental conditions and stocking density have an important impact on the growth performance of ducks and the conditions in which the birds are raised will affect the design of the diets offered. Early rapid growth requires due recognition in the birds mineral nutrition and the application of exogenous phytases can be useful in achieving appropriate phosphorus and calcium nutrition.

Pekin ducks have specific nutritional requirements for amino acids at particular stages of growth and the adoption of a 3 stage feed programme tailored to these stages will prevent over and under feeding of, particularly, TSAA to ensure good feather growth. This is important to give a good yield of commercially valuable feathers and to provide protection from scratching at high stocking density and insulation in cool growing conditions.

There are differences in raw material and nutrient digestibility between ducks and other poultry species, but until there are broad and robust databases specific to ducks we need to continue using the data for chickens and adapt our diet specifications and raw material usage limits accordingly.

REFERENCES

ADEOLA, O. (2010) Phosphorus equivalency value of an *Escherichia coli* phytase in the diets of White Pekin ducks *Poultry Science* **89**:1199-1206

ADEOLA, O. (2018) Phytase in starter and grower diets of White Pekin ducks *Poultry Science* **97**:592-598

AVIAGEN http://eu.aviagen.com/tech-center/download/13/Ross-308-Broiler-PO-2014-EN.pdf

BONS, A., TIMMLER, R. and JEROCH, H. (2002) Lysine requirement of growing male Pekin ducks. British Poultry Science **43**:677-686

CHEN, X. and APPLEGATE, T.J. (2016) Meat duck nutrition – formulation considerations across genetic and feedstuff resources. **L45** Proceedings of the World Poultry Congress, Beijing, 2016

CHERRY, P. and MORRIS, T. (2008) Domestic Duck Production Science and Practice (CABI) ISBN 0-85199-054-5

CHERRY VALLEY https://www.cherryvalley.co.uk/enquiriesandspecifications

COBB-VANTRESS http://www.cobb-vantress.com/docs/default-source/cobb-500-guides/Cobb500_Broiler_Performance_And_Nutrition_Supplement.pdf

DUGGAN, B.M., HOCKING, P.M., SCHWARTZ, T. and CLEMENTS, D.N. (2015) Differences in hindlimb morphology of ducks and chickens: effects of domestication and selection. *Genetics Selection Evolution* **47**:88

EVONIK (2012) Facts & Figures Poultry No. 15105 Lysine requirement of finishing Pekin Ducks from 35 to 49 days of age.

EVONIK (2016) AMINODat 5.0 Recommendation Report – Pekin Ducks, Evonik Nutrition & Care GmbH

GRIMAUD http://www.grimaudfreres.com/en/products/pekin-ducks/breeders/

HELMBRECHT, A. (2012) Amino acid nutrition in ducks *14th World's Poultry Congress Salvador-Bahia-Brazil*

JIANG, Y., UZMA, M, TANG, J., WEN, Z.G., HOU, S.S., HUANG, W. and Xie, M. (2016) Effects of dietary protein on threonine requirements of Pekin ducks from hatch to 21 days of age. Animal feed Science and Technology **217**: 95-99

JIANG, Y., TANG, J., XIE, M., WEN, Z.G., QIAO, S.Y. and HOU, S.S. (2017) Threonine supplementation reduces dietary protein and improves lipid metabolism in Pekin ducks. British Poultry Science **58,6** :687-693

KLUTH, H. and RODEHUTSCORD, M. (2006) Comparison of Amino Acid Digestibility in broiler Chickens, Turkeys, and Pekin Ducks. Poultry Science **85**:1953-1960

KONG, C. and ADEOLA, O. (2013) Comparative amino acid digestibility for broiler chickens and White Pekin ducks. Poultry Science **92**:2367-2374

LIU, Y., YUAN, J.M., ZHANG, L.S., ZHANG, Y.R., CAI, S.M., YU, J.H. and XIA, Z.F. (2015) Effects of tryptophan supplementation on growth performance, antioxidative activity, and meat quality of ducks inder high stocking density. Poultry Science **94**:1894-1901

MATEOS, G.G., CAMARA, L., SALDANA, B., GUZMAN, P. and LAZARO, R. (2015) Evaluating the energy content of ingredients in poultry diets – an update. pp57-64 Proceeding 20th European Symposium on Poultry Nutrition Prague, Czech Republic

ORVIA http://www.orvia.fr/en/OUR-PRODUCTS/Pekin-Duck/Canard-Pekin-PEKIN-DUCKLING-ST5-HEAVY-Commerciaux

RODEHUTSCORD, M. (2005) Towards an optimal utilisation of phosphorus sources in growing meat ducks. Proceeding of the 15th European Symposium on Poultry Nutrition, Balatonfured, Hungary, pp 84-93

RODEHUTSCORD, M. (2015) Differences in amino acid and phosphorus digestibility between broiler chickens, turkeys and ducks. pp124-129 Proceeding 20th European Symposium on Poultry Nutrition Prague, Czech Republic

TIMMLER, R. and RODEHUTSCORD, M. (2003) Dose-Response Relationships for Valine in the Growing White Pekin Duck. Poultry Science **82**:1755-1762

VAN WYHE, R.C., APPLEGATE, T.J., LILBURN, M.S. and KARCHER, D.M**.** (2012) A comparison of long bone development in historical and contemporary ducks *Poultry Science* **91**:2858-2565

WANG, C., XIE, M., HUANG, W., XIE, J.J., TANG, J. and HOU, S.S. (2013) Arginine requirements of White Pekin ducks from 1-21 days of age. Poultry Science **92**:1007-1010

WEN, Z.G., RASOLOFOMANANA, T.J., TANG, J., JIANG, Y., XIE, M., YANG, P.L. and HOU, S.S. (2017) Effects of dietary energy and lysine levels on growth performance and carcass yields of Pekin ducks from hatch to 21 days. Poultry Science **96**:3361-3366

XIE, M., ZHANG, L., WEN, Z.G., TANG, J., HUANG, W. and HOU, S.S. (2014) Threonine requirement of White Pekin ducks from hatch to 21 d of age. British Poultry Science **55:4** 553-557

XIE, M., JIANG, Y., TANG, J., WEN, Z.G., ZHANG, Q., HUANG, W. and HOU, S.S. (2017) Effects of low-protein diets on growing performance and carcass yield of growing White Pekin ducks Poultry Science **96**:1370-1375

XIE, M,. BORTOLUZZI, C., ANGEL, C.R., LI, W., NOY, Y and APPLEGATE, T.J. (2016) Effects of dietary calcium, nonphytate phosphorus, and phytase on growth performance of Pekin ducks *Poultry Science Association Annual Meeting 2016*

ZENG, Q.F., ZHANG, Q., CHEN, X., DOSTER, A., MURDOCH, R., MAKAGON, M., GARDNER, A. and APPLEGATE, T.J. (2015a) Effect of dietary methionine content on growth performance, carcass traits, and feather growth of Pekin duck from 15-35 days of age. Poultry Science **94**:1592-1599

ZENG, Q.F., CHERRY, P., DOSTER, A., MURDOCH, R., ADEOLA, O. and APPLEGATE, T.J. (2015b) Effect of dietary energy and protein content on growth and carcass traits of Pekin ducks. Poultry Science **94:**384-394

ZHOU, Y.F., LIU, Y.Q., Wei, H.K. and PENG, J. (2016) Estimation of the optimal digestible lysine level for Cherry Valley ducks. Poultry Science **00**:1-6

Premises and possibilities of reducing the use of minerals in turkey nutrition

Krzysztof Kozłowski[1], Jan Jankowski[1], Zenon Zduńczyk[2], Katarzyna Ognik[3]

[1]Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn (Poland), [2]Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn (Poland), [3]Department of Biochemistry and Toxicology, University of Life Science in Lublin (Poland)

ABSTRACT

The premises and possibilities of reducing the use of minerals in poultry nutrition can be viewed from two perspectives: exploiting the full genetic potential of young birds and minimizing the environmental impact of trace mineral excretion. The dietary inclusion levels of macroelements and microelements recommended by poultry breeding companies exceed the nutrient requirements established by academic and research institutions such as the National Research Council (NRC). Over the past decades, increased concentrations of minerals in diets have contributed to increasing the effectiveness of turkey meat production on the one hand, and to deterioration in bird welfare standards and higher excretion levels of unutilized nutrients on the other.

The results of recent experiments indicate that modern hybrid turkeys do not need high-dose supplementation with calcium (Ca), phosphorus (P), sodium (Na), copper (Cu), zinc (Zn) and manganese (Mn), recommended by the European poultry breeding companies.

INTRODUCTION

Modern poultry lines, including turkeys, have the genetic potential for rapid growth and high meat yield. Today, the body weight (BW) of commercial hybrid turkeys at 16 weeks of age is two-fold higher than that achieved by their counterparts in the 1970s. However, accelerated growth and weight gain have been accompanied by undesirable consequences such as an increased incidence of leg weakness (Kestin et al., 1992) and painful skeletal disorders (Duncan et al., 1991; Julian, 1998; Danbury et al., 2000). To minimize those adverse effects, the mineral content of practical poultry diets was increased in the 1990s. The dietary inclusion levels of calcium (Ca) and phosphorus (P) as well as other minerals were increased: copper (Cu) - for maintenance of vascular integrity (Stenzel et al., 2008), zinc (Zn) - for reducing the severity of footpad dermatitis - FPD (Youssef et al., 2012), manganese (Mn) - for preventing perosis (Liu et al., 2015) and sodium (Na) - for stimulating feed intake (Mushtaq et al., 2013). However, the dietary supply of supplemental minerals is often associated with their limited utilization and increased excretion (Bao et al., 2007). According to the European Food Safety Authority (EFSA, 2014), the concentrations of heavy metals, mostly Zn and Cu, exceeded the maximum permissible levels in 90% of turkey feed samples analyzed in 22 European countries. One of the reasons is an increase in the mineral content of plant raw materials, caused by environmental pollution. Excessive dietary supplementation with minerals can lead to their excess accumulation in tissues, toxic effects on cells and antagonistic interactions with other macroelements and microelements, disrupting their absorption and - in consequence - numerous metabolic reactions (Angel, 2007; Dibner et al., 2007; Leeson, 2009; Ognik et al., 2018). An increase in the content of minerals, including biologically active metals, in animal excreta has been well documented (Bao et al., 2007; Inal et al., 2001; Wang et al., 2008; Powers and Angel, 2008; Bratz et al., 2013). In view of the above, the current nutrient requirements and practical nutrition guidelines for fast-growing birds, including turkeys, should be revised and updated. This report summarizes the results of studies investigating the biological effects of reducing the mineral content of turkey diets.

MINERAL REQUIREMENTS OF TURKEYS

Table 1 data show that the content of macroelements and microelements in complete turkey diets is similar in both the latest (1994) and previous (1984) editions of the Nutrient Requirements of Poultry published by the National Research Council (NRC). In comparison with the 1966 edition, the recommended levels of Ca, P and Na were decreased in diets for turkeys at 9 weeks of age and older, the recommended levels of microelements were increased in diets for younger turkeys, and the respective guidelines for older turkeys were developed. The dietary inclusion levels of minerals recommended by turkey breeding companies are much higher (Table 2), but it should be noted that these recommendations focus only on supplemental trace elements added to turkey diets, and do not account for the mineral content of feed ingredients. In the feed industry, dietary mineral levels are similar to those recommended by poultry breeding companies, and substantially exceed the values recommended by research centers. In practical turkey diets, the concentrations of Ca and P are comparable with those recommended in the 1960s (Havenstein et al., 1994). The extensive use of phytase contributes to reducing the amount of limestone and phosphates in turkey diets. The greatest differences between breeding companies and research centers have been found in the Na and microelement requirements of turkeys.

Table 1. Mineral content of turkey diets according to the successive editions of the Nutrient Requirements of Poultry published by the National Research Council (NRC, 1966, 1984 and 1994)

Element	**NRC, 1966**		**NRC, 1984**		**NRC, 1994**	
	1 - 8 wk	**9 wk and older**	**1 - 8 wk**	**9 wk and older**	**1 - 8 wk**	**9 wk and older**
Ca, %	1.2	1.2	1.2 - 1.0	0.85 - 0.55	1.2 - 1.0	0.85 - 0.55
Pp, %	0.8[1)]	0.8[1)]	0.6 - 0.5	0.42 - 0.28	0.6 - 0.5	0.42 - 0.28
Na, %	0.15	0.15	0.17 - 0.15	0.12	0.17 - 0.15	0.12
Mn, mg/kg	55	?	60	60	60	60
Zn, mg/kg	70	?	75 - 65	50 - 40	70 - 65	50 - 40
Cu, mg/kg	6	?	8	8 - 6	8	6

[1)] total phosphorus, including minimum 0.5% from inorganic sources

Table 2. Macroelement content of turkey diets and the addition of supplemental microelements as recommended by breeding companies

Element	**B.U.T. (2013)**		**Hybrid (2019)**	
	1 - 8 wk	**9 wk and older**	**1 - 8 wk**	**9 wk and older**
Ca, %	1.40 - 1.19	1.19 - 0.77	1.40 - 1.25	1.10 - 0.83
Pp, %	0.71 - 0.65	0.59 - 0.39	0.75 - 0.62	0.55 - 0.42
Na, %	0.16	0.16 - 0.14	0.17 - 0.18	0.18 - 0.18
Mn, mg/kg	130 - 110	110 - 110	100	100 - 90
Zn, mg/kg	100	100 - 80	100	100 - 90
Cu, mg/kg	12	12 - 10	15	15 - 12

In Europe, the amounts of microelements added to turkey diets (per kg feed) vary widely, from low values (53-44 mg Mn, 44-35 mg Zn, 13-5 mg Cu) recommended by the German Society of Nutrition Physiology - GfE (2004) to much higher levels (120-80 mg Mn, 90-60 mg Zn, 20-25 mg Cu) recommended by the Polish Nutrient Requirements of Poultry (Smulikowska and Rutkowski, 2005), which however were considerably reduced in the latest edition, to 100-60 mg Mn, 70-5 mg Zn and 10 mg Cu (Smulikowska and Rutkowski, 2018).

BIOLOGICAL RESPONSES OF TURKEYS TO DIFFERENT DIETARY INCLUSION LEVELS OF CALCIUM AND PHOSPHORUS

Among all minerals, Ca and P are added to turkey diets at the highest rates. Due to concerns over skeletal development, the recommendations of research centers and poultry breeding companies regarding the addition of supplemental Ca and P do not differ considerably, in particular in the first stage of rearing. At high inclusion levels of limestone and phosphates, dietary fat content must be increased to balance the concentration of metabolizable energy (ME), which increases feed cost. Moreover, a decrease in the P content of diets provides environmental benefits.

In one of our experiments (Jankowski et al., 2006; Tatara et al., 2011), male Big 6 turkeys raised to 20 weeks of age were fed diets with 4 inclusion levels of Ca and P in each of the 5 four-weeks periods, i.e. 85, 95, 105 and 115% of the values recommended by the NRC. The final BW of turkeys increased with increasing dietary Ca content, and significant differences were found between treatments NRC_{85}, NRC_{95} and NRC_{115}. In 20-week-old turkeys, no significant differences were noted in BW or skeletal system properties such as tibia length, tibia volume, volumetric bone mineral density, mean relative wall thickness, maximum elastic strength, and the ultimate strength of tibia. In 4-week-old and 8-week-old turkeys, selected parameters of the skeletal system deteriorated in group NRC_{85}, whereas mortality and culling rates were comparable in all experimental groups.

In another experiment performed on male Hybrid Converter turkeys (Laurin et al., 2014a), the Ca and P content of diets was reduced by 10% and 15% from 7 and 10 weeks of age, respectively (experimental group E) relative to the levels recommended by Hybrid Turkeys Inc. (2013), which were administered in the control group (C). At 20 weeks of age, the average BW of birds was similar in both groups (group C - 20.46 kg, group E - 20.60 kg), and the value of FRC was 1.13% lower in turkeys fed diets with reduced Ca and P content. Feed cost per kg live weight was 2.23% lower in group E than in group C.

In yet another experiment with male Hybrid Converter turkeys from 7 weeks of age, conducted at the Department of Poultry Science at the University of Warmia and Mazury in Olsztyn (Laurin et al., 2014b), dietary Ca and P levels in the control group (C) were identical to those in group E in the above-described experiment (i.e. reduced by 10 and 15%). In groups E-1, E-2 and E-3, in weeks 7-9, Ca and P content was reduced by 10%, whereas in successive feeding phases, the concentrations of Ca and P were decreased as follows: group E-1 - by 22.5%, group E-2 - by 30%, group E-3 - by 30% (weeks 9-11), 35% (weeks 12-15) and 40% (from week 16) relative to the values recommended by Hybrid Turkeys Inc. (2013). The most important results of this experiment are presented in Table 3.

Table 3. Selected results of a study investigating the possibility of reducing the calcium and phosphorus content of diets fed to turkeys raised to 20 weeks of age

Specification	Groups[1]			
	C	E- 1	E- 2	E-3
Body weight, kg	20.38	20.63	20.28	20.53
FCR, kg/kg	2.57	2.57	2.55	2.56
Mortality, %	4.14	6.45	1.84	4.60
Carcass, %	76.07	76.82	75.98	76.58
Tibia ash, %	64.14	63.43	63.63	62.44
Feed cost per kg live weight, %	100[2]	99.15	98.43	98.56

[1] 7 replicates per group, 31 turkeys per replicate, 217 turkeys per group, a total of 868 turkeys

[2] in a previous experiment, feed cost per kg live weight was 2.23% lower in the experimental group where Ca

and P content was reduced by 10 and 15% relative to the levels recommended by Hybrid Turkeys Inc. (2013), compared with the control group

It can be concluded that the Ca and P content of diets fed to turkeys at 7 weeks of age and older can be safely reduced relative to the levels recommended by Hybrid Turkeys Inc. (2013). The major benefit is a decrease of up to 3.8% in feed cost per kg live weight.

An experiment with an identical design was also performed on female turkeys. It was found that, similarly to males, the experimental factor had no significant effect on the growth performance of birds or carcass quality parameters.

BIOLOGICAL RESPONSES OF TURKEYS TO DIFFERENT DIETARY INCLUSION LEVELS OF SODIUM

Sodium metabolism is regulated by complex hormone-enzyme interactions, which also control circulating blood volume and the concentrations of Na and potassium ions in bodily fluids (Daniels et al., 2007). Increased dietary Na concentrations can reduce endogenous amino acid flow and influence the physiological response to the ingestion of phytic acid by broiler chickens (Cowieson et al., 2011). Increased dietary levels of Na are associated with increased water consumption and ultimately higher litter moisture (Mushtaq et al., 2013). Wet litter is the main reason for FPD in turkeys (Youssef et al., 2012). Excessive Na intake can lead to aortic rupture in turkeys (Shivaprasad et al., 2004), whereas Na deficiency is related with nervousness and aggressive behaviors, particularly in turkey toms.

Among all macroelements, the recommended Na requirements of turkeys vary over the broadest range, in particular in the last stage of rearing. The highest Na intake (0.18%) is recommended by Hybrid Turkeys Inc. (2013), and the lowest (0.09%) by GfE (2003).

Our team have conducted 5 experiments aimed to determine the biological responses of turkeys to various dietary levels and sources of Na, and their results have been summarized in a review article (Zduńczyk and Jankowski, 2014).

Our research demonstrated that an increase in the Na content of turkey diets from 0.07% to approximately 0.22% led to minor changes in the gastrointestinal tract of birds, limited to increased hydration and decreased viscosity of small intestinal digesta, and that it had no significant influence on fermentation processes in the cecum. Dietary Na contributed to the development and severity of FPD in turkeys, despite its non-significant effect on excreta moisture content. Blood electrolyte concentrations were similar in turkeys fed Na-deficient diets (0.08% and less) and diets containing an excess of Na (0.17% - 0.22%). No differences were observed in the immune responses of birds (percentages of T-cell subpopulations). Moderate levels of dietary Na (0.13% and 0.17%) improved tibia mineralization compared with diets containing lower Na levels (0.12% and less). Nonetheless, an increase in Na inclusion rates from 0.17% to 0.22% and from 0.13% to 0.28% did not improve the parameters of tibia growth, mineralization or breaking strength. Turkeys fed a diet with low Na content (below 0.1%) were characterized by a slower growth rate, and the noted trend was much more pronounced in the first 8 weeks than at the end of the rearing period. Dietary Na inclusion levels higher than those recommended by NRC (1994) did not increase the final BW of turkeys. The recommendations of GfE (2004) more adequately reflect the actual Na requirements of turkeys than the recommendations of the NRC (1994) and poultry breeding companies. In diets for younger turkeys, dietary Na levels can be lowered to 0.13%, 0.12% and 0.10% at 1-4, 5-8 and 9-12 weeks of age, respectively, and in older birds the Na content of diets can be maintained at 0.09%. The results of one experiment that examined the physiological effects of Na sources alternative to NaCl in turkey diets do not justify far-reaching conclusions. Further research is needed to confirm the suitability of $NaHCO_3$ and Na_2SO_4 as alternatives to NaCl in turkey nutrition.

BIOLOGICAL RESPONSES OF TURKEYS TO DIFFERENT DIETARY INCLUSION LEVELS OF COPPER, ZINC, AND MANGANESE

According to the current NRC (1994) recommendations, the Cu content of diets for growing turkeys should range from 6 to 8 mg/kg feed. However, the dose of supplemental Cu in practical turkey diets is close to the values recommended by poultry breeding companies or even higher, i.e. 20 mg/kg feed, without accounting for the Cu content of feed ingredients. Our studies reveled that in basal diets, the concentration of Cu supplied only by feed ingredients ranged from 6 to 13 mg/kg (Kozłowski et al., 2018; Jankowski et al., 2019a, d). Thus, the total Cu content of turkey diets, including Cu contained in feed ingredients and a supplemental Cu dose of around 20 mg/kg, may reach 33 mg/kg. According to the EU recommendations, the Cu content of poultry diets should not exceed 25 mg/kg feed (EFSA, 2016). Our findings indicate that a decrease in the dietary inclusion levels of Cu from 20 to 10 and 2 mg/kg increased Cu digestibility (24.7 vs. 32.9 and 46.3%, respectively) and reduced the amount of undigested Cu (21.2 vs. 12.2 and 6.95 mg, respectively). It was also found that the Cu content of breast meat increased with decreasing dietary Cu levels (2.57 vs. 3.18 and 4.33 mg/kg, respectively) (data not published). A decrease in the dietary inclusion levels of Cu from 20 to 10 and 2 mg/kg did not compromise the growth performance of turkeys: at 98 days of age, average BW reached 9.55 vs. 9.49 and 9.50 kg, FCR - 2.19 vs. 2.22 and 2.21 kg/kg, and % mortality - 2.32 vs. 0.46 and 0.93 (Jankowski et al., 2019a). The results of many experiments with broiler chickens, summarized in a review article by Leeson (2009), suggest that a considerable increase in dietary Cu dose, as great as 20-fold their requirements, may deliver health benefits, and stimulate the antioxidant and immune systems. There are a limited number of similar studies involving turkeys. We have found that a decrease in the dose of supplemental Cu from 20 to 10 mg/kg does not compromise the antioxidant status of turkeys, whereas a decrease from 20 to 2 mg/kg could be a risk factor for deterioration in the antioxidant and immune systems (Jankowski et al., 2019a; Otowski et al., 2019).

According to the NRC (1994), Zn requirement is 40 - 70 mg/kg feed in growing turkeys. However, a report published by EFSA (2017) revealed that feed producers in most European countries add nearly 100 mg Zn/kg feed. The concentration of Zn derived from plant-based ingredients ranges from 40 to 50 mg/kg feed (Jankowski et al., 2019b), resulting in the total content of 140 - 150 mg Zn/kg in complete turkey diets. In our study, a decrease in the dose of supplemental Zn from 100 to 50 and 10 mg/kg did not reduce plasma Zn levels (72.6 vs. 76.5 and 70.1 μmol/L, respectively). It should be noted that a decrease in the dietary Zn dose from 100 to 50 mg/kg did not reduce the coefficient of Zn digestibility (47.4 vs. 45.4), whereas a further decrease to 10 mg/kg decreased the intestinal digestibility of Zn (47.4 vs. 35.1). A decrease in the dietary inclusion levels of Zn did not compromise the growth performance of turkeys: at 98 days of age, average BW reached 8.69 vs. 8.78 and 8.59 kg, FCR - 2.44 vs. 2.50 and 2.51 kg/kg, and livability - 99.0 vs. 97.1 and 100% (Jankowski et al., 2019a). A decrease in the dose of supplemental Zn from 100 to 50 mg/kg had no influence on the plasma antioxidant status and lipid profile of birds, whereas a further decrease to 10 mg/kg had a negative effect on the lipid profile (Jankowski et al., 2019a).

According to NRC recommendations (1994), diets for growing turkeys should be supplemented with Mn at 60 mg/kg feed throughout the rearing period. Similarly to Zn, European feed producers add up to 120 mg Mn/kg feed. As a result, the total Mn content of complete turkey diets exceeds the maximum limit set by EFSA (2014) at 150 mg/kg. The results of our studies show that a decrease in the dietary inclusion levels of Mn from 100 to 50 and 10 mg/kg reduced Mn digestibility (22.69 vs. 18.55 and 16.69%, respectively), but did not compromise the growth performance of turkeys: at 98 days of age, average BW reached 9.29 vs. 9.33 and 9.29 kg, FCR - 2.31 vs. 2.31 and 2.34 kg/kg, and livability - 99.3 vs. 100 and 99.0% (Jankowski et al., 2018; Jankowski et al. 2019c). A decrease in the dose of supplemental Mn from 100 to 50 and 10 mg/kg increased Mn concentration in the blood plasma and other tissues of turkeys, including the liver, breast muscles and skin (Jankowski et al., 2019c). Moreover, lower dietary Mn content had a beneficial influence on the immune system of birds and limited lipid peroxidation in breast muscles (Jankowski et al., 2019c). However, in young turkeys (6 weeks of age), a decrease in the dietary Mn dose could promote protein oxidation and DNA methylation (Ognik et al., 2019).

In an experiment summarizing the above studies, where the doses of supplemental Cu, Zn and Mn were reduced to 50 and 10% of the respective values recommended by poultry breeding companies, or completely eliminated from turkey diets (Jankowski et al. 2019d, data not published), the applied dietary treatments had no adverse effects on the growth performance or antioxidant and immune responses of birds. However, our earlier studies (Kozłowski et al., 2018; Jankowski et al., 2019a,b,c,d; Otowski et al., 2019; Ognik et al., 2019) revealed certain metabolic changes, and deterioration in the antioxidant status and immune status of turkeys when the dietary inclusion levels of Cu, Zn and Mn were decreased to 10% of their respective requirements established by poultry breeding companies. We must therefore be cautious when evaluating the possibility of decreasing the dose of supplemental Cu to 2 mg/kg, and the dose of supplemental Zn and Mn to 10 mg/kg, although the growth performance parameters of turkeys were similar in all dietary treatments (Table 4).

It can be concluded that dietary supplementation with 10 mg Cu/kg, 50 mg Zn/kg and 50 mg Mn/kg can be sufficient for achieving satisfactory performance in turkeys, and can contribute to reducing trace mineral excretion to the environment.

Table 4. Effects of decreasing the inclusion levels of Cu, Zn and Mn in turkey diets

Trace element	Addition (mg per kg feed)	Effect	References
Cu	20	control (reference) group	*Kozłowski et al., 2018; Jankowski et al., 2019a; Otowski et al., 2019a - in press*
	10	- no effect on growth performance - ↓ intestinal digestibility of Cu - ↑ amount of undigested Cu - ↑ Cu accumulation in tissues - ↑ Cu concentration in tissues - ↑ antioxidant status in tissues - ↑ lipid and protein metabolism	
	2	- no effect on growth performance - ↑ intestinal digestibility of Cu - ↓ amount of undigested Cu - ↑ Cu accumulation in tissues - ↑ Cu concentration in tissues - ↓ antioxidant status in tissues - ↓ immune status	
Zn	100	control (reference) group	*Jankowski et al., 2019b - in press; Otowski et al., 2019b*
	50	- no effect on growth performance - no effect on antioxidant status	
	10	- no effect on growth performance - ↓ intestinal digestibility of Zn - no effect on antioxidant status - ↓ lipid metabolism	
Mn	100	control (reference) group	*Jankowski et al., 2018; Jankowski et al., 2019c; Ognik et al., 2019*
	50	- no effect on growth performance - ↓ intestinal digestibility of Mn - ↑ Mn concentration in tissues - ↓ intestinal digestibility of Zn - ↓ lipid peroxidation - ↓ cell apoptosis - ↑ immune status	
	10	- no effect on growth performance - ↓ intestinal digestibility of Mn - ↑ Mn concentration in tissues - ↓ lipid peroxidation - ↑ protein and DNA oxidation - ↓ antioxidant status in tissues - ↑ immune status	
Cu, Zn, Mn	20, 100, 100	control (reference) group	*Jankowski et al., 2019d - in press*
	10, 50, 50	- no effect on growth performance - no negative effect on immune and antioxidant status	
	2, 10, 10	- no effect on growth performance - no negative effect on immune and antioxidant status	
	0, 0, 0	- no effect on growth performance - no negative effect on immune and antioxidant status - ↓ lipid peroxidation in tissues	

↑ - increase/improvement ↓ - decrease/deterioration

control group - doses of supplemental Cu, Zn and Mn recommended by Hybrid Turkeys Inc. (2016)

REFERENCES

Angel, R., 2007: Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. J. Appl. Poult. Res. 16, 138-149.

Bao, Y.M., Choct, M., Iji P.A., Bruerton K. 2007: Effect of Organically Complexed Copper, Iron, Manganese, and Zinc on Broiler Performance, Mineral Excretion, and accumulation in Tissues. *J. Appl. Poult. Res.* 16, 448-455.

Bratz, K., Golz, G., Riedel, C., Janczyk, P., Nockler, K., Al-Ter, T. 2013: Inhibitory effect of high-dosage zinc oxide dietary supplementation on *Campylobacter coli* excretion in weaned piglets. J. Appl. Microbiol. 115, 1194-1202.

British United Turkeys (B.U.T.). Aviagen Turkeys, 2013: Management Guidelines for Raising Commercial Turkeys (accessed 20.10.13) https://www.aviagenturkeys.com/media/183481/aviagen commercial guide.pdf.

Cowieson, A.J., Bedford M.R., Ravindran V., Selle P.H. 2011: Increased dietary sodium chloride concentrations reduce endogenous amino acid flow and influence the physiological response to the ingestion of phytic acid by broiler chickens Br. Poult. Sci. 52, 613-624.

Danbury, T.C., Weeks C.A., Chambers J.P., Waterman-Pearson A.E., and Kestin S.C. 2000: Self-selection of the analgesic drug carprofen by lame broiler chickens. Veter. Rec. 146, 307-311.

Daniels, D., Yee D.K., Fluharty S.J., 2007: Angiotensin II receptor signaling. Exp. Physiol. 92, 523-527.

Dibner, J.J., Richards J.D., Kitchell M.L., Quiroz M.A. 2007: Metabolic challenges and early bone development. J. Appl. Poult. Res. 16, 126-137.

Duncan, I.J.H., Beatty E.R., Hocking P.M., Duff S.R.I. 1991: Assessment of pain associated with degenerative hip disorders in adult male turkeys. Res. Vet. Sci. 50, 200-3.

GfE (Gesellschaft für Ernährungsphysiologie, Ausschuss für Bedarfsnormen). 2004: Empfehlungen zur Energie und Nährstoffversorgung der Mastputen. Proc. Soc. Nutr. Physiol. 13, 199-233.

Havenstein, G.B., Ferket, P.R., Scheideler, S.E., Larson, B.T. 1994: Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poult. Sci. 73, 1785-1794.

Inal, F., Coskun, B., Gulsen, N., Kurtoglu, V. 2001: The effects of withdrawal of vitamin and trace mineral supplements from layer diets on egg yield and trace mineral composition. Brit. Poult. Sci. 42, 77-80.

Jankowski, M., Zduńczyk, Z., Jankowski, J., Juśkiewicz, J., Mikulski, D., Wróblewska, M. 2006: Effect of calcium level on properties of gastrointestinal digesta in turkeys in different growth periods. Pol. J. Natur. Sci. 3(Suppl. 1), 403-408.

Jankowski, J., Ognik, K., Stępniowska, A., Zduńczyk, Z., Kozłowski K., 2018: The effect of manganese nanoparticles on apoptosis and on redox and immune status in the tissues of young turkeys. PloS One 13, e0201487.

Jankowski, J., Kozłowski, K., Ognik, K., Zduńczyk, Z., Otowski, K., Sawosz, E., Juśkiewicz, J. 2019a: Redox and immunological status of turkeys fed diets with different levels and sources of copper Ann. Anim. Sci. DOI: 10.2478/aoas-2018-0054

Jankowski, J., Kozłowski, K., Ognik, Otowski, K., Juśkiewicz, J. Zduńczyk, Z. 2019b: The effect of the dietary inclusion levels and sources of zinc on the performance, metabolism, redox and immune status of turkeys. Anim. Feed Sci. Technol. (after review).

Jankowski, J., Ognik, K., Stępniowska, A., Zduńczyk, Z., Kozłowski, K., 2019c: The effect of the source and dose of manganese on the performance, digestibility and distribution of selected minerals, redox, and immune status of turkeys. Poult. Sci. 98, 1379-1389.

Jankowski, J., Ognik, K., Kozłowski, K., Stępniowska, A., Zduńczyk, Z. 2019d: Effect of different levels and sources of dietary copper, zinc and manganese on the performance and immune and redox status of turkeys Animal Feed Sciences and Technology (under review).

Julian, R.J., 1998: Rapid growth problems: ascites and skeletal deformities in broilers. Poult. Sci. 77, 1773-1780.

Kestin, S.C., Knowles T.G., Tinch A.E., Gregory N.G. 1992: Prevalence of leg weakness in broiler chickens and its relationship with genotype. Veter. Rec. 131, 190-194.

Kozłowski, K., Jankowski, J., Otowski, K., Zduńczyk, Z., Ognik K., 2018: Metabolic parameters in young turkeys fed diets with different inclusion levels of copper nanoparticles. Pol. J. Vet. Sci. 21, 245-253.

Laurin, D.E., Jankowski, J., Kozłowski, K. 2014a: Mieszanki o obniżonej zawartości wapnia i fosforu w żywieniu indyków rzeźnych Hybrid Converter. Indyk Polski. 1, 10-12.

Laurin, D.E., Jankowski, J., Kozłowski, K. 2014b: Żywienie indorów rzeźnych Hybrid Converter dietami o zmniejszonej zawartości wapnia i fosforu, doświadczenie 2. Indyk Polski. 4, 24-26.

Leeson, S., 2009: Cooper metabolism and dietary needs. World's Poult. Sci. J. 65, 353-366.

Liu, R., Jin C., Wang Z., Wang Z., Wang J., Wang L. 2015: Effects of manganese deficiency on the microstructure of proximal tibia and OPG/RANKL gene expression in chicks. Vet. Res. Commun. 39, 31-37.

Mushtaq, T., Pasha T.N., Mushtaq M.M.H., Parvin R. 2013: Electrolyte, dietary electrolytes balance and salts in broilers: an updates review on growth performance, water intake and litter quality. World's Poult. Sci. J. 69, 789-802.

National Research Council (NRC). 1994: Nutrient Requirements of Poultry. Edn. 9th rev. National Academy Press, Washington, D.C. USA.

Ognik, K., Sembratowicz, I., Cholewińska, E., Jankowski, J., Kozłowski, K., Juśkiewicz, J., Zduńczyk Z., 2018: The effect of administration of copper nanoparticles to chickens in their drinking water on the immune and antioxidant status of the blood. Anim. Sci. J. 89, 579-588.

Ognik, K., Kozłowski, K., Stępniowska, A., Szlązak, R., Tutaj, K., Zduńczyk, Z., Jankowski J., 2019: The effect of manganese nanoparticles on performance, redox reactions and epigenetic changes in turkey tissues. Animal DOI:10.1017/S1751731118002653

Otowski, K., Kozłowski, K., Ognik, K. 2019: Growth rate, metabolic parameters and carcass quality in turkeys fed diets with different inclusion levels and sources of supplemental copper. J. Anim. Feed Sci. (under review).

Powers, W. and R. Angel. 2008: A review of the capacity for nutritional strategies to address environmental challenges in poultry production. Poult. Sci. 87, 1929-1938.

Shivaprasad, H.L., Crespo, R., Puschner, B. 2004: Coronary artery rupture in male commercial turkeys. Avian Pathol. 33(2), 226-232.

Smulikowska, S., Rutkowski, A. 2005: Nutrient Requirements of Poultry. Feeding Recommendations and Nutritive Value of Feed. The Kielanowski Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences, Jabłonna, Poland. (in Polish).

Stenzel, T., Tykałowski B., Koncicki A. 2008: Cardiovascular system diseases in turkeys. Pol. J. Vet. Sci. 11, 245-250.

Tatara, M.R., Krupski, W., Jankowski, M., Zduńczyk, Z., Jankowski, J., Studziński, T. 2011: Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkeys. Brit. Poult. Sci. 52, 718-729.

Wang, Z., Cerrate, S., Yan, F., Sacakli, P., Waldroup, P.W. 2008: Comparison of different concentrations of inorganic trace minerals in broiler diets on live performance and mineral excretion. Inter. J. Poult. Sci. 7, 625-629.

Youssef, I.M.I., Beineke A., Rohn K., Kamphues J. 2012: Influences of increased levels of biotin, zinc or mannan-oligosaccharides in the diet on foot pad dermatitis in growing turkeys housed on dry and wet litter. J. Anim. Physiol. Anim. Nutr. 96, 747-761.

Zduńczyk, Z., Jankowski, J. 2014: The effects of inclusion level and source of sodium in diets for growing turkeys. A review. J. Anim. Feed Sci. 23, 3-12.

Updates in Broiler Breeder Trace Mineral Nutrition

Dr. Leonardo Bretas Linares

Zinpro Corporation, USA.

Broiler breeder research is becoming rare, as lengthy trials, and thus increased costs for facility usage, decreases the affordability of these studies. Accomplishing trace mineral (TM) research can be even more difficult, as TMs are not always given the high-priority they deserve in the industry. Compared to commercial layers, the degree of importance that TMs play in the diet of broiler breeders is phenomenal, as basal requirements for these minerals are higher. Supplementing with TMs can assist birds in achieving optimal performance in terms of egg production, immune response, fertility rates, and ability of the broiler to supply embryos with the quantity of nutrients needed to increase successful hatching rates, thus influencing their lifetime performance as broilers.

Genetic breeding companies, such as Aviagen (2016) and Cobb-Vantress (2018) release their nutrient recommendations as a reference (Table 1), typically based on current published research and from internal studies. These recommendations, however, do not account for specific environmental or challenge conditions, progeny performance, legislated maximum inclusion rates, or any opportunity to improve performance through the use of so-called organic trace minerals (OTM), which will be addressed in the last portion of this review.

Table 1. Suggested Trace Mineral Supplementation Levels for Broiler Breeder Layer Diets, ppm

Mineral	**Rostagno, 2017**	**Aviagen, 2016**	**Cobb-Vantress, 2018**	**NRC, 1994**
	ppm			
Cu	9.97	10	10 to 15	-
Fe	45.8	50	40 to 55	60
Zn	65.1	110	110	45
Mn	70.0	120	120	20
Se	0.30	0.30	0.30	0.06
I	1.0	2.0	2.0	0.1

More recently, the broiler breeder research group of Federal University of Rio Grande do Sul, Brazil, has determined broiler breeder requirements for Zn, Cu, and Fe in diets supplemented with inorganic mineral sources (Mayer et al., 2018; Berwanger et al., 2018; Taschetto et al., 2017). Mayer et al. (2018) evaluated several breeder performance variables in 33- to 44-week-old Cobb 500 broiler breeders, using zinc sulfate ($ZnSO_4$) in a basal diet with 19 to 171 ppm Zn, under quadratic polynomial (QP), broken line quadratic (BLQ), and exponential asymptotic (EA) models. The average Zn requirement estimates obtained by EA and BLQ models in the Mayer et al. (2018) study was 72.28 ppm or 11.1 mg/hen/d. Considering the variables of total eggs and total settable eggs produced per hen, Zn requirements were estimated as 75.7 and 64.7 ppm (R^2=0.42), and 56.5 and 41.5 ppm (R^2=0.57), respectively, for BLQ and EA models. The variables of alkaline phosphatase and eggshell percentage led to Zn requirement estimates of 161.8 and 124.9 ppm (R^2=0.17 and 0.19), and 126.1 and 122.4 ppm (R^2=0.91), respectively, using QP and BLQ models. The maximum response of Zn deposition in the egg yolk, for periods of 37 to 40 and 41 to 44 weeks-of-age (woa) were 71.0 and 78.1 (R^2=0.76), and 64.5 and 59.6 ppm (R^2=0.79), respectively, using BLQ and EA models. Eggshell breaking strength had Zn requirements estimated at 68.0 and 96.7 ppm (R^2=0.23), whereas eggshell palisade layer and thickness were maximized with 67.9 and 67.9 ppm (R^2=0.78), and 67.7 and 64.4 ppm (R^2=0.66), respectively, for BLQ and EA models. Kidd et al. (1992) did not find any source or level differences when broiler breeders were supplemented with 72 and 152 ppm Zn from Zn oxide (ZnO) or Zn-Methionine (Zn-Met), respectively, on egg and chick weight, egg production, livability, and humoral or cellular response of breeders, which supports the current work of Mayer et al. (2018). However, Kidd et al. (1993) did observe an improvement in the immune response of broiler breeder offspring supplemented with either ZnO or Zn-Met.

Following the same approach as above, Berwanger et al. (2018) studied the requirements for copper, using copper sulfate ($CuSO_4$) in a basal diet with 2.67 to 20.2 ppm Cu. The average of all variables studied revealed the Cu requirement for Cobb 500 broiler breeders, from 25 to 44 woa, as estimated to be 12.5 ppm. Copper requirements for hen day egg production and total settable eggs per hen were 6.2, 7.3, and 12.9 ppm (R^2=0.43, 0.43, and 0.41), and 8.1, 9.0, and 13.4 ppm (R^2=0.13, 0.13, and 0.11), respectively, using EA, BLQ, and QP models. The QP model was the only one with a fit for total eggs per hen of 13.1 ppm Cu (R^2=0.21), as a requirement. Maximum responses for egg yolk Cu deposition, eggshell membrane thickness, and eggshell mammillary layer were obtained with 15.0, 16.3, and 15.7 ppm (R^2=0.11, 0.11, and 0.10), 7.3, 7.8, and 14.0 ppm (R^2=0.16, 0.16, and 0.11), and 10.6, 10.1, and 14.4 ppm Cu (R^2=0.68, 0.70, and 0.66), using EA, BLQ, and QP models, respectively.

From the same aforementioned group in Brazil, Taschetto et al. (2017) estimated the iron requirements for 47- to 70-week-old Cobb 500 broiler breeders, using an Fe-deficient mash diet (basal Fe content 24.6 ppm), supplemented with different levels of $FeSO_4$ to reach up to 148 ppm Fe in the complete feed. The average of all Fe requirement estimates obtained in the Taschetto study was 106 ppm Fe, whereas averaged values for BL, QP, and EA models were 107, 113, and 97 ppm Fe, respectively, with high R^2. When breaking down the variables studied, requirements obtained for total hatching eggs and egg yolk Fe content were 96.8, 76.4, and 97.9 ppm (R^2=0.88, 0.89, and 0.87), and 97.1, 89.3, and 111.0 ppm Fe (R^2=0.90, 0.88, and 0.82), using QP, BL, and EA models, respectively. Requirements for hen hematocrit and hemoglobin were 130.6 and 120.0 ppm (R^2=0.82 and 0.80), and 135.0 and 133.8 ppm Fe (R^2=0.86 and 0.85), using QP and BL models, respectively. Lastly, requirements for chick hematocrit and hemoglobin were 122.6 and 125.0 ppm (R^2=0.85 and 0.82), and 128.4 and 95.0 ppm Fe (R^2=0.89 and 0.86), using QP and BL models, respectively.

The utilization of OTM sources in broiler breeder diets has become common practice, notable for effects not only in breeder performance, but also in benefits for progeny (Favero et al., 2013b). There are many different forms of OTMs, and it can be difficult to differentiate between them. If the organic ligand does not remain bound through low pH conditions of the stomach, then biologically, the animal will encounter a mineral that is no different from an inorganic source. Amino acids (AA) have a much higher binding affinity that other ligands and have also been shown to be absorbed through AA transporters. Additionally, not all AAs are the same, especially glycine, which has no side chain and is known to have poor absorption via AA transporters.

From Richards and Packard (1996), the yolk has the highest concentration of TM in the egg, and that is very important in supporting early and late embryonic development, especially skeletal formation and ossification. Yair and Uni (2011) showed how TMs (Zn, Cu, Fe, Mn) and phosphorus (P) quickly disappear from the yolk sack, after day 11 of incubation, with almost 100% of their utilization occurring at hatching. The residual yolk sack is then mostly depleted of TM reserves for the first days of life, and chicks need to have quick access to mineral-supplemented feed, so they can perform normally.

Favero et al. (2013a) fed 22- to 68-week-old Cobb 500 broiler breeders a supplemented Control diet with: 1) 100, 100, and 10 ppm Zn, Mn, and Cu sulfates, respectively; 2) a partial replacement (ISO) of 40, 40, and 7 ppm Zn, Mn, and Cu, respectively, from AA-complexed (AAC); and lastly 3) the same concentration of AAC from treatment 2 on-top of the Control (OT). No differences were detected for egg production, however, feeding ISO or OT diets compared to the Control, significantly increased eggshell weight and thickness, and decreased early embryonic mortality, improving hatchability of fertile eggs (HOF). As a continuation of this study, Favero et al. (2013b) fed the ISO treatment to broiler breeders and, compared with the Control, showed increased Zn content of the yolk and albumen blend. At E14, ISO and OT treatments were observed to have a trend for increased tibia calcification, and on E18 birds fed ISO and OT had 2% thicker tibias, compared with the Control, regardless of hen age.

Moraes et al. (2011) fed Cobb 500 breeders from 56 to 62 woa with either inorganic (ITM) or OTM as DL-2-hydroxy-4-(methylthio) butanoic acid (*HMTBA*), to replace 30% of Zn, Mn, and Cu from a total 125, 125, and 25 ppm, respectively. It was reported that progeny of breeders fed diets with 100% ITM had faster development of gizzard/proventriculus and gut. Using the same treatments as Moraes et al. (2011), Oviedo-Rondon et al. (2013) observed that partial replacement of ITM with HMTBA positively affected humoral immune response of progeny, according to levels of serum antibody against Newcastle disease virus, noted after vaccination. Torres (2013) reported that maternal TM supplementation with Zn, Mn, and Cu as HMTBA at reduced levels (50, 60, and 10 ppm Zn, Mn, and Cu, respectively) supported egg yolk mineral deposition levels and bone development to the same extent as did industry levels of Control ITM (100, 120, and 10 ppm Zn, Mn, and Cu, respectively, from sulfates). Increasing TM levels to 140 ppm Zn, 160 ppm Mn, and 30 ppm Cu, as either a combination of Control ITM plus 40 ppm Zn, 40 ppm Mn and 20 ppm Cu as OTM, or Control ITM plus 40 ppm Zn, 40 ppm Mn and 20 ppm Cu as sulfates had no effect on egg mineral content or embryonic bone traits relative to the Control ITM.

Li et al. (2015) fed 45-week-old Ross 308 broiler breeders a Zn-deficient (20 ppm) or adequate (50 or 300 ppm) diet supplemented with either $ZnSO_4$ or Zn-HMTBA for 6 weeks. Authors reported that the maternal diet of 300 ppm OTM Zn increased mucin-2 abundance and secretory Immunoglobulin A production in the jejunum of progeny, and decreased abundance of TNF-α and anti-inflammatory protein A20, that suppress ubiquitin-dependent nuclear factor κb (NFκb) signaling.

Sun et al. (2012) reported that Zn, Mn, and Cu as HMTBA (50, 60, and 8 ppm, respectively), plus 0.3 ppm Se yeast added broiler breeder diets in place of ITMs had a positive effect on lipid metabolism. The authors observed decreasing plasma cholesterol and triglycerides, increasing yolk triglycerides via increased high-density lipoprotein (HDL), along with decreased low-density lipoprotein (LDL) and very low-density lipid protein (VLDL) cholesterol in plasma, protecting breeders from lipid peroxidation. Additionally, a reduction in yolk Cu deposition was noted, which can negatively affect embryonic mortality rate, especially in the first 3 to 4 days of incubation (Savage, 1968).

Virden et al. (2003) fed broiler breeders supplemental Zn and Mn from AAC (average analyzed content in the diets were 95 and 83 ppm, respectively) in varying combinations with sulfate TMs, and reported that AAC-containing diets improved early livability of progeny from eggs of 37-week-old hens, without affecting growth or carcass characteristics.

Two studies evaluated the use of the same source of OTM (Mn-proteinate) compared to $MnSO_4$, in Arbor Acres broiler breeders. Xie et al. (2014) supplemented up to 240 ppm Mn and reported an inhibitory effect of OTM on activation of gonadotropin-releasing hormone-I (GnRH-I), where the ITM supplementation doubled GnRH-I expression. Zhou et al. (2015) supplemented up to 120 ppm Mn and observed increasing levels of Mn in bones, only where OTM was used.

A study verifying the effects of dietary Fe on Cobb 500 broiler breeders and the performance of their progeny was accomplished by Ebbing et al. (2019). These birds were fed diets supplemented with either 40 ppm Fe sulfate, 40 ppm Fe as AAC , or 40 ppm Fe as AAC on-top of 50 ppm $FeSO_4$. Breeder performance was evaluated from 40 to 67 woa, and eggs were separated by coloring as either pale or dark. Hens fed Fe-AAC produced more eggs per hen housed and had improved fertility, compared to hens fed $FeSO_4$. Broilers chicks originating from dark eggs and hens fed diets containing Fe-AAC had improved growth performance along with carcass and breast weight.

When collecting semen samples of Indigenous broiler breeder roosters, Aghaei et al. (2010) observed a positive correlation between Cu and Zn concentrations of seminal plasma and spermatozoa motility.

Selenium is an essential trace mineral important in maintaining antioxidant defenses and preventing tissue damage. This mineral is an integral part of the glutathione peroxidase (GPX) enzyme, which helps to control free radical levels produced during normal physiological activity. Selenium supplementation is important, not only to protect the broiler breeder against oxidative stress, but also for maintaining the antioxidant system of the developing embryo. The metabolism of a chicken embryo is almost exclusively fueled by lipids, mainly in the form of triglycerides. As the embryo mobilizes polyunsaturated fatty acids for tissue growth, there is an increase in free radical production, which can cause damage to cells. However, the chicken embryo protects itself by activating several enzymes including the Cu-, Zn-, and Mn-superoxide enzyme, Fe-catalase, and Se-GPX. These enzymes complement rather than substitute one another, which demonstrates the importance of feeding hens the source of trace mineral known to increase egg mineral storage, and thus guarantee the embryo antioxidant function is working well. Organic Se sources, such as seleno-methionine (Se-Met) and selenium yeast, increase Se egg storage to a greater extent than inorganic Se. For instance, supplementing 0.3 ppm zinc-selenium methionine (Zn-Se-Met) to broiler breeders increased egg Se storage by 84%, compared to hens fed similar levels as sodium selenite (Reis et al., 2009). When Se was supplemented at 0.4 ppm, increased chick body weight at hatch was observed (Urso et al., 2015).

In yet another study, embryos from hens feed a basal diet low in Se and supplemented with 0.15 ppm selenium-methionine had the greatest glutathione peroxidase type 1 activity in the liver, which was followed by a parallel decrease in free radical production and lipid peroxidation, when compared to embryos fed 0.15 ppm Se as sodium selenite. It is important to note these embryos were heat-stressed at 17 days of incubation (39.5 °C for 6 hours; Xiao et al., 2016). Therefore, during physiological stress, when there is an increased antioxidant demand, Se storage in the egg plays an important role. When egg stores of Se are built-up prior to a challenge, the animal can mobilize stored Se, allowing them to balance supply and demand necessary for antioxidant defense. This would not work in an optimal manner if a Se storage was low.

CONCLUSION

From this review, it can be concluded that the majority of recommendations suggested by genetic breeding companies are in line with the experimental data we have presented, with the exception of Zn and Fe. The values presented in Table 2 below are average requirements in the complete diet suggested by researchers, and recommendations from breeder companies in Table 1 are supplemental levels. It is likely then that supplemental levels of Zn can be reduced or fit to specific challenge situations. The concentration of Fe in poultry diets and its bioavailability varies with source and concentration (source examples include plant-based ingredients, animal byproducts, or mineral supplements, suggesting recommended levels of supplemental Fe can vary widely). Sources of limestone and phosphates contain Fe in ferrous and ferric forms (Abbaspour et al., 2014) and, according to Henry and Miller (1995), Fe oxide has a relative bioavailability of 10%, when compared to Fe sulfate monohydrate (set at 100%) in poultry. There are also opportunities to set chromium (Cr) requirements for broiler breeders, as little research has been done in this area.

Table 2. Average Trace Mineral Requirement in Complete Broiler Breeder Layer Diets and Suggested Supplemented Levels, ppm

Mineral (ppm)	Average in Complete Diet	Supplemented		
		Non-challenge	Challenge	Breeder Lines
Cu	12.5	10	12	10 to 15
Fe	106	40	50	40 to 55
Zn	72.3	70	90	110
Mn	-	-	-	120
Se	-	-	-	0.3
I	-	-	-	2
Cr	-	-	-	-

The utilization of OTM can also affect recommended levels of TMs, where their bio-efficacy might allow nutritionists to lower supplemental levels, potentially reducing trace mineral excretion. It is worth mentioning that, according to the data, not all products considered OTMs will show a performance response in broiler breeders, and many of these products lack published data.

It can be concluded that OTMs, especially those AAC types, have consistently presented results in broiler breeders, with relation to immune response and performance, production of hatching eggs, improvement in eggshell quality and hatchability, reduced early and late embryo mortality, and improved chick quality though skeletal development and mineralization, enhancing broiler performance from early on, until market weight is attained.

REFERENCES:

Abbaspour, N., R. Hurrell, and R. Kelishad. 2014. Review on iron and its importance for human health. J. Res. Med. Sci. 19:164-174.

Aghaei, A., S. Tabatabaei, and M. Nasari. 2010. The correlation between mineral concentration of seminal plasma and spermatozoa motility in rooster. J. Anim. Vet. Adv. 9:1476-1478.

Aviagen. 2016. Ross 308 Parent Stock: Nutrition Specifications. Aviagen, Huntsville, AL, USA.

Berwanger, E., S.L. Vieira, C.R. Angel, L. Kindlein, A.N. Mayer, M.A. Ebbing, and M. Lopes. 2017. Copper requirements of broiler breeder hens. Poult. Sci. 96:3920-3927.

Cobb-Vantress. 2018. Cobb 500 Breeder Management Supplement. Cobb-Vantress, Siloam Springs, AR, USA.

Ebbing, M.A., S.L. Vieira, C. Stefanello, E. Berwanger, A. Mayer, D.D. Maria, and A.K. Fireman. 2019. An investigation on iron sources fed to broiler breeder hens and the corresponding color of laid eggshells on the performance of the resulting progeny. J. Appl. Poult. Res. 28:184-193.

Favero, A., S.L. Vieira, C.R. Angel, F. Bess, H.S. Cemin, and T.L. Ward. 2013a. Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. J. Appl. Poult. Res. 22:80-91.

Favero, A., S. Vieira, C. Angel, A. Bos-Mikich, N. Lothhammer, D. Taschetto, R. Cruz, and T. Ward. 2013b. Development of bone in chick embryos from Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid complexed sources. Poult. Sci. 92:402-411.

Henry, P., and E. Miller. 1995. Iron bioavailability. Pages 169–199. in Bioavailability of nutrients for animals: Amino acids, minerals and vitamins. C. B. Ammerman, D. H. Baker, and A. J. Lewis. (eds) Academic Press, New York, USA.

Kidd. M., N.B. Anthony, Z. Johnson, and S. Lee. 1992. Effect of zinc methionine supplementation on the performance of mature broiler breeders. J. Appl. Poult. Res. 1:207-211.

Kidd. M.T., N.B. Anthony, L.A. Newberry, and S.R. Lee. 1993. Effect of supplemental zinc in either a corn-soybean or a milo and corn-soybean meal diet on the performance of young broiler breeders and their progeny. Poult. Sci. 72:1492-1499.

Li, C., S. Guo, J. Gao, Y. Guo, E. Du, Z. Lv, and B. Zhang. 2015. Maternal high-zinc diet attenuates intestinal inflammation by reducing DNA methylation and elevating H3K9 acetylation in the A20 promoter of offspring chicks. J. Nutr. Biochem. 26:173-183.

Mayer, A.N., S.L. Vieira, E. Berwanger, C. R. Angel, L. Kindlein, I. Franca, and T.L. Noetzold. 2019. Zinc requirements of broiler breeder hens. Poult. Sci. 98:1288-1301.

Moraes, V.M.B., E.O. Oviedo-Rondon, N.S.M. Leandro, M.J. Wineland, R.D. Malheiros, and P. Eusebio-

Balcazar. 2011. Broiler breeder trace mineral nutrition and feeding practices on embryo progeny development. Avian Biol. Res. 4:122-132.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th rev. Ed. National Academy Press, Washington, DC, USA.

Oviedo-Rondon, E.O., N.M. Leandro, R. Ali, M. Koci, V. Moraes, and J. Brake. 2013. Broiler breeder feeding programs and trace minerals on maternal antibody transfer and broiler humoral immune response. J. Appl. Poult. Res. 22:499-510.

Reis, R.N., S.L. Vieira, P.C. Nascimento, J.E. Pena, R. Barros, and C.A. Torres. 2009. Selenium contents of eggs from broiler breeders supplemented with sodium selenite or zinc-L-selenium-methionine. J. Appl. Poult. Res. 18:151-157.

Richards, M.P., and M.J. Packard. 1996. Mineral metabolism in avian embryos. Poult. and Avian

Biol. Rev. 7:143-161.

Rostagno, H.S., L.F.T. Albino, M.I. Hannas, J.L. Donzele, N.K. Sakomura, F.G. Perazzo, A. Saraiva, M.L.T. Abreu, P.B. Rodrigues, R.F. Oliveira, S.L.T. Barreto, and C.O. Brito. 2017. Brazilian Tables for Poultry and Swine Feedstuff Composition and Nutritional Requirements, 4th Ed. Vicosa, MG, Brazil. UFV/DZO., pp. 436.

Savage, J.E. 1968. Trace minerals and avian reproduction. Fed. Proc. 27:927-931.

Sun, Q., Y. Guo, S. Ma, J. Yuan, S. An., and J. Li. 2012. Dietary mineral sources altered lipid and antioxidant profiles in broiler breeders and posthatch growth of their offsprings. Biol. Trace Elem. Res. 145:318-324.

Taschetto, D., S.L. Vieira, C.R. Angel, C. Stefanello, L. Kindlein, M.A. Ebbing, and C.T. Simoes. 2017. Iron requirements of broiler breeder hens. Poult Sci. 96:3920-3927.

Torres, C.A. 2013. Influence of maternal flock age, mineral nutrition and incubation temperature on bone development of embryos and chicks. PhD Diss. Univ. Alberta, Edmonton.

Urso, U.R., F. Dahlke, A. Maiorka, I.J. Bueno, A.F. Schneider, D. Surek, and C. Rocha. 2015. Vitamin E and selenium in broiler breeder diets: Effect on live performance, hatching process, and chick quality. Poult. Sci. 94:976-983.

Virden, W.S., J.B. Yeatman, S.J. Barber, C.D. Zumwalt, T.L. Ward, A.B. Johnson, and M.T. Kidd. 2003. Hen Mineral Nutrition Impacts Progeny Livability. J. Appl. Poult. Res. 12:411-416.

Xiao, X., D. Yuan, Y.X. Wang, and X.A. Zhan. 2016. The protective effects of different sources of maternal selenium on oxidative stressed chick embryo liver. Biol. Trace Elem. Res. 172:201-208.

Xie, J., C. Tian, Y. Zhu, L. Zhang, L. Lu, and X. Luo X. 2014. Effects of inorganic and organic manganese supplementation on gonadotropin-releasing hormone-I and follicle-stimulating hormone expression and reproductive performance of broiler breeder hens. Poult Sci. 93:959-969.

Yair, R., and Z. Uni. 2011. Content and uptake of minerals in the yolk of broiler embryos during incubation and effect of nutrient enrichment. Poult. Sci. 90:1523-1531.

From yolk to intestine: nutrient transfer for better-quality chicks

Zehava Uni

Department of Animal Science, Faculty of Agriculture, The Hebrew University, Israel, zehava.uni@mail.huji.ac.il

ABSTRACT

Decreased age to market of commercial poultry increased the importance of incubation period. While extensive knowledge exists for poultry nutrition related to the growing period, the available information on nutritional processes and requirements of the broiler embryo during the incubation period is minor. It is clear by now that knowledge about processes and organs involved in digestion and absorption of egg nutrients during incubation period and at the pre-post hatch period is essential since proper broiler embryo development leads to optimal hatchability and better hatchlings' quality.

Yolk nutrients are consumed by the embryo in a differential pattern along incubation period; The yolk sac tissue (YST) expresses genes, usually expressed by intestinal cells, and are related to CHO, peptides, amino acid, and minerals digestion and absorption from the yolk contents to the embryo's blood circulation. Furthermore, the yolk is a major gluconeogenic and glycogenic extra-embryonic organ that stores CHO for provision to the embryo in the days prior to hatch, and to the hatchlings until first feed will arrive.

Chick embryos derive most essential nutrients from transported yolk molecules during incubation, and then following hatch, convert to intestinal absorption processes. Research shows that digestion and absorption of egg nutrients during incubation period and in the pre-post hatch period is accomplished by a) the yolk sac tissue (YST) which functioned from first week of incubation until 2-3 days' post hatch, and by b) the small intestine which develops and 'become operated' 3-4 days before hatch. Towards the end of incubation, the small intestinal undergoes rapid maturation processes in order to transit from uptake of egg nutrients to external feeding

INTRODUCTION

Different from mammalians, the growth and development of the avian embryo and hatchling are dependent upon the nutrient deposits in the fertile egg. While the fertile egg has a defined nutrient composition, our knowledge regarding the rate and mechanism of absorption of these nutrients by the embryo is partial. Furthermore, the mechanisms of egg nutrients digestion and absorption during incubation have not been fully investigated.

Based on the increased importance of incubation period to the chick quality there is a need to answer the following questions: What are the amounts of fat, protein, carbohydrates (CHO), water, mineral and vitamins that are stored in the different egg compartments (albumen, yolk, shell) and are 'served' as nutrients stores for the chicken embryo? What is the consumption rate of these nutrients by the embryo during incubation? Is it subjected to environmental (i.e. incubation) conditions? What are the mechanisms by which nutrients are digested, absorbed and delivered from the egg compartment to the embryo blood stream? Do broiler breeder eggs have sufficient nutrients for normal embryo development, especially in light of the increased metabolic rate in today's commercial embryos? …and how does a nutritional deficiency status in the egg affect the development of the embryo and the post-hatch chick? Can a nutritional deficiency be compensated by changing the broiler breeder diet? Which tissues are responsible for egg nutrients uptake? Can we affect their functionality for better digestion and absorption?

Answering these questions will help us to better understand the process of egg nutrients absorption, as well as clarify what are the nutritional needs of the developing embryo throughout incubation and at post hatch period. The current presentation will answer some of these questions

NUTRIENTS RESOURCES AVAILABLE TO THE CHICKEN EMBRYO DURING INCUBATION

The fertile laid egg provides a closed environment with the nutrient requirements needed for the developing embryo. Unlike mammals, where a continuous transport of nutrients takes place from the mother to the embryo through the placenta, the chick embryo is separated from the hen, and derives all of its nutrients during incubation from the contents of the egg that were deposited by the hen- the albumen and the yolk (Moran, 2007;

Sheng and Foley, 2012). The ratio between these two egg compartments, their integrative relationship during incubation, and their nutrient composition, have a major role in the optimal development of the embryo. The albumen compartment represents about 65 to 75% of the egg's total content, and consists of approximately 88% water and 12% protein. The yolk compartment consists of approximately 50% water, 15% protein, less than 1% carbohydrates, and 33% fat, which is only found in the yolk (Romannof, 1960; Shenstone, 1968). The composition of the fertile egg is not fixed and is greatly dependent upon egg weight, genetic strain, and hen age (O'Sullivan et al., 1991; Vieira and Moran, 1998; Peebles et al 2000; Suk and Park 2001; Yadgary et al 2010).

FAT

The utilization of fat from the yolk sac (YS) by the chick embryo provides the main energy source for tissue development during the last week of incubation (Noble and Cocchi, 1990). In agreement with previous studies (Noble and Ogunyemi,1989; Noble and Cocchi, 1990), we found that only a small amount (less than 10%) of the yolk fat had been absorbed by the chick embryo by E13, whereas from E15 to E21 more than 50% of YS fat was utilized for the growing nutritional needs of embryo (Yadgary et al 2010; 2013). We also observed that between E15 and E19, long chain poly-unsaturated fatty acids (22:6, 20:4) were more rapidly utilized from the YS as compared to all other examined fatty acids (Yadgary et al 2014). To elucidate these results, we examined possible processes and mechanisms by which YS lipids are transferred to the embryo: the absorption and transport of lipoproteins and free fatty acids by apical membrane receptors and the lysosomal digestion of lipids and their emulsification by bile acids. Analysis by high throughput gene expression analyses revealed that a vast variety of lipoprotein receptors mediate lipoprotein entrance to yolk sac membrane (YSM) epithelial cells (Yadgary et al 2014). Among these receptors is VLDLR, which has a binding domain that recognizes VLDL and vitellogenin. VLDLR has been previously suggested to have a major role in YSM lipoprotein uptake (Hermann et al., 2000), however we showed that VLDLR had relatively low levels of expression in the YSM. On the other hand LRP2, a receptor for a range of lipoproteins (Kozyraki and Gofflot, 2007), had the highest expression levels among all lipoprotein receptors with an increase from E13 to E17 and a decrease from E17 to E21. Expression levels of several other lipoprotein receptors in YSM epithelia (LRP1, 5, 6, 12) imply that LRP2 does not have an exclusive role in YSM lipoproteins uptake.

To elucidate the differential utilization of yolk fatty acids during incubation, we examined the expression of genes responsible for absorption and transport of free fatty acids and found that different membrane fatty acids transporters were expressed in YSM epithelia, as well as cytosolic fatty acids binding proteins that also play a role in uptake of long chain poly-unsaturated fatty acids. Among them was FABP5 which exhibited a substantial up-regulation between E15 and E21 (Yadgary et al 2014). Inside YSM epithelial cells, lipoproteins are transferred to lysosomes for digestion (Murray et al., 1999; Powel et al., 2004; Bauer et al., 2013). The utilization of YS fat is affected, among other, by the digestive capacity of YSM lysosomes, nevertheless YSM lysosomal pathways have not been fully described yet. In our study we found that several lysosomal digestion related genes were among the 50 most highly expressed genes in YSM epithelial cells. Prosaposin, which facilitates the catabolism of glycosphingolipids in lysosomes, was the highest expressed gene from E15 to E21. In addition, many lysosomal proteases such as cathepsin A and B that can digest lipoproteins, were highly expressed in the YSM (Yadgary et al 2014).

These results provide support to the hypothesis that yolk lipoproteins are hydrolyzed in the lysosomes of YSM epithelial cells into free fatty acids, partial glycerides and glycerol. We attained further support for this hypothesis from the activity of the digestive enzyme lipase in the YSM (Yadgary et al 2013): Total YS lipase activity relative to fat content (U per g YSM fat) increased from E15 to E21. Lipase activity in the YSM is probably associated with the lysosomal digestion of lipoproteins. Furthermore, we found that this digestion is apparently aided by bile acids that could serve as an emulsifier of lipids in the lysosomes of the YSM (Yadgary et al 2013 ;2014).

To our knowledge, our studies are the first to verify the hypothesis that bile acids are found in the YSM of the chick embryo. This may be the reason for the typical green color of the bile which is observed in the yolks of 18-19 E embryos. It had been previously suggested that the origin of YS bile may be from the transfer of intestinal bile into the yolk sac through the yolk stalk (Surai and Speake, 1998 Speake and Teale, 2006), however our gene expression analyses point out for the first time that the epithelial cells of the YSM synthesize bile as well as enzymes involved in the conjugation of bile acids.

Lipids are transported from the epithelial cells of the YSM to the embryo as newly formed lipoproteins (Kanai et al., 1996; Murray et al., 1999; Powel et al., 2004). An integral part of these new lipoproteins are apolipoproteins that serve as structural proteins and cell surface receptors. Apolipoproteins genes (apoA1, A2 A4, B, C3,) were among the most highly abundant in the YSM. Our results reflect the intensive re-synthesis of lipoproteins in the YSM and indicate that both HDL and LDL are produced in the YSM and are then secreted to the embryonic circulation as previously suggested by Kanai et al. (1996).

PROTEIN

The fertile egg yolk, before setting in the incubator, contains approximately 15% protein (Romannof, 1967; Shenstone, 1968). The uptake of protein from the yolk sac is difficult to evaluate because in agreement with previous studies. In agreement with Sugimoto et al 1989;1999) we observed that protein and water pass from the egg albumen compartment and from the amniotic sac to the yolk sac during incubation (Yadgary et al 2010). Nevertheless, we attempted to estimate the YSM's absorptive capacity of yolk protein by examining mechanisms of yolk protein break-down and peptide or amino acid uptake by the YSM.

Lipoproteins are made up of approximately 10% protein and therefore their uptake provides one transport mechanism for proteins through the YSM. However, other than endocytosis of lipoproteins, no study has elucidated the molecular and cellular mechanisms in the YSM that are involved in nutrient uptake by the epithelial cells. We therefore examined expression patterns of nutrient transporters and digestive enzymes such as the digestive enzyme APN (aminopeptidase N), the di- and tripeptide transporter PepT1, and the cationic amino acid transporter CAT1 (Yadgary et al 2011). The expression patterns of APN and PepT1 genes, which were up-regulated between E13 and E17 and down-regulated between E17 and E21, may be indicative of a change in the YSM's capacity to digest and transport yolk peptides during the second half of incubation. Expression pattern of the CAT1 transporter differed from that of APN and PepT1. This difference may be associated with the transporters' locations in the absorptive cells: CAT1 at the basolateral membrane, and APN and PepT1 at the brush-border membrane.

CARBOHYDRATES

Towards the end of incubation, the high demand for energy to support the dramatic physiological changes of the hatching process drives the embryo towards catabolism of glucose. Previous studies determined that during chick embryonic development, the liver performs most of the essential processes involved in carbohydrates metabolism and in the supply of glucose to muscle tissues (Christensen VL 2001; De Oliveira et al 2008). However based on our publications (Yadgary et al 2010; 2014), which examined the role of the YSM in the supply of carbohydrates to the chick embryo during incubation, it can be concluded that YSM has the major role in producing glycogen stores for the embryo during incubation period.

Our studies showed that the levels of glucose in the yolk increased between E11 and E19, and then decreased until E21. Only trace amounts of glycogen were found in the yolk of the fresh laid fertile egg (E0), whereas on E11 glycogen amount in the yolk was 25 mg, and then increased by ten-fold between E13 and E19. Between E19 to E21, glycogen levels decreased by 100 mg. Liver carbohydrates amount had a similar pattern compared to the YS, yet the liver had significantly lower levels of glycogen (20-50 folds lower) because of its substantially smaller size as compared to the YS (Yadgary et al 2012).

The increased levels of glucose and glycogen in the YS, which far exceeded the amount of carbohydrates in the yolk on E0, indicated that glucose is synthesized in the YS during embryonic development. To elucidate whether glucose is synthesized in the YS, gene expression of enzymes involved in gluconeogenesis and glycogenesis were characterized in the YSM (Yadgary and Uni 2013). Expression levels of genes coding for enzymes (FBP1, PEPCK, G6PC2, PEPCK-C) that are exclusive to the gluconeogenesis process indicated that glucose is synthesized in the YSM from amino acids and glycerol. In addition, the YSM tissue was clearly seen to express the key enzyme involved in glycogen synthesis—GYS2.

Thus, it can be postulated that during the last week of chick embryonic development, the YS serves as the main organ synthesizing glucose and storing it in the form of glycogen, with a quantity 20 times greater than in the liver on E19. Between E19 and E21, YS glycogen is intensively broken down to glucose 6-phosphate which is probably further converted by G6PC2 to free glucose. The free glucose might be subsequently released into the blood to be used in the days prior to hatch and during the hatching process as an additional source of energy (to β-oxidation of fatty acids), and as the major source of energy in the form of carbohydrates. Our hypothesis is that the major part of glycogen-derived glucose during incubation is released from the YS into the blood for delivery to the embryonic tissues.

DIGESTION AND ABSORPTION ORGANS IN THE CHICKEN EMBRYO AND HATCHLING

Generally, the transport of nutrients into a digestive tissue is necessary for nutrient assimilation and growth. Several proteases act to break down proteins and peptides into free amino acids and small peptides, which are then transported into cells by amino acid and peptide transporters. Similarly, carbohydrates are broken down by carbohydrates to yield monosaccharides, which are then transported into cells by monosaccharide transporters.

Research have shown that during incubation the YST and the embryonic intestine serve these functions (Speier et al 2012 ;Yadgary et al 2014): The digestive enzymes (APN, SI), amino acid and peptide transporters (B0AT, EAAT3, CAT1, PepT1), and monosaccharide transporters (SGLT1, GLUT5) showed a diverse array of developmental gene expression profiles in the YST and in the neonatal small intestine. All genes had a tissue by embryonic day interaction. Although the YST and embryonic small intestine are a continuous entity throughout development, their roles in nutritional assimilation differ significantly as they have different expression pattern along incubation

Towards the end of incubation both amnion and albumen are totally consumed by the embryo, but not all of the Yolk sac nutrients. Two days before hatch, from E19, the residual yolk begins to be internalized into the embryo's body cavity, and at hatch, it constitutes about 10 to 20% of the chick's body weight, providing the hatchling with immediate nourishment until exogenous feed is given in the brooder house. After hatch the yolk sac supplies nutrients not only to the blood circulation through the YST, but also directly from the Yolk sac to the small intestine through the yolk stalk (Esteban et al., 199l; Noy et al., 1996).

Initially, the yolk of the fertile egg is surrounded by the vitelline membrane, which had been laid down during follicular maturation (Speake et al., 1998 a,b). In the initial phase of incubation, the vitelline membrane gradually disintegrates and the YSM, which develops rapidly from the hind gut of the embryo, replaces it. The advancing edge of the YST contains a nonvascular region of simple columnar endodermal cells which increases in area and spreads over the surface of the yolk, concomitant with the formation of an outer supportive ectodermal layer (Mobbs and McMillan, 1981; Moran, 2007). The increase in endodermal surface area leads to the formation of villus-like folds into which mesodermal cells migrate, between the ectodermal and endodermal cells, and differentiate into blood vessels, blood cells and connective tissue (Romannof 1960; Sheng and Foley, 2012).

By E10 the entire yolk is surrounded and vascularized, and the endodermal epithelial cells (EEC) of the YST have developed into columnar epithelial absorptive cells in close contact with the yolk (Noble and Cocchi, 1990). These endodermal epithelium cells function as the absorptive and digestive cells of the YST and are the mediators for the transport of nutrients from the yolk content to the developing embryo (Nakazawa et al 2011). Nutrients are absorbed into the EEC from the yolk content , undergo different metabolic, digestive and re-assembly processes, and are then secreted to the blood circulation of the embryo (Mobbs and McMillan, 1981; Kanai et al., 1996).

In the second half of incubation, after all extraembryonic tissues had been fully developed and the YST had totally surrounded the yolk, the chick embryo begins its rapid growth. The transition from early embryogenesis to the second phase of incubation is characterized by prompt utilization of nutrients by the embryo. Yadgary et al 2013 found that total area of the YST and its absorptive area increased up to E17 and then start to decreased, probably by apoptosis, until the end of the first week post hatch. These dynamics in YST dimensions is relate to the increasing demand for yolk nutrients from mid-incubation period and later on, the assimilation of the yolk sac into the embryo's body cavity toward hatch.

Bauer et al (2012) who investigated the mechanism for acquisition of chick yolk sac function by receptor expression, lipoprotein secretion, and lipid droplet metabolism in endodermal epithelial cells (EECs) cells concluded that vascularization and gain in function of the developing yolk sac are coordinated processes and that the changes in gene expression during differentiation of EECs are critical for yolk sac maturation. His findings suggest a differentiation process that orchestrates the vascularization of the developing YS with the induction of yolk uptake and lipoprotein secretion by EECs to ensure embryo nutrition.

Form the beginning of the third week of incubation (at E15-E16), the chick embryos start transition to intestinal absorption of nutrients. The uptake of nutrients is mediated by a variety of membrane-bound transporter proteins. Speier et al 2012 determined the expression profiles of nutrient transporters and digestive enzymes during incubation in the yolk sac tissue (YST) and embryonic intestine of egg-laying (Leghorn) and meat-producing (Cobb) chickens. The examined transporters included the peptide transporter PepT1, the glutamate/

aspartate (EAAT3), cationic (CAT-1) and neutral (B0AT) amino acid transporters, and the fructose (GLUT5) and glucose (SGLT1) transporters. Digestive enzymes included aminopeptidase N (APN) and sucrase-isomaltase (SI). Expression of these genes were assessed by real-time PCR using the absolute quantification method embryonic day (E) 11, 13, 15, 17, 19, 20, and day of hatch (DOH) and intestine at E15, 17, 19, 20, and DOH. The findings were that The PepT1 and APN gene expression in the YSM increased until E15 and then decreased until DOH, whereas expression in the intestine increased from E15 to DOH. The B0AT showed a similar pattern in both YSM and intestine with greatest expression in the YSM occurring at E17/E19.

The CAT1 and GLUT5 genes showed decreased expression in the YSM and increased expression in the intestine until E17/E19 and then a decrease until DOH. Expression of SGLT1 and EAAT3 showed increased gene expression over time in both the intestine and YST. Expression of SI showed little to no gene expression in the YSM, whereas the intestine exhibited consistently high levels of gene expression. In YST and intestine, SI expression was greater in Leghorn than Cobb, whereas CAT1 and GLUT5 expression was greater in Cobb than Leghorn. Expression of the APN, CAT1, and SI genes was greater in embryos from young flocks than old flocks in YSM and intestine.

These results demonstrate that the YST expresses many of the digestive enzymes and nutrient transporters typically associated with the intestine and that these genes show tissue- and development-specific patterns of expression.

The analysis of expression levels of these genes in neonatal intestine compared with YSM elucidate their role in embryonic chick nutrient assimilation and growth and show potential differences in growth rate and nutrient utilization of embryos from different genetic lines and laying flock ages.

FACTORS AFFECTING YOLK UTILIZATION

The transport of nutrients from the yolk to the chick embryo is controlled by the embryonic demands for nutrients. These demands are dictated by the genetic potential of the embryo to build tissues. Yolk sac nutrient utilization by the chick embryo is also affected by environmental factors and nutritional factors.

Inadequate environmental conditions in the incubator (for example: above optimal temperature or CO_2 level) have deleterious effects on physiological processes and biochemical reactions in embryonic and extraembryonic tissues of the chick, thus reducing YS utilization and embryo development (Burnham et al., 2001, Piestun et al., 2009; maatjens et al 2014; Reijrink et al., 2010); affects glucose metabolism (Molenaar, et al 2010), decrease growth performance and increase incidence of ascites in broilers (Molenaar, et al 2011)

A variety of nutritional factors contribute to the dietary composition of the egg and thus influence utilization of nutrients from the egg. These include egg size, breeder age, maternal nutrition, and in-ovo nutrient supplementation. Eggs from young and old breeder hens differ in their composition- older hens lay larger eggs with a larger yolk to albumen ratio, compared to young breeder hens. Hence, eggs from breeder hens of different ages provide an excellent model to study the effect of egg composition on YS utilization. Several studies have investigated YS fat utilization by embryos from young and old breeder hens (Noble et al., 1986; O'Sullivan et al., 1991; Burnham et al., 2001; Yalcin et al., 2008), and some of which have associated higher late mortality of young hens' embryos to a reduced transfer of yolk lipids.

Embryos and chicks of different strains have a different genetic potential and body composition, and therefore may have different nutritional demands to build body tissues. However, the effect of the nutritional demands of the embryo on yolk utilization has hardly been studied, due to the difficulty to separate this factor from other factors affecting YS utilization such as egg weight, egg content, incubation conditions, genetic strain, breeder hen diet and age.

THE IMPORTANCE OF EMBRYO NUTRITION DURING INCUBATION PERIOD

The avian embryo is dependent upon the nutrient deposits in the fertile egg (Romanoff, 1960). These nutrient resources and their utilization by the chick embryo have a direct effect on embryonic development, hatchability, hatchlings quality and subsequently on chicken performance. Thus, the nutrition of the developing chick embryo affects not only hatchling weight and vitality but also the broiler's weight at market age. The importance of an adequate nutrition of the embryo during the 21 days of incubation increased in the last decades as broiler chickens reach market age earlier every year due to the intense artificial selection for body weight performed in the breeding companies for the past 6 decades. Consequently, the 21-day incubation period of the embryonic chick makes up approximately one-third of the broiler growing period. Therefore, data on embryo nutrient utilization, the developmental profiles and capacities of the physiological, cellular and molecular mechanisms that digest and absorb nutrients during incubation need to be further investigate. In light of the increased metabolic rate recorded in today's commercial embryos, research related to the nutrition of the broiler embryo can clarify questions which have implications to both poultry broiler breeder nutrition and to hatchery management.

REFERENCES

BAUER, R., PLIESCHNIG, J. A., FINKES, T., RIEGLER, B., HERMANN, M., & SCHNEIDER, W. J. (2013). The developing chicken yolk sac acquires nutrient transport competence by an orchestrated differentiation process of its endodermal epithelial cells. Journal of Biological Chemistry, *288*(2), 1088-1098.

BURNHAM, M. R., PEEBLES, E. D., GARDNER, C. W., BRAKE, J., BRUZUAL, J. J., & GERARD, P. D. (2001). Effects of incubator humidity and hen age on yolk composition in broiler hatching eggs from young breeders. Poultry science,80(10), 1444-1450.

DE OLIVEIRA, J. E.; UNI, Z.; FERKET, P. R. (2008) Important metabolic pathways in poultry embryos prior to hatch. *World's* Poultry Science, 64: 488-499.

ESTEBAN, S., J. RAYO, M. MORENO, M. SASTRE, R. RIAL, AND J. TUR. (1991). A role played by the vitelline diverticulum in the yolk sac resorption in young post hatched chickens. J. Comp. Physiol. B 160:645–648.

HERMANN, M., M. G. MAHON, K. A. LINDSTEDT, J. NIMPF, AND W. J. SCHNEIDER. (2000). Lipoprotein receptors in extraembryonic tissues of the chicken. J. Biol. Chem. 275:16837–16844.

KANAI, M., T. SOJI, E. SUGAWARA, N. WATARI, H. OGUCHI, M. MUSTASABRA, AND D. C. HERBET. (1996). Participation of endodermal epithelial cells in the synthesis of plasma LDL and HDL in the chick yolk sac. Microsc. Res. Technol. 35:340–348.

KOZYRAKI, R., & GOFFLOT, F. (2007). Multiligand endocytosis and congenital defects: roles of cubilin, megalin and amnionless. Current pharmaceutical design, *13*(29), 3038-3046.

MAATJENS, C. M., REIJRINK, I. A. M., MOLENAAR, R., VAN DER POL, C. W., KEMP, B., & VAN DEN BRAND, H. (2014). Temperature and CO2 during the hatching phase. I. Effects on chick quality and organ development. Poultry science,93(3), 645-654.

MOBBS, I. G., & MCMILLAN, D. B. (1981). Transport across endodermal cells of the chick yolk sac during early stages of development. American Journal of Anatomy, 160(3), 285-308

MORAN JR., E. T. (2007). Nutrition of the developing embryo and hatchling. Poultry Science. 86:1043–1049.

MOLENAAR, R., HULET, R., MEIJERHOF, R., MAATJENS, C. M., KEMP, B., & VAN DEN BRAND, H. (2011). High eggshell temperatures during incubation decrease growth performance and increase the incidence of ascites in broiler chickens. Poultry science, 90(3), 624-632.

MOLENAAR, R., MEIJERHOF, R., VAN DEN ANKER, I., HEETKAMP, M. J. W., VAN DEN BORNE, J. J. G. C., KEMP, B., & VAN DEN BRAND, H. (2010). Effect of eggshell temperature and oxygen concentration on survival rate and nutrient utilization in chicken embryos. Poultry science, *89*(9). s. Poultry Science, 2001, 80.12: 1729-1735.

MURRAY, A. M., R. DENIS, AND B. K. SPEAKE. (1999). Acyltransferase activities in the yolk sac membrane of the chick embryo. Lipids 34:929–935.

NAKAZAWA, F., ALEV, C., JAKT, L. M., & SHENG, G. (2011). Yolk sac endoderm is the major source of serum proteins and lipids and is involved in the regulation of vascular integrity in early chick development. Developmental Dynamics,240(8), 2002-2010.

NOBLE, R. C., & OGUNYEMI, D. (1989). Lipid changes in the residual yolk and liver of the chick immediately after hatching. Neonatology, 56(4), 228-236.

NOBLE, R. C., AND M. COCCHI.(1990). Lipid metabolism and the neonatal chicken. Prog. Lipid Res. 29:107–140

NOY, Y.; UNI, Z.; SKLAN, D. (1996) Routes of yolk utilization in the newly-hatched chick. British poultry science, 37(5): 987-996.

O'SULLIVAN, N. P., DUNNINGTON, E. A., & SIEGEL, P. B. (1991). Relationships among age of dam, egg components, embryo lipid transfer, and hatchability of broiler breeder eggs. Poultry Science, 70(10), 2180-2185.

PEEBLES, E. D., ZUMWALT, C. D., DOYLE, S. M., GERARD, P. D., LATOUR, M. A., BOYLE, C. R., & SMITH, T. W. (2000). Effects of breeder age and dietary fat source and level on broiler hatching egg characteristics. Poultry science, 79(5), 698-704.

PIESTUN, Y., HAREL, M., BARAK, M., YAHAV, S., & HALEVY, O. (2009). Thermal manipulations in late-term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. Journal of Applied Physiology, *106*(1), 233-240.

POWELL, K. A., E. A. DEANS, AND B. K. SPEAKE. (2004). Fatty acid esterification in the yolk sac membrane of the avian embryo. J. Comp. Physiol. B 174:163–168.

REIJRINK, I. A. M., BERGHMANS, D., MEIJERHOF, R., KEMP, B., & VAN DEN BRAND, H. (2010). Influence of egg storage time and pre incubation warming profile on embryonic development, hatchability, and chick quality. Poultry science, 89(6), 1225-1238.

ROMANOFF, A. L. (1960). The Avian Embryo. Structural and Functional Development. MacMillan Co., New York,

ROMANOFF, A. L. (1967). Biochemistry of the Avian Embryo. Macmillan, New York.

SHENG, G., & FOLEY, A. C. (2012). Diversification and conservation of the extraembryonic tissues in mediating nutrient uptake during amniote development. Annals of the New York Academy of Sciences, 1271(1), 97-103

SHENSTONE, F. S. (1968). The gross composition, chemistry and physico-chemical basis of organization of the yolk and white. Egg Quality: A Study of the Hen's Egg. TC Carter, ed. Oliver and Boyd, Edinburgh, UK, 26-58.

SPEAKE, B. K., A. M. MURRAY, AND R. C. NOBLE. (1998a). Transport and transformations of yolk lipids during development of the avian embryo. Prog. Lipid Res. 37:1–32.

SPEAKE, B. K., R. C. NOBLE, AND A. M. B. MURRAY. (1998b). The utilization of yolk lipids by the chick embryo. World's Poult. Sci. J. 54:319–334.

SPEAKE, B.K., AND M.C. TEALE. (2006). Metabolism and functions of omega-3 fatty acids during development of the avian embryo. Omega 3 Fatty Acid Research.

SPEIER, J.S., L. YADGARY, Z. UNI AND E. A. WONG. (2012). Gene expression of nutrient transporters and digestive enzymes in the yolk sac membrane and small intestine of the developing embryonic chick. Poultry Science. 91:1941–1949.

SUGIMOTO, Y. , A. SAITO, T. KUSAKABE, K. HORI, AND K. KOGA. (1989). Flow of egg white albumin into the yolk sac during embryogenesis. Biochim. Biophys. Acta992:400–403

SUGIMOTO, Y. , S. SANUKI, S. OHSAKO, Y. HIGASHIMOTO, M. KONDO, J. KURAWAKI, H. R. IBRAHIM, T. AOKI, T. KSUSAKABE, AND K. KOGA. (1999). Ovalbumin in developing chicken eggs migrates from egg white to embryonic organs while changing its conformation and thermal stability. J. Biol. Chem. 274:11030–11037.

SUK, Y. O., & PARK, C. (2001). Effect of breed and age of hens on the yolk to albumen ratio in two different genetic stocks. *Poultry Science*, *80*(7), 855-858.

SURAI P.F., B.K. SPEAKE. (1998). Selective excretion of yolk-derived tocotrienols into the bile of the chick embryo. Biochem. Physiol. B Comp. Biochem. 121: 393–396.

UNI, Z., L. YADGARY, AND R. YAIR. (2012). Nutritional limitations during poultry embryonic development. The journal of Applied Poultry. Research. 21: 175–184.

VIEIRA, S. L., & MORAN, E. T. (1998). Eggs and chicks from broiler breeders of extremely different age. The Journal of Applied Poultry Research, 7(4), 372-376.

YADGARY, L., A. CAHANER, O. KEDAR, AND Z. UNI. (2010). Yolk sac nutrient composition and fat uptake in late-term embryos in eggs from young and old broiler breeder hens. Poultry. Science. 89:2441–2452.

YADGARY, L., AND Z. UNI. (2012). Yolk sac carbohydrate levels and gene expression of key gluconeogenic and glycogenic enzymes, as compared to the liver, during chick embryonic development. Poult. Sci. 91:444–453.

YADGARY, L., R. YAIR, AND Z. UNI. (2011). The chick embryo yolk sac membrane expresses nutrient transporter and digestive enzyme genes. Poult. Sci. 90:410–416.

YADGARY, L., WONG, E. A., & UNI, Z. (2014). Temporal transcriptome analysis of the chicken embryo yolk sac. BMC genomics, *15*(1), 690.

YALÇIN, S., ÇABUK, M., BRUGGEMAN, V., BABACANOĞLU, E., BUYSE, J., DECUYPERE, E., & SIEGEL, P. B. (2008). Acclimation to heat during incubation. 1. Embryonic morphological traits, blood biochemistry, and hatching performance. *Poultry science*, *87*(6), 1219-1228.

Effects of corn kernel hardness, grain drying temperature, and amylase in diets for broiler chickens

E. O. Oviedo-Rondón[1], H. A. Córdova-Noboa[1], A. Ortiz[1], Y. Matta[1], J. O. B. Sorbara[2], and A. J. Cowieson[2]*

[1]Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA; [2]DSM Nutritional Products, Kaiseraugst, Switzerland

***Corresponding author:** edgar_oviedo@ncsu.edu

The variability in corn nutrient and energy content has great impact on broiler live performance. Corn kernel hardness and drying temperature are important factors influencing this variation. Kernel hardness, starch structure, vitreousness and protein solubility are closely related. These properties can also affect average particle size and distribution after grinding. All these factors affect digestibility, gastro intestinal organ development, body weight gain, feed conversion and flock uniformity. Exogenous amylase can improve the digestibility of corn starch when grain has been dried under certain conditions, improving live performance. This presentation will describe these relationships and propose possible strategies to overcome the negative effects of some post-harvest processing conditions of corn.

Keywords: corn hybrids; kernel hardness, drying temperature; particle size; NIRS; amylase; broilers.

INTRODUCTION

Corn is the most common feed ingredient for poultry worldwide, responsible for 55 to 75% of total dietary energy. However, great variability in corn energy values is observed causing inconsistency in animal live performance. The MEn values could vary up to 400 kcal/kg due to genetics, agronomic conditions, pre- and post-harvest processes (CARVALHO *et al*., 2004; MALUMBA *et al*., 2014). Starch structure and prolamine concentration vary between corn hybrids with different endosperm hardness conferring different degrees of vitreousness (COWIESON, 2005; ODJO *et al*., 2015; YIN *et al*., 2017). Variation on starch structure and protein solubility can lead to differences in digestibilities and thus undigested fractions (MOUGHAN *et al*., 2014; PLUMIER *et al*., 2015; WANG *et al*., 2015). Salt-soluble protein (SSP) content and vitreousness assays have been developed (AFNOR, 2008; JANAS *et al*., 2010; KACZMAREK *et al*., 2014) to measure and identify unaccounted corn quality traits disregarded by current grading systems. These tests are well correlated with energy utilization (GEHRING *et al*., 2012). Considering that corn hybrids with similar proximate composition could have diverse vitreousness and SSP, it is important to evaluate its effects on overall nutrient composition by rapid methods like NIRS, mechanical properties during grinding, intestinal health, nutrient digestibility, energy utilization, and broiler live performance.

Drying temperature of corn can influence its nutritional quality, but whether this is influenced by endosperm hardness is not completely clear. It is known that elevated drying temperatures change starch structure, increases gelatinization and retrogradation (COWIESON, 2005; WANG *et al*., 2015). These changes may have an impact on how corn kernel brakes during grinding (ABASI and MINAEI, 2014) and on final geometric mean particle size (Dgw) and its distribution. On practical terms, it is important to determine if these changes in corn nutrient content are detected by near infrared reflectance spectroscopy (NIRS), since nowadays this is the most common rapid tool in feed mills to obtain values for feed formulation adjustments.

Corn biochemical and physical variability influences digestibility, gastrointestinal organ development, and live performance. The addition of exogenous enzymes (COWIESON and ADEOLA, 2005; TANG *et al*., 2014; KACZMAREK *et al*., 2014; AMERAH *et al*., 2016) have been reported to improve corn nutritional value, but they may have different responses depending on corn kernel hardness, nutrient transformation during the drying process, particle size post grinding, and hydrothermal processing during pelleting (GEHRING *et al*., 2012). Tests using amylase on diverse corn hybrids are scarce.

The objectives of this project were: 1) To evaluate the effects of drying temperatures for two corn hybrids differing in endosperm hardness; 2) To detect the impact on nutrient concentration detected by NIRS; 3) To measure effects on particle size after grinding in hammer and roller mills, and on broiler live performance; and finally, 4) To assess the effects of an exogenous amylase added to diets containing corn of these diverse traits.

MATERIALS AND METHODS

In the project described herein, two corn hybrids (Dekalb 68-05 and Dekalb 65-20) varying in kernel hardness (average and hard, respectively) were cultivated under identical agronomic conditions. Later, corn was harvested at similar moisture content and maturity, and dried at three temperatures (35, 80, and 120°C) to investigate the effects on its nutritional content. Subsequently, five samples of whole and grounded corn per treatment were collected and read in a Foss (DS2500) NIR. The non-standardized spectrum of each sample was sent to three commercial companies (A, B, and C) to use their respective calibration models.

Corn was ground in hammer and roller mills. The Dgw and standard deviation (Sgw) of particle size were assessed by the sieving method with three replicates per treatment. In roller-milling, four combinations of roller openings were studied: 0-0, 25-20, 30-30, and 35-35. Settings investigated in the hammer mill were 900, 2,400 and 3,600 rpm, at a constant 12-12 screen. For the nutrient composition measured by NIRS and particle size evaluation, six treatments resulted from a 2 x 3 factorial arrangement of two corn kernel hardness and three drying temperatures. Data were analyzed in a completely randomized design using two-way ANOVA and mean separation with Tukey's and student's t test.

Finally, one experiment was conducted to investigate the effects of corn kernel hardness and drying temperatures without or with amylase (Ronozyme HiStarch®) supplementation (0 *vs.* 133 g/ton) on live performance of Ross 708 male broilers raised up to 3.3 kg. A total of 1,920 male-chicks were randomly allocated to 96 floor pens (20 chicks/pen). Chickens were fed corn-soybean diets with DDGS and poultry fat. All diets contained phytase and ionophore, but no growth promoting antibiotics were added. Starter diets were fed in crumbles, while grower and finisher diets were offered in pellets. For this experiment, 12 dietary treatments resulted from a 2 x 3 x 2 factorial arrangement of two corn kernel hardness, three drying temperatures and amylase supplementation respectively. Pen distribution inside the broiler house was considered random effect. Data were analyzed using three-way ANOVA in a randomized complete block design. Mean separation was performed using Tukey's and student's t test.

RESULTS AND DISCUSSION

Not all NIRS calibration models were developed for whole grain. Results of NIRS analyses indicated that whole grain samples of non-dried grain with hard kernel had greater ($P < 0.05$) moisture in all models, vitreousness (A: 89.17 *vs.* 83.44%), starch (A: 83.57 *vs.* 78.71%), and fat (B: 4.28 *vs.* 3.88%) compared with the corn with average hardness. In contrast, no differences ($P > 0.05$) were observed on protein with these samples. However, once the corn hybrids were dried, results indicated that both grain dried hybrids had the lowest ($P < 0.01$) protein at 80°C and higher ($P < 0.01$) fat content at 35°C in grounded samples. At 35°C higher ($P < 0.01$) content of Lys, Met+Cys, and Thr were observed in corn with average hardness. Fatty acid content was greater ($P < 0.001$) in corn with hard kernel compared with the corn of average hardness and effects of drying temperatures also were detected. Hard kernel corn had less starch ($P < 0.001$) than corn with average hardness when dried at 80 and 120°C in all models. NIRS technology detected the expected differences on nutrient content and these results could affect the predicted energy value for feed formulation (CARVALHO *et al*., 2004). Similar variations on nutrients has been previously reported (BHUIYAN *et al*, 2010; MALUMBA *et al*., 2014).

In particle size analyses, results obtained in roller mill at 0-0 and 35-35 opening showed that the hard corn kernel had higher ($P < 0.001$) Dgw than corn with average kernel hardness when dried at 35°C. Similar response was observed when the corn was dried at 120 °C. In addition, the Sgw was reduced ($P < 0.001$) when using corn with hard kernel dried at 80 and 120°C and milled at 0-0 (2.39 *vs.* 2.53%), 30-30 (2.41 *vs.* 2.65%), and 35-35 (2.34 *vs.* 2.69%), respectively. In contrast, no effects ($P > 0.05$) were observed on Sgw when the corn dried at 120°C was grinded in the hammermill. Higher ($P < 0.001$) Sgw was detected in the corn with average hardness and dried at 35 and 80°C compared with corn with hard kernel, when milled at 2,400 and 3,600 rpm (3.84 *vs.* 3.45; and 3.21 *vs.* 3.03 respectively). The Dgw was higher ($P < 0.001$) in corn dried at 120°C and with hard kernel compared to the grain with average hardness when they were grinded at 2,400 (744 *vs.* 594 μm) and 3,600 rpm (556 *vs.* 424 μm) respectively (Figure 1). Generally, corn with hard kernel and dried at 120 °C had higher Dgw and corn with average hardness had greater Sgw independently of the grinding method. Drying temperatures affected the mechanical properties of dried corn as other reports had indicated (ABASI and MINAEI, 2014), but responses vary according to corn kernel hardness.

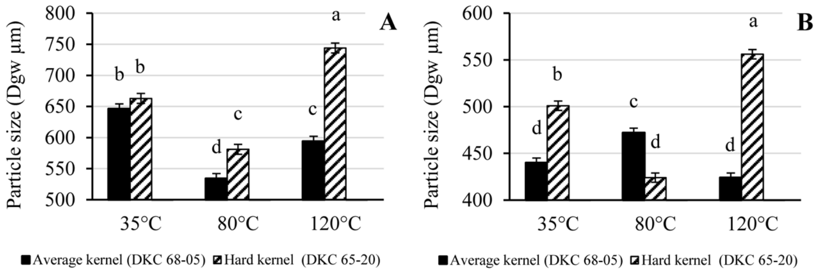


Figure 1. Effect of kernel harness and drying temperature on particle size (Dgw) in hammermill at 2,400 (**A**) and 3,600 rpm (**B**) in a 12-12 screen.

In the broiler experiment, an interaction effect of treatments between drying temperature and enzyme supplementation on body weight (BW) gain and BW ($P = 0.07$) was observed at 14 d of age. Chicks fed non-supplemented diets with corn dried at 35°C were lighter (507 g) than chicks fed diets supplemented with amylase and containing corn dried at 35°C and 120°C (517 g). At 28d a three-way interaction ($P < 0.05$) was detected on BW gain. Only for chickens fed corn with average kernel hardness and dried at 35°C, the addition of amylase resulted in greater BW gain than broilers fed non-supplemented diets (1,770 *vs.* 1,699 g). At 40 d, interaction effects ($P < 0.05$) of drying temperature and amylase supplementation were observed on BWG, BW, feed conversion ratio (FCR), and flock uniformity.

Supplementation with exogenous amylase resulted in heavier broilers, better FCR and flock uniformity, only in the diets based on corn dried at 35°C. Additionally, interaction effects were observed on FCR due to kernel hardness and drying temperature ($P < 0.01$), kernel hardness and enzyme supplementation ($P < 0.001$), and drying temperature and amylase supplementation ($P < 0.05$). Exogenous amylase addition to the diet based on corn with an average endosperm hardness improved FCR up to 2 points (1.49 *vs.* 1.51 g:g) whereas there was no effect of amylase on FCR of birds that received the diet based on corn with a hard endosperm.

These results suggested variations on the efficacy of amylase depending on corn kernel hardness that have not been observed in prior reports feeding other enzyme combinations (AMERAH *et al.*, 2016; KACZMAREK *et al.*, 2014; TANG *et al.*, 2014). The FCR of broilers (Figure 2) fed diets based on corn with average endosperm hardness was improved by drying at 120°C (1.49 *vs.* 1.51 g:g) whereas opposite effect was observed for corn with a hard endosperm (1.50 *vs.* 1.49 g:g). Feed intake was not affected ($P > 0.05$) by treatments throughout the whole experiment.

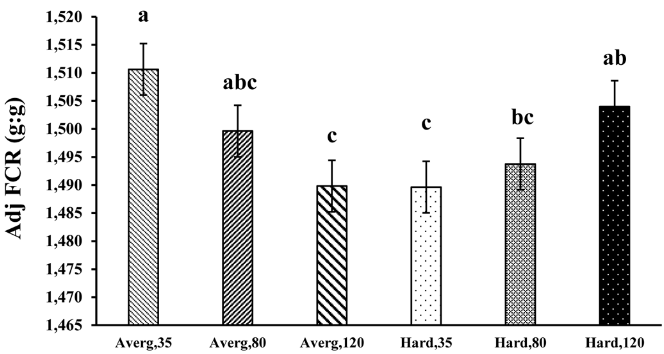


Figure 2. Effect of corn kernel hardness (Average or Hard) and grain drying temperature (35, 80, and 120 °C) on FCR of Ross 708 male broilers at 40 d

CONCLUSIONS

In conclusion, corn hybrids differed in nutrient content, vitreoussness, and potentially in energy value. NIRS was able to detect differences between corn hybrids dried at different temperatures independently of the calibration model, but there was variation in results among models. Kernel hardness of corn and grain drying temperatures affected the particle size during grinding in roller and hammermill. Corn endosperm hardness, drying temperature and exogenous amylase influenced the live performance of broilers. However, these factors are not independent and so must be manipulated strategically to improve broiler performance.

REFERENCES

ABASI, S., S. MINAEI, 2014: Effect of drying temperature on mechanical properties of dried corn. Drying Tech. **32**, 7, 774-780.

AFNOR (Association Française de Normalisation), 2008: Méthode Promatest d'évaluation de la dénaturation des proteins thermosensibles. NF-V03–741. AFNOR, La Plaine Saint- Denis, France.

AMERAH, A.M., L.F. ROMERO, A. AWATI, V. RAVINDRAN, 2016: Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/ soy diets. Poult. Sci. **95**,1–10.

BHUIYAN, M.M., A.F. ISLAM, P.A. IJI, 2010: Variation in nutrient composition and structure of high-moisture maize dried at different temperatures. S. Afr. J. Anim. Sci. **40**, 3, 190-197.

CARVALHO, D., C. DE OLIVEIRA, L.F.T. ALBINO, H.S. ROSTAGNO, J.E. OLIVEIRA, J.G. VARGAS, R.S. TOLEDO, C.H.R. COSTA, S.R.F. PINHEIRO, R.M. SOUZA, 2004: Chemical and energy composition of corn sample under different drying temperature and storage periods. R. Bras. Zootec. **33**, 2, 358-364.

COWIESON, A., 2005: Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Tech. 119, 293-305.

COWIESON, A., O. ADEOLA, 2005: Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. Poult. Sci. **84**, 1860–1867.

GEHRING, C.K., M.R. BEDFORD, A.J. COWIESON, W.A. DOZIER III, 2012: Effects of corn source on the relationship between in vitro assays and ileal nutrient digestibility. Poult. Sc. **91**, 1908–1914.

JANAS, S., S. BOUTRY, P. MALUMBA, L. VANDER-ELST, F. BÉRA, 2010. Modelling dehydration and quality degradation of maize during fluidized-bed drying. J. Food Eng. **100**, 527-534.

KACZMAREK, S.A., A.J. COWIESON, D. JÓZEFIAK, A. RUTKOWSKI, 2014: Effect of maize endosperm hardness, drying temperature and microbial enzyme supplementation on the performance of broiler chickens. Anim. Prod. Sci. **54**, 956–965.

MALUMBA, M., S. ODJO, C. BOUDRY, S. DANTHINE, J. BINDELLE, Y.B.F. BÉRA, 2014. Physicochemical characterization and in vitro assessment of the nutritive value of starch yield from corn dried at different temperatures. Starch **66**, 7-8, 738-748.

MOUGHAN, P.J., V. RAVINDRAN, J.O. SORBARA, 2014: Dietary protein and amino acids. Considerations of the undigestible fraction. Poult. Sci. **93**, 2400-2410.

ODJO, S., P.K. MALUMBA, Y. BECKERS, F. BERA, 2015. Impact of drying and heat treatment on the feeding value of corn. A review. Biotechnol. Agron. Soc. Environ. **19**:301-312.

PLUMIER, B.M., M.C. DANAO, K.D. RAUSCH, V. SINGH, 2015: Changes in unreacted starch content in corn during storage. J. Stored Products Res. 61, 85-89.

TANG, D., S. HAO, G. LIU, F. NIAN, Y. RU, 2014: Effects of maize source and complex enzymes on performance and nutrient utilization of broilers. Asian Australas. J. Anim. Sci. **27**, 1755-1762.

WANG, S., C. LI, L. COPELAND, Q. NIU, S. WANG, 2015: Starch Retrogradation: A Comprehensive Review. Food Sci. Food Safety **14**, 568-585.

YIN, D., J. YUAN, Y. GUO, L.I. CHIBA, 2017: Effect of storage time on the characteristics of corn and efficiency of its utilization in broiler chickens. Anim. Nutr. **3**, 252-257.

Update: The WPSA phosphorus digestibility protocol

P sub-committee of Working Group No 2 –Nutrition– of the European Federation of Branches of WPSA

Correspondence: markus.rodehutscord@uni-hohenheim.de

Maintaining phosphorus (P) resources in view of finite global phosphate rock stores has been identified to be one of the greatest challenges for sustainable food production (Gross 2010, Neset and Cordell 2012). This implies special challenges for all livestock industries. In awareness of these challenges, the Working Group No 2 –Nutrition– of the European Federation of Branches of WPSA made harmonisation of P evaluation and P requirement a major objective of their activities. Based on the work of a sub-committee of experts from academia and industry, the Working Group No 2 developed a standard protocol for the determination of precaecal digestible P (pcdP) in broilers (WPSA 2013), initiated data collection to compile a feedstuff table (Shastak and Rodehutscord 2015), and suggested a model for pcdP requirement of broilers (Khaksar et al. 2017).

When publishing the protocol for determination of pcdP, the group stated the protocol to be an initial step and 'open for improvement. Several details had to be set in spite of a lack of experimental evidence and they should be clarified in future.' Hence, researchers were invited to contribute answers to unresolved questions (WPSA 2013).

The sub-committee has reviewed recent publications with linkages to the protocol and received suggestions and comments from the community. Main aspects of discussion were the following. Based hereupon, a refined protocol is in preparation for publication.

1. Following indications from results of the international P digestibility ring test (Rodehutscord et al. 2017), the refined protocol will pay more attention to the pre-experimental period (i. e. the period from placement of hatchlings until introduction of the experimental diets). In its current version, the protocol defines the starter diets only in a general way, stating that they are adequate in all nutrients. Refinement of the protocol includes the absence of coccidiostats and antibiotics in the starter diet, specifies concentrations of Ca, P, and vitamin D_3, use of phytase, and it calls for reporting ingredient composition of the diet.

2. The standard protocol implies that a low-P basal diet and diets with graded levels of a test P source are used and the pcdP of test source is calculated by linear regression. Because several recent studies showed the existence of interactions between the release of phytate-P in the digestive tract and supplements of mineral P and Ca sources, ingredient composition of the basal diet and how much phytate it contains is important for the outcome of digestibility studies. Digestibility of mineral and animal P sources appears to be under-predicted when included in a phytate-containing basal diet because the added P reduces release of P from phytate. After careful consideration of possible alternatives, it was concluded the protocol will not be changed in regard to the composition of basal diets suggested to test such P sources. The main reason is that poultry diets in the industry contain phytate in considerable amount. Hence, using phytate-containing basal diets when testing P sources of mineral and animal origin reflects the digestibility effects that the P sources will have when mixed into industry-type diets. However, the protocol allows to identify differences in digestibility between different P sources. When pcdP of plant P sources is tested, diets should not contain any mineral P source, and the protocol will be revised accordingly.

3. Standardisations on calcium are very important in P digestibility studies. The protocol uses a constant Ca:P ratio (1.3:1 – 1.4:1) in the diets. It has been suggested that using a constant Ca level of 0.35 % in all test diets could be advantageous over the constant ratio (Perryman et al. 2017). One argument was that estimated endogenous P losses are more meaningful. However, estimates of endogenous losses are outside the scope of the protocol. They are difficult to combine with digestibility estimates when the extrapolation to zero P intake is wide. Using a constant Ca concentration in the diet impairs the risk of making Ca the limiting factor for P retention with increasing P supplementation and for reduced P absorption at low P concentration of the diet. The committee concluded after extensive discussion that using a constant Ca:P ratio in the diets is the preferred way to study P digestibility and the protocol will not be changed in this aspect. Limestone is a common Ca supplement with variation in particle size distribution, which can interfere with P digestibility (Kim et al. 2018). For this reason, the protocol aimed at standardisation by specifying that 'finely ground limestone' is used in the diets. The refined protocol will contain a more precise definition of the target mean particle size and encourage to determine and report the particle size distribution of limestone used in each P digestibility study.

REFERENCES

GROSS, M. (2010) Fears over phosphorus supplies. *Current Biology* 20: R386.

KHAKSAR, V., MEDA, B. and NARCY, A. (2017) Updating the available P requirements of broilers. Proceedings of the 21st European Symposium on Poultry Nutrition, Salou/Vila-seca, Spain, pp. 124-129.

KIM, S. W., LI, W., ANGEL, R. and PROSZKOWIEC-WEGLARZ, M. (2018) Effects of limestone particle size and dietary Ca concentration on apparent P and Ca digestibility in the presence or absence of phytase. *Poultry Science* 97: 4306-4314.

NESET, T.-S. S. and CORDELL, D. (2012) Global phosphorus scarcity: Identifying synergies for a sustainable future. *Journal of the Science of Food and Agriculture* 92: 2-6.

PERRYMAN, K. R., MASEY O'NEILL, H. V., BEDFORD, M. R. and DOZIER III, W. A. (2017) Methodology affects measures of phosphorus availability in growing broilers: Effects of calcium feeding strategy and dietary adaptation period length on true ileal phosphorus digestibility and predicted endogenous phosphorus losses. *Poultry Science* 96: 611-621.

RODEHUTSCORD, M., ADEOLA, O., ANGEL, R., BIKKER, P., DELEZIE, E., DOZIER III, W. A., UMAR FARUK, M., FRANCESCH, M., KWAKERNAAK, C., NARCY, A., NYACHOTI, C. M., OLUKOSI, O. A., PREYNAT, A., RENOUF, B., SAIZ DEL BARRIO, A., SCHEDLE, K., SIEGERT, W., STEENFELDT, S., VAN KRIMPEN, M. M., WAITITU, S. M. and WITZIG, M. (2017) Results of an international phosphorus digestibility ring test with broiler chickens. *Poultry Science* 96: 1679-1687.

SHASTAK, Y. and RODEHUTSCORD, M. (2015) Recent developments in determination of available phosphorus in poultry. *Journal of Applied Poultry Research* 24: 283-292.

WPSA [Working Group Nutrition of the European Federation of Branches of WPSA]. (2013) Determination of phosphorus availability in poultry. *World's Poultry Science Journal* 69: 687-698.

The members of the P sub-committee of Working Group No 2:

Mike Bedford, Franco Calini, Evelyne Delezie, Dieter Feuerstein, Maria Francesch, Pierre-André Geraert, Marinus van Krimpen, Agnès Narcy, Markus Rodehutscord, Sanna Steenfeldt

Abstracts

Content

Session 2: Nutrition of layers 171

Poster session 2: Feed additives 174

Session 1: Future protein sources

Oral presentations

Understanding the impact of soybean meal trypsin inhibitor on protein digestion in broiler chickens: Peptide characterization of jejunal digesta

L. Haahr[1], T. Vitved Jensen[1], J. O. Sorbara[2], R. Angel[3]

[1] Novozymes A/S, Bagsvaerd, Denmark, [2] DSM Nutritional Products, Kaiseraugst, Switzerland, [3]University of Maryland, College Park, MD, USA; ltvh@novozymes.com

Trypsin inhibitor (TI) levels in processed soybean meal (SBM) varies according to processing conditions. High TI levels pose a challenge to animal nutrition. Exogenous proteases, which are not inhibited by TI, are believed to aid protein digestion under high TI levels, either by degrading TIs or by effectively hydrolyzing feed protein. The objective of this study was to evaluate the effect of varying concentrations of TI in SBM in the presence of graded concentrations of a commercial protease (RONOZYME® ProAct) on the levels of free amino acids (AA) and small peptides in jejunum digesta samples. Jejunal samples were obtained from an in vivo standardized ileal amino acid digestibility (SIAAD) experiment in a 4 by 5 factorial arrangement with 5 SBM batches with varying TI concentration and 4 protease concentrations. Samples were freeze-dried and extracted followed by peptide analysis using AccQ-Tag derivatization and liquid chromatography mass spectrometry (LCMS). Increasing levels of TI (0.64 to 3.46 mg/g feed) significantly decreased the jejunal concentration of free AA, di- and tripeptides ($P<0.05$). In addition, supplementation with increased levels of protease (0 to 150,000 PROT/kg feed) significantly induced the jejunal concentration of free AA, di- and tripeptides ($P<0.01$), reflecting the activity of the exogenous protease in the animals. For example, free AA increased 23% with protease concentration going from 0 to 150,000 PROT/kg. No interaction between TI and protease was observed on the level of free AA, di- and tripeptides in jejunal samples.In conclusion, supplementation with exogenous protease increases the concentration level of free AA and small peptides in jejunum of broilers.

Effect of total substitution of soybean meal in a low-protein diet fed to finishing broilers

B. Méda[1], S. Tesseraud[1], W. Lambert[2], E. Tormo[3], H. Juin[4], M. Lessire[1]

[1] BOA, INRA, Nouzilly,[2] Ajinomoto Animal Nutrition Europe, Paris, [3] Terres Univia, Paris,[4] EASM, INRA, St Pierre d'Amilly; bertrand.meda@inra.fr

In order to further improve protein self-sufficiency of broiler supply chains, the effect of a total substitution of soybean meal in a low-protein diet has been studied. 1104 Ross PM3 male chickens were distributed in 24 pens of 3 m² and fed with one of the 3 experimental diets (8 pens/diet) between 22 and 36d of age. Metabolisable energy and digestible lysine levels in the diets were 3150 kcal/kg and 10g/kg respectively. Diets were formulated with the same ideal protein profile. They differed by the protein level (D1: 18.8% vs. D2-D3: 16.5%) and the incorporation soybean meal (D1-D2) or not (D3). In the D3 diet, soybean meal was replaced by dehulled beans, French sunflower (HiPro) and rapeseed meals. At 36d, no significant difference in body weight was observed but a significantly higher feed conversion ratio was observed for the D2 and D3 diets. No significant difference in breast meat yield was observed between the D1 and D2 diets while it was significantly lower in the D3 diet. The reduction in dietary protein (D2, D3) significantly increased in carcass fattening. Regarding the quality of the meat, the ultimate pH of breast meat in the D2 and D3 diets was the same but significantly lower than in the D1 diet, which led to higher water losses storage and cooking. It is therefore possible to fully replace imported soybean meal in low-protein in diets fed to finishing broilers with no negative effect on performance.

Processed animal proteins can replace soybean meal in broiler diets

M. van Krimpen[1], P. Bikker[1], J. van Harn[1]

[1] Wageningen Livestock Research, PO Box 338, NL-6700 AH, Wageningen, The Netherlands; marinus.vankrimpen@wur.nl

Two experiments were conducted to i) determine nutrient digestibility and AME_n of two different porcine protein meals in broilers, and ii) to assess the effect of replacing soybean meal (SBM) by these PAPs on growth performance, gut health, bone quality and blood characteristics related to Ca and P metabolism. Both PAPs contained 62% crude protein, but PAP1 had a higher fat content but lower contents of Ca and P than PAP2. In exp. 1, 3 treatments (basal diet, basal+18% PAP1, basal+7% PAP2) were replicated in 6 pens with 12 Ross_308 male broilers per pen from 14 – 23d. The performance study comprised 3 treatments with starter, grower, and finisher diets without PAPs (control), with 8.0, 8.5, and 9.0% PAP1, and with 8.0, 7.0, and 6.0% PAP2, respectively. Each treatment was replicated in 8 pens with 10 Ross_308 male broilers per pen from 0 – 42d. PAP2 had a higher precaecal digestibility of dry matter (76.6 vs. 60.9%) and crude protein (68.8 vs. 60.9%), but a lower digestibility of calcium (40.3 vs. 48.8%) and phosphorus (49.9 vs. 56.4%) compared to PAP1. PAP2 had a higher total tract digestibility of organic matter (67.1 vs. 55.0%) and ash (52.2 vs. 42.3%), but a lower digestibility of crude fat (47.2 vs. 79.6%). AME_n content of PAP1 and PAP2 was 11.6 and 9.3 MJ/kg, respectively. In the performance study, feed intake and BWG of PAP1 fed birds was higher than in control or PAP2 fed birds. FCR corrected to a similar BW of 3850 g was not affected by dietary treatments. PAP1 fed birds had a higher villus/crypt ratio compared to the other treatments. The current study provided up to date nutrient characteristics of the tested PAPs, while performance data showed that these PAPs can partly replace SBM without compromising growth performance, gut health, or bone quality.

Comparison of the effects of 3 faba bean varieties with different tannin, vicine and convicine contents on laying hens' performance, egg and feces quality

S. Klein[1], S. Toussaint[1], M. Bournazel[1], A. Mathiaud[1]

[1] MIXSCIENCE; stephanie.klein@mixscience.eu

Faba bean (FB; Vicia faba L.) is an interesting protein source for poultry, which could be used as an alternative for soybean meal. Despite its good nutritional profile, this feedstuff contains anti-nutritional factors, tannin (T), vicine (V) and convicine (C), known to reduce laying hen performance. The objective of this study was to compare the effects of 3 FB varieties with different T and VC contents on laying hens' performance, egg and feces quality. 96 ISA Brown laying hens aged 52 to 61 weeks, allocated to a randomized complete block design in 48 cages in a French experimental station, received one of the following feeds: NC (negative control, with no FB), T+VC+ (15% Espresso FB, with high T and VC content), T+VC- (15% Fabelle FB with high T but low VC content) and T-VC- (15% Mandoline FB, with low T and VC content). Laying performance, feed intake and egg quality were recorded weekly. Feces quality was scored at 52, 57 and 61 weeks. T+VC+ decreased significantly egg weight ($p<0.05$) compared to NC and the other FB varieties T+VC- and T-VC-, and increased feed conversion ratio, suggesting a negative impact of VC on these criteria. Performances with T-VC- were similar to NC. There was neither effect on feed intake, nor on feces quality. Downgraded eggs were decreased with T+VC- compared to the control ($p<0.05$). Dirty eggs were reduced with T+VC+ compared to the other groups ($p<0.05$). Egg albumen Haugh unit was improved at the end of the trial with T+VC+, compared to NC and T+VC- ($p<0.05$), with T-VC- showing intermediate value. This Haugh unit increase with FB inclusion is in agreement with previous studies. Overall, our results suggest that FB with low VC concentrations can be used in laying hens diets at 15% without negative impact on performance or egg quality.

Black Soldier Fly Larvae in broiler diets did not affect performance but decreased cellular immune parameters

J. de Souza Vilela[1], N. Andronicos Andronicos[2], M. Hilliar[1], N. Andrew[3], R. Swick[1], I. Ruhnke[1]

[1] School of ERS, Faculty of SABL, University of New England, Armidale, Australia, [2] Biomedical Sciences, School of Science and Technology, University of New England, Armidale, Australia, [3] Insect Ecology Lab, Zoology, University of New England, Armidale, Australia; jdesouz2@myune.edu.au

To evaluate the effects of different inclusion levels of dried Black Soldier Fly larvae (BSF) in broiler diets, five inclusion levels of BSF were investigated in the starter diets (0, 2.5, 5, 7.5 and 10%), and the grower and finisher diets (0, 5, 10, 15 and 20%). Diets were fed to a total of 400 broilers, placed in cages with 8 replicates per treatment. For the starter (2d to 10d), there was no significant difference in total broiler performance across the treatments. However, during the grower period (11d to 21d), means feed intake (FI), body weight (BW), and feed conversion ratio (FCR) were significantly different across five treatments (ANOVA, $P < 0.05$); broilers with 10% larvae inclusion diet had the highest FCR and the highest FI. Furthermore, orthogonal polynomial analysis reveals that the FI has a negative quadratic response to graded levels of BSF inclusion ($P < 0.05$). Means white blood cell and lymphocytes were also significantly different in broilers across five treatments (ANOVA test $P < 0.05$). Those birds with 15% and 20% larvae diets had significantly lower lymphocytes and white blood cell count compared to the control group (Tukey post hoc test, $P < 0.001$). In the finisher period, there was a significant ($P < 0.05$) four-fold decrease in cluster of differentiation 3 (CD3+) lymphocytes and a significant 9.7 fold decrease in the population of CD3+CD8+ intestinal cytotoxic T lymphocytes in birds fed a 20% BSF larval diet compared to the control group. These findings suggest that inclusion of BSF may improve performance and affect the immune status in broilers.

Poster 1.1: The effects of pre-pellet cracked maize and phytase inclusions in maize-soy diets with three levels of crude protein (22, 19.5 and 17%) evaluated via a Box-Behnken response surface design

A. Moss[1], P. Chrystal[2], Y. Dersjant-Li[3], S. Liu[1], P. Selle[1]

[1] Poultry Research Foundation within The University of Sydney, NSW, Australia, [2] Baiada Poultry Pty Limited, NSW, Australia, [3] Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK; amy.moss@sydney.edu.au

Reducing crude protein (CP) levels by replacing soybean meal with complementary amino acids and grain in diets for broilers may generate economic, environmental and flock welfare benefits; however, broiler performance is usually compromised. Phytase (PHY) has been reported to enhance amino acid digestibility and absorption which may be facilitated by enhanced gizzard functionality. Thus, the effects of pre-pellet cracked maize (CM; 0, 15 and 30%) and PHY (0, 750 and 1500 FTU/kg) in iso-energetic maize-soy diets with three levels of CP (22, 19.5 and 17%) were evaluated via a 13 treatment Box-Behnken design. Where appropriate, CM (mixed into diets prior to pelleting) replaced ground maize and PHY (Buttiauxella sp. expressed in Trichoderma reesei; Axtra® PHY, Danisco Animal Nutrition) was added over the top. CP levels were reduced and complementary lys, met, thr, trp, val, arg, ile were included. Treatments were offered to 6 replicate cages (6 birds/cage) of male Ross 308 broiler chicks from 7-28 days post-hatch. Model prediction and response surface plots were generated in R. According to model predictions; weight gain, feed intake and FCR were all influenced by CM, PHY and CP level ($P < 0.001$). Reductions from 22% to 17% dietary CP in non-supplemented diets reduced weight gain, feed intake, relative gizzard weight and contents but improved FCR ($P < 0.001$). However, 30% CM inclusion to 17% CP diets increased gizzard weight and 1500 FTU PHY inclusion to 17% CP diets increased gizzard content. CM and PHY inclusion in tandem to 17% CP diets increased weight gain, feed intake and FCR; however, this FCR was still more efficient than broilers offered the non-supplemented 22% CP diet. Therefore, PHY and CM in tandem may improve performance of broilers offered reduced CP diets.

Poster 1.2: Effect of metabolizable energy and balanced essential amino acids with reduced crude protein levels on egg production performances and egg composition of brown laying hens

K. Soisuwan[1], N. Chauychuwong[1]

[1] Department of Animal Science, Faculty of Agriculture, Rajamagala University of Technology Srivijaya, Thungsong, Nakhon Si Thammarat, Thailand 80110; ksoisuwan52@gmail.com

An experiment was conducted to determine the influence of dietary metabolizable energy and balanced essential amino acids with reduced crude protein levels on egg production performances and egg composition of brown laying hens. The experiment was designed as 2x2x3 factorial arrangement with 2 dietary energy levels (2,800; control and 2,900 kcal of ME/kg) and 2 levels of digestible lysine-methionine ratio (0.75:0.68; control and 0.85:0.77%) and also with 3 levels of crude protein levels (15, 16 and 17; control; %). The study lasted 16 weeks. Isa brown hens (n=540) in 28 weeks of age were randomly divided into 12 treatments (5 replicates of 45 hens per treatment). Performances and egg composition were evaluated in the fourth 28-d periods from the 28th to 45th weeks of age. The results shown that no interacttions between metbolizable energy, essential amino acids and crude protein levels for any traits. The increasing dietary metabolizable energy and crude protein levels did not improve performances and egg composition. However, increased digestible lysine-methionine ratio from 0.75:0.68% to 0.85:0.77% improved ($P<0.05$) egg production performances in term of egg weight, egg mass and feed conversion ratio also with improved ($P<0.05$) egg composition in term of albumen percentage. It is concluded that the levels of 2,800 kcal of ME and 15% protein and also with 0.85:0.77% of digestible lysine-methionine ratio were sufficient for brown laying hens without decreasing their performances and egg composition.

Poster 1.3: Effect of low protein diets on litter quality and incidence of footpad dermatitis in broilers

L. Perić[1], K. Dublecz[2], D. Žikić[1], M. Đukić Stojčić[1], L. Pál[2]

[1] University of Novi Sad, Faculty of Agriculture, [2] University of Pannonia, Georgikon Faculty; lidija.peric@stocarstvo.edu.rs

The study was conducted to evaluate the impact of low protein diets on litter quality and consequently on the occurrence of footpad dermatitis (FPD) in broilers. A total of 592 Ross 308 broilers were allocated to two treatments with four replicates. Birds were fed either a standard feed (C) or a 2% protein reduced feed (LP), containing the same ileal digestible amounts of the first limiting amino acids (MET, M+C, LYS, THR, VAL). The level of crude protein in starter feed (1-12 days) was 23% (C) or 21% (LP), in grower feed (13-33 days) 21% (C) or 19% (LP) and in finisher feed (34-42 days) 19% (C) or 17% (LP). The nitrogen content in feces, litter quality (moisture content, pH, litter score) and incidence of footpad dermatitis were evaluated at the age of 4 and 6-weeks. The litter quality was evaluated by scoring from 1-dry and friable litter to 5-totally caked and wet. The severity of FPD was measured by the method described by Eichner (2007). Reduction of the crude protein content in diets significantly decreased ($P<0.01$) the nitrogen content in feces (%) at 4 (0.95 vs. 0.79) as well as at 6 weeks of age (1.20 vs. 0.93). The moisture content in litter (%) was significantly lowered in LP group at 6 weeks of age (52.35 vs. 43.27, $P<0.05$). The pH of the litter was lowered in LP group at 4 weeks of age (8.14 vs. 7.01, $P<0.01$) and at 6 weeks of age (7.85 vs. 7.39). The litter score was significantly lower ($P<0.05$) in LP group at 4 (3.75 vs. 3.00) but not at 6 weeks (4.25 vs. 4.00). The FPD score was significantly lowered in groups with LP diets (0.96 vs. 0.76 at 4 weeks and 2.34 vs. 1.35 at 6 weeks of age).In conclusion, the results showed that reduction of protein content in feed improved the litter quality and significantly lowered the incidence of FPD in broilers.

Poster 1.4: Influence of trypsin inhibitors in soybean meal on protein digestion in broiler chickens: amino acid digestibility

R. Angel[1], N. Ruiz[2], J. O. Sorbara[3]

[1] University of Maryland, [2] Nelson Ruiz Nutrition LLC, [3] DSM Nutritional Products; rangel@umd.edu

The objective of the trial was to determine the effect of different concentrations of trypsin inhibitor (TI) in soybean meal (SBM) on amino acid (AA) digestibility in the presence of graded concentrations of a commercial protease. SBM of different TI concentrations were produced from a batch of soy beans and 5 batches selected with KOH solubilities >80%, containing 0.64, 0.94, 1.67, 2.24, 3.46 mg/g of TI. The trial consisted of 21 treatments (Trt) (4x5 factorial arrangement of 5 TI and 4 protease concentrations (0, 15, 75, 150 {PROT/g) plus one purified diet). One batch of a purified basal diet was mixed and batches of SBM replaced 40% of the basal in the SBM Trt diets. Each batch of SBM Trt diet was divided into 4 and protease added to achieve desired concentrations. Each Trt was fed to 8 pens of Cobb 500 broilers from 14 to 16d of age. Broilers were euthanized after 48 h and distal ileal samples collected. There were main effects of TI and protease on Ala, Arg, Cys, Glu, Gly, His, Iso, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val ($P<0.01$) but no interaction ($P>0.05$) on standardized ileal digestibility (SID). Effects of protease were driven by improvements in SID between 0 and 15 mg/g protease with no further improvements at higher protease inclusions. For example, SID Thr were 0.77, 0.82, 0.82, 0.83 g/kg when 0, 15, 75, and 150 PROT/g of protease were added. SID for all AA were lower ($P<0.5$) when TI was 0.64 and 3.46 mg/g. The effect of TI appears to be confounded with effects of over processing at 0.64 mg/kg TI. Overall, addition of protease at 15 PROT/g improved digestibility of all AA. Further work is needed to understand what in processed SBM is impacting digestibility differently and how addition of a protease is helping improve digestibility.

Poster 1.5: The impact of starch, protein and fat digestive dynamics on growth performance in broiler chickens from 7-28 days

S. Liu[1], P. Chrystal[2], A. Moss[1], P. Selle[1]

[1] Poultry Research Foundation, The University of Sydney, Australia, [2] Baiada Poultry Pty Limited, Pendle Hill, Australia; sonia.liu@sydney.edu.au

Amino acids and glucose should be available in appropriately balanced quantities at the sites of protein synthesis for efficient protein deposition and growth performance; previous studies have suggested that feed conversion efficiency may be enhanced by rapidly digestible protein or to a lesser extent, slowly digestible starch. A 3-factor-3-level Box-Behnken response surface study was conducted to determine the influence of rapidly digestible starch (wheat, 0, 300, 600 g/kg), rapidly digestible protein (whey protein, 0, 50, 100 g/kg) and tallow (0, 25, 50 g/kg) inclusions on growth performance from 7-28 days post-hatch in male Ross 308 broiler chickens offered sorghum-soybean meal based diets. All diets were formulated to be iso-energetic and contain the same digestible lysine and essential amino acids. Each of the 13 diets was offered to 30 birds in 5 replicated cages (6 birds per cage). High tallow and wheat inclusions led to increased relative fat pad weight. There were quadratic relationships between weight gain and wheat, whey protein and tallow inclusions which suggested a balance between the extent and rate of starch, protein and fat digestion is important.

Poster 1.6: Application of near-infrared technology to predict wheat apparent metabolisable energy on adult roosters

M. Doret-Aubertot[1], J.-F. Faivre[1], N. Bernard[1], S. Toussaint[1], M. Bournazel[1], S. Klein[1]

[1] Mixscience; stephanie.klein@mixscience.eu

Each year in France, wheat harvest starts in June and special attention is given to characterise its nutritional value. Harvest quality for poultry feeding is assessed by measuring and predicting apparent metabolisable energy (AME). This value is usually obtained by performing in vivo digestibility trials on adult roosters and by updating predictive equations based on wheat analytical data. Near-Infrared (NIR) analysis is a fast and cheap alternative to predict wheat AME. The aim of this study was to develop a NIR calibration for rooster AME on wheat. A total of 98 wheats were studied through digestibility trials conducted from 2006 to 2017 according to the protocol of Bourdillon and al. (1990). NIR wheat spectra were made using a MPA (Multi Purpose Analyzer, Bruker Optics SARL). A calibration was developed to predict AME from spectra according to in vivo trials. This specific calibration has several advantages: it is economical (no additional analytical cost), considerably time saving and approximately 15% more accurate than the equation-based method to predict wheat AME. In addition, application of this new calibration allows highlighting inter-harvests variability (n=1002 wheat samples) and intra-harvest variability (n=580). In conclusion, the development of this calibration proves importance of a continuous reassessment of nutritional value of wheat and possibility to observe new trends during harvest earlier thanks to NIR technology. It opens prospects such as real-time wheat nutritional quality assessment in feed plants.

Poster 1.7: Effect of dietary inclusion of rice-DDGS on performance and nutrient retention of WL layers

M. V. L. N. Raju[1], R. N. Chatterjee[1], S. V. Rama Rao[1], B. Prakash[1], S. S. Paul[1]

[1] ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad 500030, Telangana, India; mvlnraju@gmail.com

The rice based Distillers Dried Grains with Solubles (DDGS), a by-product of alcohol production from rice grain fermentation, was tested in the diet of White Leghorn laying chickens (BV 300) at graded levels (0, 7.5 and 15%) on iso-caloric and iso-nitrogenous basis. A total of 4752, 24-weeks old laying chickens were divided at random into 54 replicate groups of 88 birds each and housed in an open sided 3-tiered California cage house. Each of the three experimental diets was fed to chickens in 18 replicate groups from 24 to 47 weeks of age.The response of chickens to diets was assessed in terms of egg production, feed conversion efficiency, egg weight, egg shell quality and nutrient retention. The overall response of the chickens during the whole experimental period indicate that the egg production was not affected at 7.5% DDGS, but was significantly ($P<0.05$) depressed at 15%. Feed intake at 15% DDGS was significantly ($P<0.05$) higher than in control and was intermediate in the group fed 7.5% DDGS. Feed intake for one egg and unit egg mass was significantly ($P<0.05$) higher with 15% DDGS, while no difference was observed between control and 7.5% DDGS. Egg weight decreased with both levels of DDGS, but egg mass (g/bird/day) decreased only at 15% level of DDGS. Egg shell quality was not affected, while mortality showed a trend of increase with DDGS inclusion in diet. Nutrient retention (dry matter, energy and nitrogen) was significantly ($P<0.05$) lower at 15% DDGS, whereas at 7.5%DDGS, the values were intermediate. From the overall results it is concluded that rice based DDGS could be fed to laying chickens at 7.5% in diet without any adverse effect, while 15% DDGS was detrimental.

Poster 1.8: In vitro degradation of soybean and sorghum anti-nutritional factors (ANFs) by a commercial mono component protease

T. Vitved Jensen[1], L. Tvedebrink Haar[1]

[1] Novozymes A/S; tvit@novozymes.com

The level of Antinutritional Factors (ANFs) in processed soyabean and sorghum varies according to processing conditions. ANFs work by different mode of actions to decrease nutrient uptake and animal growth performance. The objective of this study was to evaluate the effect of a commercial protease (RONOZYME® ProAct) by SDS-PAGE for its ability to degrade the soy protein ANFs Lectin, The Kunitz trypsin inhibitor (KTI) and Bowman-Birk trypsin inhibitor (BBI), glycinin and beta-conglycinin under physical conditions relevant for the gastrointestinal tract of mono-gastric animals.

Additionally, the potential inhibitory effect of Condensed Tannins (CT) from sorghum on activity of RONOZYME® ProAct was tested using a soybean/maize meal (30:70) diet by an in vitro gastrointestinal model.The results showed that RONOZYME® ProAct completely degrades glycinin and beta-conglycinin to smaller units, which would likely eliminate the antigenic properties of these ANFs. RONOZYME® ProAct also effectively degraded approximately 95% of both lectin and KTI and approximately 65% of the BBI. Only minor degradation of these ANFs was detectable by trypsin and chymotrypsin.

The in vitro analysis of tannic acid showed that it does not affects the activity of RONOZYME® ProAct. There is a negative effect on solubilization of protein by pepsin and pancreatin with addition of tannic acid. However, ProAct uplifts the protein solubility to the same level as the reference without tannic acid.

In conclusion, RONOZYME® ProAct has the potential to reduce the pressure on the pancreatic proteases by counteracting the negative effect of soybean and sorghum ANFs, and thereby reducing the sensitivity of animal performance to low quality soybean meal and sorghum.

Poster 1.9: Effect of extraction methods on protein profiles of paddy and germinated paddy rice

W. Likittrakulwong[1], P. Poolprasert[2], T. Incharoen[3]

[1] Animal Science Program, Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Phitsanulok, Thailand, [2] Biology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, Thailand, [3] Department of Agricultural Science, Faculty of Agriculture, Naresuan University, Phitsanulok, Thailand; wirotliki@psru.ac.th

This study determined the functional properties and different processes for rice protein isolation from paddy (PR) and germinated paddy rice (GPR). It was found that GPR exhibited better nutritional qualities than PR. GPR and PR exhibited similar results except in some properties. They contained high Crude protein (CP), Crude fiber (CF) and Nitrogen free extract (NFE). Rice protein was isolated by SDS and alkaline (0.2% NaOH) extraction followed by neutralization sedimentation was compared with that isolated by starch degradation by alpha-amylase method. There were no major differences in protein composition among SDS, alkaline and alpha-amylase degradation which contain proglutein, globulin and prolamin protein. In addition, protein profiles result showed similar in both of GPR and PR. These results strongly indicated that alkaline extraction was the best method to isolate the rice protein from PR and GPR because it is very easy, rapid and clearly protein profiles.

Poster 1.10: Feeding high-oleic peanuts and/or oleic acid to layer hens enhances egg yolk color and egg nutrition in shell eggs

O. Toomer[1], T. Vu[1], E. Sanders[2], R. Malheiros[2], K. Anderson[2]

[1] Market Quality & Handling Research Unit, ARS, USDA, [2] Prestage Dept. of Poultry Science, North Carolina State University; Ondulla.Toomer@ars.usda.gov

For decades, the US southeast has led poultry production. However, the need for poultry feed components such as corn and soybean far exceeds the ability to produce these ingredients locally. Thus, we aimed to examine the use of high-oleic peanuts, an oilseed crop abundantly grown within the US southeast as a layer hen feed ingredient. We aimed to determine the effect of high-oleic (HO) peanuts, or oleic fatty acids on layer hen performance, nutritional content, quality and sensory attributes of the eggs produced. Thirty-three layer hens were fed either a soybean meal + corn control diet (SBM), a HO peanut + corn diet or a control diet spiked with oleic acid oil (SBM+OA) for 8 weeks. Body, feed and egg weights were collected weekly. Egg samples were analyzed for quality, lipid analysis and sensory attributes. There were no differences in hen performance, egg quality or number of eggs produced between the treatment groups. Eggs produced from layer hens fed the HO peanut diet had greater yolk color, HO fatty acid and β-carotene levels in comparison to eggs of the other treatment groups. Eggs produced from layer hens fed the conventional diet (SBM) and SBM + OA diet had significantly greater content of saturated fatty acids (palmitic and stearic) in comparison to eggs produced from layer hens fed the HO peanut diet. Additionally, 100 consumer panelist preferred the sensory attributes of eggs produced from layer hens fed the HO peanut diet equally to shell eggs produced from layer hens fed a conventional SBM diet. This study identifies whole HO peanuts with the skin intact as an abundant global commodity that could be used as a valuable layer hen feed ingredient to enhance the nutrition of the eggs produced.

Poster 1.11: Inclusion of Neurospora intermedia in starter diet to broilers with immediate or delayed access to feed post hatch

E. Ivarsson[1], J. Wåhrn[1], M. Boyner[1], P. Ellström[2], H. Wall[3]

[1] Department of Animal Nutrition and Management, Swedish University of Agicultural Sciences, [2] Department of Medical Sciences, Uppsala University, [3] Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences; Emma.Ivarsson@slu.se

The filamentous fungi Neurospora intermedia cultured in thin stillage from bio-ethanol production is a novel protein source containing about 50% crude protein with an amino acid profile resembling that of soy bean. In this study, 288 chickens were used in a 43-day trial to study the effect of inclusion of 10% N. intermedia (N) in a starter diet, compared to a control diet based on soybean meal (C). The diets were formulated to be iso-nutrient. Eggs were hatched on-farm during days 19-21 post incubation and chickens were continuously distributed into 24 pens. Chicks in half of the pens had immediate access to feed and water (I) post hatch, while the others got access after 48 h (D). The D groups resembled conventional hatching. The pens in each hatching treatment (I and D) were randomly distributed to the 2 dietary treatments. At day 5, 9 and 43, two chickens per pen were killed and length and weight of organs were measured. Within the I group, chickens fed C had a higher feed intake than chickens fed N at 48 h post-hatch, (10.9 vs. 8.4 g/chicken, ±0.73; p=0.042), while there were no effects on body weight. From day 5 and onwards, no effect of diet was observed for any parameter. At day 5, feed intake and body weight were higher (p< 0.001) in I than D chicken. At day 9, there was a tendency for higher feed intake (p=0.053) in I than D chickens, but there were no effects (p=0.128) on body weight. From day 9 and on, all chickens were fed the same grower diet, and no further effects of starter diet or hatching treatment were observed on growth performance or feed intake. Organ development was affected neither by hatching treatment or starter diet. In conclusion, 10% N. intermedia, can be used as a protein source in starter diets, independent of hatching treatment.

Poster 1.12: Effect of yellow lupine seeds (Lupinus luteus L.) and Tenebrio molitor larvae meal in the diet for broiler chickens on production parameters and sensory quality of the meat

S. Orczewska-Dudek[1], M. Pietras[1], W. Szczurek[1], M. Pieszka[1]

[1] National Research Institute of Animal Production, Department of Nutrition Physiology; sylwia.orczewska@izoo.krakow.pl

The aim of the study was to determine the effect of lupine seeds and larvae meal as a replacements of soybean meal in diets for broiler chickens on growth performance, carcass parameters and sensory quality of the meat. The experiment was carried out using 120 Ross 308 broilers. At the age of 21 days the birds were randomly assigned to three experimental feeding groups. Chickens of the control group (I) were fed with a grower-type diet based on corn and soybean meal. The chickens from group II received diet with a 20% seeds of yellow lupine (Lupinus luteus L.), and in group III the Tenebrio molitor larvae meal (17%) and yellow lupine seeds (20%) as a protein source instead of soybean meal were used. At 42 days of age, the 8 broilers from each group were weighed and slaughtered. There was no significant effect of dietary factors on the final body weight and on daily average body weight gains. Broilers fed a diet containing larvae meal had a lower feed intake (P≤0.01) and a lower feed conversion ratio (P≤0.05), compared with other groups. The content of dry matter in breast meat of birds from the control group (I) was significantly lower (P≤0.05) compared to the experimental groups II and III. The content of crude fat in leg muscles was significantly higher (P≤0.01) in groups II and III. There was a significant increase (P≤0.01) in the gizzard mass in group III. Sensory evaluation of the meat did not show any significant differences among the dietary treatments. The obtained results indicate that in a grower-type diets soybean meal can be replaced by Tenebrio molitor larvae meal and yellow lupine seeds as alternative protein source for growing broilers without negative impact on the performance parameters and sensory quality of the meat.

Poster 1.13: The effect of increasing level of repassed meal in diet on laying hens performance and egg shell thickness

M. Hejdysz[1], S. A. Kaczmarek[1], M. Kubiś[1], K. Perz[1], A. Rutkowski[1]

[1] Poznan University of Life Sciences; marcin.hejdysz@up.poznan.pl

A 16-week feeding trial was conducted to examine the effect of three different levels on rapeseeds meal on performance and eggshell thickness of laying hens. One hundred and twenty Lohman brown laying hens were randomly divided into four dietary treatments. Each treatment was replicated ten times with three hens per replication in a completely randomized design. Four isonitrogenous and isocaloric corn-soybean meal based diets were formulated to contain 0, 15, 25, 35% of rapeseeds meal. Parameters were evaluated including egg production, egg weight, feed intake, feed conversion ratio, and eggshell thickness. Results showed that egg production till 41 weeks of age was affected by rapeseeds meal level. Birds fed diets with 15, 25, 35% of rapeseeds meal were characterized by significant higher egg production than control groups. There was no difference in egg production between birds fed diets with rapeseeds meal. The value of egg weight, feed intake, feed conversion ratio did not differ significantly in comparison to the control. The use of repassed meal in 35% significantly increased eggshell thickness. Conclusion, repassed meal can be used as a valuable source of protein in laying hens.

Poster 1.14: Nutritional analysis of solid-state fermented canola meal (an improved protein source for broilers)

O. Olukomaiya[1], C. Fernando[1], R. Mereddy[2], X. Li[3], Y. Sultanbawa[1]

[1] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Brisbane, Australia, [2] Department of Agriculture and Fisheries, Queensland, Australia, [3] Poultry Science Unit, School of Agriculture and Food Sciences, The University of Queensland, Gatton, Australia; o.olukomaiya@uqconnect.edu.au

Canola meal contains significant amounts of protein. However, its dietary use is restricted to low inclusion due to the presence of anti-nutritional factors such as fibre, phytic acid, glucosinolates and tannins which have adverse effects on broiler performance. In the present study, the effect of solid-state fermentation on the nutrient composition, phytic acid and total phenolic contents of canola meal was evaluated. Single culture fermentation (Aspergillus sojae and Aspergillus ficuum) and mixed culture fermentation of both strains were conducted under aerobic conditions. Fermented and unfermented canola meals were analyzed for proximate composition (dry matter, crude protein, metabolizable energy, crude fat, crude fibre, acid insoluble ash, total sugar (sucrose), starch, calcium and phosphorus) as well as for pH, phytic acid and total phenolic contents. Crude protein, dry matter, calcium and phosphorus were improved in the solid-state fermented canola meals. The amount of crude fibre and total sugar were decreased by solid-state fermentation. Phytic acid concentration was reduced by 14.1% in Aspergillus sojae-fermented canola meal, 20% in Aspergillus ficuum-fermented canola meal and 18% in mixed culture fermented canola meal. Similarly, total phenolic contents were reduced by 74%, 79.8% and 77.3% in Aspergillus sojae-fermented canola meal, Aspergillus ficuum-fermented canola meal and mixed culture fermented canola meal, respectively. The pH of canola meal increased at the end of the fermentation period. Solid-state fermentation could induce positive changes in the nutrient composition of canola meal. Solid-state fermentation may influence bioavailability of nutrients, nutrient digestibility and feed efficiency of canola meal in broilers.

Poster 1.15: Silages as a novel feed ingredient for poultry?

M. Lourenço[1], E. Delezie[1], C. van Poucke[2], L. Sobry[3], E. Wambacq[4], J. Latré[4]

[1] ILVO-Animal Sciences Unit, [2] ILVO-Technology and Food Science Unit, [3] Inagro, [4] University College Gent; marta.lourenco@ilvo.vlaanderen.be

The presence of vicine (V) and convicine (CV) in faba beans (FB) limits their inclusion in poultry diets. Ensiling moist FB with cereals could be a solution. To assess V and CV reduction by ensiling one FB winter variety (Axel, intercropped with triticale (Tricanto)) and seven FB spring varieties (Bioro, Cartouche, Fanfare, Imposa, Melodie, Taifun and Tiffany, intercropped with wheat (Feeling)) were used. Crops were harvested, beans and grains collected and ground (winter crop) or crushed (spring crops) before ensiling. To each variety one of three silage additives was added: no additive, a mixture of propionic and formic acid (ratio 1:1, OA) or lactate bacteria (Pediococcus pentosaceus, Lactobacillus plantarum and L. buchneri). Plant material was packed into micro-silages and kept in the dark at ambient temperature for 90 days. After, silages were opened and samples were taken to determine silage quality and reduction of V and CV content. Silage V and CV content was reduced for all varieties tested, but to a different extent depending on the FB bean variety. Convicine reduction was highest in silages without additive, independent of FB variety. Within the different varieties, Axel resulted in the biggest reduction of CV, followed by Taifun and Tiffany, independent of the silage additive added. All varieties resulted in good quality silages, with Axel resulting in higher ammonia, lactate and ammonia fraction and lowest pH and crude protein content. Within the spring varieties there was no difference for all silage parameters measured. Silages prepared with OA resulted in the lowest ammonia, lactate and ethanol and in the highest propionate contents. These results are promising for the valorization of FB as protein source for organically produced laying hens.

Poster 1.16: Functional protein rich ingredient increases broiler performance independent of Eimeria challenge

H. Schulze[1], D. Haese[2], H. V. Masey O'Neill[1]

[1] Agilia/ AB Agri, [2] Centro de Tecnologia Animal; hagen.schulze@abagri.com

Early chick development and growth is known to be influential on later stage growth and can be influenced by high quality nutrition in the first week. The aim of this study was to evaluate a functional protein ingredient of co-processed soy and yeast (Alphasoy Gold, DK; ASG), applied in starter diets for broiler, on final growth performance, with or without an Eimeria sp. challenge.

Seventy-two groups of 30 male broiler chicks (Cobb 500) were allocated to one of 3 dietary treatments, containing either 0, 5 or 15% ASG during the starter phase (d 1–14), and equally distributed over 2 identical rooms (challenge vs non challenge). The challenge comprised of placing the birds on litter collected from a previous Eimeria challenge study, further enriched with a cocktail of Eimeria sp. ASG levels were formulated into corn/ soybean meal (SBM) / soy protein concentrate (SPC) based starter diets,replacing SPC and SBM to be iso-caloric and iso-nitrogenous. Thereafter, all broiler were fed common commercial diets.The diets were free of coccidiostats and antibiotics.

Eimeria challenge significantly increased overall (d 1-42) FCR by 2.2 points, whereas final body weight (BW) and overall feed intake (FI) were not affected. However, up to d 33, the challenge significantly reduced BW (84 g) and FI (50 g), and increased FCR by 4.6 points. The inclusion of ASG significantly increased BW of the birds during the starter period by up to 18 g and overall by up to 57g, whereas FCR significantly improved 6.3 points during the starter phase only. No 'challenge x ASG' interactions were found for any of the parameters measured. It can be concluded, that independent of an Eimeria challenge, the inclusion of AlphaSoy Gold in broiler (pre-)starter diets improves overall bird performance parameters.

Poster 1.17: Influence of flaxseed and antioxidants association on egg quality

C. Hamelin[1], *N. Hubert*[1], *M. Mireaux*[1], *M. Panheleux-Lebastard*[1]

[1] CCPA Group; chamelin@ccpa.com

Flaxseed is a good source of linolenic acid, essential fatty acid of the omega 3 family. Supplemented in the diet of hens it increases the content of omega-3 fatty acids in eggs. However, enrichment in omega 3 fatty acids increases susceptibility to oxidation of the yolk fat. So, it is interesting to study the effect of a combination of antioxidants (AOX) from plants extracts and vitamin E on the egg quality and the susceptibility to oxidation. ISA Brown laying hens were divided in 4 groups each of 8 cages of 2 hens. The groups were: G1 = control (10 ppm Vit E=10 IU AOX), G2 = G1+4.5% flaxseed (10 IU AOX same than G1), G3= G1+ AOX (eq to 75 IU-AOX), G4= G2+AOX (75 IU-AOX same than G3). Feed were formulated on the same nutritional basis (energy, protein, minerals, aminoacids). The eggs were collected and classified by week (dirty eggs) for 56 days. Yolks pools of the eggs collected at the end of the trial were analysed. No significant difference was observed on the laying rate (AVG = 97.8%), nor on the weight of eggs (AVG=56.2g). The rate of linolenic acid (C18:3) in yolks was increased with flaxseed (G1=54 ; G2=623 ; G3=65 ; G4=628 mg C18:3/100g egg yolk). The sensitivity to oxidation of egg yolks (TBARS) was significantly higher for groups with flaxseed (302 nmol MDA/g in G1 vs 520 in G2 ; $p<0.05$) and significantly decreased with the addition of AOX (148 and171 in G3 & G4). The rate of dirty eggs was significantly decreased in the presence of AOX (3.74, 5.22, 2.28 and 1.89% in G1, G2, G3 & G4 ; $p<0.05$). The addition of AOX is interesting as part of a enriched diet with flaxseed. Based on the reduction of dirty eggs, the return on investment is € 1.8 to 2.7 for € 1 invested (depending on the egg price 7 or €10 / 100 eggs).

Poster 1.18: Requirements of digestible lysine and total sulfur amino acids in White Leghorn laying hens of Babcock strain under tropical condition

S. V. Rama Rao[1]

[1] ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad-500030, Telangana, India; svramarao1@ gmail.com

An experiment was conducted with WL layers to determine optimum requirements of digestible lysine (dLys) and sulfur amino acids (dSAA) in diets containing sub-optimal protein. Five diets were formulated to contain graded concentrations of dLys (0.700, 0.665, 0.630, 0.593 and 0.563%) and dSAA at fixed ratio to that of dLys (88:100), but similar levels of protein (15%) and energy (2450 kcal ME/kg). A total of 3520 hens (26 wk of age) were randomly divided into 40 replicates of 88 birds in each and housed in open sided colony cage house. Each diet was offered ad libitum to eight replicates from 27 to 74 wks of age. The performance data were grouped into three phases, that is early (27-38wks), mid (39-58wks) and late laying phase (59-74wks) for fitting statistical models (Linear broken-line, quadratic broken line, exponential and quadratic polynomial). Egg production (EP) and feed efficiency (FE) were significantly influenced by the dLys level during the early and mid laying phases but not during the late phase. Feed intake (FI) was significantly influenced by dLys levels during mid (order 4 response) and late laying phases (quadratic response) but not during early laying phase. The egg weight (EW) was not influenced by dLys levels. Based on average estimates of best fitted models on four response variables (EP, FE, EW and FI), dietary requirements of dLys and dSAA worked out to be 0.698 and 0.614%, 0.631 and 0.555%, and 0.586 and 0.515%, respectively in early, mid and late phase. Based on observed FI data, WL layers require 741 and 651 mg/d of dLys and dSAA, respectively during the early and mid laying phases, but require 674 and 592 mg/d, respectively of dLys and dSAA during the late laying phase when fed diet with 15% CP under tropical condition.

Poster 1.19: Effect of the inclusion of an enzymatically treated soybean protein in feed on the intestinal epithelium morphology and performance of broiler chickens

N. Q. Thieu[1], A. Blanch[2], C. Broekner[2], S. H. Rasmussen[3]

[1] Nong Lam University, Hochiminh City, Vietnam, [2] Hamlet Protein A/S, Horsens, Denmark, [3] Hamlet Protein A/S, 8700 Horsens, Denmark; abl@hamletprotein.com

It is hypothesized that partly replacement of soybean meal (SBM) by a low antigen soy product in the starter diet would affect intestine epithelium development and long term performance. A total of 300 day-old Luong Phuong chicks (mixed sex) were assigned to 3 treatments (10 replicates per treatment, 10 chicks per replicate): T1, maize-SBM control diet (d0-28); T2, 5% replacement of SBM by enzymatically treated soybean meal (HPA) (d0-7) followed by the same diet as in T1 (d8-28); T3, 5% HPA replacing SBM (d0-28). All birds were fed the same diet from d29 to 60 of life. Body weight, feed intake were recorded, feed conversion ration (FCR) calculated on d0 at d28 and d60. At d28 and d60, 5 and 10 birds per treatment were selected for duodenum/ileum morphology assessment. The addition of HPA from d0 to 28 significantly (P=0.023) increased villus height/crypt depth ratio in ileum compared to T2 birds, T1 chickens showed no difference. At d60 of life, T3 chickens showed significantly (P=0.029) higher villi than T1 chickens. HPA supplementation showed numerically higher body weight (BW) compared to T1. With regard to FCR, T3 birds showed significantly lower FCR than T1 birds, whereas T2 birds showed intermedium FCR. Regarding the total experimental period, T3 resulted in 4 points lower FCR than T1 and T2 birds. There was no difference in mortality between experimental groups. In conclusion, the supplementation of HPA from 0 to 28 days allows chickens to better develop their gastrointestinal tract, which is crucial for the gastrointestinal function throughout the bird's life. Furthermore, chickens that eat HPA in the first period of life show better performance in later phases.

Poster 1.20: Comparision of the absorption kinetics of synthetic and protein-bound methionine in broiler breeder hens

M. Zamani[1], M. Zaghari[1]

[1] Tehran University; mzaghari@ut.ac.ir

Two experiments (exp.) were conducted to compare the absorption kinetics of synthetic methionine (DL-Met) and protein-bound methionine (PB-Met) in broiler breeder hens. In all exp. day old chicks were fed with common starter and grower diets in mash form for 38d and subsequently allotted to treatment diets in a completely randomized design (5 chicks per pen, 4 replications) until 60d. Dietary treatments were formulated to have increasing levels of DL-Met (0, 0.4, 0.8, 1.2, 1.6, 2 and 2.4 g/kg) in exp.1 and replacement of PB-Met with increasing levels of DL-Met (2:0, 1.6:0.4, 1.2:0.8, 0.8:1.2, 0.4:1.6, 0:2 and 0:2.4 g/kg) in exp.2. At 59d, blood samples were taken via wing vein of 2 birds per replicate before feeding (0h) and then hourly until 3h after feeding for plasma Met analysis with time repeated measurement. The efficiency of free and protein-bound Met retention in whole carcass was also measured. Results indicated that in exp.1 and 2, the intractive and main effects (P<0.0001) of experimental factors were observed on plasma Met concentration. In exp.1, the highest blood Met level was observed at 1h postprandial in chicks received diet containing DL-Met. In exp.2 in treatments 1, 2 and 3 with higher level of PB-Met, plasma Met concentration tended to increase at 2h after feeding, but in treatments 4, 5, 6 and 7 with higher level of DL-Met, the higher level of plasma Met was observed at 1h postprandial. It is concluded that the absorption kinetics and retention of free and protein-bound Met is completely different due to more rapid absorption of free compared with protein-bound Met. Therefore, source of AA should be considered in diet formulas (synthetic vs. protein-bound AA).

Oral presentations

Effects of pre-lay dietary calcium and pullet strain on calcium utilization and femur quality of hens at 1st through to 50th egg

T. Khanal[1], T. Widowski[1], G. Bedecarrats[1], E. Kiarie[1]

[1] University of Guelph; tkhanal@uoguelph.ca

To study the effects of pre-lay dietary Ca and strain on Ca utilization and femur quality at 1st through to 50th egg, 30 Lohmann Brown (LB) and 30 Lohmann LSL-Lite (LSL) pullets (14 wks of age, woa) reared under same management regimen were used. Six pullets per strain were necropsied for baseline femur study. The rest of the pullets (24 per strain) were placed in individual cages (65 cm x 30 cm x 45 cm) and fed 1% Ca developer diet for 2 wks. At 16 woa, all the pullets were weighed and allocated within strains to pre-lay diets (2.5 vs. 4.0% Ca) effectively creating a 2 x 2 factorial arrangement (n=12). The pullets were offered pre-lay diets for 2 wks and switched to layer diet (4% Ca) at 18 woa. The diets contained TiO_2 to determine apparent retention (AR) of Ca. The age, BW and feed intake (FI) at 1st, 25th and 50th egg was recorded. Excreta samples were taken during pre-lay, at 1st and 25th egg, and 4 hens per treatment were necropsied for femur samples at 1st, 25th and 50th egg. There was no interaction (P>0.05) between pre-lay Ca and strain on Ca intake, bone mineral density (BMD), mineral content (BMC), breaking strength (BBS), and total femur ash (TFA) content at 1st, 25th and 50th egg. At 25th egg, pre-lay Ca interacted with strain on AR of Ca (P=0.014) such that at 4.0% pre-lay Ca level, LSL hens retained more Ca than LB hens. Pre-lay Ca had no effect (P>0.05) on BMD, BMC, BBS and TFA at 1st, 25th and 50th egg lay. Compared with LB hens, LSL hens had higher BMD (0.30 vs. 0.19 g/cm2; P=0.010) and TFA (51.9 vs. 42.5 %; P<0.01) at 1st egg, and BBS (259.4 vs. 173.8 N, P<0.01) at 25th egg. In conclusion, except at 25th egg, pre-lay Ca and strain had independent effects. The data highlighted importance of strain on Ca utilization and skeletal quality in hens.

The effect of limestone particle size, phytase inclusion, and time post oviposition on ionized blood calcium levels in commercial laying hens

M. Sinclair-Black[1], R. Angel[2], W. Li[3], P. Plumstead[4]

[1] Chemunique PTY LTD, Randburg, South Africa, University of Pretoria, Hatfield, Pretoria, [2] University of Maryland, College Park, Maryland, [3] DuPont Industrial Biosciences, Wilmington, DE, [4] Chemunique PTY LTD, Randburg, South Africa; Micaela@chemunique.co.za

Blood ionized calcium (iCa) is an indication of the calcium (Ca) that is physiologically active and available for the hen to utilize for bone remodeling and eggshell formation. A study was conducted to determine the effects of limestone (LS) particle size (PS) and phytase added to diets, on changes in blood iCa following oviposition (OP). Amberlink hens (64) at 31 weeks of age were used. A basal diet was mixed to contain no limestone or inorganic P, (1.14g/kg Ca and 3.6g/kg P). The basal diet was then used to mix four dietary treatments (T1-T4) in a 2x2 factorial design containing 2 particle sizes of limestone, grit (1.5mm geometric mean diameter (GMD)), or fine (0.2mm GMD) and two levels of added phytase from Buttiauxella spp. (0 or 600 FTU/kg). LS was analyzed for Ca and 35.8 g/kg Ca from LS added to each basal diet. Each hen received a meal of 130g of feed each day and the exact time of OP recorded. For the same hen, blood collection commenced within 5 minutes of OP, and in 3-h intervals for 24h following OP. iCa and blood pH were measured using an i-STAT point-of-care laboratory system (Abbott Point of Care, East Windsor, NJ). The effects of PS, phytase, sampling timepoint and their interactions on blood pH and iCa concentrations were tested by SAS MIXED model using repeated measurement (SAS, 9.4). Individual hens were treated as random effect and pre-trial average daily feed intake and body weight included in the model as covariates. Blood pH and iCa of hens changed (P<0.05) over the 24h period and were not affected by phytase. An interaction (P<0.05) of LS PS and time was observed with hens fed LS grit having higher blood iCa from 12 to 24 hours post OP. This suggests LS grit does increase iCa during the time the next egg is in the shell gland.

Unsupervised exploratory cluster analysis of free range laying hens to determine the use of aviary feed chains and range access

T. Zimazile Sibanda[1], M. Welch[1], M. Kolakshyapati[1], D. Schneider[1], I. Ruhnke[1]

[1] University of New England; terencezs123@gmail.com

Clustering is a common data mining methodology used for improved subject understanding. The aim of this study was to identify sub-populations of laying hens housed in an aviary system to understand the use of feeding chains which can affect hen performance and welfare. A total of 5,641 Lohmann Brown free-range laying hens placed amongst 3 commercial flocks equipped with a 3-tier aviary system were individually monitored using radio- frequency identification (RFID) technology. Individual body weights of all hens were obtained at 16, 22, and 72 weeks of age. K-Means cluster analysis optimised with the Calinski-Harabasz Criterion was performed. Hens of cluster 1 (n=2442 hens) spent significantly more time on the lower tier feeding chain (14.5 ± 2.36 hours/hen/day) compared to hens of cluster 2 (n=2083; 6.9 ±2.4 h/hen/day) and hens of cluster 3 (n=1116; 2.0 ± 1.9 h/hen/day), respectively ($P < 0.05$). Hens of cluster 3 spent 10.9 ± 3.6 h/hen/day at the top tier feeder chain compared to hens of cluster 1 and 2 (0.9 ± 1.1 and 3.6 ±2.1 h/hen/day respectively; $P < 0.05$). Hens of all clusters were of comparable body weight distributions at week 16, 22 and 72 weeks of age. Hens of cluster 3 spent the least time on the range and the most time on the upper tier feed chain of the upper tier ($P < 0.05$), however there was no significant impact on weight gain between 16 and 72 weeks. We conclude that several subpopulations of hens can be identified in the aviary system and that these subpopulations result in an uneven load on the resources (e.g feed chains). Further analysis of the data using classification models based on support vector machines, artificial neural networks and decision trees is warranted to predict other parameter of hen performance.

Feeding insects to laying hens during peak of lay: effects on production performance, behaviour and welfare

J. Heerkens[1], M. Ruis[2], H. Kuipers[2], J. Katoele[3], L. Star[1]

[1] Aeres University of Applied Sciences, De Drieslag 4, 8251 JZ Dronten, The Netherlands, [2] VHL University of Applied Sciences, Department of Animal Management, Agora 1, 8934 CJ Leeuwarden, The Netherlands, [3] Wadudu Insect Centre, Noordveen 1, 9412 AG Beilen, The Netherlands; j.heerkens@aeres.nl

Aim of the study was to measure effects of feeding Black Soldier Fly larvae (BSF, Hermetia illucens) and mealworm (MW, Tenbrio molitor) on egg production, body weight (BW) and behaviour in Bovans Brown laying hens. BSF and MW larvae were fed alive from 20-37 wks of age, additionally to ad lib access to mash feed and water. Three treatments were used (20 hens/pen). T1: only mash feed (Control; N=6 pens); T2: BSF larvae crawling out slatted floor after development in manure (N=7 pens); T3: adult larvae (BSF or MW) provided daily in feed tube (5-10% of daily ration; N=7 pens). Production performance was measured from 24 to 37 wks. At 18, 28 and 36 wks, hens were individually weighed, feathers damage was scored, and all pecking behaviours, other active (scratching, eating, drinking, preening, dustbathing, walking) and inactive (resting, nesting) behaviours were scored through 1-min scan sampling. Laying period was divided in four periods (P1=24-26 wks; P2=27-29 wks; P3=30-33wks; P4=34-37wks). For repeated measures, mixed models were used, with treatment, period and interactions as predictors. For single measures one-way ANOVA was used. Laying rate, FCR, and mortality were not affected by treatment. T3 showed a tendency for lower feed intake. In P4, egg weight was lower in T2 (60.4±0.3; $P<0.05$) than in T3 (61.4±0.5). At 36 wks, BW was lower in T2 (1.90±0.01; $P<0.05$) than in T1 (1.93±0.01) and T3 (1.94±0.01), and T3 showed more active behaviours (91.7±1.1%; $P<0.05$) than T2 (86.9±1.5) and T1 (86.0±1.7). T2 and T3 showed a tendency for less feather damage. Results showed that feeding larvae stimulated active behaviours (T2 and T3), suggesting a better welfare, but production was only guaranteed when compensated by sufficient intake of larvae as observed for BW and egg weight.

Energy efficiency of commercial free-range laying hens of different body weight and ranging activity

M. Kolakshyapati[1], S. Wu[1], T. Zimazile Sibanda[1], I. Ruhnke[1]

[1] University of New England; mkolaksh@myune.edu.au

Within a free-range flock, some hens prefer to spend majority of their time in the shed, while others frequently access the range. Development of these sub-populations may be associated with different hen performance. Evaluation of energy efficiency of laying hens provides important knowledge for optimal egg production. The aim of this study was to determine net energy utilisation of these subpopulations obtained from a free-range system. Forty-eight Lohmann brown hens at 72weeks of age were selected from a commercial free-range farm and classified as heavy or light body weight (average 2.01kg and 1.68kg respectively, n=24) and as rangers (accessed the range on 84.1% of the available days; n=24) or stayers (accessed the range on 7.17% of available days; n=24). The energy efficiencies were evaluated in a close-circuit respiratory chamber system. Stayers had significantly higher metabolisable energy intake/bird/d/$BW^{0.75}$ (0.852±0.019 vs 0.798±0.016 MJ/g; P=0.025), heat production/$BW^{0.75}$ (0.637±0.069 vs 0.607±0.057 MJ; P=0.005), heat increment/$BW^{0.75}$ (0.267±0.007 vs 0.237±0.006 MJ;P=0.005) and retained nitrogen/bird/d (1.59±0.02 vs 1.46±0.03g; P=0.023) compared to rangers. Light hens had significantly higher metabolisable energy intake/bird/d/$BW^{0.75}$ (0.854±0.019 vs 0.796±0.016 MJ/g; P=0.018), net energy intake/bird/d/$BW^{0.75}$ (0.595±0.013 vs 0.551±0.012 MJ/g; P=0.032), retained energy/bird/d/$BW^{0.75}$(0.225±0.013 vs 0.181±0.012 MJ; P=0.032) and lower heat production/bird/d (0.936±0.011 vs 1.003±0.012 MJ; P=0.002) than heavier hens. In conclusion, stayers required higher maintenance energy than rangers, and light rangers are more energy efficient than light stayers. Future research on determination of ideal body weight of layers in the free-range system would be beneficial.

Poster session 2: Feed additives

Poster 2.1: Effects of phytase supplementation on prececal amino acid digestibility of different oilseed meals in broilers

J. Krieg[1], W. Siegert[1], J. Bock[1], D. Feuerstein[2], M. Rodehutscord[1]

[1] University of Hohenheim, Institute of Animal Science, Emil-Wolff-Straße 10, 70599 Stuttgart, Germany, [2] BASF SE, E-ENE/LW, 68623 Lampertheim, Germany; markus.rodehutscord@uni-hohenheim.de

Literature is not consistent in regard to phytase supplementation effects on prececal (pc) amino acid (AA) digestibility in broilers. Contradictory results might be caused by variation in feed composition between studies, especially the use of phytate-rich oilseed meals. The objective of this study was to investigate phytase supplementation effects on pc AA digestibility in different oilseed meals: rapeseed meal (RSM), soybean meal (SBM), and sunflower meal (SFM) using the regression approach. The basal diet contained 300 g corn starch/kg, which was substituted by the respective oilseed meal in two inclusion levels, each. Each diet was mixed without or with supplementation of 1500 FTU phytase (Natuphos E)/kg, resulting in a total of 14 diets. Experimental diets were provided for ad libitum consumption for 5 days until day 21 post-hatch. Each diet was allocated to five pens of 15 birds each, with the exception of the basal diets that were allocated to seven pens each. On day 21, digesta of the posterior half of the ileum was sampled and analyzed for AA and TiO_2. According to regression analysis, the mean increase in essential AA digestibility was 7 percentage points (pp) for RSM, 1 pp for SBM, and 2 pp for SFM. For non-essential AA, the mean increase was 7, 2, and 4 pp, respectively. While numerical increases were considerable for most AA, statistical significance was determined only for Arg (6 pp), Ile (14 pp), Lys (8 pp), Val (11 pp) and Pro (10 pp) in RSM, and Cys in SBM (8 pp) and SFM (10 pp). Phytase effects were more pronounced in RSM than SBM and SFM. We conclude that the protein source used in the diet can cause divergent AA digestibility responses to supplemented phytase.

Poster 2.2: Barley in broiler starter diets: Effects of particle size and enzyme supplementation

W. N. U. Perera[1], M. R. Abdollahi[1], F. Zaefarian[1], V. Ravindran[1]

[1] Monogastric Research Centre, School of Agriculture and Environment, Massey University, Palmerston North, 4442, New Zealand; M.Abdollahi@massey.ac.nz

The influence of barley particle size and carbohydrase and phytase addition, individually or in combination, on growth performance, energy and nutrient utilisation in broilers was investigated. Two barley particle sizes (fine and coarse) and four enzyme treatments [non-supplemented (control), carbohydrase (Ronozyme multigrain; 0.15 g/kg of feed; C), phytase (Ronozyme HiPhos 0.10 g/kg; P) and combination of carbohydrase and phytase (C+P)] were evaluated in a 2 × 4 factorial arrangement of 8 dietary treatments. Fine and coarse particles were achieved by grinding the whole barley in a hammer mill to pass through 2.0 and 8.0 mm screens, respectively. The diets, in pellet form, were offered ad libitum from 1 to 21 days post-hatch. Weight gain was not influenced ($P > 0.05$) by either main effects or the interaction. Coarse grinding of barley and carbohydrase supplementation in the diet (both C and C+P) lowered feed per gain ($P < 0.05$) and improved apparent metabolisable energy ($P < 0.05$). Feeding coarse particles increased ($P < 0.05$) the ileal digestibility of dry matter and nitrogen. Enzyme supplementation, both individually or in combination, enhanced ($P < 0.05$) the dry matter digestibility. Neither particle size nor enzyme supplementation influenced starch digestibility or jejunal digesta viscosity ($P > 0.05$). In conclusion, coarse grinding of barley and supplementation of carbohydrases are beneficial to improve the utilisation of nutrients and energy, and feed efficiency.

Poster 2.3: Insects full-fat meals as functional feed additives affect broiler chickens' growth performance and immune system traits

A. Benzertiha [1], B. Kierończyk[1], P. Kołodziejski[2], E. Pruszyńska-Oszmałek[2], M. Rawski[3], D. Józefiak[1], A. Józefiak[4]

[1] Poznań University of Life Sciences, Department of Animal Nutrition, [2] Poznań University of Life Sciences, Department of Animal Physiology and Biochemistry, [3] Poznań University of Life Sciences, Division of Inland Fisheries and Aquaculture, [4] Poznań University of Life Sciences, Department of Preclinical Sciences and Infectious Diseases; basset@up.poznan.pl

This study was conducted to investigate the effect of insect full-fat meals (Tenebrio molitor and Zophobas morio larvae), added "on top" of a complete diet or calculated into diets, on the growth performance, and level of certain immunoglobulins which may have functional consequences reflected in the immune response of broiler chickens. 1000 one-day old female Ross 308 broiler chicks were used in two independent trials. In the first trial, the birds were randomly assigned to 6 groups (NC, PC, TM02, ZM02, TM03, ZM03) i.e., NC (negative control) – no additives; PC (positive control) – NC + salinomycin (60 mg/kg diet); TM02 - NC + 0.2% T. molitor full–fat meals; ZM02 - NC + 0.2% Z. morio full–fat meals; TM03 - NC + 0.3% T. molitor full–fat meals; and ZM03 - NC + 0.3% Z. morio full–fat meals. In the second trial, 4 treatments (NC, PC, TM03, ZM03) were set: NC (negative control) - no additives; PC (positive control) - NC + salinomycin (60 mg/kg diet); TM03 - NC with 0.3% T. molitor full fat meals; and a ZM03 - NC with 0.3% Z. morio full-fat meals. Insect full-fat meals significantly increased the BWG and FI (Exp. 1; $P = 0.024$, $P = 0.022$, respectively), and no effect on the FCR was recorded. In addition, in groups fed insect full-fat meals and PC comparing to NC decreased the IgY ($P = 0.045$) and IgM, ($P < 0.001$) levels significantly. In second experiment, IgM levels were also decreased significantly ($P < 0.001$) in groups fed insect meals. Moreover, the IgM levels were negatively correlated to the BWG ($r = -0.4845$) and the FI ($r = -0.4986$), with significant values ($P < 0.001$). In conclusion, the current results confirmed that both T. molitor and Z. morio may be applied as functional feed additives to achieve improved growth performance and changes in the immune system. This work was supported by the funds of Poznań University of Life Sciences; TEAM TECH/2016–2/11– 0026 project under the title: Insects as novel protein sources for fish and poultry, financed by the Foundation of Polish Science (POIR 4.4); as well as funds of the National Center for Research and Development, no POIR.01.01.01–00–0828/15, entitled: InnSecta: innovative technology of feedstuffs production based on insect biomass.

Poster 2.4: Application of low crude protein concept in common Hungarian broiler diets

A. Barna[1], M. Mueller[1], K. Dublecz[2], N. Such[2]

[1] Evonik Nutrition & Care GmbH, Rodenbacher Chaussee 4, Hanau, Germany, [2] University of Pannonia, Georgikon Faculty, Department of Animal Science, 8361 Keszthely, Hungary; andrea.barna@evonik.com

Feeding broilers with low protein diets supplemented with amino acids may reduce feed cost and allow using alternative feed ingredients. High crude protein (CP) levels increase the nitrogen and water excretion, which can reduce litter quality and may elevate the incidence of footpad lesions. High CP level is also reported as a predisposing cause for necrotic enteritis. The aim of the trial was to analyze the possibility of lowering the CP levels using by-products and amino acids without impairing the birds' performance. A total of 576 one-day-old male broilers (Ross 308) were randomly distributed to 3 dietary treatments consisting of 8 replicates with 24 birds each. Three diets were fed: basal diet (control) and two experimental diets with 2% less CP: LPSB (soybean meal as main protein source) and LPBP (soybean meal, corn DDGS and sunflower meal). The highest values for body weight (BW) were achieved in group LPSB (2,992 g), followed by LPBP with 2,925 g. Control group showed the lowest BW (2,833 g). Feed conversion rate (FCR) in control group amounted to 1.57 kg/kg and was significantly higher than for LPSP (1.52 kg/kg) and LPBP (1.53 kg/kg). The difference in FCR between LPSP and LPBP was not significant. Reduction of dietary CP with the help of supplemented amino acids and usage of alternative ingredients such as DDGS and sunflower meal, improved core performance parameters. Earnings (per 100 birds) after feed and day old chick costs amounted to 83.5 EUR in control group, 107.8 EUR in LPSP group, and 100.7 EUR in LPBP group, respectively.

Poster 2.5: The Lipid Evaluation Test: an exclusive service provides insights in lipid nutrition from an extensive field trial

K. Bierinckx[1], E. Witters[1], M. Jansen[1], M. di Benedetto[1]

[1] Kemin Europa N.V.; karen.bierinckx@kemin.com

Numerous industrial by-products enter the lipid market. Hence, nutritionist observe decreased animal performance due to an inaccurate allocated dietary energy value as well as induced metabolic stress by oxidised lipids. In 2014, Kemin designed the exclusive lipid evaluation test (LET). In total ten chemical characteristics are assessed. Two qualitative parameters estimate the degree of lipid oxidation and estimation of sensitivity for future oxidation and recovery of antioxidants, including tocopherols. Three nutritional parameters are evaluated, the level of free fatty acids, the ratio of unsaturated over saturated fatty acids and the level of short chain fatty acids. These parameters are used in the Wiseman model to estimate the dietary energy contribution of lipids in feed for broilers and pigs. As an extra factor, LET incorporates three energy-diluting factors: moisture (M), impurities (I) and unsaponifiables (U).

Since its launch, more than 750 lipid samples have been analysed in the LET test. Almost 66% of this dataset can be represented by 5 groups: soybean oil, animal fat, acid oils, sunflower oil and poultry oil. LET's exclusive dataset shows that in two out of three cases signs of oxidation are detected. Most samples tested showed oxidation in its initial phase, except sunflower oil where more evolved oxidation was registered. LET also confirms the presence of a large variation in dietary energy values, even between identical fat sources. Inclusion of MIU factor demonstrated a more excessive variation on nutritional value of lipids. It is confirmed that the LET test gives a correct estimation of the metabolisable energy content and oxidative quality of a lipid and helps to fully understand the nutritional and oxidative quality of fats and oils.

Poster 2.6: Supplementation with a dry or liquid synergistic mixture of lysophospholipids, monoglycerides and emulsifier improves nutrient digestibility in broilers

K. Bierinckx[1], G. A. Papadopoulos[2], A. L. Wealleans[1], P. Fortomaris[2], M. di Benedetto[1], M. Jansen[1]

[1] Kemin Animal Nutrition and Health, Herentals, Belgium, [2] Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece; karen.bierinckx@kemin.com

Lysophospholipid based absorption enhancers are added to broiler diets to enhance performance and nutrient digestibility. To assess the impact of product form on efficacy, two forms of an absorption enhancer (LYSOFORTE EXTEND Liquid (LEL) and LYSOFORTE EXTEND Dry (LED)) were tested in a broiler trial for digestibility evaluation. One-day old Ross 308 broiler chicks (2 birds per pen; 6 replicate pens per diet) were assigned to 4 dietary treatments: A corn-based broiler diet was formulated as: Positive control (PC), a lowered energy (70 kcal/kg) diet serves as the negative control (NC), NC + LEL at 350 ppm and NC + LED at 500 ppm. Diets contained TiO_2 as a marker. All birds were raised until the 42nd day of age. Feed and excreta content were analysed for moisture, nitrogen and fat according to the standard methods (AOAC, 2003). Dry matter retention was found to be significantly lower in LEL and LED treatments compared to NC (36.74% NC, 31.19% LEL, 31.20% LED; $P<0.05$) and equivalent to PC (33.25%). LEL and LED showed a trend in improved N retention (49.99% NC, 46.62% LEL, 43.57% LED; $P<0.1$). A positive trend in crude fat digestibility was observed with LEL (64,36% NC, 71.98% LEL; $P<0.1$). Significant improvements in AMEn were seen in both LEL and LED compared to the NC (2867 kcal/kg NC, 3140 kcal/kg LEL, 3106 kcal/kg LED; $P<0.05$). This study demonstrates that supplementation of dry and liquid absorption enhancers improves the apparent total tract coefficient of digestibility and AMEn of broiler feeds.

Poster 2.7: Analytical assessment of 750 field samples shows a large variation in nutritional and oxidative characteristics of fats and oils

K. Bierinckx[1], E. Witters[1], M. Jansen[1], M. di Benedetto[1]

[1] Kemin Animal Nutrition and Health, Toekomstlaan 42, 2200 Herentals; karen.bierinckx@kemin.com

Today, nutritionist observe decreased animal performance due to inaccurate allocated dietary energy values and induced metabolic stress by oxidised lipids. 750 fat and oil samples collected from the entire EMENA region were scrutinized through a portfolio of 10 chemical characteristics (Lipid Evaluation Test, LET). Two qualitative parameters estimate the degree of lipid oxidation and estimation of sensitivity for future oxidation and recovery of antioxidants, including tocopherols. Three nutritional parameters are evaluated, the free fatty acids content, the ratio of unsaturated over saturated fatty acids and the level of short chain fatty acids. These parameters were used in the Wiseman model to estimate the dietary energy contribution of lipids in feed for broilers and pigs. Three energy-diluting factors: moisture (M), impurities (I) and unsaponifiables (U) were incorporated.

Almost 66% of the samples is represented by 5 groups: soybean oil (SO), animal fat (AF), acid oils (AO), sunflower oil (SFO) and poultry oil (PO). The assessment showed that in 66% of the cases signs of oxidation were detected. Initial oxidation (PV<5meq.kg^{-1}) was determined in SO at 64%, AF at 42%, AO at 43% and PO at 9% except for SFO where 93% of the samples showed strong primary oxidation (PV>10 meq.kg^{-1}). The data confirmed a large variation in dietary energy up to 40% between identical fat sources (AF). Inclusion of MIU factor demonstrated a more excessive variation (230% AF) on nutritional value.

The analytical assessment gives insight in the large variation present between different lipid sources. The compiled data helps to fully understand the huge variability in nutritional and oxidative quality of fats and oils.

Poster 2.8: Use of a capsicum based additive improved performance of broilers under summer conditions in Spain

M. Blanch[1], G. Tedó[1], L. Mur[2], F. Bacha[3]

[1] Lucta S.A., Innovation Division, UAB Research Park, 08193 Bellaterra, Spain, [2] UVE S.A, 31500 Tudela, Spain,[3] UVE S.A, 31500 Tudela, Spain and Nacoop S.A., San Sebastián de los Reyes, Spain; marta.blanch@lucta.com

A previous study showed that the use of a capsicum oleoresin based additive (Luctarom Convert - LOM, Lucta S.A.) was able to improve dietary fat digestibility in broilers. The present study aimed to evaluate the effect of LOM on growth performance of broiler chickens under summer conditions in Spain, where animals are fed with highly concentrated energy diets. A total of 720 1 d-old male Ross 308 broilers were used until 32 d of age. A mash commercial diet based on cereal-soya and soybean oil was used comprising two feeding phases: starter (1-21 d, 2980 Kcal/kg, 1.31% dLys, 0.6% dMet, and 4.9% EE) and grower (22-32 d, 3150 Kcal/kg, 0.99% dLys, 0.48% dMet, and 6.6% EE). Animals were randomly distributed into 12 pens (60 birds/pen), and allotted to 2 experimental treatments (6 pens/treatment): control (CON, without additive) and LOM (250 ppm and 125 ppm, in starter and grower diets, respectively). Each treatment had 6 replicates of 60 birds/pen. Body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were recorded at 7, 14, 21 and 32 d of age. Data were analyzed using a mixed-effects model with repeated measures (SAS software). A significant interaction between treatment and time was observed for ADG ($P < 0.001$). ADG was improved in LOM compared CON (+4.79%) during the grower period (121.7 vs 127.5 g/d, CON and LOM, respectively); this gain in ADG was accompanied with a numerical improvement ($P=0.18$) in FCR (1.38 vs 1.36, CON and LOM, respectively). In conclusion, the use of LOM showed positive effects in growth performance during the grower phase, when animals received high energy diets.

Poster 2.9: Acaricidal activity of essential oils blend on *Dermanyssus gallinae* under in vitro conditions

J. Bošnjak-Neumüller[1]

[1] PATENT CO. DOO; jasna.bosnjak@patent-co.com

A series of experiments were conducted to evaluate the toxicity of essential oils blend (EOB) on different stadiums of Dermanyssus gallinae, hematophagous ectoparasite of laying hens. EOB consists of oils from thyme, coriander, mint, lavender, and eucalyptus plants. For testing the effect of EOB on D. gallinae, a methodology based on guidelines from EUROPEAN COMMISSION, 2011, was used in this study after minimal modifications. For determining LC90 (Probit Analysis), the concentration of EOB, varied from 0.001; 0.0012; 0.0025; 0.003; 0.005 and 0.015 µl/ml. The results of toxicity test show that 90% of adult and juvenile stadiums of D. gallinae will not survive after 24 hours of exposure with 0.0112 µl/ml concentration of tested EOB. After a period of 96 hours, the group subjected to treatment with LC90 concentration of tested EOB hatched 12.78% eggs, while 83.89% eggs were hatched in control group. When tested LC90 concentration of EOB over a period of 4, 8, 24 and 48 hours, the mortality of mites exceeded 20%, 40%, 70% and 90%, respectively. In contrast, the mortality of untreated D. gallinae individuals occurred not earlier than 48 hours after the commencement of the experimental procedure. The efficacy of the LC90 concentration of the EOB was tested in simulated conditions on different substrates. Twenty-four hours after the application of the tested EOB, the mortality of experimental individuals on the wooden substrate ranged from 86.67 to 100%, on the cardboard substrate it ranged between 86.67% and 93.33% and between 96.67 and 100% on the non-porous plastic substrate. The results of this study suggest that tested EOB can be used as an alternative to synthetic acaricides in controlling D. gallinae.

Poster 2.10: Non-chemical control of poultry red mites (Dermanyssus gallinae De Geer, 1778): phytogenic feed additives

J. Bošnjak-Neumüller[1]

[1] PATENT CO. DOO; jasna.bosnjak@patent-co.com

Infestation of laying hens with poultry red mite, Dermanyssus gallinae (De Geer 1778) is a great economical threat to the egg production industry in Europe. The objective of our research was to evaluate impact of phytogenic feed additive on D. gallinae population under field conditions. Applied feed additive consists of a blend of essential oils that predominately originate from Thymus vulgaris and Origanum vulgare. The self-controlled trial lasted 8 weeks on a farm of 17588 laying hens of line Hy-Line Brown. Modified Avivet (MA) and corrugated cardboard (CC) traps were used to monitor presence of adult, nymph, larvae and egg stadium of D. gallinae. The traps were placed on -7, 14, 35 and 42 day of the trial and collected 7 days after placement. From day 0 to 28, the additive was administered at a dose of 0.5 kg/ton of feed. The significant difference ($p<0.01$) between average number of blood-fed adults on day 0 (188) and day 49 (18.8) was detected in MA traps, as well as in CC traps on day 0 (195.95) and day 49 (13.22). On contrary, the significant ($p<0.01$) increment of the average number of partially blood-fed adults on day 0 in AV (0) and CC (0) occurred in comparison to day 49 in AV (56.15) and CC (52.72) traps. Considering that during the trial the total number of mites did not change noticeably, and the number of blood-fed adults decreased significantly, their proportion also decreased in both traps. Interestingly, the average number of eggs also decreased significantly ($p<0.01$) in both traps when comparing day 0, in AV (131.16) and in CC (134.95) to day 49, in AV (31.61) and in CC (38.44) traps. These results suggest that phytogenic feed additive has no acaricidal effects, but it could be used as a natural reppelent, to prevent blood feeding activity of mites.

Poster 2.11: What are the drivers of organic selenium forms efficacy: a comparison in broiler studies

T. Guillou[1], M. de Marco[1], M. Briens[1]

[1] Adisseo France S.A.S.; mickael.briens@adisseo.com

The role of Selenium (Se) is well established as well as the high efficacy of organic Se forms, but comparison within those sources remain controversial. Two experiments (Exp) were performed in broiler chickens (Exp1= male Ross PM3; Exp2= male Ross 308) to investigate the drivers of efficacy based on tissue Se transfer. Birds were raised from 1 to 7 d with 3 pen replicates of 14 or 25 birds in Exp1 and Exp2, respectively. Birds were fed a control diet (CT; no supplemental Se) or CT completed with sodium selenite (SS), Se-yeast (65% or 35% of Se as selenomethionine; SY65, SY35), pure forms of selenocysteine (methylselenocysteine, SeMSC; selenocystine, SeC) or hydroxy-selenomethionine (HMSeBA). Endogenous Se level of CT was 0.15 and 0.11 mg Se/kg in Exp1 and 2, respectively. All sources were supplemented at 0.3 mg Se/kg in Exp1 comparing: SS, SY65, SeMSC, SeC and HMSeBA. In Exp2 comparing: SS, SY65, SY35 and HMSeBA, plus a treatment SY65+SeC with both sources added at 0.3 mg Se/kg each, to reach 0.6 mg Se/kg. On day 7, breast muscle of 6 or 9 birds per treatment were collected and analyzed for total Se. Results expressed as mg Se/kg of dry matter muscle were in Exp 1, CT: 0.56; SS: 0.73; SeC: 0.70; SeMSC: 0.68; SY65: 1.52; HMSeBA: 1.65 (p-value < 0.001). In Exp2 results were, CT: 0.34; SS: 0.53; SY35: 0.84; SY65: 1.22; SY65+SeC: 1.18; HMSeBA: 1.33 (p-value < 0.001). Statistics confirmed the higher efficacy of organic forms (SY35, SY65, HMSeBA) compared to SS and the higher efficacy of HMSeBA compared to SY65 and SY35. It also showed that the efficacy of Se-yeasts is driven by their SeMet content, and not by their selenocysteine as SeC or SeMSC treatments showed the same efficacy as SS and SY65+SeC lead the same result as SY65 alone (P>0.05).

Poster 2.12: Anticoccidial sensitivity tests for broiler eimeria: results over the last 5 years

E. Capron[1], A.-L. Ledoux[1], D. Vancraeynest[1], E. Uyttebroek[1]

[1] Zoetis veterinarian specialized for poultry; elise.capron@zoetis.com

Coccidiosis is one of the economically most important diseases affecting in broilers. Prevention of coccidiosis depends largely on the use of in-feed anticoccidials. Poultry producers can choose between different anticoccidials on the market. For this choice, the most important parameter should be efficacy of the product. At present, the only tool to directly compare efficacy of different anticoccidials are anticoccidial sensitivity tests (AST). These are in-vivo experiments evaluating the performance of a range of anticoccidials by challenging chicks with field isolates of Eimeria parasites. A first study done in 2011 analysed 112 ASTs performed between 2002 and 2010. A decrease of efficacy was shown over time that suggesting some intensive use of certain classes of anticoccidials. We analyze here the ASTs over the last 5 years. In the ASTs, the maximal authorized treatment dosage for each of the tested molecules was applied. Statistical analysis was conducted with 32 ASTs performed between 2012 and 2017. Field isolates were collected from different farms in Europe, the Middle-East and Africa. The statistical analysis was done with objective data, based on weight and oocyst excretion. Least square means have been calculated for body weight at 22/23 days, body weight gain, feed consumption, Feed Conversion Ratio (FCR), Oocysts per gram (OPG) and mortality. The performances of the monovalent ionophores are still under those of the divalent ionophore. This analysis suggests once again the over-use of some anticoccidials resulting in lack of performances and demonstrating the need for rotation between different classes of anticoccidials.

Poster 2.13: The effect of physicochemical properties of ZnO on the bioavailability of zinc in broilers

D. Cardoso[1], A. Romeo[2], N. Meme[3], Y. Chevalier[4], A. Narcy[3]

[1] University of Lyon/ INRA, [2] Animine, [3] INRA, [4] University of Lyon; dcardoso@animine.eu

The bioavailability of a trace mineral is related to its in vivo solubility, which in turn is determined by its physicochemical properties. It is still not clear which characteristics are more relevant in affecting solubility and bioavailability of minerals. Zinc oxide (ZnO) is a common feed additive used to supplement zinc in poultry diets. However, different sources have shown different responses on bioavailability. This study hypothesized that different sources of feed grade ZnO have various physicochemical features that lead to distinct bioavailability values. Over 40 samples of ZnO have been collected from the feed industry worldwide. Samples were analyzed for density, tapped density, particle size, shape, specific surface area and dissolution kinetics. A principal component analysis (PCA) was performed to define the most relevant characteristics and categorize the samples into groups. Representative products from each family were selected for in vivo trial to measure the effect of their characteristics on the zinc bioavailability. 135 one-day-old Cobb male broilers were fed a standard starter diet from day 1 after hatching up to day 7. At day 8, animals were allocated in individual cages and fed one of each treatment. Treatments consisted of a basal diet with 23 ppm of Zinc and 7 diets with supplement ZnO or sulfate at 6 or 12 ppm. Each treatment had 9 replicates. Animals were slaughtered at day 22 and 23. Bone zinc was used to analyze zinc bioavailability. The bioavailability of the different sources varied from 49 to 160% from zinc sulfate. In conclusion, physicochemical properties of ZnO can partly explain the variability observed in terms of Zn biological value. Further works are needed to precise the most accurate set of predictors of Zn bioavailability.

Poster 2.14: An innovative source of natural yellow carotenoids as a substitute to synthetic pigment for broiler skin pigmentation

M. Castillo[1], C. Cousin[1], S. Peris[1], C. Alleno[2]

[1] Novus Europe S.A./N.V.- Edifici CEPID, Avda. Cambra del Comerç, 42,43204, Reus, Spain, [2] Zootests, Parc technologique du zoopôle, 5 Rue Gabriel, Calloet Kerbrat, 22440 Ploufragan, France; marisol.castillo@novusint.com

The use of Colortek® Yellow, a 10% natural yellow pigment, delivers a consistent and uniform coloration of egg yolks. A new trial was designed to study the efficacy of this natural yellow pigment for broiler skin coloration versus apo-ester. 1120 1-d-old male broiler chickens were divided in 8 treatments. The animals were fed ad libitum a diet with identical composition except source and level of supplemented carotenoids – apo-ester at 12 and 25 ppm for grower and finisher, respectively, or natural pigment at a ratio of 1:1, 1.25:1 and 1.5:1 versus apo-ester in both phases; canthaxanthin was used as red pigment, at 2 or 5 ppm; 8 treatments in total. Shanks pigmentation was measured at d21 and d35 (Egg Yolk Color Fan) and breast skin color at d35, on 15 animals/treatment (Broiler Color Fan and Minolta colorimeter). There were not statistical differences on growth and feed intake. Animals fed with 10% natural pigment at a ratio of 1.5 versus apo-ester showed a significantly higher coloration in shanks and breast skin at d35 (6.29 and 102.2, respectively; $P<0.001$) than the other groups receiving the same carotenoids source at lower doses, and equivalent to the apo-ester group, when using canthaxanthin at 2 ppm (5.89 and 102.1, respectively). Same results were observed when using canthaxanthin at 5 ppm; values being 6.66 and 6.95 in shanks, and 102.2 and 102.3 in breast skin, for the 10% natural yellow pigment at 1.5 dose ratio and for apo-ester, respectively. Results demonstrate that 10% natural pigment at 1.5 dose ratio versus apo-ester concentration equals the coloration of shanks and breast skin in broiler chickens versus apo-ester when combined with canthaxanthin, either at 2 or at 5 ppm.

Poster 2.15: Use of a natural antimicrobial to treat and prevent Clostridium perfringens induced necrotic enteritis in broilers

J. Pié[1], D. Díez[1], C. Domenech[1], M. E. Rosemberg[2]

[1] Biovet, S.A. (Spain), [2] Universidad Científica del Sur (Lima, Peru); ddiez_biovet@yahoo.com

Alquermold Natural (AMN) is a natural antimicrobial based on cimenol ring (botanical origin) effective against virus, bacteria and fungi. It works as an intestinal biocide to control the balance of the digestive flora. An in vivo experiment was conducted to evaluate AMN as a prevention and treatment for necrotic enteritis (NE) in broilers. Productive performance, mortality, uniformity, carcass yield and Clostridium perfringens (CP) intestinal colonies were evaluated. 504 broilers were divided into four treatment groups: non-infected control (T1); infected control (T2); infected group receiving AMN as a prevention at 0.5 kg/t through feed, continuously during all the trial (T3); infected group receiving AMN as a treatment at 1 ml/l through water for one week, starting its administration the day after the infection (T4). Birds in T2, T3 and T4 were challenged with CP in drinking water on days 19, 20 and 21 of age with 10^8 UFC/bird. Data were analyzed using PROC GLM procedures of SAS 9.1 and P value less than 0.05 was set as statistically significant.Results showed that AMN as a preventive (T3) was significantly effective in controlling NE (91.51 more grams/bird, 1.17% feed conversion reduction; compared to T2, $P<0.05$). AMN as a treatment (T4) also resulted effective in counteracting the negative effects of NE (65.34 more grams/bird, 0.58% feed conversion reduction; compared to T2), although less efficient in maintaining productive performance, carcass yield and controlling the proliferation of CP, compared to T3. In conclusion, AMN in feed can be used as a preventive for NE in poultry. Additionally, farms with clinical NE can be treated with AMN in the water. Results were corroborated in a similar trial in the USA.

Poster 2.16: Zeolites in diets for broilers

T. Egorova[1], T. Lenkova[1], I. Sysoeva[1]

[1] Federal Scientific Center "All-Russian Research and Technological Institute of Poultry" of Russian Academy of Sciences; eta164@yandex.ru

Natural zeolites are widely used in various industries and agriculture because of their unique sorptive, sieving, and catalytic properties and long periods of action. The positive effect of their use is propelled by a number of factors, principally by the composition of the zeolite tuff determining their physical and chemical properties and unique action.The aim of the study presented was the evaluation of efficiency of a mineral zeolite-based additive with high clinoptilolite content (85%) in a diets for broilers. The broilers (Cobb-500, 5 replicates of 7 as-hatch birds per treatment) were kept in cage batteries from 1 to 36 days of age. Control treatment (Trt) 1 was fed crumbled vegetable diets without zeolite; Trt 2 was fed the same diets with 2.0% of zeolite in the form of grits (<1 mm); Trt 3 and 4 were fed the same diets with 0.2 and 0.5% of micronized zeolite, respectively. Mortality in all treatments was 0%. The efficiency of the additive depended on the particle size. Live bodyweight at 36 days of age in Trt 2 was higher by 1.6% in compare to control, feed conversion ratio (FCR) lower by 0.6%. Live bodyweight in Trt 3 and 4 was insignificantly higher by 2.4 and 3.1% and FCR lower by 1.8 and 3.0%, respectively, in compare to control. Digestibility of basic nutrients in Trt 4 was higher by 2.8-3.4% in compare to control, while digestibility of nitrogen, calcium, and phosphorus was higher by 2.7; 0.5 and 0.8%, respectively. The zeolite supplements did not absorb dietary vitamins A, E and B2; to the contrary, there was a trend in Trt 2-4 towards improved absorption and accumulation of vitamins A (by 2.1-14.7%) and E (by 24.1-37.1%). Histological investigation of the intestine also evidenced certain zeolite-induced improvements in villus heights and crypt depths.

Poster 2.17: Effects of an endo-1,4-β-xylanase on energy metabolisability and performance of laying hens fed wheat-based diets

M. Francesch[1], J. Broz[2]

[1] IRTA, [2] DSM Nutritional Products; maria.francesch@irta.cat

Two studies were conducted to evaluate the effects of an endo-1,4,-β-xylanase derived from Thermomyces lanuginosus (RONOZYME WX) on AMEn of the diet and performance of laying hens fed wheat-based diets. A total of seventy-two 26-wk-old hens (Hy-line brown) were used in a balance study and kept in digestibility cages (2 hens/cage and replicate). The AMEn value of the diet was determined by the European reference method. In a 24-wk performance trial, a total of 576 hens (23-wk-old Hy-Line brown) were allocated into 72 replicate groups of 8 birds each. Wheat-soy protein concentrate (trial 1) and wheat-rye-soybean meal (trial 2) based diets were formulated to provide 16% crude protein and 2600 kcal/kg AME. Two dietary treatments were compared in each trial: T-1, a negative control fed the basal diets, and T-2, receiving basal diets supplemented with xylanase at 100 FXU/kg feed. Each treatment was assigned either to 18 replicates (AME study) or to 36 replicates (performance trial). Egg production (%), egg weight (g), egg mass production (g/day), feed intake (g/day) and feed conversion ratio (feed/egg mass) were monitored in the performance trial. The AMEn value was numerically increased by 42 kcal/kg in the enzyme supplemented diet when compared to the control one, and nitrogen digestibility was increased from 74.9 to 76.2% ($P>0.05$). Xylanase supplementation resulted in significant increases of both egg production (92.4 vs. 94.0%, $P<0.05$) and egg mass (55.0 vs. 56.3 g/d, $P<0.01$) during the overall trial period of 24-wk, and particularly in an improvement of the feed conversion ratio (2.113 vs. 2.046, $P<0.001$). In summary, xylanase supplementation to a wheat-rye based diet increased egg production and improved feed conversion of laying hens from 23 to 46 wk of age.

Poster 2.18: Effects of inorganic P sources and low doses of phytase on P and Ca retention and bone mineralisation in broiler

M. Francesch[1], P. Ader[2]

[1] IRTA, Animal Nutrition, Centre Mas Bover, 43120 Constantí, Spain, [2] BASF SE, Technical Marketing Segment Animal Nutrition, 68623 Lampertheim, Germany; maria.francesch@irta.cat

The study evaluated the effects of inorganic P sources (granulated monocalcium phosphate -GMCP or fine dicalcium phosphate –FDCP) and low doses of phytase (PHY; Natuphos E) on performance, P and Ca retention and bone mineralisation of chickens. Eight treatments in a factorial arrangement with 2 P sources x 4 diets were used (n=12; 3 birds/n). Diets were: PC and NC (9.0 or 5.8 g/kg Ca; 4.4 or 2.1 g/kg non-phytate-P), and NC supplemented with 125 and 250 FTU/kg of PHY. Performance (1-22 d), P and Ca balance (18-22 d), and tibia mineralisation (22 d) were measured. Weight gain (WG), feed intake (FI), P retention (mg/d), and tibia dry weight, ash and P weight were greater with GMCP relative to FDCP ($P<0.001$). The P and Ca retention relative to P and Ca intake was greater with FDCP ($P<0.05$). The NC diets resulted in lower WG, FI, P retention (mg/d), and tibia dry weight, ash percentage, and P and Ca weight ($P<0.05$), but in greater P and Ca retention (expressed as % of intake) relative to PC diets ($P<0.05$). Both doses of PHY increased WG and P retention, regardless of P source. The high dose of PHY also increased Ca retention and tibia mineralisation traits ($P<0.05$). Finally, there was a P source x diet interaction for Ca retained (mg/d) ($P<0.001$). Ca retained from the PC diets was greater with GMCP ($P<0.05$) and greater with FDCP in the NC diets ($P<0.05$). Both doses of PHY increased the amount of Ca retained with GMCP ($P<0.05$), whereas with FDCP the highest dose was required. In summary, the source of inorganic P affected performance, P and Ca retention and tibia mineralisation traits. Phytase at low doses increased WG, and absolute and relative amount of P retained, regardless of the P source. Phytase at 250 FTU/kg also improved bone mineralisation traits.

Poster 2.19: The effect of cereal type, pelleting temperature and enzyme (xylanase+beta-glucanase) supplementation on performance, nutrient digestibility and digesta viscosity in broiler chickens

M. Francesch[1], A. Troescher[2]

[1] IRTA, Animal Nutrition, Centre Mas Bover, 43120 Constantí, Spain, [2] BASF SE, Technical Marketing Segment Animal Nutrition, 68623 Lampertheim, Germany; maria.francesch@irta.cat

A 2x2x2 factorial study was conducted to evaluate interactions between cereal type (CER) (wheat -W vs. maize -M), conditioning temperature (TEMP) (65°C vs. 90°C), and enzyme (xylanase+beta-glucanase) supplementation (ENZ; with or without Natugrain TS) on performance (1-24 d); dry matter, nitrogen, fat and energy apparent total tract digestibility (ATTD) and AMEn (from 21-23 d); and ileal digesta viscosity (24 d) in broilers. There were 8 treatments, 8 replicates, with 3 male Ross 308 chickens each. There was a three-way interaction for average daily gain (ADG) and average daily feed intake (ADFI) ($P<0.05$). ENZ increased ADG and ADFI, but only with W diet pelleted at 65°C. Maize diets resulted in better FCR relative to W ($P<0.05$). Dry matter and energy ATTD and AMEn were greater with M relative to W diets ($P<0.001$), and greater in diets pelleted at 90°C relative to 65°C ($P<0.05$). ENZ increased ATTD of dry matter (+2.5 percent point), energy (+1.5 percent point) and AMEn (+3.5%) ($P<0.001$). There was a CER x TEMP interaction for nitrogen ATTD ($P<0.05$), and a TEMP x ENZ interaction for fat ATTD ($P<0.05$). Pelleting at 90°C tended to increase nitrogen ATTD for M diets, and ENZ increased nitrogen ATTD but only for M diet pelleted at 65°C ($P<0.05$). ENZ increased fat ATTD only with W diet pelleted at 65°C ($P<0.05$). There was a CER x ENZ interaction for ileal digesta viscosity ($P<0.001$); ENZ reduced digesta viscosity of chickens fed W diets and had no effect on those fed M diets. In conclusion, ENZ supplementation increased, on average, AMEn of W and M based diets by 109 kcal/kg and 71 kcal/kg, respectively. Pelleting at 90°C also increased the energy value of the diet. The effect of the ENZ on performance, fat and nitrogen ATTD was dependent on the CER and the TEMP.

Poster 2.20: Effect of an algae-clay complex on the performance of broiler chickens using a corn-soybean meal based diet

M. Gallissot[1], M. Rodrigez[2], L. Albino[2], M. Angeles Rodriguez[1]

[1] Olmix Group, [2] University of Viçosa; mgallissot@olmix.com

This study was set up to evaluate the effect of supplementing an algae-clay complex (MFeed+) on growth performance of broiler chickens fed with a corn-soybean diet. The experiment was conducted in the poultry facilities of the Departamento de Zootecnia at the Universidade Federal de Viçosa, Minas Gerais, Brazil. Six hundred and sixty 1-day-old Cobb 500 male chicks were randomly distributed to 3 treatments with 10 replicates per treatment and 22 chicks per experimental unit, allocated to 1 of 3 groups receiving different diets: the standard diet (C), the test diet 1 (T1), containing the standard diet supplemented with 0.1% of algae-clay complex, and the test diet 2 (T2), containing the standard diet supplemented with 0.2% of algae-clay complex. Two different feeds were distributed from D0-D22, starter feed, and D23-D35, grower feed. Group weighing of the animals (D0, D21, and D35) and daily feed intake were measured. Results were submitted to analysis of variance (ANOVA). Results show a higher weight gain for groups T1 and T2 compared to control from D23 to D35, +4% and +5% respectively, though it was not statistically significant ($P=0.065$). There were no significant differences in the whole period, despite a 2.4% increase in T1 and T2 values compared to control. There was a significant decrease of feed conversion rate for both test groups during grower period (-4% and -5% respectively, $P=0.043$), also during the whole period: -3% in both tested groups compared to control ($P=0.038$). No dose effect of the algae-clay product was observed. In the end, this study shows a positive effect of the algae-clay complex on feed efficiency of broiler chickens. Its use at 0.1% of the feed seems the most interesting to improve performance and profitability.

Poster 2.21: Evaluation of xylanase alone or in combination with xylo-oligosaccharides on performance of broiler chickens fed corn- or wheat-based diets

G. Gomes[1], T. Santos[1], R. Ten Doeschate[1], U. Aftab[1], S. Srinongkote[2], D. Creswell[3]

[1] AB Vista, Marlborough, UK, [2] ARC, Thailand, [3] Creswell Nutrition, Australia; gilson.gomes@abvista.com

This study evaluated the effects of xylanase alone (XYL) or a combination of xylanase plus fermentable xylo-oligosaccharides (XYL+XOS) on broiler performance fed energy-deficient diets based on corn or wheat. Day-old male Ross 308 broiler chicks were randomly allocated to 6 pens per treatment, with 12 chicks per pen, and assigned to a factorial arrangement (2 x 4): positive control (PC); negative control (AME reduced by 0.63 MJ/kg; NC); NC+XYL; NC+XYL+XOS, with diets based on either corn or wheat as main cereals. All diets contained 1,000 FTU/kg phytase, and were fed as pellets in three dietary phases. Feed and water were available ad libitum throughout the trial. Body weight gain (BWG) and feed intake (FI) were measured from 0-40 d. Feed conversion ratio was calculated after correcting for mortality and adjusting to equal body weights (FCR). Data was submitted to two-way ANOVA, and means separated using Student's T-test ($P<0.05$). No differences in liveability were seen throughout the experiment ($P>0.05$). There were no significant interactions or cereal effect throughout the whole experimental period ($P>0.05$). FI ($P=0.08$) and BWG ($P=0.10$) tended to be affected by diet. NC-fed birds showed the highest FI and the lowest BWG when compared to all other diets. BWG was highest in birds fed PC and NC+XYL+XOS. Birds fed PC diets had a better FCR than those fed NC diets ($P<0.05$). Birds fed XYL+XOS improved FCR in NC-fed birds ($P<0.05$), while birds fed NC+XYL did not differ from either NC or NC+XYL+XOS ($P>0.05$). Birds fed diets supplemented with XYL+XOS showed improved performance when compared to NC-fed birds, recovering bird performance to similar levels as PC-fed birds.

Poster 2.22: Effects of a diet supplemented with a phytobiotic mixture, on the gene expression profile in the intestinal mucosa of broiler chicken

M. Olvera-Garcia[1], A. Sanchez-Flores[2], G. Villar[1], A. Casarin[1], L.-M. Gomez-Osorio[1]

[1] Research and Development Unit, Grupo Nutec, Mexico, [2] Unidad Universitaria de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología, UNAM, Mexico; lmgomez@gponutec.com

A feeding trial was conducted to evaluate the effect of a phytobiotic mixture (mainly capsicum essential oil, PROFLORA®), on the intestinal transcriptome profile of broiler chickens, through next-generation sequencing of mRNA (RNA-seq). One-day-old Ross 308 broilers (n= 50), were randomly allocated to one bird per pen, during 21 days. One basal starter mash diet (St, 0 to 21 d) was formulated, resulting in two treatments (Trt). Trt 1 was a control (C) and Trt 2 was a C + 500 g/ton of phytobiotic. At 1 and 21 d, (10 chickens from each Trt) were euthanized, weighed and samples of ileum were taken for RNA extraction. RNA-seq libraries were prepared for sequencing according to the Illumina sequencing protocol. The obtained reads were mapped against the Gallus gallus transcripts obtained from the ENSEMBL database. The differential expression analysis was performed using 3 different software packages to compare the expression between the control and the phytobiotic added diet after 21 days. A total of 218 genes were identified for being differentially expressed related to the phytobiotic feed additive (128 up and 90 down). Mainly, the differentially expressed genes were related to the immune response, gut physiology and metabolism. In particular, scavenger receptor MARCO (macrophage receptor with collagenous structure) and interleukins (IL) such as IL-7, IL-8, IL-12, IL-13 and IL-22 were overexpressed. Genes related to feed efficiency and gut health were up-regulated, such as thyroid hormone receptor beta (cTR) which can affect the animal growth, development and homeostasis. The obtained results shed some light on the effect of phytobiotics in the immunity, nutrition and health of broiler chickens and its positive impact in poultry production

Poster 2.23: Optimizing feed cost and performance in broiler diets based on alternative protein sources and supplemented with a protease enzyme

G. Tactacan[1]

[1] Jefo Nutrition Inc.; gtactacan@jefo.ca

The supplementation of protease to maximize the use of alternative protein raw materials can be a viable strategy to lower feed cost. A study was conducted to determine the effects of a protease in broiler diets formulated with traditional and alternative protein sources. A total of 1200 day-old male Ross 308 were randomly divided in 4 dietary treatment with 10 replicates of 30 birds each. The first group (Control) received a traditional diet based on corn and soybean meal. The second group (Alt-Diet) received an alternative diet where soybean meal was partially replaced by rapeseed meal and meat and bone meal with standard CP and AA content as the Control. The third (Normal-PT) and fourth group (Alt-Diet-PT) received respectively the Control and Alt-Diet supplemented with protease (125 g/t) and formulated with recommended matrix for CP and AA. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and feed cost per kilogram of weight gain (FC/KG) were measured for 35 days. There were no statistical differences between the groups regarding BWG, FI and FCR, although the Control group had numerically higher BWG and lower FCR. FC/KG was increased in Alt-Diet compared to the Control, however, the use of protease reduced feed cost per unit weight gain for both types of diet. Overall, protease can be used to lower feed cost per unit weight gain while allowing nutritionists to work on a wider range of protein sources.

Poster 2.24: Gut microbial profile and broiler performance with matrix protected blend of organic acid and essential oil

G. Tactacan[1]

[1] Jefo Nutrition Inc., gtactacan@jefo.ca

The increasing public concern about the use of antibiotics in commercial poultry production has changed the ways in which producers manage the birds' overall health. Currently, additives with anti-microbial and growth promoting effects are added in poultry feeds to prevent and control gastro-intestinal tract infections that adversely affects performance. This study was conducted to determine the effects of protected organic acids (OAs) and essential oils (EOs) on performance and gut microbiota of broiler chickens. A total of 612 Ross 308 day old chicks were assigned to receive 1 of 3 treatments for 28 days: 1) basal diet with no antibiotic (T1), 2) T1 + 300 ppm of protected OAs and EOs (T2), and 3) T1 + 1500 ppm of protected OAs and EOs (T3). A completely randomized design with 3 treatments and 12 replicates with 17 birds was used. The bodyweight (BW) of birds in T2 and T3 at d 21 was increased relative to T1 ($P<0.02$,), as was the BW of birds in T2 at d 28 ($P<0.05$). Though not significant, a trend towards improved feed conversion ratio (FCR) at d 21 in T2 ($P<0.09$) and T3 ($P<0.06$), as well as at d 28 in T2 ($P<0.06$) was observed. Sequencing data at d 14 and 28 revealed retained complexity and overall structure of the ileal and cecal microbiota across treatments. However, compared to T1, significant changes in abundance of Lactobacilluswithin the cecum of birds in T2 and T3 were found at d 28. Overall, the supplementation of protected OAs and EOs had no adverse effect on microbial diversity of the intestine and appears to offer benefits on gut health and productivity of broiler chickens.

Poster 2.25: Metabolomics analysis reveals potential benefits of organic acids in broilers exposed to Salmonella pullorum challenge

D. Dai[1], G.-H. Qi[1], J. Wang[1], Y.-Y. Wu[2], Y.-M. Han[2]

[1] Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, [2] Trouw Nutrition R&D, The Netherlands; qiguanghai@caas.cn

The objective of this study was to determine the protective effects of organic acids (OA) in broilers exposed to experimentally induced Salmonella pullorum challenge at early stage, and to explore the potential benefits of OA by metabolomics analysis. A total of 672 newly hatched male Arbor Acre broiler chicks were randomly allocated into one of the 6 treatments, including non-challenged group supplied with the control diets, S. pullorum-challenged group with the control diets, challenged group with 0.2% virginamycin, challenged group receiving the drinking water added with OA blend, challenged group with OA feed additives, and challenged group receiving the combination of acidified drinking water and OA feed additives. Each group consisted of 8 replicates of 14 birds each. Results showed that early Salmonella challenge induced an acute systemic infection of broilers in starter phase, followed by the grower phase without triggering clinical signs. OA supplementation in the combination of feed and water alleviated the adverse effects of challenge on growth performance during the starter phase, and exerted growth-promoting effects during the grower phase. This positive effects were comparable to virginamycin supplementation in challenged birds. Furthermore, the abnormal levels of a variety of circulating metabolites involved in amino acid and steroid metabolism, steroid hormone biosynthesis, antioxidant and immune defense, antibiotics synthesis and xenobiotic degradation in challenged birds were reversely changed by OA supplementation. This data suggest that OA could modulate the systemic metabolic perturbation caused by salmonella challenge, which may partly explain the potential benefits of OA in birds.

Poster 2.26: The association of adipogenic transcriptional and adipocyte cellularity in broiler chicken fed with conjugated linoleic acid

S. Ramiah[1], Y. Meng Goh[1], M. Ebrahimi[2]

[1] Institute of Tropical Agriculture and Food Security, University Malasia, [2] Faculty of Veterinary Medicine; s_kumari@upm.edu.my

Conjugated linoleic acids (CLA) act as an important ligand for nuclear receptors that are crucial in adipogenesis and fat deposition in mammals and avian species. Most documented effects such as changes in cellular adiposity and lipid metabolism are well reported in the mammalian (rodent) models, or cell lines of mammalian origin. This study aimed to determine whether similar effects are plausible on avian abdominal fat adipocyte size, as well as abdominal adipogenic transcriptional level that contributed to improved leanness in the broiler chicken. CLA was supplemented at different levels, namely (i) basal diet without CLA (5% palm oil) (CON), (ii) basal diet with 2.5% Low CLA (LCLA), (iii) basal diet with 5% High CLA (HCLA). The content of cis-9, trans-11 CLA were between 1.69 to 2.3 fold greater ($P<0.05$) than that of trans-10,cis-12 CLA in the abdominal fat of the LCLA and HCLA group. The adipogenic capacity of the abdominal fat depot in LCLA and HCLA fed chicken is associated with a decreased proportion of adipose cells. The transcriptional level of aP2 (adipocyte Protein 2) and peroxisome proliferator-activated receptors gamma (PPAR γ) were down-regulated by 1.08 to 2.5 fold in CLA supplemented diets respectively. Cis-9, trans-11 CLA or trans-10, cis-12 CLA contents increased linearly with the increasing the level of CLA in the feed. CLA content in the feed influences the fatty acid composition of abdominal fat. Our data suggested that CLA down-regulated PPAR γ and aP2, which subsequently resulted in a decrease in adipocyte size, number and area of abdominal fat cells.

Poster 2.27: Effect of in-ovo supplementation of betaine on production & immunity in heat stressed broiler chickens

J. Rokade[1], M. Gopi[1], V. K. Saxena[2], S. K. Bhanja[2], K. Gautham[1]

[1] Scientist, ICAR-CARI, [2] Principal Scientist, ICAR-CARI; jaydeepvet@gmail.com

The fertile eggs of broiler breeder (CARIBRO-VISHAL) were set into an incubator and on 18th day of incubation candling was performed. Then, the fertile embryos were injected by in-ovo injection with pre-decided doses of betaine i.e, T1 (20mg/egg), T2 40 (mg/egg), T3 (60mg/egg), T4 (control). Broiler starter (ME 2896 kcal/kg & CP 22%) and finisher (ME 3000.35Kcal/kg & CP 19%) diet were given for 42 days. There was no difference in the weight of egg and chick, between and within the injected or control group. In 0-3 weeks, significant increase ($P<0.01$) in feed intake, was observed in group T_1followed by T_3. The feed intake in group T_2 and T_4 were comparable and lowest among all.In 0-3 weeks, significant increase ($P<0.01$) in body weight observed in T_3 followed by T_1. The body weight gain in T_2 and T_4were comparable and lowest among all. In 0-3 weeks, significant improvement ($P<0.05$) in FCR was observed in group T_3 (T_3- 1.55) followed by control (T_4-1.72) andT_2 (T_2-1.74) groups. However, the FCR was comparatively poor in groupT1 (T_1-1.84). In 4 to 6 weeks of age as well as in overall trial period of six weeks, the in-ovo supplementation of betaine did not cause significant improvement in feed intake, body weight gain & FCR. Moreover, In-ovo betaine injection could not produce significant change in HI antibody titre against NDV. Birds also did not show significant change in foot pad index after PHA-P injection. Though, in-ovo betaine supplementation did not cause significant change in overall hatchability, day old chick weight, growth performance, immune status and serum biochemical profile, but 60 mg of in-ovo betaine injection have produced the beneficial effects, like improves the body weight during starter phase of broiler chicken.

Poster 2.28: Improvement of broiler breeders performance by adding a potentiated ZnO source compared to standard ZnO

A. Romeo[1], M. Barzegar[2], E. Darsi Arani[2], M. Zaghari[2]

[1] Animine, [2] University College of Agriculture and Natural Resources of Tehran; aromeo@animine.eu

Zinc (Zn) is an essential trace element which plays various roles in bird metabolism. In this study, two zinc oxide (ZnO) sources were compared at two dosages in broiler breeders. A total of 200 females and 24 males (Ross 308) were used in a 84-d experiment. The trial started at 54 and ended at 65 weeks of age. Hens were assigned to 20 experimental pens and males to 4 pens. Prior to start of the dietary treatments, all birds were fed a depletion diet (without added zinc) for two weeks. Basal diets were supplemented with 70 and 100 mg/kg zinc from standard ZnO, and 70 and 100 mg/kg zinc from a potentiated ZnO source (HiZox®). Egg production and egg quality were evaluated every week. Fertility was determined with eggs incubated at the end of week 59. Hatchability was measured at the end of the experiment. For roosters, semen quality was evaluated and a sperm penetration assay was performed. Zn dosages and sources did not affect hen day egg production, egg and yolk weight. Percentage of settable egg during whole experimental period was numerically higher with potentiated ZnO. Egg shell thickness decreased following the depletion period. Supplementation of breeder diets with potentiated ZnO significantly improved mean shell thickness during the whole period of trial ($P<0.01$).

Comparing two sources of ZnO reveal that fertility of egg ($P<0.01$) and number of sperm penetrated to the perivitelline layer ($P<0.05$) were higher with potentiated ZnO compared to regular ZnO. Roosters fed potentiated ZnO recorded an increase in sperm numbers compared with those fed ZnO ($P<0.01$). Results showed that potentiated ZnO source was more efficient than regular ZnO in improving egg shell quality, and reproductive performance of male and female broiler breeders.

Poster 2.29: Effect of mentha piperita (peppermint) extract and its juice on performance, egg quality traits, hematological and biochemical parameters in laying hens

A. Rahman[1], E. E. Gultepe[2], C. Uyarlar[2], I. Sadi Cetingul[2], I. Bayram[2], A. R. Asif[1]

[1] Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore (Jhang Campus)-Pakistan, [2] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar/TURKEY; abdurrehman@uvas.edu.pk

A total of 252 Babcock laying hens were divided into 7 groups and each group was further divided into 4 subgroups having 9 hens in each. Group A served as a control while rest 6 groups as treatments groups. Group A was fed basal diet without any supplementation. Group B, C and D was offered diets supplemented with mentha oil extract at 50, 100 and 200 mg/kg of feed while groups E, F and G were having same dose of mentha juice in drinking water. The results of the study showed non-significant difference in all groups for body weight, mean weekly egg weight, mean weekly egg mass, egg production, feed intake while FCR was observed significantly better ($P<0.05$) in groups C and D and poor in groups G and F as compared with control group. Regarding egg quality traits egg shell breaking strength remained unaffected, yolk color index was better ($P<0.05$) in all supplemented groups, haugh unit showed no difference ($P>0.05$) between all the groups although a significant declining ($P<0.05$) trend was noticed among the group analysis with respect to time in groups A, E and F, and similarly no effect ($P>0.05$) was seen on internal egg quality traits during storage at 4°C for 0, 15 and 30 days. Hematological parameters and serological parameters including blood glucose, cholesterol, HDL, LDL, liver enzymes AST-ALT, total protein, IgG, phosphorus level, calcium level and egg cholesterol showed no significant differences ($P>0.05$). It is concluded that mentha oil and juice supplementation had shown better effect on FCR and egg quality especially, egg yolk, in treated groups while other parameters remained unaffected. It has been observed too that, generally, mentha oil and juice exhibited similar results which depicts the similar potential of juice as of oil.

Poster 2.30: Effect of hydroxychloride and inorganic sources of copper, zinc and manganese on performance of broilers reared under two stocking density conditions

Y. Ruangpanit[1], L. Pineda[2], D. Konkawat[1], Y. Han[2]

[1] Kasetsart University, Thailand, [2] Trouw Nutrition R&D, The Netherlands; ysungwa@hotmail.com

In a 2×2 arrangement, the effect of trace mineral source (Hydroxychloride, HyC vs Inorganic, INO) and stocking density (Normal,22 birds/pen vs High,32 birds/pen) was investigated on performance and health of broilers. Birds (n=1944) were reared in floor pens (1.5m×2m) and fed diets supplemented with 150 ppm Cu, 100 ppm Zn and 80 ppm Mn. HyC increased body weight gain by 2% in the starter phase ($P=0.01$) and enhanced feed intake during the grower phase and overall period (+2%, $P<0.05$). The birds from both treatments showed equal performance in terms of FCR in all phases ($P>0.05$). HyC tended to improve dressing percentage (+0.4%, $P=0.06$) and numerically increased breast yield, bacteria counts and mortality rate ($P>0.05$). Increasing stocking density negatively influence body weight gain (−3.2%, $P=0.001$) and feed intake (−7.3%, $P<0.0001$), but not the FCR during the overall period. The Lactobacillus decreased with high stocking density (−log 4.1, $P=0.01$), while the E coli counts, mortality rate and carcass yield were not influenced by stocking density ($P>0.05$). At d35, birds raised under normal density have a good foot-pad integrity compared to those in high stocking density. A significant interaction was observed on foot-pad score at d21, with birds given HyC and reared at normal or high stocking density, and those with INO at normal density tended to have a better score ($P=0.07$) and thereby a healthy foot-pad ($P=0.004$). The results suggest that HyC supplementation is essential when keeping more birds per pen to promote a healthy foot-pad. The effect of HyC on growth was not stable over the entire rearing period; however, HyC enhanced feed intake and positively influencing the carcass yield. Stocking density strongly influenced growth and the number of beneficial bacteria.

Poster 2.31: Effect of dietary alkaline protease supplementation on the performance of broilers and layers

M. Tempra[1]

[1] Nuevo Milenio, Inc; miriam@nuevomilenioinc.org

Partial characterization of two alkaline protease preparations and two feeding trials were conducted to determine the effect of dietary protease supplementation on the performance of broilers and layers. Proteases A and B had the same optimum pH of 9.0. The pH stability of protease A ranged from 5.0 to 10.0 while protease B ranged from 6.5 to 10.0. Protease activity was optimal at 45°C for protease A and 65°C for protease B. Thermal stability was up to 30°C and 55°C for protease A and B, respectively. In broiler study, 320 day-old broiler chicks were randomly assigned to four treatments with eight replications of 10 birds each. In layer study, 120 individually caged 20-week old pullets were randomly distributed to four treatments following a completely randomized design. Each treatment was replicated 30 times with an individually-caged pullet per replicate. The dietary treatments for broilers and layers were essentially the same. Treatment 1, basal diet; Treatment 2, reduced crude protein and amino acids (RCP/aas) diet without protease supplementation; Treatment 3, RCP/aas diet with 125 ppm protease A; Treatment 4, RCP/aas diet with 200 ppm protease B. The broiler study lasted for 42 days while the layer study lasted for 12 weeks. Results indicate that 10% reduction in crude protein and amino acid contents of the diet adversely affected the performance of broilers but not of the layers. Supplementation of the RCP/aas diets with protease A or B did not compensate for the reduction in nutrient contents of the broiler diets, which consequently decreased the profitability of broiler production. Supplementation of the RCP/aas diets with protease A or B slightly increased the profitability of table egg production.

Poster 2.32: Effect of phytase on real-time gastric acid Secretion and calcium solubility in the gizzard of laying hens

S. Lee[1]

[1] AB Vista; sophie.lee@abvista.com

An experiment was conducted to evaluate the effect of phytase on real-time gizzard pH and calcium solubility in laying hens during peak-lay. A total of 50 laying hens (33wk) were housed individually and fed a nutrient-adequate diet with or without 1500 FTU/kg Quantum Blue phytase. At 36wk, following a dietary adaption period, pH capsules (Heidelberg Medical) were administered to 4 hens per treatment for each 3h monitoring period from 6 to 22h post-egg lay (32 hens in total). Subsequent to capsule dosing, birds were humanly euthanised and a spear-tip probe used to measure gizzard pH. Real-time pH capsule readings showed a significant interaction between the time period post-egg lay and phytase ($P<0.001$), whereby pH in the gizzard was lower ($P<0.05$) in phytase fed birds at 10-14h post-egg lay compared to the control. Conversely, spear-tip probe readings were not significantly affected by time post-egg lay or phytase supplementation. Irrespective of the time post-egg lay, phytase increased ($P<0.05$) blood Ca concentrations compared to the non-supplemented control, suggesting better digestion and absorption of Ca in the diet. Phytase had no effect on soluble Ca in the gizzard, however it tended ($P<0.1$) to increase soluble Ca in the small intestine. These findings suggest that phytase increased blood Ca as a result of improved solubility and absorption of Ca in the small intestine. Increased Ca solubility could be attributed to the synergistic effect of phytase on phytate degradation and gastric acid secretions.

Poster 2.33: Synbiotic as eco-friendly feed additive in diets of laying hens during the late laying period

A. A. A. Abdel-Wareth[1]

[1] Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt; a.wareth@agr.svu.edu.eg

This study was conducted to determine the effects of supplementation of synbiotic in laying hen diets on laying performance, egg quality, nutrient digestibility, and serum metabolic profile. A total of two hundred Bovans Brown laying hens (60-week-old) were assigned to five treatment diets including synbiotic at 0, 250, 500, 750, or 1000 mg/kg, respectively, for 12 weeks. Each treatment had eight replicates with five hens each. The results revealed that incremental dietary synbiotic linearly increased ($P<0.01$) feed intake, egg production and egg mass from 60-64, 64–68, 68–72 and 60–72 weeks of age. Moreover, feed conversion ratio was significantly decreased (linear, $P<0.01$) with increasing levels of synbiotic. Egg shell thickness and haugh unit were improved ($P<0.01$) with increasing levels of synbiotic at 60-72 weeks of age. However, synbiotic supplementation did not influence other egg quality characteristics like albumen and yolk percentages. Digestibility of crude fiber, crude protein, ether extract, calcium and phosphorus linearly increased ($P<0.001$) with increasing levels of supplementation. Notably, serum cholesterol significantly decreased ($P<0.001$) with the increasing levels of synbiotic. In conclusion, inclusion of synbiotic at 250, 500, 750, and 1000 mg/kg had improved productive performance, haugh unit, shell quality, nutrient digestibility, and decreased serum cholesterol levels and can be used as an effective feed additive in laying hens during the late laying period.

Poster 2.34: Bio-equivalence of performance of laying hens fed different nutrients density diets and dietary probiotic

D. Mikulski[1]*, V. Demey*[2]*, M. Mikulska*[1]*, J. Jankowski*[1]

[1] University of Warmia and Mazur, [2] Lallemand SAS; vdemey@lallemand.com

The aim of this trial was to study the effect of nutrients density and probiotic (Pediococcus acidilactici MA18/5M) supplementation on performance of layers. Two hundred hens (Hyline-Brown) were randomly assigned to 4 dietary treatments: 2 levels of energy (2,650(SE) and 2,550 kcal (LE) of ME/kg) and 2 levels of probiotic (0(C) and $1x10e^6$ CFU/kg feed (PA)). Each group counted 50 replicates. Hens were fed a wheat-corn-soybean meal diet (mash). Production performance parameters (laying rate, exported egg mass, egg weight and feed conversion ratio (FCR)) were recorded during 16 weeks. To evaluate the effect of reducing the energy density of the diet and the effect of the addition of PA, the concept of bio-equivalence was applied. The treatment receiving standard energy without PA supplementation (SE-C) was chosen as the reference to which each of the other treatments were compared. Bio-equivalence was considered demonstrated if the 90% confidence intervals of the difference between two treatments lie in the range of the equivalence interval (EI). EI was defined as ± 3% of the Least Square Means of SE-C. Results show that reducing the energy content (LE-C) resulted in non-bio-equivalence with SE-C for most performance parameters except laying rate. Supplementation of the probiotic to this reduced energy diet (LE-PA) seems to correct for this. Performance of all parameters of this group, except egg weight, were found bio-equivalent for the SE-C group. Adding the probiotic on top of the standard diet (SE-PA) resulted in non-bio-equivalence for the egg mass, egg weight and FCR. In this case the performances were improved compared to the SE-C group. Results of this trial suggest that by adding PA to a LE diet bio-equivalent performance can be obtained then with a SE diet.

Poster 2.35: A novel phytase improves production performance, tibia ash content, P in egg and faecal P retention in laying hens

L. Star[1], C. Kwakernaak[1], R. Santos[1], D. Feuerstein[2]

[1] Schothorst Feed Research, P.O. Box 533, 8200 AM Lelystad, the Netherlands, [2] BASF SE, Animal Nutrition, Lampertheim, Germany; lstar@schothorst.nl

Efficacy of a novel phytase (Natuphos® E 5000 = NE) was tested at two dose levels on performance, tibia ash content, phosphorus (P) content in egg and apparent faecal P retention (ret P) in Dekalb White layers. Four dietary treatments with 8 replicate pens were tested. Negative control (NC) diet was low in P without added inorganic P (2800 kcal AMEn/kg, 162 g CP/kg, 3.4 g P/kg, 2.3 g IP/kg. NC was supplemented with 100 or 200 FTU NE/kg. A diet with 5.4 g P/kg served as positive control (PC) based on addition of MCP in exchange for diamol/limestone. Diets were maize-soya based (3.5 g TiO_2/kg included as inert marker) starting at 28 weeks of age during 35 days. Hens were housed in balance cages (16 hens per 2 m^2) with a wire-floor. Performance was measured from D0-28. Droppings were collected semi-quantitatively at D33+34+35 to determine ret P. Tibia were collected from 4 hens per pen at D35 to determine ash content. Ten eggs per pen were collected at D34+35 to determine P content in egg components. Phytase activity (FTU) was analysed according AOAC method. Results were analysed by ANOVA.

Addition of 100 and 200 FTU NE significantly ($P<0.05$) improved ret P by 7.4% and 10.6%, respectively, compared to NC diet. Tibia ash content was improved by addition of 200 FTU/kg compared to NC ($P<0.05$). Compared to NC, addition of 200 FTU NE improved feed intake, egg weight and egg mass to the level of the PC ($P<0.05$). P content in yolk and albumen was not affected, whereas P content in egg shell was lower for NC diet compared to the other diets ($P<0.01$).

To conclude, addition of 200 FTU NE to a NC diet low in P enhanced production performance, tibia ash content, and P content in egg shell to the level of the PC treatment by an improved ret P.

Poster 2.36: Influence of the dietary buckthorn meal in high polyunsaturated fatty acids layer diets on egg quality

T. D. Panaite[1], R. D. Criste[1], V. Nour[2], M. Ropota[1], P. R. Socoliuc[1], M. Saracila[1], C. Soica[1]

[1] National Research Development Institute for Animal Biology and Nutrition, Calea Bucuresti nr. 1, Balotesti, Ilfov, 077015, Romania, [2] Department of Horticulture & Food Science, University of Craiova, 13 AI Cuza Street, 200585, Craiova, Romania; tatiana_panaite@yahoo.com

A 6-week feeding trial was conducted on 84, Lohmann Brown layers (43 weeks) assigned randomly to 2 groups (C and E). The birds were housed in an experimental hall with controlled microclimate (16h light/24h; $T=18.66\pm1.71^0C$ and humidity=57.78±4.58%). The corn-soybean meal basal diet (2780 kcal/kg ME and 17.8% CP), was common for the two groups. The diet formulation for group E included (unlike diet C) 2% buckthorn meal and 6% flaxseed meal, which increased the concentration of carotenoids (12.05 mg/kg lutein + zeaxanthin /kg diet) and of α-linolenic acid (8.92 g%g total fatty acids) in this diet. Eighteen eggs per group were collected during the final experimental day, and physical measurements were performed on the eggs. These eggs were used to form 6 yolk samples/group (3 eggs/sample), assayed for the fatty acids and lutein + zeaxanthin. Further 18 eggs/group were also collected during the final experimental day and stored at 4^0C for 28 days. After this period of storage, 6 yolk samples/group (3 eggs/sample) were formed and assayed for oxidation stability over time. The concentration of lutein + zeaxanthin (12.76±1.26 mg/kg) in the yolk of E group eggs was almost 2-fold higher than in group C (6.48±1.77 mg/kg). Also in group E, where the concentration of α-linolenic acid (1.57 g%g total fatty acids) increased significantly ($P<0.05$) compared to group C (0.74 g%g total fatty acids), after 28 days of storage, the level of yolk malondialdehyde (1.786 mg/kg) was lower than in group C (1.885 mg/kg). The buckthorn meal supplementation of high polyunsaturated fatty acids layer diets inhibited the onset of lipid peroxidation in the yolk of the eggs enriched in polyunsaturated fatty acids.

Poster 2.37: A low level of C-reactive protein as a potential marker for lower inflammation in laying hens

A. Khadem[1], V. Slausgalvis[2], V. Kliseviciute[3], C. Gougoulias[2], J. Alsaifi[2]

[1] Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium, [2] Innovad® NV/SA, Cogels Osylei 33, 2600 Berchem, Belgium, [3] Department of Animal Sciences, Lithuanian University of Health Sciences, Veterinary Academy, Tilzes str. 18, LT-47181 Kaunas, Lithuania; A.Khadem@innovad-global.com

Earlier, it was hypothesized that growth promoting effects of Antimicrobial Growth Promoters (AGPs) in poultry feed are attributed to their non-antibiotic anti-inflammatory properties. This hypothesis predicts that alternatives to AGP can be selected by an in-vitro tests. The aim of the present study was to confirm the anti-inflammatory properties of a commercial blend of the SCFAs, MCFAs, essential oils and polyphenols (Lumance®) in an in-vitro model of inflammation and correlate it with in-vivo growth promotion in laying hens using plasma C-reactive protein (CRP) as a potential marker of inflammation. In vitro, the known AGP oxytetracycline (OTC) and Lumance® had an anti-inflammatory effect with an IC_{50} of 88 and 1400 ppm, respectively. In-vivo, Sixty Hisex Brown laying hens at the age of 30 weeks were divided into five dietary treatments (Lumance® at 0, 0.5, 1, 1.5 and 2 ppm) with three replicates of four birds each. At weeks 38, three laying hens from each treatment were euthanized and plasma level of CRP was measured. The plasma level of CRP decreased linearly by increasing the concentration of Lumance® from 0 to 2000 Kg/tone ($P = 0.0109$). We have reported previously the beneficial effects of Lumance® on production and egg quality of these laying hens. Regarding the blood parameters, change in the concentration of plasma CRP, is one of the major hallmarks of inflammation, which are associated with growth performance and several economically important parameters. These results suggest that Lumance® can reduce the inflammation in in-vitro and in-vivo model and consequently can improve the production and egg quality of laying hens. Thus, a combination of different ingredients with anti-inflammatory effect hold the promising approach to replace AGPs.

Poster 2.38: Quails as a model for egg yolk color prediction in laying hens

B. Vilà[1], S. Peris[2], V. Montoya[1], D. Ferrús[3], M. Castillo[4]

[1] IRTA, Animal Nutrition, [2] NOVUS, Head Tech. Services EMEA, [3] NOVUS, Specialist Product Research, [4] NOVUS, EMEA R&D Coordinator, Sr. Tech. Services; borja.vila@irta.cat

Using quails as a model for poultry production was already evaluated by Wilson et al (1961), and by Nelson (1966) specifically for egg yolk color. More recent references of quails as a model for chickens' egg yolk color have not been found. This work updates the one of Nelson with present birds' strains and pigments to predict chickens' egg yolk color from quails' egg yolk color. The studies were conducted on 54 cages with 8 quails and 9 cages/treatment (Tr); and on 48 cages with 3 hens and 8 cages/Tr; experimental unit was 1 cage in all cases. Basal diets were wheat-based without xanthophylls addition, that met birds' requirements for each species. A period of xanthophyll depletion was implemented, followed by the experimental period when xanthophylls were added to the basal diets. Studies had a RCBD with 1-wk previous laying rate as blocking effect. There was a total of 6 Tr, the same for both studies, that arose from the addition of canthaxanthin (CTX) combined with apo-ester (APO) or ColorTek Yellow® (NOUVS, CTY) at different levels (ppm of total xanthophylls) to cover commercial pigments used and egg yolk colors targets as follows: a) CTX1.5+APO2.5; b) CTX1.5+CTY3.1; c) CTX1.5+CTY3.8; d) CTX3.0+APO3.0; e) CTX3.0+CTY3.8; f) CTX3.0+CTY4.5. Egg yolk color was assessed by a Minolta CR300 colorimeter and DSM Yolk Color Fan (YCF) on >1000 eggs from each study (from 176 to 241 eggs/Tr); proc means, corr and glm of SAS 9.4 were used. Both YCF and ratio redness/yellowness (ab, Galobart et al, 2004) from quails' eggs yolk were well correlated ($R^2 \geq 0.9$, $P<0.01$) with the values in chickens' eggs yolk, though quails had lower values and higher variability. Chickens' eggs YCF could be predicted using quails' eggs as: $YCF_{chickens}=10.1+12.3\times ab_{quails}$ (CV=2.3%)

Poster 2.39: Similar laying hens' performance between methionine sources under tropical conditions

S. V. Rama Rao[1], M. V. L. N. Raju[1], D. Nagalaksmi[1], B. Prakash[1], D. I. Batonon-Alavo[2], Y. Mercier[3]

[1] Sri Ramadhoota Poultry Farm Pvt Ltd Kothur 501 359; India, [2] Adisseo France S.A.S. Anthony park II, 92160 Antony, [3] Adisseo France S.A.S. Anthony park II, 92160 Antony; svramarao1@gmail.com

Heat stress is known to impair performance and to induce oxidative stress in poultry. Apart from its role in protein synthesis, methionine also plays role in antioxidant defense as the precursor of bioactive compounds such as glutathione and taurine. A study was performed to compare the effects of dietary supplementation of DL-Methionine (DL-Met) or Hydroxy-Methionine (OH-Met) at the requirement in sulfur amino acids on laying hen's performance under tropical summer season. Four-hundred and forty-eight Babcock layers were randomly allocated based on their body weight in two treatments of 14 replicates each (16 hens per replicate). Hens were offered either DL-Met or OH-Met at the requirement from week 19 to 31 weeks of age. Temperature (from 27 ± 3°C at 9 am to 30 ± 5°C at 9 pm) and relative humidity (from 62 ± 20% at 9 am to 51 ± 21% at 9 pm) varied widely along the day and throughout the experimental period. Body weight increased with age and was not significantly different between treatments. Feed intakes were lower than breed standards, as influenced by heat stress but were similar between the two sources of methionine. There were no significant differences between egg mass, egg weight of hens fed OH-Met or DL-Met. Feed conversion ratio was not different between DL-Met and OH-Met. Egg density, eggshell strength and Haugh units were not significantly different between methionine sources. This study demonstrated that DL-Met and OH-Met are similar in laying hens under tropical conditions.

Poster 2.40: Effects of dietary Flos Lonicerae-Baikal Skullcap supplementation on laying performance, immunoglobulin and antioxidant status in laying hens challenged with Salmonella Pullorm

S.-G. Wu[1], H.-J. Jia[1], Xi.-C. Wang[1], H.-J. Zhang[1], J. Wang[1], G.-H. Qi[1]

[1] Feed Research Institute, Chinese Academy of Agricultural Sciences; wushugeng@caas.cn

A study was conducted to evaluate the effect of Flos Lonicerae-Baikal Skullcap (FLBS, flos lonicerae extract: baikal skullcap extract =3:2,w: w) in controlling the oral administration of Salmonella Pullorm (C79-13) in laying hens. A total of 360 hens were randomly divided into 5 treatments with 6 replicates of 12 layers each. Five treatments were employed: 1) basal diet (negative control diet, NC), 2) basal diet (positive control diet, PC), 3) based diet + 250 mg/kg FLBS (250 mg/kg FLBS), 4) based diet + 500 mg/kg FLBS (500 mg/kg FLBS), and 5) based diet + 1 000 mg/kg FLBS (1 000 mg/kg FLBS). The feeding trial included 2 phases: the first phase was feeding 5 diets for 4 wks. The second phase was the layers form 2 to 5 treatments were oral 1 ml Salmonella (1×10^9 CFU/ml) each hen for two consecutive days, and then feeding 5 diets lasted for 4 wks. The PC group decreased the EP, EW, and ADFI as compared to NC group from wk 5 to 8, without improving F/E. While the EP and ADFI were increase in the FLBS groups compared to PC group, and F/E was decreased. At wk 8, hens fed PC diet exhibited the lowest the eggshell strength, while the 500 and 1 000 mg/kg FLBS dies exhibited the higher eggshell strength. Serum IgG and IgM levels were increased in 500 and 1 000 mg/kg FLBS groups compared with PC group after oral 1 ml Salmonella for two consecutive days, and the IgG level in serum was higher in the FLBS groups compared with PC group at wk 8. The hens fed 1 000 mg/kg FLBS diet showed significantly higher total-antioxidant capacity than the other diets, while the MDA content was reduced. Therefore, dietary 1 000 mg/kg FLBS supplementation could increase the performance, eggshell strength, serum immunoglobulins and antioxidant capacity in hens challenged with Salmonella.

Poster 2.41: Effect of layer broiler breeders diet on egg production

A. Jaroszek[1], R. Borkowski[1], A. Janowski[2], J. Janowska[2], H. Malec[2]

[1] Rettenmaier, [2] Poultry Breeding Special Units MALEC; rafal.borkowski@jrs.pl

The body weight of cocks and hens in parent flocks is determined by their nutrition. The mass of body is continually monitored. Insufficient feeding causes agitation and aggression in the birds. ARBOCEL concentrate of raw fibre is characterized by a high expansion coefficient and gives the feeling of satiety. ARBOCEL contains pure fibre without proteins, fats and carbohydrates so that the birds consume a significantly smaller amount of calories when eating an unchanged volume of feed. The birds are satiated, calm and their weight is always optimal. Objective the analysis included eggs laid in total, hatching eggs, broken eggs and double-yolk eggs produced by 3 flock(group) of birds Ross 308 over the years 2015–2018. In each group of 50000 birds.

1. July 9, 2015 until: June 19, 2016 control diet X,
2. August 23, 2016 until: June 8, 2017 control diet X,
3. October 2, 2017 until: July 15, 2018, diet with 0,8% ARBOCEL after 34th week of production

The amounts of feed and water consumed by the hens were analysed and microbiological tests were performed. The hens ate feed X without ARBOCEL over the first two years. In the 3rd year, the hens ate feed X until week 34 of egg laying, and beginning from week 35, their diet included feed Y with 0,8% ARBOCEL. The hens consumed only feed X until week 34. results the statistical analysis demonstrated that a diet had no effect on the number of eggs but the volume of feed consumed by the hens and cocks in group 3 was significantly smaller. A diet had an effect on the number of small and double-yolk eggs produced. The statistically ($P \leq 0.05$) smallest number of small and double-yolk eggs was produced in group 3, with hens consuming feed Y (compared to control groups 1 and 2). No significant differences in water consumption were observed.

Poster 2.42: Effects of hydroxychloride zinc, copper, manganese used in laying hens' diet on egg production, eggshell quality and serum biochemical traits

T. T. Hoai Nguyen[1], R. A. Swick[1], M. Toghyani[1], S. Wu[1], J. R. Roberts[1], N. K. Morgan[1]

[1] University of New England, Australia; tnguye85@myune.edu.au

Hydroxychloride trace minerals (HTM) have covalent bonds and thus are less reactive in the feed and may have higher bioavailability compared to other inorganic trace minerals with ionic bonds (ITM). A study was conducted to evaluate the effects of replacing $ZnSO_4$, $MnSO_4$ and $CuSO_4$ (ITM) with HTM sources on egg production, eggshell quality and serum biochemical characteristics in Hy-line Brown layers during post-peak production. A total of 600 Hy-line Brown layers at 45 wk old were randomly distributed into 300 cages, with 10 cages (20 birds) per replicate and 15 replicate per treatment. There were two treatments (HTM or ITM) with Zn, Mn and Cu supplemented at 80, 80 and 15 ppm, respectively for 12 weeks. Egg production were recorded daily. Shell reflectivity, eggshell ultrastructure and serum biochemical parameters were assessed. Higher hen-day egg production ($P < 0.01$) and egg mass ($P < 0.01$) were recorded in hens fed HTM, without any effect on egg weight ($P > 0.05$). Eggshell colour in the HTM group was significantly darker compared to the ITM group (18.3 % vs. 19.4 %, $P < 0.01$). There was a significant effect of treatment on eggshell ultrastructural scores. Lower cap size variability, lower cap quality score, higher incidence of early fusion and lower incidence of late fusion were observed in the HTM group compared to the ITM group ($P < 0.01$), resulting in higher shell quality in the HTM group. Serum alanine aminotransferase enzyme (ALT) level was lower in hens fed HTM compared to those fed ITM (11.1 U/L vs. 13.3 U/L, $P < 0.05$), reflecting greater hepatic function in the HTM group. The results of this study indicate benefits of HTM over ITM for improving egg production, eggshell colour, shell ultrastructure and liver health.
Acknowledgements: This study was financially supported by Trouw Nutrition, A Nutreco Company

Poster 2.43: Effects of feeding isoquinline alkaloids on the grow performance of broiler chickens under heat-stressed conditions

M. Kikusato[1], G. Xue[2], A. Pastor[2], M. Toyomizu[1]

[1] Graduate School of Agricultural Science, Tohoku University, Aramaki Aza aoba 468-1, Sendai 980-8572, Japan, [2] Phytobiotics Futterzusatzstoffe GmbH, Wallufer Straße 10a, 65343 Eltville, Germany; kikusato.m@tohoku.ac.jp

Heat stress is known to negatively impact gut barrier function and broiler´s performance. This study was conducted to evaluate the effect of feeding a standardized blend of isoquinoline alkaloids (IQs) on the growth performance and gut integrity of broiler chickens reared under heat-stressed (HS) conditions. Three hundred sixty 0-day-old Ross 308 male broiler chickens were randomly distributed to two treatment groups: (1) negative control (NC) diet, no additives, or NC supplemented with IQs (provided as Sangrovit® Extra, 100 ppm). Each group consisted of 6 replicates with 15 birds per replicate. At day 14, the chicks in each diet group were divided into two groups, each of which reared at thermoneutral (TN, 21.1-24.7 °C) or constant HS (30.0-33.3 °C) conditions groups until day 42. At day 21, HS treatment significantly decreased body weight (BW), feed intake and feed conversion ratio (FCR) compared to TN birds, and these were not alleviated by feeding IQs. At day 42, IQs supplemented birds reared at HS conditions showed significantly larger BW, feed consumption and FCR (day 21-42) compared to the non-supplemental group. HS treatment significantly induced plasma lipid peroxidation (TBARS) levels and 3-methyl histidine concentration, which is a muscle proteolysis marker, and the increases was not observed in IQs group. Intestinal barrier function was evaluated by FITC-dextran administration, and an increase in the plasma concentrations was reduced in IQs group at HS conditions, indicating an improved barrier function. The results suggest that IQs can improve the growth performance under HS conditions, in which the improvement of intestinal barrier function may be involved.

Poster 2.44: Evaluation of isoquinoline alkaloids as alternative to antimicrobial growth promotors in broiler chickens

M. Kikusato[1], G. Xue[2], A. Pastor[2], M. Toyomizu[1]

[1] Graduate School of Agricultural Science, Tohoku University, Aramaki Aza aoba 468-1, Sendai 980-8572, Japan, [2] Phytobiotics Futterzusatzstoffe GmbH, Wallufer Straße 10a, 65343 Eltville, German; kikusato.m@tohoku.ac.jp

This study was conducted to evaluate the effect of a standardized blend of plant-derived isoquinoline alkaloids (IQs) as alternative to antimicrobial growth promotors (AGPs) in broiler chickens. Two hundreds eighty eight 0-day-old Ross 308 male broiler chickens were assigned to 4 treatment groups with 6 replicates (12 birds each). Birds were randomly distributed to 4 dietary treatment groups: (1) AGP-free control diet, diets supplemented with (2) oxytetracycline -30 ppm, (3) colistin sulfate -5 ppm or (4) IQs, supplemented as Sangrovit® Extra -80 ppm. Production was split into two periods: starter (day 0-21; CP 22%, ME 3200 kcal/kg) and finisher (day 22-42; CP 19%, ME 3250 kcal/kg), diets were based on corn and soybean meal and followed breeder´s recommendations. Birds had ad libitum access to feed and water. During starter period, body weight (BW), feed consumption and feed conversion ratio (FCR) did not considerably differ between the groups tested. Colistin sulfate and IQs groups exhibited larger BW at day 42 compared to the control group ($P < 0.05$, $P < 0.07$, respectively), and no difference in the BW was observed between these two groups. IQs group exhibited a higher FCR value compared to the control and AGPs groups ($P < 0.1$). Whilst plasma ceruloplasmin concentrations were not changed by feeding AGPs and IQs, SAG group showed significantly lower plasma NO levels compared to the control group. The result suggest that IQs can be used as the alternative to AGPS in broiler production, and effects might be attributable to anti-inflammatory action.

Poster 2.45: Effect of intermittent feeding, formic acid and phytase on performance, bone mineralization and pH in digestive tract of broiler chickens

S. Kristoffersen[1], Y. Bai[1], A. Benzertiha[2], K. Itani[1], D. Józefiak[2], B. Svihus[1]

[1] Norwegian University of Life Sciences, [2] Poznań University of Life Sciences; siril.kristoffersen@fkra.no

An experiment was conducted to test the hypothesis that a reduced pH of the diet and increased retention time in the crop by intermittent feeding would improve the efficacy of phytase. Male Ross 308 chickens (8 pens/10 birds per treatment) were given ad libitum or intermittently a diet with a suboptimal P content, high phytate-P and no inorganic P added. In addition, the diet had either no acid or 1% formic acid added, and no phytase or 500 FTU phytase added, thus resulting in a 2×2×2 factorial analyses of variance experimental design. Ad libitum fed birds had constant access to feed with 2x4 h darkness a day. Intermittently fed birds had limited access to feed from day 11 with four 1 h feeding bouts/d and one 2 h feeding bout/d. Experimental diets were fed from day 15 to 36. Intermittently fed birds were killed 1 h 20 min, 2 h 40 min and 4 h after the start of feeding. Samples from different segments of the digestive tract were collected for analyses of P digestibility and degradation. DM content in the crop was determined. Acid addition reduced crop pH significantly (5.05 vs 4.39), but not to the level expected by the difference in feed pH. Crop pH of intermittently fed birds decreased with time. Phytase increased BWG and improved FCR ($P<0.05$), while intermittent feeding reduced BWG. Acid reduced feed intake and tended ($P=0.086$) to reduce weight gain. No significant interaction effects were observed, apart from a tendency ($P=0.083$) for an interaction of acid and phytase, where reduced weight gain was only observed when no phytase was used. Phytase increased P content in the tibia, but no significant interaction effects with acid and intermittent feeding were observed. Further results will be presented later.

Poster 2.46: Effect of the addition of emulsifier and carbohydrase in a maize-wheat-SBM-tallow diet for broiler chickens on nutrient digestibility

M. Kubiś[1], M. Hejdysz[1], P. Kołodziejski[1], E. Pruszyńska-Oszmałek[1], M. Sasek[1], P. Konieczka[2], P. Górka[3], J. Flaga[3], S. A. Kaczmarek[4]

[1] Poznan University of Life Sciences, Poland, [2] The Kielanowski Institute of Animal Physiology and Nutrition, Poland, [3] University of Agriculture in Krakow, Poland, [4] Poznan University of Life Scienes, Poland; kubis.marta@gmail.com

Our earlier results (Kaczmarek et al., 2015) showed that use of exogenous emulsifiers (E) had a positive effect no only on fat digestibility but also on neutral detergent fibre (NDF) total tract digestibility. We assumed that improved fat digestibility reduced its content in digesta and, consequently, enhanced carbohydrate availability for microbe enzymes. Common practice is to use xylanase (X) to prevent high digesta viscosity and digestibility deterioration caused by wheat. Based on that, it can be assumed that the use of E and X may affect not only the digestibility of fats but also the additive effect on the use of carbohydrates by poultry. The experiment was conducted with 480 one day old ROSS 308 male chickens. Birds were randomly assigned to 4 dietary treatments (15 rep/ 8 birds). The 1st group was fed only a basal diet (BD): maize-wheat-SBM-tallow. The 2nd treatment consisted of a BD and an E additive, whereas 3rd group was supplemented by X. In the 4th group, E+X were added to the diet. The combined use of E and X resulted in a reduction in viscosity compared to the groups in which the additives were used individually ($P<0.001$). The simultaneous use of an E and X had a positive effect on NDF digestibility ($P<0.05$). The highest apparent prececal digestibility of crude fat and crude protein was found in group with the addition of E + X ($P<0.01$). This results show that the combined use of E & X affects the digestibility of nutrients to a greater extent than when used separately. Research supported by the National Science Centre, Poland, grant 2015/19/D/NZ9/03580

Poster 2.47: Effect of addition of emulsifier and carbohydrase in a maize-wheat-SBM-tallow diet for broiler chickens on the activity of the microflora in the caecum

M. Kubiś[1], M. Hejdysz[1], P. Kołodziejski[1], E. Pruszyńska-Oszmałek[1], M. Sasek[1], P. Konieczka[2], P. Górka[3], J. A. Flaga[3], S. A. Kaczmarek[1]

[1] Poznan University of Life Sciences, Poland, [2] The Kielanowski Institute of Animal Physiology and Nutrition, Poland, [3] University of Agriculture in Krakow, Poland; kubis.marta@gmail.com

Our earlier results (Kaczmarek et al., 2015) showed that use of emulsifier (E) had a positive effect no only on fat digestibility but also on NDF total tract digestibility. We assumed that improved fat digestibility reduced its content in digesta and, consequently, enhanced carbohydrate availability for microbe enzymes. Common feed additive in poultry feeding is also xylanase (X), which is added to prevent high digestive viscosity and decreased digestibility caused by wheat. It could be assumed that the use of E and X may have an additive effect on carbohydrate utilization by poultry by quantitative and qualitative changes in the bird's caecal microflora.The experiment was conducted with 480 one day old ROSS 308 male chickens. Birds were randomly assigned to 4 dietary treatments (15 rep/ 8 birds). The 1st group was fed only a basal diet (BD): maize-wheat-SBM-tallow. The 2nd treatment consisted of a BD and an E additive, whereas 3rd group was supplemented by X. In the 4th group, E+X were added to the diet. The addition of E increased content of Clostridium in both group, when it was used alone and in combination with X ($P<0.05$). X and E used together or separately affected the total SCFA content to the same extent ($P<0.01$). The simultaneous application of E and X resulted in an increase in the concentration of: β-glucosidase ($P<0.0001$) and β-glucuronidase ($P<0.05$) in the caecum. This results show that the combined use of E and X affects the composition and activity of the caecal microflora to a greater extent than when used alone. Research supported by the National Science Centre, Poland, grant 2015/19/D/NZ9/03580

Poster 2.48: Organic acids combined with a higher dose of medium chain fatty acids alleviates the impact of sub-clinical necrotic enteritis on performance of broiler chickens

A. Kumar[1], S. Kheravii[1], R. A Swick[1], M. Toghyani[1], S. Wu[1]

[1] School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia; akumar26@myune.edu.au

This study examined the efficacy of feed additives on growth performance and gut integrity in broilers challenged with a sub-clinical necrotic enteritis (NE). Additives were: A) blend of a phenolic compound, medium chain fatty acids, target release butyrate and organic acids (PhC+MCFA+OA); B) Higher level of OAs with MCFAs (High OA+MCFA); C) OAs combined with a higher level of MCFAs (OA+High MCFA). A total of 1404 male Ross 308 chicks were assigned to one of the six treatments: T1 - unchallenged group; T2 - challenged group; T3 - challenged + Zn bacitracin; T4 - challenged plus A at 1.5, 1.5, 0.5 g/kg feed; T5 - challenged plus B at 2.5, 2.0, 1.0 g/kg feed; T6 - challenged plus C at 2.0, 1.5, 1.0 g/kg feed in starter, grower and finisher phases, respectively. Birds were challenged with field strains Eimeria spp oocysts on d 9 and Clostridium perfringens (NE-18) on d 14. The unchallenged group had higher feed intake, body weight gain (BWG), lower FCR, and serum FITC-d compared to NE challenged groups ($P < 0.05$). Birds treated with feed additives had higher BWG compared to T2 (d 0-10; $P < 0.05$). After challenge, birds treated with C had higher BWG compared to T2 ($P < 0.05$). Birds treated with C had lower FCR than T2 (d 0-10; $P < 0.05$) but no difference was observed after the challenge ($P > 0.05$). Diet supplementation with additives mitigated the deleterious effect of challenge on overall FCR compared to T2 ($P < 0.05$). On d 16, a higher concentration of serum FITC-d was observed in T2 compared to feed additives groups ($P < 0.05$). These findings suggest that, additive C (OA+High MCFA) was more effective in alleviating the impact of NE as indicated by higher BWG and enhanced gut integrity during challenge period, albeit all the additives improved overall FCR. Acknowledgements: Trouw Nutrition, A Nutreco Company financially supported this study.

Poster 2.49: Adaptation time affects faecal P and Ca digestibility/retention of young broiler chickens

C. Kwakernaak[1], Y. Dersjant-Li[2]

[1] Schothorst Feed Research, [2] DuPont Animal Nutrition/ DuPont IB; ckwakernaak@schothorst.nl

A diet low in P is fed as negative control (NC) to broilers in phytase efficacy studies. NC supplemented with (more) inorganic P is used as a positive control (PC). The objective was to study their effect on apparent faecal digestibility (=retention) coefficient of P (dc P) and Ca (dc Ca) after a short (2 d), intermediate (8 d) and a long (13 d) adaptation time. Four dietary treatments were fed as pellets to Ross 308 male broilers housed in 6 replicate balance cages per treatment (20 birds/cage), from 5-20 days of age. NC was a maize-soya based diet (2900 kcal/kg AMEn, 210 g/kg CP, 4.7 g/kg P, 2.6 g/kg IP and 7.5 g/kg Ca). Monocalcium phosphate (MCP) was added to NC diet as +0.6, +1.2 and +1.6 g P/kg diet to obtain PC1, PC2 and PC3, respectively, keeping Ca at 7.5 g/kg by exchange of only $CaCO_3$ and diamol. Feed (3.5 g/kg TiO_2 included as inert marker) and water were freely available. Droppings were collected qualitatively at D7+8, D13+14 and D18+19+20. Results were analysed by ANOVA using time and diet as treatment factors. Dc P and dc Ca were strongly affected (P<0.001) by diet and time but also by their interaction. Dc P and dc Ca increased with time for NC but this was smaller or not present for PC diets. Calculated dc P of the added MCP-P in PC diets was on average 85, 69 and 48% at D8, D14 and D20, respectively. Although time is confounded with age of bird and thus their physiological status, the results indicated that birds fed diets with P level below requirements can adapt over time and increase the dc P, while that is not needed or to a lesser extent in diets with higher P level. This means that adaptation time affects measured response of a supplemented inorganic P source or of a phytase. The impact of this for efficacy trials should be further studied.

Poster 2.50: Adaptation time and access to litter influence P retention of hens fed a diet low in P

C. Kwakernaak[1], L. Star[1], R. Santos[1], D. Feuerstein[2]

[1] Schothorst Feed Research, [2] BASF SE Animal Nutrition; ckwakernaak@schothorst.nl

Objective was to determine the effect of feeding a low phosphorus (P) diet to hens on apparent (faecal) retention coefficient of P (rc P) after a short and a long adaptation time and giving hens access to litter or not. Two experimental treatments (Trt) were tested with Dekalb White laying hens. Hens were fed a maize-soya based diet without added inorganic P and thus low in P (2800 kcal/kg AMEn, 162 g/kg CP, 3.4 g/kg P, 2.3 g/kg phytate-P and 40 g/kg Ca) starting at 28 weeks of age (94% laying rate) for 35 days. Hens were housed in balance cages (16 hens per 2 m^2). For Trt 1, hens were housed on a wire floor, while for Trt 2 hens were housed on 1 m^2 wire floor plus 1 m^2 with wood shavings as bedding material and thus had access to litter. Droppings were collected semi-quantitatively at D5+6 of Trt 1 and at D33+34+35 of Trt 1 and 2 to determine rc P. Pelleted feed (3.5 g/kg TiO_2 included as inert marker) and water were freely available. Each Trt had eight replicates. Performance was measured over 35 days. Results were analysed by ANOVA using time (rc P at D6 vs. D35 of Trt 1) and litter access (Trt 1 vs. Trt 2) as treatment factors. Adaptation time affected rc P (P<0.001). Feed intake (FI) and rc P were affected by access to litter (P<0.001). Extending adaptation time from 6 to 35 days increased rc P from 6 to 21% in hens housed on a wire floor. Having access to litter decreased rc P from 21 to 16% at D35 and FI with 11 g/h/d. The diet served as negative control diet (NC) in a phytase efficacy trial. Results indicate that adaptation time and litter access (housing) may affect the determined absolute response of NC diet supplemented with phytase or inorganic added P. This should be further studied and considered when guidelines are set for methods in P efficacy trials.

Poster 2.51: Dose response of a phytase on (phytate) phosphorus, protein and ash digestibility and on bone ash in broilers

S. Leleu[1], E. Delezie[1], M. Lourenço[1], L. Nollet[2]

[1] ILVO (Research Institute for Agriculture, Fisheries and Food), Animal Sciences Unit, Scheldeweg 68, 9090 Melle, Belgium, [2] Huvepharma, Uitbreidingstraat 80, 2600 Berchem, Belgium; saskia.leleu@ilvo.vlaanderen.be

The objective of this trial was to investigate the effect of different doses of a phytase (OptiPhos) on the ileal digestibility and tibia quality of male broilers (Ross308). Starter diet was the same for all animals. From day 9 on, broilers were divided into eight treatment groups (pelleted grower): a positive control group (PC; corn-soybean meal based diet) and a negative control group (NC, -1.5 g/kg available phosphorus (aP) and calcium), and six different doses of the phytase (added to the NC): 250, 500, 750, 1000, 1250, and 1500 FTU/kg (T3 to T8). On day 17, broilers were transferred to digestibility units (six replicates of four or five broilers per treatment) and on day 27 ileal digesta and right tibia were collected and pooled per digestibility unit; No significant differences were found in crude protein and crude ash digestibility. Adding the phytase (except for T5), significantly increased the total P digestibility (72.5-74.8%) compared to the PC (55.8%). Concerning the phytate P digestibility, all groups had a significantly higher digestibility (range 57.6-81.1%) compared to the PC (41.1%). Compared to the NC (57.6%) only the two highest doses (T7 and T8; 74.3% and 81.1%, respectively) were significantly higher. The tibia crude ash concentration was significantly higher for all treatments (range 39.5-45.4%) compared to the NC (36.3%), where the addition of the two highest phytase doses (T7 and T8; 43.6% and 43.9%, respectively) no longer significantly differed from the PC (45.4%). Based on these results, adding the phytase at 1250 and 1500 FTU/kg could compensate for the decrease in available phosphorus.

Poster 2.52: Effect of protease on the performance of broilers fed reduced energy, protein and amino acid diets

F. L. de Souza Castro[1], T. Shieh[2], W. Kyun Kim[1]

[1] Department of Poultry Science, University of Georgia, Athens, Georgia, [2] Vitech Ultra Bioscience Corp.; flc48066@uga.edu

The aim of the study was to investigate the effect of Vitazyme Protease (provided by Vitech Ultra Bioscience Corp.) on growth performance of broilers fed diets with different energy, protein and amino acid levels. A total of 720-d-old male chicks (Cobb 500) were distributed randomly in 6 treatments, 6 replicates of 20 birds, allocated to floor pens and grown for 42 d. The treatments were: PC- recommended energy and protein level, NC – reduction in 100 kcal/kg ME, 1% in CP and 5% in Lys, Met, Thr, Met+Cys levels, T1 – NC+50 g/T, T2 - NC+100 g/T, T3 - NC+200 g/T, and T4 - NC+400 g/T of VitaPro. At 14, 28 and 42 d, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured. One-way ANOVA was performed, and means were compared by Duncan's test ($P<0.05$). From 1-14 d, BWG was higher for birds fed PC diet compared to NC, T3 and T4 ($P=0.032$). The FCR was similar between birds feed PC and T2, and these birds had lower FCR than the ones fed NC, T3 and T4 ($P=0.002$). From 14-28 d, the FCR was lower for birds fed PC compared to NC, T1, T3 and T4 ($P=0.021$). From 28-42 d, FI was lower for PC than the other treatments ($P=0.001$), and BWG was higher for birds fed T2, T3 and T4 than PC fed group ($P=0.002$). The FCR was lower for birds fed PC, T2, T3 and T4 than NC ($P=0.01$). From 1-42 d, FI was the lowest for birds fed PC (5.11kg) ($P=0.018$), and FCR was lower for birds fed PC (1.59) and T2 (1.61) compared to NC (1.69) and T1 (1.67) ($P=0.006$). In conclusion, the reduction in nutritional levels (NC) resulted in low FI, BWG and high FCR. However, the use of 100g/T of VitaPro in NC diets was able to counteract the negative effects of low nutritional levels, leading to performance results similar to those of birds fed the positive control diet.

Poster 2.53: Performance of ross 308 broiler breeders with amino acid complexed trace minerals

L. Linares[1], D. Neves[1], M. Rebollo[1], C. Aranibar[2], J. Wilson[2]

[1] Zinpro Corporation, [2] University of Georgia, Athens, USA; llinares@zinpro.com

A total of 960 Ross 308 hens + 72 roosters (24 pens, 40 hens + 3 roosters/pen) were fed experimental diets from 24 to 40 weeks of age (woa). T1: Sulfates–S (Zn, 80 ppm; Mn, 120 ppm; Cu, 15 ppm; Fe, 50 ppm); T2: S (Zn, 40 ppm; Mn, 80 ppm; Cu, 8 ppm; Fe, 0 ppm) + Amino Acid Complexed Trace Minerals–AACTM (Zn, 40 ppm; Mn, 40 ppm; Cu, 7 ppm; Fe, 50 ppm); T3: AACTM (Zn, 40 ppm; Mn, 40 ppm; Cu, 7 pm; Fe, 50 ppm) in a Complete Randomized Design with data analyzed by GLM and SLICE methods. There were no differences (P>0.05) in overall egg production, hen weekly mortality, egg weight and fertility among treatments. In the overall production period, eggs from hens that consumed S+AACTM had the best shell quality (P<0.05) measured by specific gravity, eggshell percentage, and eggshell weight. Average of egg shell color in the production period was significantly darker (P<0.10) for treatments S and S+AACTM. At 30 woa there were no differences (P>0.05) in hatchability of set eggs (HOS), hatchability of fertile eggs (HOF), and embryonic mortality. At 35 woa, eggs from hens consuming S+AACTM had the best HOS and HOF. Eggs from hens consuming the S treatment presented higher percentage (P<0.05) of late dead embryonic mortality than those of the S+AACTM and AACTM diets treatments. Similar trends were observed for the 40 woa incubation data but they were not significant (P>0.05). Chick weight differed by breeder flock age but not by treatment. Grade A and B chicks were not different (P>0.05) among treatments, however chicks from hens consuming the S+AACTM had significantly higher hematocrit than chicks from eggs where the hens were fed AACTM, and S treatment was intermediate. In conclusion, combination of sulfates and AACTM improved reproductive performance of Ross 308 broiler breeders.

Poster 2.54: Evaluation of a novel fungal β-mannanase on the performance and digestibility of broiler chickens, across a range of enzyme activity levels in feed

S. Llamas Moya[1], N. F. Higgins[1], R. Adhikari[1], S. A. Kaczmarek[2], A. Rutkowski[2]

[1] Kerry, Global Technology and Innovation Centre, Millennium Park, Naas, Co. Kildare, Ireland; [2] Poznan University of Life Sciences, Dept of Animal Nutrition, Wolynska 33, 60-637 Poznan, Poland; sara.llamasmoya@kerry.com

The efficacy and tolerance of a novel β-mannanase from Aspergillus niger [bMAN] was evaluated based on the performance and digestibility of broilers reared to 42 days. A total of 480 Ross 308 male day-old chicks were placed in one of 60 pens. Pens were blocked and randomly allocated to one of 5 treatments (N=12). Treatments followed a dose-response design, with the inclusion of bMAN at 0, 0.25x, 0.5x, 1x or 100x-times the standard recommended enzyme activity in feed (1x=100 u/kg feed). A typical 3-phase feeding program was formulated on corn, wheat, soybean meal and rapeseed meal. Feed was pelleted at 70°C. Body weight (BW) and feed intake were determined at the change of feeding phase on day 0, 10, 21 and 42, and were used to determine feed conversion ratio (FCR). On day 21, collection trays were placed on the floor of all replicates to allow for excreta sampling and subsequent determination of nitrogen-corrected apparent metabolizable energy (AME_N). Data was statistically analysed using the general liner models of R environment. BW gain was statistically influenced by treatment (P=0.0434), with heavier broilers on the 0.5x dose. Significant differences were detected on FCR (P=0.0254), with all levels of bMAN improving efficiency in comparison to the control group. Treatment had a significant effect on AME_N (P<0.001), with the 1x and 100x dose exhibiting statistical increases in energy availability. No detrimental effects on broiler performance or AME_N were seen with 100x dose, suggesting good tolerance of bMAN by broilers. In conclusion, this novel β-mannanase from A. niger delivered improvements in broiler performance and nutrient digestibility, which were observable at the lowest dose of 0.25x the recommended enzyme activity in feed.

Poster 2.55: Effects of dietary sodium diformate on broiler performance during the starter phase

C. Lückstädt[1], S. Petrovic[1]

[1] ADDCON; christian.lueckstaedt@addcon.com

Broiler growth rate and feed efficiency are key to their economic performance through to market. Nutrition plays a crucial role, particularly during the post-hatch period, since healthy gut development affects later performance. Organic acids and their salts have proved especially effective in maintaining growth performance. Previous studies on the antimicrobial impact of organic acids and their salts, including sodium diformate (NDF), placed less emphasis on the starter period. The objective of this experiment was to evaluate the impact of dietary sodium diformate (traded as Formi NDF, Addcon) in broiler starter diets until day 21, against both a negative and positive control containing an antibiotic growth promoter (AGP). A total of 180 one-day old male broilers (Ross 308) were randomly allocated to one of 3 treatment diets with 6 replicates of 10 birds each on a research farm in Iran. Experimental treatments were: negative control; AGP and 1 kg/tonne NDF. Broiler starter diets were provided as mash feed ad libitum. Body weight, feed intake and FCR were recorded. Data were analysed and a confidence level of 95% was defined. Growth performance results revealed a positive impact of the acidifier. Dietary NDF improved body weight gain compared to both the control and AGP diets (10.9% and 4.4%, respectively; $P<0.01$). Since feed intake was unaffected by diet, FCR was also improved significantly by NDF inclusion ($P<0.05$) and varied between control (1.74) AGP (1.70) and NDF (1.56). Dietary sodium diformate improves growth performance in broilers during the crucial period of early growth, not only compared to a negative control but also compared to an AGP.

Poster 2.56: Effect of methionine and guanidinoacetic acid supplementation on performance and energy metabolites in breast muscle in male broiler chickens fed corn-soybean diets

M. Majdeddin[1], A. Golian[1], H. Kermanshahi[1], S. De Smet[2], J. Michiels[2]

[1] Ferdowsi University of Mashad, [2] Ghent University; maryam.majdeddin@ugent.be

Guanidinoacetic acid (GAA) is the single endogenous precursor of creatine, and the latter plays a critical role in energy homeostasis of cells with high energy demands. Since GAA is endogenously converted to creatine by methylation, it was hypothesized that dietary GAA would have different effects on performance and breast muscle energy metabolites depending on the methionine provision in corn-soybean based diets. A total of 540 day-old male Ross 308 broilers were allocated to 9 dietary treatments with 6 replicates in a 3×3 factorial arrangement with 3 graded levels of supplementary methionine (+0.4 g/kg per level), resulting in low, medium and high level of total sulfur amino acids, and with 3 levels of GAA (0, 0.6 and 1.2 g/kg) for 42 days. Increasing methionine enhanced performance indices in all rearing periods. Relative breast weight and protein content in muscle was linearly enhanced on d25 by graded methionine. Beneficial effects of dietary GAA were inconsistent across age of birds, as it improved FCR by reduction in feed intake in grower period, whereas in finisher, GAA at 0.6 g/kg and not at 1.2 g/kg resulted in higher BW compared to control. At the end of grower, creatine was elevated when feeding GAA at 0.6 and 1.2 g/kg (4464 and 4472, respectively, vs. 4054 mg/kg fresh muscle, in control). Importantly, an interaction between methionine and GAA demonstrated that at low and high methionine, GAA at 1.2 g/kg, negatively affected growth in finisher phase, which was mainly associated with a reduction in feed intake. To conclude, the effects of dietary GAA supplementation were influenced by the dietary Met level only in the finisher period, which urges for proper sulfur amino acid formulation of diets when feeding GAA.

Poster 2.57: Effect of increasing standardized ileal digestible threonine to lysine ratios on growth performance and intestinal health parameters of coccidiosis-challenged broilers

H. Malins[1], V. Daniel Naranjo[2], O. Abiona Olukosi[3]

[1] Evonik Nutrition & Care GmbH, Pure Offices Ltd., Lake View House, Warwick, UK; [2] Evonik Nutrition & Care GmbH, Rodenbacher Chaussee 4, Hanau, Germany,.[3] Monogastric Science Research Centre, Scotland's Rural College, Edinburgh, EH9 3JG, UK; Department of Poultry Science, University of Georgia, Athens, GA 30602, USA; holly.malins@evonik.com

Coccidiosis is a poultry disease affecting intestinal morphology and nutrient utilization, potentially increasing requirements for functional amino acids such as Threonine (Thr). Thr is the third limiting amino acid in broiler diets, essential for growth and immune responses. Two simultaneous studies were conducted to determine the optimal standardized ileal digestible (SID) Thr:Lysine (Lys) ratio for 10-24d broilers under non-challenged and coccidiosis-challenged conditions. In each study, 480 male, Ross 308 broilers at 10d of age were split into 6 dietary treatments with 8 replicate pens of 10 birds each. Treatments included a positive control with SID Lys at requirement and a SID Thr:Lys of 65% and five treatments with calculated SID Thr:Lys ratios of 57, 61, 65, 69 and 73% with SID Lys at 1% of the diet (90% of the requirement). Experimental diets were pelleted, free from coccidiostats and fed ad lib from 10 to 24d of age. At 14d of age, all birds received an oral dose of distilled water in the unchallenged study and 12x the standard oral dose of a live coccidiosis vaccine in the challenged study. Bird weight and feed consumption were measured at 24d of age. The actual SID Thr:Lys ratios based on analysed diets ranged from 65 to 73% and thus the first 2 levels were not as deficient as planned. Increasing Thr:Lys from 65 to 73%, under non-challenged conditions did not affect ($P > 0.05$) any of the growth performance parameters. Under coccidiosis challenge, FCR was linearly reduced ($P < 0.05$) from 1.377 to 1.261. SID Thr:Lys of 65% was sufficient to optimize growth performance under non-challenged while higher Thr:Lys may be necessary under coccidiosis challenge to optimize FCR. Further research is needed using wider ranges of Thr:Lys ratios under coccidiosis challenge.

Poster 2.58: Effect of grinding type, particle size and xylanase inclusion in corn based pelleted diets on performance of broiler chickens

D. M. Durán[1], J. F. Pérez[1], G. González Ortiz[2], S. Villagómez Estrada[1], H. Graham[2], M. Bedford[2], D. Solà Oriol[1]

[1] Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, 08193, Spain, [2] AB Vista, Marlborough, Wiltshire, SN8 4AN, United Kingdom; diego.melo.d@outlook.com

Seven hundred sixty eight day-old broiler chicks (Ross 308) were distributed by initial body weight in twelve dietary treatments arranged as a $2 \times 3 \times 2$ factorial. Birds were placed in 96 pens (n = 8 birds/pen). With two grinding type (hammer; H vs roller; R), three corn particle size 0,5 mm, 1 mm and 1,5 mm (fine; F vs medium; M vs coarse; C) and two levels of xylanase inclusion (without; NX vs with; X, 16,000 BXU/kg), as main factors. Diets were corn-soybean meal based and were pelleted at 2.2 mm. Animals were fed from 1 to 21 d and feed was offered ad libitum. Birds were individually weighted and feed disappearance of the pen was recorded at 1 and 21 d; average daily feed intake (ADFI), average daily gain (ADG), feed to gain ratio (FCR) and coefficient of variation of body weight (CV) were calculated. Data were analyzed by Proc GLM procedure of SAS, differences considered significant at $P \leq 0.05$. Xylanase inclusion (8.11X < 9.66NX, %) and particle size (9.63F > 9.26M > 7.76C, %) influenced the CV of body weight. Interactions between grinding and xylanase inclusion were observed in ADG (48.4RX, 48.1HX and 48.5HNX > 46.9RNX, g/d/bird) and FCR (1.20RNX > 1.17RX, 1.18HX and 1.17HNX, g/g). This trial suggests that xylanase inclusion in corn-based pelleted diets improved body weight uniformity, weight gain and FCR in roller milled diets. This could be related to the rate of passage and/or the release of encapsulated nutrients from the corn with the roller milled diets being more responsive in this regards.

Poster 2.59: Inclusion level effects of a phytogenic feed additive on broiler carcass traits, availability of dietary energy and expression of genes relevant for nutrient absorptive and metabolic functions of cell growth-protein synthesis

K. Mountzouris[1], V. Paraskeuas[1], E. Griela[1], A. Kern[2], K. Fegeros[1]

[1] Department of Nutritional Physiology and Feeding, Agricultural University of Athens, 118 55 Athens, Greece, [2] Anco Animal Nutrition Competence GmbH, Linzer Strasse 55, 3100 Sankt Poelten, Austria; kmountzouris@aua.gr

The inclusion level of a phytogenic premix (PP) gut agility activator comprised of functional flavouring substances of ginger, lemon balm, oregano and thyme was investigated for its effects on broiler performance, carcass traits, nutrient digestibility, availability of dietary energy (AMEn) and expression of intestinal nutrient transporter (SGLT1, GLUT2, PEPT1, BOAT and LAT1) genes including genes FABP2 and mTORC1 relevant for cellular fatty acid uptake and protein synthesis, respectively. One-day-old Cobb broiler chickens (n=500) were assigned into four treatments, with five replicates of 25 chickens each. Depending on PP inclusion level (i.e. 0, 750, 1000 and 2000 mg/kg diet) treatments were: Con, PP750, PP1000 and PP2000. Data were analysed by ANOVA and significant effects ($P \leq 0.05$) were compared using Tukey HSD test. Polynomial contrasts tested the linear and quadratic effect of PP inclusion levels. Growth performance responses were not improved significantly ($P>0.05$) by PP inclusion. However, carcass ($P=0.030$) and breast yield ($P=0.023$) were higher in PP1000 compared to Con. In addition, PP1000 had higher AMEn ($P=0.049$) compared to PP2000 and Con. Gene expressions from 10 chickens per treatment of SGLT1, GLUT2, PEPT1, BOAT and FABP2 were not affected by PP. However, PP affected the expression of LAT1 ($P<0.001$) in jejunum and that of mTORC1 in duodenum ($P=0.010$) and ceca ($P=0.025$). In particular, their expression increased with increasing PP inclusion level in a linear and quadratic pattern depending on the intestinal segment. Overall, carcass and meat yield improvements by PP inclusion at 1000 mg/kg could be explained by the increased dietary energy available to the birds and the preliminary evidence for an improved muscle protein synthesis function.

Poster 2.60: Carbohydrase enzymes can improve feed energy in broiler diets

S. Musigwa[1], N. Morgan[1], P. Cozannet[2], R. Swick[1], S. Wu[1]

[1] School of Environmental & Rural Science, University of New England, Armidale, 2351, Australia; [2] Adisseo France SAS; smusigwa@myune.edu.au

Carbohydrase enzymes are feed additives that can potentially improve nutrient digestion, thereby increasing energy utilisation, particularly in low crude protein (CP) diets. This study examined the influence of carbohydrase enzymes (Rovabio Advance) on net energy (NE) in broiler diets containing normal and low CP diets. The study was designed as a 2×2 factorial array with two levels of CP (normal CP: 19.7% and low CP: 16.2%) and two levels of enzymes (without or with). Two batches of as hatched Cobb 500 chicks (n=32 per batch) were DNA sexed and allocated to four isoenergetic dietary treatments replicated eight times with two birds (one male and one female) housed in each respiratory chamber. The NE run was conducted in closed respiratory chambers within three consecutive days from d25 to d28 of age. The enzymes increased metabolisable energy (ME), ME/gross energy (GE) and NE ($p<0.01$, $p<0.01$ and $p<0.05$ respectively) of diets. The correlation between ME and NE values of diets is $R = 0.929$ ($p<0.001$). The ratio NE/ME was neither affected ($p>0.05$) by the enzymes nor by the level of CP in diets. Birds fed diets containing normal CP showed lower ME/GE ($p=0.001$) compared to those fed the low CP diet. Birds fed the low CP diets had decreased ($p<0.05$) body weight, feed intake, body weight gain (BWG), ME intake, NE intake, heat production, retained energy and heat increment compared to those fed normal CP. Birds fed the low CP diet had increased ($P<0.05$) feed conversion ratio, ME/BWG, NE/BWG and respiration quotient compared to those fed the normal CP diet. No interactions between the enzymes and CP level was detected ($P>0.05$). Overall, the findings from this study suggest that the enzymes effectively improve feed energy in broiler chickens in both protein levels investigated.

Poster 2.61: The influence of a phytogenic feed additive on broiler performance and immune response

A. Möddel[1], *M. Wilhelm*[1]

[1] Dr. Eckel Animal Nutrition GmbH & Co. KG; a.moeddel@dr-eckel.de

The natural defence activity (NDA) of broilers is an often-disregarded parameter despite the fact that it can give indications about animals' performance, health and immune responses. Defence activity supposedly plays a key role in animals' ability to withstand pathogens such as Eimeria—a foundation for good performance. The purpose of this trial was to investigate the effects of a phytogenic feed additive on the growth performance and NDA of broiler chicks. A total of 727 broiler chicks (Hubbard ISA) were divided into two groups. The chicks (5d – 42d) had two different feeding phases. Two different experimental diets were formulated for each phase: a basal diet without additive (NC) and a basal diet with a phytogenic additive (400 g/t Anta®Phyt; PFA). After 42 days, the broilers were weighed and slaughtered, and blood samples were taken. It was observed that the phytogenic additive has a tendency to improve performance in terms of growth rate (NC: 56.5 g vs PFA: 58.8 g) and FCR (NC: 1:1.79 vs PFA: 1:1.72). The analysis of the NDA from the blood samples showed significant differences ($p < 0.05$) between the PFA and the NC group. Each time, the phagocytic activity of neutrophils (NC: 62.2% vs PFA: 71.6%), T lymphocytes (NC: 49.4% vs PFA: 58.6%) and B lymphocytes (NC: 31.6% vs PFA: 37.0%) was significantly higher in the PFA group. This also affects lysozyme activity (NC: 47.3% vs PFA: 56.3%). Increased serum lysozyme activity can improve the cellular and innate immune response. The results of this trial indicate that the investigated phytogenic feed additive Anta®Phyt has a beneficial effect on the NDA of broilers. PFA can therefore be a contributing factor to the improvement of conditions for the performance and immune defence of broilers.

Poster 2.62: Comparison between an E. coli and a Buttiauxella phytase in broilers

L. Nollet[1]

[1] Huvepharma NV; lode.nollet@huvepharma.com

The objective of this trial was to compare an E. coli derived phytase (EC) versus a Buttiauxella derived phytase (BF) at two inclusion levels on technical performance of broilers. Broilers were randomly assigned to 6 treatment groups with 54 pens and 9 birds per pen. Broilers were fed a starter, grower and finisher diet. Feeds were corn/wheat/soy based and provided as a rough mash. Ca and P composition of the basal feeds was for (a) starter (0-14 d): 0.90 % Ca 0.45 % aP), (b) grower (14-21d): 0.75 % Ca, 0.34 % aP and (c) finisher (21-42d): 19.1 %, 0.65 % Ca, 0.31 % aP. A negative control feed (NC) was produced by reducing the positive control feed Ca and P levels by 0.15 % in all feeds replacing MCP and balancing Ca with limestone. To this negative control feed, EC was added at 250 or 500 OTU/kg while BF was added at 500 and 1000 FTU/kg. Reducing the Ca and P level reduced end weight significantly by 141 g (2562 vs 2421 g; $P < 0.05$) and increased feed conversion by 0.11 (1.67 vs 1.78; $P < 0.05$). Final body weight for the EC group was 2498 and 2535 g at 250 and 500 OTU/kg ($P < 0.05$ vs NC), while feed conversion was 1.70 and 1.65 respectively. Final body weight for BF was 2492 and 2519 g at 500 and 1000 FTU/kg ($P < 0.05$ vs NC) while feed conversion was 1.73 and 1.69 respectively ($P < 0.05$ vs NC). Technical performance was better at the double dose phytase inclusion level for both phytases. EC gave numerically higher final body weight (+ 6 g at single and + 16 g at double dose) and lower FCR (- 0.03 at single and - 0.04 at double dose) compared to BF (not sign.). It can be concluded from this trial that EC and BF do not give significant difference in performance but EC tended to give the highest technical and economic performance, both at single and double inclusion dose.

Poster 2.63: Effect of a xylanase based enzyme complex on broiler production: performance and economics

L. Nollet[1]

[1] Huvepharma NV; lode.nollet@huvepharma.com

The aim of the study was to evaluate the effect supplementing a xylanase-based enzyme complex on broiler performance in a feed already containing a probiotic. In total 160 ROS male birds were distributed over 2 treatments of 10 pens per treatment. Diets were corn/wheat/soy based including phytase (OptiPhos®, 250 OTU/kg) and a probiotic Bacillus spp. at $1.6*10^9$ CFU/kg (B-Act®). A 3-phase feed strategy was imposed: starter (day 0-10; 21.3 % crude protein, 1.05 % dig. Lys, 2296 kCal MEpoultry), grower (day 10-21; 20.0 % crude protein, 0.98 % dig. Lys, 3000 kCal MEpoultry and finisher (day 21-42; 20.3 % crude protein, 0.95 % dig. Lys, 3050 kCal MEpoultry). Feed was supplemented with our without a xylanase based enzymes complex (Hostazym® X) at 1500 EPU/kg. Body weight & daily gain (individual), feed intake and feed conversion (per pen) was recorded. Based on this parameters an economic performance was calculated per 1000 broilers produced. In general, performance of broilers was very good reaching an end weight of 2278 g with a feed conversion of 1.70. The addition of Hostazym® X resulted in a 48 g increase in final body weight (2826 g, $p< 0.05$)) with a reduced feed conversion rate of 0.03 (from 1.70 to 1.67). Based on a broiler live weight price ranging from 0.8 to 1.2 €/kg, the addition of Hostazym® X results in a financial gain of 32 € to 51 € per 1000 broilers produced. This trials demonstrates again the beneficial effect of Hostazym® X on broiler performance of which performance was already quite good.

Poster 2.64: Effect of a xylanase-based complex and a multi-enzyme cocktail broiler performance and economics

L. Nollet[1]

[1] Huvepharma NV; lode.nollet@huvepharma.com

A broiler trial was set up to compare a xylanase-based enzyme complex versus a multi-enzymes cocktail in broiler production. In total 363 ROSS 308 male broilers were distributed in 33 pens of 11 birds. Birds were provided a pelleted feed, in 3 phases: starter (d 0-10; 21.0 % crude protein, 1.07 % dig. Lys, 2985 kCal MEpoultry), grower (d 10-21; 19.8 % crude protein, 0.99 % dig. Lys, 3111 kCal MEpoultry) and finisher (d 21-35; 18.4 % crude protein, 0.92 % dig. Lys, 3158 kCal MEpoultry). The treatments were: (1) control, (2) control + endo-1,4 beta-xylanase complex at 1500 EPU/kg and (3) control + endo-1,4-beta-xylanase at 1100 VU/kg + endo1,3(4)-betaglucanase at 760 VU/kg. Body weight and daily gain, feed intake and feed conversion were measured per pen per feeding phase. General performance of broilers was very good yielding 2483 g end body weight at 35 days (control group). The addition of the endo-1,4 beta-xylanase complex (treatment 2) improved end weight to 2496 g and reduced feed conversion (corrected for 2.5 kg end weight) from 1.539 to 1.520 (not sign.). The addition of multi-enzyme cocktail (treatment 3) yielded a final body weight to 2472 g while reducing feed conversion to 1.522 (not sign.). Considering a broiler sales price ranging from 0.8 to 1.2 €/kg, the addition of both enzymes improved financial income with 8 to 20 € per 1000 broilers produced. It can be concluded that despite the fact that both enzymes did not improve technical performance significantly, that the addition of a NSPase still give financial benefit in broiler production when technical results are already good.

Poster 2.65: Comparison between 3 different phytases on the in vitro degradation of phytate of soybean and rapeseed meal

L. Nollet[1]

[1] Huvepharma NV; lode.nollet@huvepharma.com

The effect of the addition of 3 different phytases (E. coli phytase, EP), (Citrobacter phytase, RP) and Buttiauxella phytase (BP) at two inclusion levels on the phytate degradation in soybean and rapeseed meal was investigated in vitro. In this in vitro simulation of the stomach function, 1 g of either soybean meal or rapeseed meal (milled < 1 mm) was mixed with 25 ml of pepsin-HCl solution (pH 3.0) and incubated during 1.5 h at 37° C with or without the phytase at two different inclusion levels (low and high). The inclusion levels of the 3 phytases were 250 and 500 OTU/kg for EP, 500 and 750 FTU/kg for BP and 1000 and 1500 FYT/kg for CP. The trials were conducted 3 times to obtain 3 repetitions per treatment. Results were statistically evaluated by using a two-tailed t-test.

P release was increased significantly by the addition of all three phytases ($P < 0.05$). On soybean meal, EP and CP showed similar P release patterns over time for low and high inclusion doses (0.80 and 0.82 mg P/g at low and 1.22 and 1.05 mg P/g at high inclusion level after 1.5 h incubation for EP and CP respectively). However the P release by BP was much lower (0.42 and 0.61 mg P/g at low and high inclusion doses). EP at 250 OTU/kg, RP at 1000 FYT/kg and BP at 500 FTU/kg incubated with rapeseed meal released 0.81 mg, 0.91 and 0.76 mg P/kg respectively. EP at 500 OTU/kg and CP at 1500 FYT/kg incubated with rapeseed meal both released 1.07 mg P per g, while this was only 0.99 mg P per g for BP at 750 FTU/kg.

It can be concluded from this in vitro experiment that (a) P release from soybean meal and rapeseed meal by the E. coli and Citrobacter phytase was quite similar and (b) the Buttiauxella phytase released the least P from both protein sources at low and high phytase inclusion levels.

Poster 2.66: Comparison of a endo-1,4β-xylanase based base enzyme complex and a multi-enzyme cocktail on performance, carcass yield and economics in heavy weight broilers

L. Nollet[1]

[1] Huvepharma NV; lode.nollet@huvepharma.com

The intent of this trial was to evaluate an endo-1,4-β-xylanase based enzyme complex vs a multi-enzyme cocktail containing endo-1,4-β-xylanase, α-amylase and protease (on broiler production. A trial was conducted using Cobb x Cobb 500 males distributed over 35 birds per pen, 10 pens per treatment. Feeds were corn/soy based (pelleted): starter (d 0-14; 22.0% CP, 2956 kCal MEp), grower (d 15-28; 20.1 % CP, 3025 kCal MEp), finisher I (d 29-42; 19.0% CP, 3091 kCal MEp) and finisher II (d 43-49; 18.4% CP, 3113 kCal MEp). Treatments were: (1) control, (2) control + endo-1,4-β-xylanase complex (1500 EPU/kg) and (3) control + endo1-4-β-xylanase (2000 U/kg), α-amylase (200 U/kg) and protease (5000 U/kg). Technical performance was followed during each feeding phase and carcass characteristics were determined at slaughter. Results indicated that both enzymes improved technical performance, however only tr. 2 showed significant effects versus the control at d 42 and 49 resp. (+ 80 g and +130 g body weight and -0.06 and -0.08 on feed conversion (FCR) vs control. ($P < 0.05$)). Difference between tr. 2 and tr. 3 was + 70 g and + 90 g body weight, while FCR was -0.03 and -0.05 lower at day 42 and 49 resp. ($p<0.05$). WOG (breast+ wings + legs) of broilers of tr. 2 were higher vs control and tr. 3 (78.7 % vs 78.2 % and 78.1 % resp.). White meat yield of birds from tr. 2 was higher vs the control group and tr. 3 (26.0 % vs 24.9 % and 25.0 %). The economic calculation showed that an extra net saving of 0.06 to 0.10 euro per chicken produced could be obtained at 49 d slaughter by feeding the enzyme complex instead of enzyme cocktail. It can be concluded, that the enzyme complex outperforms the multi-enzyme cocktail on technical and economic performance.

Poster 2.67: Comparison between two phytases (E. coli derived or Pseudomonas produced) in broiler production

L. Nollet[1]

[1] Huvepharma NV lode.nollet@huvepharma.com

The objective of this trial was to compare the effect of two phytases at two inclusion levels on broiler performance. Broilers were randomly assigned to 2 treatment groups with 54 pens and 9 birds per pen. Broilers were fed a 3 phase feed strategy (starter, grower and finisher). Feeds contained 0.9 %, 0.75 % and 0.65 % Ca and 0.45 %, 0.34% and 0.31 % P, respectively (positive control). A negative control (NC) feed was produced by reducing the Ca and P levels of the positive control feed (PC) by 0.15 % in starter, grower and finisher, replacing MCP and limestone. To this NC, either an E. coli derived 6-phytase (EP) was added at 250 and 500 OTU/kg or a Pseudomonas 6-phytase (PP) was added at levels of 500 and 1000 FTU/kg. Reducing the Ca and P level reduced end weight by 141 g (2562 vs 2421 g) and increased feed conversion (FCR) by 0.11 (1.67 vs 1.78) ($P<0.05$). Final body weight for the EP group was 2498 and 2535 g, while FCR was 1.70 and 1.65 for single and double dose respectively. EP at single dose gave a higher end weight (+77 g) and a lower FCR (- 0.08) than the NC whereas PP at single dose resulted in very low improvement in final body weight (+6 g) but yielded a reduced FCR (- 0.08). PP at double dose increased end weight to 2546 g but did not reduce FCR compared to single dose while EP at double dose did increase final weight vs the single dose (+37 g) and reduced FCR further by 0.05. Assuming a broiler price between 0.8 and 1.2 €/kg live weight, a net financial profit of EP vs PP was calculated to be 27 to 56 € per 1000 broilers produced. It can be concluded from this trial that the E. coli derived phytase outperforms the Pseudomonas produced phytase on technical and economic performance both at single and double inclusion dose.

Poster 2.68: Plant extracts to strengthen the natural defenses of poultry: Development of a selection tool

A. Travel[1]*, E. Pampouille*[1]*, F. Alleman*[2]*, A. Roinsard*[3]*, O. Tavares*[3]*, D. Bellenot*[4]*, B. Lemaire*[4]

[1] ITAVI,[2] UniLaSalle, [3] ITAB, [4] Iteipmai; travel@itavi.asso.fr

In order to reduce antibiotics use, plant extracts supplementaion to strengthen the animal natural defences is becoming increasingly important in poultry production. However, their development as additives is hampered, in part, by the lack of robust and repeatable data related. Our aim was to design and test a method to assist in selection, from literature, plant extracts potentially interesting to strengthen natural defences of poultry. We were inspired by Anses methodology that aims to evaluate scientific publications and results (Saisine 2013-SA-0122 – Fév. 2018). We have established, with help of scientists and practitioners, two reading grids. The first allows level of reliability of bibliographic resources to be noted by verifying 1.that extract studied is correctly characterized and 2.that experimental design and results analysis are relevant to conclude about effect of extract tested. Second grid evaluates effects of extract studied on indicators of immune, inflammatory and antioxidant status of poultry. For each article, reliability score of resource is cross-referenced with effect score of extract, thus highlighting the most effective extracts. Our method has been proven. A fairly extensive bibliographic research phase on plant extracts affecting poultry natural defences, led to the selection of 917 articles, representing 48 plants. 8 of them were selected because 1.mentioned in book Bruneton "Pharmacognosie-Phytochimie-Plantes médicinales " as having effects on immunity, inflammation and oxidative stress, 2.mentioned in at least 5 articles 3.cultivable in France. Evaluation grid and methodological considerations were allowed to select astragalus, echinacea, ginseng, nigella as interesting plant extracts to strengthen poultry natural defences.

Poster 2.69: Isoquinoline alkaloids further improve performance in high-yielding broiler chickens

A. Pastor[1], R. Santos[2], L. Star[2]

[1] Phytobiotics Futterzusatzstoffe GmbH, [2] Schothorst Feed Research; a.pastor@phytobiotics.com

The aim of the study was to evaluate the effect of a standardized blend of plant-derived isoquinoline alkaloids (IQs, Sangrovit® Extra) in high-performing broiler chickens. 320 male broiler chicks (Ross 308) were kept in floor pens for a trial duration of 35 days (D0-35). Birds received standard vaccinations. Feed was based on corn, soybean meal, and wheat and contained a coccidiostat, mirroring commercial-type broiler diets. Production was split into three periods: starter (D0-14), grower (D14-28), and finisher (D28-35). Birds had ad libitum access to feed and water. Animals were randomly distributed to two treatments: 1) Negative Control (NC), no feed additive; 2) IQ: IQs (45 ppm, D0-35). Each group consisted of 8 replicates with 20 birds per replicate. No difference was found for final body weight between NC and IQ (2634g and 2638g, respectively). NC and IQ birds displayed a 300 g higher body weight than stated in the breeder´s performance objective, underlining the high performance of the animals used in the trial. For the overall trial period, FCR was improved numerically, if IQs were fed (1.39 and 1.36, respectively). This observation was significant for the starter period (1.16 and 1.09, respectively, $P \leq 0.05$). Viability was improved in IQ fed birds by 1.25 units compared to birds of the NC ($P > 0.05$, not significant). Consequently, a higher European Broiler Index was achieved in IQ supplemented birds (520 and 540, respectively).

In conclusion, IQs improved performance in high-yielding broiler chickens and can therefore support an economic and sustainable broiler production.

Poster 2.70: Effect of feeding synthetic carotenoid on immunity and shank color in broiler breeder

G. Prabakar[1], M. Gopi[1], R. Jaydip Jaywant[2], G. Kolluri[2], J. Singh Tyagi[2], J. Mohan[2]

[1] ICAR- Indian Veterinary Research Institute, Izatnagar, India 243122, [2] ICAR- Central Avian Research Institute,Izatnagar,India 243122; gopsgopi72@gmail.com

A biological experiment was carried out to assess the effect of carotenoids supplementation on body weight change, immune response (cellular and humoral) and pigmentation (yolk and skin) in broiler breeders during the hot-dry season (Temp: 42ºC; RH: 48%). Broiler breeders (N=72) of 30 weeks old were allocated to three groups (n=24) with three replicates each consisting of 8 birds. The groups are control and two levels of the combination of two synthetic carotenoids (Canthaxanthine and Apocarotenoid) supplemented each at 6 and 12 mg/kg feed. The feeding experiment was carried out for a period of 12 weeks. Blood (n=9) was collected three times at weekly interval and serum was separated to assess the humoral immune response against Newcastle disease virus vaccination at 8 weeks of feeding. The cellular immune response was evaluated at 10 weeks of feeding using a 0.1ml intra-dermal injection of Phytohaemagglutinin-P@500μg/ml at interdigital spaces. The yolk and shank colour was evaluated in all the birds at four weeks interval using DSM yolk and broiler skin colour fan. No change in body weight of the breeders was observed due to the supplementation. The cell-mediated immunity was significantly higher in birds supplemented with 12 mg/kg group followed by 6 mg and control group. The carotenoids supplementation (@6 and 12mg/kg) exhibited higher ($P < 0.05$) serum antibody titre levels than the control. The yolk colour was significantly ($P < 0.05$) improved in 12 mg/kg group followed by 6 mg/kg and control group. Shank colour was higher ($P < 0.05$) in 12 mg/kg fed group when compared to other groups (6 mg and control). In conclusion, the supplementation of the combination of synthetic carotenoids at 12 mg/kg each improved the immune response and pigmentation in broiler breeders.

Poster 2.71: Effect of supplementation broiler chicken diet with emulsifier and carbohydrases on gut enzyme activity

E. Pruszyńska-Oszmałek[1]

[1] Department of Animal Nutrition, Poznan University of Life Sciences, Poznań, Poland; ewaprusz27@gmail.com

Feed additives are used to improve the performance of broiler chickens. Due to the fact that the chicken gastrointestinal tract is an aqueous environment, transport and absorption of hydrophobic substances such as free fatty acids is difficult. Therefore, emulsifiers in a natural way mediate and improve these processes. However, the consequence of reduced fat content in digestion is to improve the availability of carbohydrates for the intestinal microflora which may adversely affect the digesta viscosity or/and digestibility deterioration. It is believed that the addition of carbohydrases may prevent this phenomenon. The aim of present study was to examine the effect of feed additives such as emulsifiers and carbohydrases in various types of broiler diet on the activity of digestive enzymes.

Two experiments were conducted, in the first experiment ROSS 308 male (n=10 birds per group) chickens received a maize-triticale diet, in the second one broilers were fed maize–rapeseed meal diet. In each experiment, birds were divided into four groups: control, emulsifier, carbohydrases and emulsifier + carbohydrases (in exp. 1. Econase XT, in exp. 2. Superzyme OM). The effect of additives was more visible in experiment one. We observed significant differences in the amylase activity determined in the pancreas between the groups receiving feed additives and the control group. In the duodenum content, lipase activity decreased after using an emulsifier, while the activity of amylase increased after adding xylanase to the diet. The changes of trypsin activities in duodenum content were also found. However, we did not find a more beneficial effect of adding both additives at the same time than using them separately.

The research was supported by Poland NCN 2015/19/D/NZ9/03580

Poster 2.72: A modelling approach to understanding the interactions between calcium, non-phytate phosphorous, phytate phosphorus and phytase in broiler nutrition

F. Salisbury[1], *A. Cowieson*[2], *R. Gous*[1]

[1] University of KwaZulu-Natal, [2] DSM Nutritional Products; salisburyf@gmail.com

A modelling approach to broiler nutrition allows more precise feeding to meet the specific objectives of the producer. This paper describes the initial stages of development of a model for calcium and phosphorus growth. This is based on an existing model which accurately predicts broiler growth and feed intake. The relationship between the potential growth of ash and that of body protein was assumed to be isometric and the calcium and phosphorus content of the ash were fixed at 22 and 18% respectively. The potential growth of these minerals as calculated from the model was compared with data from previous studies, many of which included the application of phytase to improve phytate phosphorus availability. This comparison highlighted the challenges which face nutritionists in providing sufficient calcium and phosphorus for skeletal development: the interactions between calcium, non-phytate phosphorus and phytate phosphorus may prevent birds from consuming and absorbing sufficient amounts of these nutrients. Supplying larger amounts in feed may exacerbate rather than mitigate these effects. The potential of phytase to improve digestibility will also be taken into account as the interactions between calcium and phosphorus are modelled in the digestibility module. This will allow the growth model to work in conjunction with feed formulation and objective optimization to provide an optimal feeding strategy for the producer.

Poster 2.73: The effect of feeding Diamond V fermentation metabolites on reducing salmonella prevalence, numbers, and antibiotic resistance in fecal samples from commercial layers in Canada

E. Gingerich[1], D. Smith[1], W. Michael[1], M. Farmer[1], S. Riggs[1], A. Byrd[1], S. Carlson[2], F. Portela[1], H. Pavlidis[1]

[1] Diamond V, [2] Iowa State University; dsmith@diamondv.com

A field study was conducted in commercial laying hens to determine the effects of feeding the fermentation metabolites of Original XPC Ultra (FM) on reducing the prevalence, numbers, and antibiotic resistance of Salmonella in naturally challenged layers. Two layer flocks within a single company were evaluated. Novogen White layers were reared in a common growing unit and fed a commercial standard pullet diet from hatch to 18 weeks. At 18 weeks, the flock was split into one of two lay houses. One house remained on a commercial standard layer diet (CON) and one house was fed the CON diet containing FM at 0.625 kg/MT. At 49 weeks of age, individual cloacal swabs were taken from 400 birds per house. Cloacal swabs were analyzed for prevalence and numbers of Salmonella, with recovered colonies tested for antibiotic resistance using a panel containing 19 different antibiotics. Data were analyzed in SAS with feeding treatment as the main effect and significance considered at $P < 0.05$. FM significantly reduced Salmonella prevalence in layer cloacal swabs compared to CON (31.0% vs. 43.0%, respectively). A significant reduction was also observed for cloacal swab Salmonella numbers in FM fed birds vs. CON (5,644.9 vs. 35,311.2 CFU/g, respectively). For antibiotic resistance testing, a total of 9,921 individual Salmonella isolates were tested, and the inclusion of FM in the laying hen diets resulted in a significant reduction in the resistance of Salmonella isolates to 18 of the 19 antibiotics tested when compared to the CON fed layers. Overall these data suggest that the addition of FM to the diet of commercial layers can be an effective intervention for the reduction of Salmonella prevalence and numbers as well as restoring antibiotic sensitivity of pathogenic bacteria.

Poster 2.74: A multi-enzyme complex with ferulic acid esterase improves nutrient digestibility and apparent metabolisable energy of broiler diets

D. Gonzalez Sanchez[1], L. Thijs[1], I. Somers[1]

[1] Kemin Europa N.V.; David.GonzalezSanchez@kemin.com

Feed enzymes (especially xylanase, cellulase and beta-glucanase) are commonly added to broiler diets to counter the possible antinutritional effects caused by non-starch polysaccharides (NSP). Esterified (di)ferulic acids, present in the complex structure of the plant cell wall, limit the access of these exogeneous hydrolases to their respective substrates. Feruloyl esterase (FAE) catalyzes the cleavage of these ester bonds, enhancing the degradation of the plant cell wall by the main chain hydrolases. The present study was conducted to evaluate the effect of a multiple enzyme preparation containing xylanase, cellulase, beta-glucanase, amylase and FAE on the nutrient digestibility and AME_n of corn-soybean meal based broiler diets. Ross 308 male broilers (10 per pen and 8 replicates per treatment) were assigned to three dietary treatments: a positive control (PC) diet and a negative control (NC) diet with and without the addition of the multi-enzyme product at 250 ppm. The NC diet was formulated with a reduction of 65 kcal/kg of AME_n and 1.5% less amino acids and crude protein than the PC diet. Nutrient digestibility and AME_n were determined using a titanium dioxide tracer method. The addition of the multi-enzyme product to the NC diet significantly increased dry matter digestibility (68.7% vs 67.0 %; $P<0.05$), nitrogen retention (60.0% vs 56.8%; $P<0.05$) and starch digestibility (97.4% vs 93.2%; $P<0.05$). The multi-enzyme product significantly increased the AMEn of the NC diet (3,015 kcal/kg vs 2,826 kcal/kg; $P<0.05$), similar as those obtained with the PC diet (3,034 kcal/kg). This study demonstrates that adding multiple enzyme preparations containing FAE could improve the nutrient digestibility and AME_n of energy and protein low density broiler diets.

Poster 2.75: Efficacy of synbiotic application on health status and performance of broilers compared with antibiotic growth promoters

B. Syed[1]*, M. Mohnl*[1]

[1] BIOMIN Holding GmbH, Erber Campus 1, Getzersdorf, Austria; basharat.syed@biomin.net

Synbiotics and probiotics have gained considerable interest in the poultry industry as alternatives to antibiotics due to the concerns regarding antibiotic resistance. This study compared the effects of dietary inclusion of a multi-species poultry specific synbiotic product alone or in different combinations with Bacitracin and Colistin as AGPs, on performance parameters in broilers. A 42 days study trial was conducted with 1260 Ross 308 male day old broiler chicks assigned randomly to seven treatments, each comprising of 6 replicates with 30 birds per replicate.

The dietary treatments included a corn-soybean based control diet without growth promoter (T1) and the treatment diets containing either Bacitracin, (100 ppm, T2), Colistin (10 ppm, T3), Synbiotic (PoultryStar® me, 0.5 kg/t, T4) or a combination of synbiotic and Bacitracin (T5), Synbiotic and Colistin (T6) and Synbiotic, Bacitracin and Colistin (T7). Synbiotic application resulted in significantly higher body weight gain (BWG) than its combination with Bacitracin (P<0.05) during the critical phase of rearing from 0-9 days. None of the other treatments showed any significant improvement in BWG, feed intake (FI) or feed conversion ratio (FCR) compared to the Synbiotic application (T4) during the overall rearing period from 0-42 days (P<0.05). FCR was lower in the synbiotic group (T4, 1.87) than control (T1, 1.93) during overall rearing period. Bird mortality was also lower in the synbiotic group (T4, 1.11%) than control (T1, 2.78%). The synbiotic evaluated (PoultryStar® me) could serve as a replacement and an effective alternative to AGP's like Bacitracin and Colistin in broiler feeds. The synbiotic thus, can serve this purpose without any combination with these AGP's for a cost effective broiler production.

Poster 2.76: Effect of a new formulation of essential oil compounds on growth performance and some aspects of gastrointestinal functionality in broiler chickens

J. Zentek[1]*, A.-L. Mary*[2]*, A.-A. Séon Simon*[3]

[1] Department of Veterinary Medicine, Institute of Animal Nutrition, Freie Universität Berlin, 14195 Berlin, Germany, [2] DSM Nutritional Products Ltd, BP 3255, CH-4303 Kaiseraugst, Switzerland, [3] DSM Nutritional Products France, Research Center for Animal Nutrition & Health, 68128 Village Neuf, France; Juergen.Zentek@fu-berlin.de

This study evaluated the impact of a new formulation of pure essential oil compounds (thymol, eugenol, piperine: Crina® Digest) on broiler's growth performance and some aspects of gastrointestinal functionality. During the 35 days experiment a total of 480 days-old healthy male broiler chickens (Cobb 500) were randomly allocated to two treatments with 12 replicates per treatment. Each replicate consisted of 20 birds per pen. Birds were fed a pelleted basal diet with or without addition of Crina® Digest at the recommended commercial use level (40 mg/kg feed). Zootechnical impact was determined on performance (i.e. body weight, body weight gain, feed intake, feed conversion ratio), health status, and litter quality (i.e. scoring, moisture content, pH, and nitrogen content). Results demonstrated that overall body weight gain increased by 1.2% compared to birds fed without supplementation. Benefits of Crina® Digest on body weight gain resulted in a significantly reduced adjusted with final body weight feed conversion ratio in comparison to the control group (-2.1%; p=0.042). Crina® Digest seemed to be effective on improving litter quality, as characterized by a significant reduction of pH and slightly diminished nitrogen contents in litters at day 21 and day 35 on trial. Moreover, the treatment birds had low incidence rate on foot pad dermatitis. These findings suggest that supplementation of Crina® Digest had positive impacts on broiler's performance and litter quality.

Poster 2.77: Insoluble fibre in nutrition of reproductive flocks of meat hens

R. Borkowski[1], A. Jaroszek[1], A. Janowski[2], J. Janowska[2], H. Malec[2]

[1] Rettenmaier, [2] Poultry Breeding Special Units MALEC; rafal.borkowski@jrs.pl

Fibre is an important ingredient of feed. The structure of fibre affects it's physiological functions and that's why fibres must be correctly classified. Solubility is the principal property used to distinguish available types of fibre. Analyses of intestinal peristalsis and associated health topics have demonstrated advantages of insoluble fibre compared to soluble fibre. Traditional sources of fibre are frequently contaminated with mycotoxins and with low content of insoluble fibre. The Arbocel concentrate contains at least 65% of raw fibre, being 100% insoluble fibre. Arbocel is obtained in a fibrillation process designed to produce long and thin particles: diameter of 25-30µm and a length of 200-300 µm, characterized by capillary action that improves their capability of biding water. Such fibers promoting efficient functioning of enzymes and micro-organisms. TRIAL The tests were performed at a meat-hen farm in the egg-laying period -3 flocks of 50000 birds.0,8% ARBOCEL was given in weeks 35 to 56.Our analyses included: consumption of feed[g], water intake [ml per egg], the number of double-yolk eggs and the number of broken eggs in egg-laying periods in the three most recent production cycles. The first analysis weeks 21 to 35 of the egg-laying period with no ARBOCEL given. The second analysis included data obtained in weeks 35 to 56 of the egg-laying period when feed with an ARBOCEL addition was given. RESULTS The analyses demonstrated that an addition of ARBOCEL fibre resulted in a greater number of eggs, a smaller number of broken and double-yolk eggs, an improved quality of bedding material and a lower incidence of footpad dermatitis. An extended time of feed intake, improved survivability and better uniformity of body weights within the flock were observed

Poster 2.78: Effect of matrix encapsulated butyrates as performance enhancers for broiler chickens

P. Vervenne[1], K. Kozłowski[2], B. Boomsma[1], J. Jankowski[2], H. van den Bighelaar[1]

[1] Palital Feed Additives,[2] Olsztyn University of Warmia and Mazury; p.vervenne@palital.com

Introduction: The study was carried out at the University of Warmia and Mazury, Poland. The goal of the study was to test the dose equivalence of a 50% and a 70% butyrate product on the performance of broilers. Intest-Plus Aeon is a 50% butyrate, Intest-Plus Quattro is a 70% butyrate. Both products are matrix encapsulated and contain sodium and calcium butyrate. Material and methods: The trial consisted of 5 treatments of 10 pens each. Each pen contained 9 healthy Ross 308 male broilers. A control starter diet and 2 regimes of both the 50% and 70% butyrate (1000, 600 g/T) were tested to day 14. The finisher feeds contained either 0 or 300 g/T of the butyrate formulations. The trial duration was 35 days. Feed and water were available ad libitum. Data was analysed by ANOVA with pen as experimental unit.

Results: All dosages of butyrate improved the performance of the broilers compared to the control group. Statistical significance was set at $P<0.05$. At day 35, higher dosing of the 50% butyrate gave a higher BW, as did both regimens of the 70% butyrate. In these treatments, day 35 BW increased from 1.96 kg to over 2.06 kg. The higher starter dose of the 70% butyrate also gave a FCR reduction from 1.56 to 1.50 kg/kg at day 35. Feed intake did not differ between treatments. Conclusions: Generally, increased dose levels of butyrate formulations can be progressively beneficial for feed efficiency. This is consistent with the notion that butyrate can boost gut and flora development and thus aid feed utilisation. A higher dose of butyrate formulation in starter feeds can be beneficial for performance in unchallenged rearing conditions, as indicated by the equivalent performance of the 600 g/T of 70% butyrate and 1000g/T of 50% butyrate treatments.

Poster 2.79: Comparing the ileal amino acid digestibility of barley, winter oats and spring oats and the effect of using beta glucanase with broiler chickens

M. A. Rawash[1], N. Such[1], I. Koltay[1], L. Pál[1], L. Wágner[1], J. Poór[1], Á. Mezőlaki[2], A. Márton[1], A. Molnár[1], K. Dublecz[1]

[1] University of Pannonia, [2] Agrofeed Ltd.; mohamedali@post.agr.cu.edu.eg

A digestibility trial was carried out to compare the amino acid digestibilities of oats and barley. One barley, one winter oat and two spring oat varieties were incorporated into a corn, soybean and maize starch containing basal diet at 40% on the expense of starch, and the ileal digestibility of amino acids of the cereals was calculated with linear regression (Kluth and Rodehutscord, 2006). Five week old broilers were kept in individual cages and fed the experimental diets in 6 replicates. After 3 days adaptation period, the daily feed intake of birds were measured. After the 6th day, chickens were asphyxiated with CO_2 and the terminal two third of the ileal contents were removed. TiO2 was used as a marker. The beta glucanase activity of the enzyme supplemented diets was 20.000 beta glucanase unit (Econase GT 200 P). The results were evaluated by a two factorial ANOVA (cereal grain, enzyme). The comparison of the true ileal amino acid digestibility of the experimental diets showed that the cereal and enzyme effects were amino acid dependent. Neither factor caused significant differences in the digestibility of MET and HIS. Only the enzyme resulted in improvement in the absorption of THR and ILE, while only cereal effect was found in the case of VAL, PHE and LYS. Both factors modified the digestibility of CYS, LEU, ARG and TYR. The interaction between the two factors was not significant in any case. The digestibilities of ILE, LEU, MET of oats were close to the table values (NRC, 1994; EVONIK, 2010), however the measured digestibility of ARG, HIS, LYS and PHE were 6 to 13% lower. In the case of barley, the measured digestibility coefficients were lower than the table values in most cases. The differences were the highest in the case of HIS, ILE, LYS and VAL.

Poster 2.80: Zinc amino acid complexes improve performance of broilers in heat stress conditions

A. De Grande[1], S. Leleu[2], E. Delezie[2], C. Rapp[3], R. Ducatelle[1], F. van Immerseel[1]

[1] Ghent University, [2] Institute for Agricultural and Fisheries Research, [3] Zinpro Corporation; Annatachja.DeGrande@UGent.be

Heat stress (HS) is a common problem in (sub)tropical and temperate countries during summer months. Higher metabolic body heat generation and increased susceptibility to HS is the result of intensive selection for increased production. HS impairs animal welfare, performance and meat quality, which results in large economic losses in the poultry industry. Nutritional interventions through supplementation of minerals or vitamins (e.g. zinc or vitamin E (VE)), might alleviate the negative impact of HS. This study aimed to compare the effect of supplying Zn either from ZnSO4 or from zinc-amino-acid complexes (ZnAA) in a chronic cyclic HS model (32°C±2°C, 55-65% RH, 6h daily) which was applied from d28-36. A 2x2 factorial design was used with 9 replicates of 34 male broilers (ROSS 308) per treatment. Birds were fed a wheat-rye based diet supplemented with 60 ppm zinc either as ZnAA or as ZnSO4 combined with a normal (50IU/kg) or high level (100IU/kg) of VE from d1-36. Performance and meat quality were assessed. Broilers supplemented with ZnAA and a normal level of VE showed a significantly increased body weight (p=0.02) and showed a trend for increased growth (p=0.06) at slaughter age and a lowered FCR (p=0.09) during HS (d28-36) compared to the three other groups. Breast meat yield was increased (p=0.02) in broilers supplemented with ZnAA. Meat derived from birds that were fed a diet supplemented with ZnAA was characterized by decreased drip loss (p=0.03) and thawing loss (p=0.03), indicating improved meat quality. This study shows that providing Zn as ZnAA instead of ZnSO4 improved performance and meat quality parameters when subjected to chronic cyclic HS for the last 9 days of the grow-out period, while supplementing higher levels of VE didn't show a positive impact.

Poster 2.81: The resilience of broilers challenged with re-used litter can be improved with phytogenic feed ingredients

M. De Laet[1], *A. Dierickx*[1], *F. Dias*[1]

[1] Delacon Biotechnik; manu.delaet@delacon.com

Challenge conditions for broilers can have a big impact on production performance, resulting in a lower body weight and a higher FCR. Phytogenics are known to improve resilience in birds by different modes of action: reducing pathogenicity by quorum sensing inhibition and decreasing inflammation. Moreover, certain bioactive compounds can facilitate protection against protozoa like the Eimeria species. The objective of this study is to evaluate the effect of dietary inclusion of a blend of quillaja saponins and essential oils from the myrtaceae and asteraceae plant families on performance of broilers raised on clean or re-used litter (to induce a challenge). All diets were supplemented with Maxiban® at recommended dose. The trial was performed in the Scotland's Rural College, UK. 4 treatments were tested: 1) a control with clean litter, 2) a control with re-used litter, 3) and 4) re-used litter and treated with a phytogenic blend at 2 different dosages of 300 and 400 g/t (IBP 300 and IBP 400). Each treatment had 12 replicates with 12 bird per pen. Performance was measured at 35 days of age. Data were analyzed by analysis of variance (ANOVA). Use of re-used litter decreased performance, resulting in a lower body weight (2160 g vs 2211 g) and higher FCR (1.433 vs 1.422) compared to clean litter. Results from the treated groups show that IBP 300 and IBP 400 can have an additional effect on top of Maxiban®, resulting in a significant ($P<0.05$) higher body weight for IBP 400 (2316 g) and a significant ($P<0.05$) lower FCR for IBP 300 (1.380) and IBP 400 (1.374) compared to both control groups. This study shows that resilience of the birds can be improved by feeding this phytogenic blend, resulting in better performance results under field alike challenge conditions.

Poster 2.82: Effect of reduced calcium and phosphorus and phytase supplementation on performance and tibia ash of broilers

E. Delezie[1], *S. Leleu*[2], *M. Lourenço*[1], *D. Feuerstein*[3]

[1] Research Institute for Agriculture, Fishieries and Food / ILVO,[2] Research Institute for Agriculture, Fishieries and Food / ILVO, [3] BASF; evelyne.delezie@ilvo.vlaanderen.be

The objective of this study was to provide efficacy data for a phytase to be used in broiler diets. The control treatment (PC) consisted of a corn-soybean diet which met all dietary recommendations. A negative control diet was formulated by reducing the Ca and P content with 30% (NC). For both, the PC as well as the NC diet the Ca/P ratio was kept at 1.4. This reduction in the nutrient content versus the positive control was the same for starter, grower and finisher diet. Phytase (Natuphos E) was supplemented at 125 (T2) and 250 FTU/kg (T3). Body weight at 9, 25 and 39 d of age was significantly affected by dietary treatments (all $p < 0.001$). Lowest and highest body weight was obtained when broilers were given the NC and PC, respectively. Supplementing phytase significantly increased the body weight of the broilers compared to the broilers fed T2. However, this increase in body weight was insufficient to obtain a comparable body weight with the broilers fed the PC. The higher supplementation level of phytase did result in a further increase of body weight, although not significant. Same results were obtained for feed intake and body weight gain. The most favourable feed conversions were obtained when broilers were fed the PC or the high phytase dosage. Highest and lowest tibia ash values were obtained for the PC and NC, respectively. Adding phytase even at the highest dosage could not compensate for this reduction. No significant effect of dietary treatment on mortality percentage was observed. To conclude, a reduction of Ca and P levels by 30% combined with the highest dosage of the phytase did not affect performance parameters but resulted in a lower tibia ash content.

Poster 2.83: Effect of xylanase and glucanase mixtures on performance, litter quality and foot pad dermatitis of broilers

E. Delezie[1], S. Leleu[2], M. Lourenco[2], D. Feuerstein[3]

[1] Flanders Research Institute for Agriculture, Fisheries and Food, Animal Sciences Unit, [2] ILVO; [3] BASF; evelyne.delezie@ilvo.vlaanderen.be

The objective of this study was to provide efficacy data of non-starch polysaccharides (NSP) degrading enzymes for use in broiler diets. For this purpose a mixture of xylanase and glucanase at different levels was introduced in a wheat-rye diet. The experiment consisted of 3 dietary treatments with 8 replicates of 30 birds. A wheat-rye-soybean meal diet (T1) was used as control diet. The enzyme mixture (xylanase/glucanase) was supplemented at 560 TXU/ 250 TGU (T2) and 1120 TXU/ 500 TGU (T3).

Body weight was significantly affected by dietary treatments ($p < 0.001$). In general, lowest body weight and body weight gain was obtained when broilers were fed the wheat rye diet without supplementation of the mixture. Supplementing the enzymes significantly increased these performance results. However, supplementation level of xylanase and glucanase mixture had no significant effect on body weight and body weight gain. Same results were obtained for feed conversion ratio as no significant effect of the enzymes was found for feed intake. Enzyme supplementation improved the litter quality as moisture content numerically decreased compared with the non-supplemented group. Moreover, a significant effect of diets was observed concerning the prevalence of footpad dermatitis (FPD) at d39 ($P = 0.023$). Supplementing the xylanase and glucanase mixture decreased the incidence of FPD and by increasing the dosage of enzymes a further reduced prevalence of FPD was observed. From these results it can be concluded that 1) enzyme supplementation increased performance, litter quality and decreased occurrence of lesions but 2) no significant effect of dosage was observed for the measured parameters.

Poster 2.84: Field evaluation of the effect of live yeast on broiler performances

V. Demey[1], J.-B. Soula[1], A. Sacy[1]

[1] Lallemand SAS; vdemey@lallemand.com

The objective of this field trial was to study the effect of the supplementation of the live yeast Saccharomyces cerevisiae boulardii CNCM I-1079 (LY) on performance of broiler chickens reared under commercial conditions. A total of 11 commercial broiler buildings situated in Poland and Czech Republic participated in the trial. Five of the buildings received a standard 3-phase broiler feed (C). The other 6 buildings received the same feed but supplemented with the probiotic LY at the level of $1x10^9$ CFU/kg feed (LY).Duration of the trial was standardized to 40 days. Zootechnical performances of each building were recorded (final body weight (BW), average daily gain (ADG), feed conversion ratio (FCR) and mortality). The European Production Efficiency Factor was calculated. Data were analysed using a Mixed Model with treatment as fixed effect and study as a random effect. Results show that the addition of the LY to the feed significantly increased growth performances: +3.1% final BW ($P<0.05$) and +4.5% higher ADG ($P<0.05$). FCR tended to be improved (-2.6%). The EPEF was significantly augmented (391 vs 424; $P<0.05$). Mortality was lower in LY group (C: 4.0 vs LY: 2.4%). It can be concluded that the addition of the live yeast Saccharomyces cerevisiae boulardii CNCM I-1079 to broiler chickens feed is beneficial on their performances.

Poster 2.85: Mannan Oligo Saccharides and its effect on broiler performance: a multi-analysis

V. Demey[1], F. Barbé[1]

[1] Lallemand SAS; vdemey@lallemand.com

The usage of Mannan Oligo Saccharides (MOS) in general in poultry has been well documented in literature. The objective of this multi- analysis is to evaluate the effect of one specific MOS (AgriMOS ®) in broilers by assembling the results of the publications evaluating the effect of this prebiotic. A total of 6 studies have been identified comparing the performances of poultry with or without the supplementation of this MOS. Each publication reports at least the two main performance criteria 1) average daily gain (ADG) and 2) feed conversion ratio (FCR). When reported, mortality was also included in the database (n=4). Birds, from different genetics (Arbor Acres plus, Ross 308 and Cobb 500), all entered the trials as day old chicks. All trials had a duration of 35 days. MOS dosages ranged from 1000 g/T to 2000 g/T. Data are analyzed using a Mixed Model (SPSS 24.0) with treatment as fixed effect. Study was added as a random effect to the model. The multi-analysis shows a beneficial effect of the prebiotic on the performances of broiler chickens: a significant higher growth (3.3% ADG, $P<0.05$) and improved FCR (3.4% better FCR, $P<0.05$) are recorded. Mortality was numerically lower for chickens receiving MOS. Results from this multi-analysis support the benefit of a supplementation with this specific MOS to improve the performances of broiler chickens.

Poster 2.86: Evaluation of xylanase and a fermentable oligosaccharide on performance of broiler chickens fed energy and amino acid deficient diets

G. González-Ortiz[1], M. I. García[2], J. Sanchez[2], M. R. Bedford[1]

[1] AB Vista,[2] Imasde Agroalimentaria; gemma.gonzalez@abvista.com

The objective of this study was to evaluate the effect of a product consisting of a xylanase and a fermentable oligosaccharide (Signis) on performance of broiler chickens fed energy and amino acid deficient diets. Day-old male Ross 308 broiler chicks were randomly allocated to 8 pens per treatment, with 25 chicks per pen. Forty percent of the litter was reutilized from the previous flock. Treatments based on wheat-corn-soybean meal diets were arranged in a 3 x 2 factorial: a positive control that met or exceeded nutrient recommendations (PC), a negative control diet with a 50 kcal/kg AME reduction (NC1) and a NC2 further reducing amino acids (AA) by 3%, each with or without supplementation of 100 g/t of Signis. Starter, grower and finisher diets and water were available ad libitum. Body weight gain (BWG), feed intake (FI), feed conversion ratio corrected for mortality (FE) and the European Production Efficiency Factor (EPEF) were recorded from 0-42 days. Statistical comparisons were performed using a two-way ANOVA. A significant interaction was observed for BWG and FI ($P<0.001$). The energy and AA dilution reduced ($P<0.05$) BWG when compared to the PC (NC1=1722 vs. NC2=1592 vs. PC=1978 g/bird), and although Signis improved BWG in all diets (NC1=+451 vs. NC2=+314 vs. PC=+176 g/bird; $P<0.05$), the effect was greater in the NC1 birds. No interactions were observed on the EPEF or FE; however, Signis supplementation improved EPEF (230 vs. 278; $P<0.001$) and FE (1.766 vs. 1.608 g/g; $P<0.001$) irrespective of the energy reduction or AA density. Signis supplementation improved performance of broiler chickens fed all diets, particularly those deficient in energy and AA. When added to energy and energy plus AA deficient diets Signis recovered performance to similar levels to the PC.

Poster 2.87: Effect of feeding glycerol esters of butyric and valeric acid on broiler performance

S. Vaessen[1], J. M. Ros[1], O. Casabuena[2], C. Millán[2], M. I. Gracia[2]

[1] Perstorp Waspik BV, [2] Imasde Agroalimentaria, S.L.; mgracia@e-imasde.com

An experiment was conducted in order to evaluate the effect of different feed additives (ProPhorce SR 130: glycerol esters of butyric acid (BA), and ProPhorce Valerins: glycerol esters of valeric acid (VA)) added to the feed on broiler performance. A total of 1,056 one-day-old Ross 308 male broilers were placed in 16 floor pens, with 22 broilers/pen. There were three experimental treatments, T1: Control; T2: BA at 500 g/t from 0-28d and 250 g/t from 28-42d; and T3: BA at 500 g/t from 0-14d, VA at 1,500 g/t from 14-28d and BA at 250 g/t from 28-42d. Observations of feed intake, growth, BW, and FCR were recorded at 14, 28 and 42d. Data were analysed as a randomized complete design with treatment as main effect. From 0-14d, broilers receiving BA at 500 g/t (T2&T3) were heavier (376.4^{b}, 396.6^{a}, 396.7^{a} g; P=0.0382) and exhibited increased growth (23.7^{b}, 25.1^{a}, 25.1^{a} g/d; P=0.0396) than T1. From 14-28d, broilers receiving VA at 1,500 g/t (T3) were heavier (1327^{b}, 1362^{b}, 1414^{a} g; P=0.0013), exhibited increased growth (67.9^{b}, 69.0^{b}, 72.8^{a} g/d; P=0.0003) and reduced FCR than the other two treatments. No significant differences between treatments were observed at 28-42d. For the global study (0-42d), the combination of BA and VA during the fattening period (T3) resulted in increased BW, growth (66.3^{b}, 67.6^{ab}, 69.2^{a} g/d; P=0.0079) and EPEF (396^{b}, 414^{b}, 436^{a} g/d; P=0.0010) and reduced FCR over the other two treatments, mainly due to the positive effect shown by VA at 14-28d. It is concluded that, the supplementation of broiler diets with glycerol esters of butyric acid improved performance in your broilers. Moreover, the combination during the fattening period of glycerol esters of butyric acid with glycerol esters of valeric acid improved growth, FCR, and EPEF in broilers.

Poster 2.88: Effect of supplementation of nano-zinc on performance and expression of HSP70 in heat stressed broiler chickens

A. Hable[1], A. Chabukswar[2], S. Jagdale[2], S. Bhagat[3]

[1] PhD Scholar, MAEER's MIP, Pune, [2] Professor, MAEER's MIP, Pune, [3] PhD Scholar, ICAR-CARI; asawaree.hable@gmail.com

The poultry industry has occupied a leading role in the agricultural segment in many parts of the world. Especially in a tropical country like India High temperature coupled with high humidity, imposes severe stress on birds and leads to reduced performance. With the use of nanotechnology, nanoparticles (NP) can be used as a supplemental source of trace minerals in diets. Zinc is one of the essential elements its deficiency impairs overall development and growth of bird. The twelve dietary treatments, following 3x4 factorial design, were formulated by employing three levels of zinc (40, 60, 80 ppm) of four different sources (inorganic, organic, green nano, and market nano) with four replicate of each. The trail was carried out in hot dry summer with THI above 88 for a trial period. The commercial broiler cob birds were kept for six weeks on standard pre-starter, starter and finisher diet. The green synthesis is an environmentally friendly method of nano synthesis production (Sandeep Uniyal et al., 2015). In the present study, the 80 ppm Zn of nano source revealed significantly (P<0.05) better growth performance and immunity of the birds. The weight of immune organs was significantly (P<0.05) higher at 80 ppm Zn of nano source. The positive response to heat stress was shown by significantly lower H:L ratio & significant (P<0.05) down-regulation of heat shock protein gene. Finally, from all the above results, it is concluded that nano zinc at the rate of 80 ppm by green synthesis is recommended to alleviate heat stress loss in broiler chicken during hot dry heat.

Poster 2.89: The effect of Spirulina Platensis on laying performance and heat stress biomarkers in Japanese quails

H. Hajati[1], M. Zaghari[1], H. Cedraz[2]

[1] University of Tehran, [2] Universidade Federal de Viçosa; h.hajati@ut.ac.ir

This study was done to evaluate the effects of Spirulina Platensis (SP) on egg production, intestinal microflora, heat stress biomarkers, and HSP70 gene expression in laying Japanese quails under heat stress condition. A total of 250 female quails were allocated to 5 treatments, 5 replicates and 10 birds each replicate in a completely randomized design. Experimental treatments included: basal diet, basal diet+ 0.03 % probiotic, basal diet+ 0.01 % SP, basal diet+ 0.03 % SP, basal diet+ 0.05 % SP. The experiment lasted 12 weeks, and the birds had free access to feed and water. The results showed that using probiotic and different levels of Spirulina had not significant effect on laying performance of Japanese quails (P>0.05). Probiotic supplement increased Lactobacil bacteria population in laying quails' ileum (P< 0.05). SP at the level of 0.05% caused the lowest blood MDA level, heterophil %, H/L ratio, and E. Coli population in quails' ileum (P< 0.05). HSP70 gene expression was lower in heart compared with liver in laying quails under heat stress (P< 0.05). In conclusion, results of present study revealed that SP at the level of 0.05 % had the potential to decrease heat stress in laying quails, so it can be considered as a probiotic alternative in the diet of laying quails suffering heat stress condition.

Poster 2.90: Effect of two bioactive extracts from Olea europaea in broiler chickens challenged with Eimeria spp vaccine

J. Herrero-Encinas[1], D. Menoyo[1], M. Blanch[2], J. Pastor[2], S. Rochell[3]

[1] Departamento de Producción Agraria, Universidad Politécnica de Madrid, ETS Ingeniería Agronómica Alimentaria y de Biosistemas, 28040 Madrid, Spain, [2] Lucta S.A., Innovation Division, UAB Research Park, Edifici Eureka. 08193 Bellaterra. Spain, [3] Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA; j.herreroe@alumnos.upm.es

The present study evaluates the effect of two bioactive pomace extracts (OEa and OEb) from Olea europaea (Lucta S. A.,Madrid, Spain) in broiler chickens challenged with an Eimeria spp vaccine. To this end, 560 1-d old male chicks (Cobb 500) were randomly assigned to 7 experimental treatments (10 cages/treatment, with 8 birds/cage). Treatments included a control group with birds fed a basal diet and not challenged (NCU) and 6 treatments in which birds were challenged with an oral gavage of live oocysts (Coccivac®-B52) and fed either the basal diet with no additives (CC) or supplemented with 500 ppm of a coccidiostat (PCC, Clinacox®), 500 or 1500 ppm of EOa (OEa500C and OEa1500C) or with 667 and 2000 ppm of OEb (OEb667C and OEb2000C). All animals were fed starter (1 to 14 d) and grower (15 to 28 d) diets ad libitum. Performance, carotenoids plasma concentration (CAR) and oocysts per gram of excreta (OPG) were measured. From 0 to 20 d of age, birds fed NCU treatment showed higher feed intake (P<0.05) and body weight gain (P<0.01) compared to CC, OEb667C and PCC. Feed conversion ratio in NCU was numerically lower than CC treatment (1.31 vs 1.38). Furthermore, performance was similar among NCU, OEa500C, OEa1500C and OEb2000C treatments. From 20 to 28 d of age, no significant differences were observed in performance among treatments. At 20 d of age, CAR was higher (P<0.05) in NCU compared to CC, OEb667C and OEb2000C. In addition, PCC showed lower (P<0.001) OPG at 7d compared to the other challenged treatments but higher than NCU (P<0.001). Finally, OPG at 14d and 21d significantly increased (P<0.001) in all challenged treatments (except PCC) compared to NCU. In conclusion, OEa and OEb2000 ameliorate the negative consequences on performance derived from a coccidian challenge.

Poster 2.91: The benefit of applying dig P, Ca, dig AA and AME matrix value of Buttiauxella phytase dosed at 1000 FTU/kg in broilers fed diets containing 2000U xylanase

B. Hillen[1], Y. Dersjant-Li[1], A.-M. Debicki-Garnier[1], K. Lipinski[2]

[1] DuPont Animal Nutrition, DuPont Industrial Biosciences, Leiden, The Netherlands, [2] University of Warmia and Mazury, Poland; Barthold-christian.hillen@dupont.com

This study evaluated the production benefit of using the contribution of AME, dig AA, P and Ca for a Buttiauxella phytase in broilers fed complex diets containing xylanase at 2000U/kg. A total of 1600 d-old male Ross 308 broilers were randomly allocated to 3 treatments with 10 replicates per treatment (53 chicks per pen at day 42). The diets were based on wheat/corn/SBM/triticale/rapeseed meal and fed in mash form ad libitum. The positive control (PC) was formulated to meet nutritional requirements. A Buttiauxella phytase was added at 1000FTU/kg to NC1 (NC1 + 1000 FTU) and NC2 (NC2 + 1000 FTU), both with reduction of 0.19% AvP, 0.16% Ca and 37 kcal ME. In addition dig AA (Lys, M+C and Thr) was reduced by 0.02-0.03% and 0.03-0.06% in NC1and NC2 representing 50% or 100% dig AA matrix value respectively. At day 10 and 42, 3 birds per pen were randomly selected for bone ash analysis. Feed intake and BW were measured at the start and end of each phase (day 10, 21, 35 and 42). Body weight gain and FCR per pen were calculated for each phase and the whole trial period. Feed cost per kg BWG was calculated based on the price of the ingredients. For each phase and overall of 42 days, phytase added to NC1 or NC2 maintained performance compared to PC (ADG 64, 65 and 64g/b/d; FCR 1.56, 1.56 and 1.57 respectively for PC, NC1+1000 FTU and NC2+1000 FTU respectively). Tibia ash was not influenced by treatments. At day 42, NC2 + 1000 FTU reduced food pad dermatitis and improved bone strength ($P < 0.05$). NC1+1000 FTU and NC2+1000 FTU reduced feed cost per kg BWG by 2.5 and 3.7% respectively vs PC. This study demonstrates that applying dig AA and ME matrix, in addition to P and Ca matrix, can lead to production and animal welfare benefit in broilers fed complex diets containing xylanase.

Poster 2.92: The benefit of applying dig P, Ca, dig AA and ME matrix value of Buttiauxella phytase in broilers

B. Hillen[1], Y. Dersjant-Li[1], A.-M. Debicki-Garnier[1], L. Pál[2]

[1] DuPont Animal Nutrition, DuPont Industrial Biosciences, Leiden, The Netherlands, [2] University of Pannonia, Georgikon Faculty, Hungary; Barthold-christian.hillen@dupont.com

This study evaluated the production benefit of using the contribution of ME, dig AA, P and Ca for a Buttiauxella phytase in broilers. A total of 888 day-old male Ross 308 broilers were randomly allocated to 4 treatments with 6 replicates per treatment (37 chicks per pen). A positive control (PC) was formulated to meet nutritional requirements; the PC was reduced by 0.16% AvP, 0.13% Ca, 62kcal/kg ME, 0.39% CP and 0.02-0.03% dig AA (Lys, M+C, Thr) contributing from and supplemented with the phytase at 500 FTU/kg (NC1 + 500 FTU); PC reduced by 0.19% AvP, 0.15% Ca, 68 kcal/kg ME, 0.71% CP, and 0.04-0.06% dig AA (Lys, M+C, Thr) contributing from 1000 FTU phytase and supplemented with the phytase at 1000 (NC2 + 1000 FTU) or 2000 FTU/kg (NC2 + 2000 FTU). The diets were based mainly on corn and SBM, with wheat (7 to 10%) and sunflower meal (4 to 7.5%), fed in mash form ad libitum. Results were analysed by one way ANOVA and feed cost/kg BWG was calculated based on the price of the ingredients when the trial was done. Feed intake and bodyweight were measured at the start and the end of each phase (1-10d, 11-24d and 25-42d). For each phase and overall of 42 days, NC1 + 500 FTU, NC2 + 1000 FTU and NC2 + 2000 FTU maintained performance compared to PC (ADG 72, 72, 71 and 73g/b/d; FCR 1.75, 1.73, 1.74 and 1.74 respectively, $P > 0.05$). In the starter/grower (1-24d) phase, NC2 + 2000 FTU tended to improve BWG by 5.5% ($P < 0.1$) vs PC. The feed cost/ kg BWG was reduced by 6.9, 7.1 and 7.3% respectively for NC1+500 FTU, NC2 + 1000 and NC2 + 2000 FTU vs. PC. This study demonstrates that applying dig AA and ME matrix, in addition to P and Ca matrix, can lead to production benefit in broilers. A high dose of 2000 FTU can further improve production benefit, especially in starter/grower phase

Poster 2.93: Effect of emulsifier supplements on xanthophyll absorption and egg yolk color in laying hens

E. Holtslag[1], A. Pérez-Vendrell[2], B. Vilà[2], J. Estevez[3], S. Carné[3], J. Brufau[2]

[1] WUR,[2] IRTA, [3] ITPSA; eline_holtslag@hotmail.com

Color plays an important role in the perception of food quality and although consumer preferences for egg yolk color vary, a dark orange color is more appreciated in several markets. For a more intense egg yolk color, xanthophylls can be included into the diet. Emulsifiers may increase xanthophyll solubility and boost their uptake in the intestine and subsequently in egg yolk. Therefore, emulsifiers may improve egg yolk color. A total of 288 Hy-line Brown laying hens (21-24 weeks of age) were divided into three diet groups: control, or with non-ionic surfactant composed by esterified fatty acids (Emulsifier A or B), with each 5 levels of red xanthophylls (2, 4, 8, 16, 32 ppm). Yolk color was determined with a Minolta CR 300 Colorimeter and resulting data was analyzed with a randomized complete block design. Emulsifier effect was evaluated by comparing non-linear regression curves of color vs red xanthophyll concentration. For each emulsifier there were 30 replicates (5 levels x 6 replicates). Significant emulsifier effect was seen for CIE values redness, redness/yellowness (r/y) and calculated Roche Yolk Fan Color (RCQO) values ($P<0.001$). Emulsifier A and Emulsifier B were significantly more efficient than no Emulsifier, ratios varying between 104% and 110% for redness, r/y and RCQO. Emulsifier B was numerically more efficient than Emulsifier A for egg yolk color variables. Although underlaying mechanisms of xanthophylls absorption are not fully understood, these results indicate that the nature of different emulsifiers might have an impact on their variable efficacy to optimize intestinal uptake of xanthophylls. Xanthophylls-emulsifiers preparations can be an effective strategy to improve pigmentation by means of increasing bioavailability of xanthophylls.

Poster 2.94: The effects of enzymes supplementation on the performance, carcass and tibia characteristics, some blood parameters and nitrogen and phosphorus bioavailability of broiler chickens

Z. S. H. Ismail[1], M. A. Metwally[2], M. F. A. Farghly[2], A. M. Inas[3], M. E. Ghonime[3]

[1] Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt, [2] Department of Poultry Production, Faculty of agriculture, Assiut University, [3] Department of Poultry Production, Faculty of agriculture and Natural Resources, Aswan University; zien7300@yahoo.com

Abstract: This experiment was carried out to investigate the effect of optizyme (multienzymes) on the productive performance, carcass and tibia characteristics, some blood parameters and nitrogen and phosphorus bioavailability of broilers chickens. One hundred and eighty birds were randomly distributed into three equal groups (60 birds each, of four replicates of 15 birds each). The first group was kept as a control – unsupplemented group 0 mg of optizyme/ kg diet, while the second group was fed the basal diet supplemented with 250 mg optizyme/ kg diet and the third group was fed the basal diet supplemented with 500 mg optizyme/ kg diet. Body weight (BW), feed intake (FI) were recorded weekly and body weight gain (BWG) and feed conversion ratio (FCR) were calculated. Mortality rate was recorded daily. At 42 days of age three birds from each replicate were taken and slaughtered to calculate carcass cut-parts and plasma samples were also taken to measure some plasma components. Also, small scale experiment was carried out to calculate nitrogen and phosphorus bioavailability. The results showed significant influences of optizyme on the parameters measured.

Poster 2.95: Evaluation of a multi-carbohydrase and phytase complex in reduced energy, amino acids, available phosphorus and calcium diet fed to laying hens

M. Jlali[1], P. Cozannet[1], R. Davin[2], A. Preynat[1]

[1] ADISSEO France SAS, 6 Route Noire, 03600 Malicorne, France, [2] Schothorst Feed Research B.V. P.O. Box 533, 8200 AM Lelystad, The Netherlands; maamer.jlali@adisseo.com

The efficacy of a multi-carbohydrase and phytase complex (MCPC) was investigated in late lay in laying hens fed diet severely reduced in ME, digestible amino acids (dAA), avP, and Ca. Three diets were fed to Lohmann Brown laying hens (n = 1,104; 48 cages x 23 birds per cage) from 55 to 81 weeks of age in a randomized complete block design. The diets were a positive control (PC) adequate-nutrients diet with 2,783 kcal ME/kg, 0.65% dLys, 3.5% Ca, and 0.35% AvP, a negative control (NC) diet reduced in ME and dAA each by 7%, and Ca and AvP each by 0.23% point without and with MCPC which provides 1,250 U xylanase, 860 U b-glucanase and 1,000 FTU per kg diet. The nutrient reduction in the NC diet significantly reduced ($P < 0.01$) the overall egg production, egg mass and final BW by 10.1%, 10.0%, and 5.2%, respectively, and significantly increased ($P < 0.0001$) feed intake, FCR and percentage of broken and shell-less eggs by 10.9%, 24.5% and 231%, respectively. MCPC supplementation completely restored the egg production, egg mass, final BW and the percentage of broken and shell-less eggs to similar levels as observed in PC hens ($P > 0.05$). In addition, whereas not reaching the PC treatment, supplementing MCPC significantly reduced the FCR of the NC hens by 6.6%. The ME and nutrient reduction in the NC also adversely influenced tibia breaking strength ($P < 0.05$) and ash content ($P < 0.01$). The MCPC addition completely restored those parameters to similar levels of PC diet fed hens ($P = 0.75$). This study shows that the MCPC addition could be an effective mean to reduce the negative impact of ME and nutrient deficiencies on performance, egg and bone quality suggesting a better bioavailability of nutrients to maintain high productivity without compromising welfare of laying hens.

Poster 2.96: Effects of a vectorized dietary betaine and antioxidants supplementation on growth performance and meat yields of broilers kept under high environmental temperatures

Q. Page[1], A. Missohou[2], C. A. Konate[3], N. Brevault[1], S. Toussaint[1], M. Bournazel[1], S. Klein[1]

[1] MiXscience, [2] EISMV, [3] NMA Sanders; quentin.page@groupeavril.com

High temperatures in poultry production can impare zootechnical performance, reduce feed intake, induce hyperventilation, behavior's problems, and meat quality degradation. Feeding betaine may reduce such negative effects thanks to its osmoprotective properties. In addition, vitamin C and other antioxidants may protect against oxidative stress. The aim of this trial was to evaluate the effect of BeTaHit®, a specific fat coated betaine with vitamins and antioxidants on broilers performance under hot temperatures. The trial took place at the Veterinary School of Dakar (EISMV) in Senegal. 540 day-old Cobb500 chicks were allocated in 10 pens and received one of the following feeds from 0 to 42 days of age: NC (negative control, no additive) or BT750 (NC + 750 g/t BeTaHit ®). Temperature during the trial were between 22°C and 27.6°C. Broilers weight, pododermatitis and litter quality score were recorded weekly. Feed and water intakes were measured daily. Carcass and breast meat were weighed at the end of the trial (n=25 per group). Body weight of BT750 chickens was significantly improved at 42 days compared to NC group (2476g vs 2321g, $P<0.05$). Intermediate weights (7, 14, 21, 28 and 35 days) were also significantly increased ($P<0.05$). BT750 had a higher feed intake (114.7 vs 113.5 g/d, $P=0.67$) and a better feed conversion ratio compared to NC group (2.03 vs 2.14, $P<0.01$). Breast meat weights were significantly higher for BT 750 (580.1 vs 546.2 g, $P<0.01$). Breast meat carcass yields were numerically increased for BT750. There was no effect of the feed on water to feed ratio, litter quality or pododermatitis score of broilers. Our results suggest that BeTaHit® has a positive effect on growth performance and meat yields under high environmental temperatures.

Poster 2.97: Efficacy of a new Bacillus subtilis strain on post-hatch broiler performance

F. Khattak[1], A.-L. Heffron[2]

[1] Scotland's Rural College (SRUC), [2] Elanco Animal Health; Farina.khattak@sruc.ac.uk

A study was designed to investigate the effect of a Bacillus subtilis (B. subtilis) strain (Correlink™ ABS-747) on post-hatch broiler growth performance. A total of 576 Ross 308 day-old male broilers were randomly allocated to 2 treatment groups (with or without B. subtilis) each having 24 replicate pens with12 birds/pen. Nutritionally adequate wheat, barley and soyabean-based diets were generated by addition of Correlink™ ABS-747 B. subtilis at 0 and 1.5 x 10^5 CFU /g of feed and manufactured as starter (d0-11), grower (d11-25) and finisher (d25-42) phases. Feed and water was available ad libitum. Body weight and feed intake were recorded at days 0, 11, 25, 35 and 42. Data were analysed using a linear mixed model (SAS v9.4) with a $P<0.05$ level of significance. Birds fed diets supplemented with B. subtilis had higher ($P<0.05$) average daily weight gain (ADG) during starter and finisher phase. The daily feed intake (DFI) was similar ($P>0.05$) between treatments during starter and grower phase but was 4.2% higher in B. subtilis fed birds during finisher phase. Birds fed diets containing B. subtilis had improved ($P<0.05$) feed efficiency (FCR) during starter phase whereas no differences ($P>0.05$) were noted in grower and finisher phase. The thinning broiler flock period (0-35d), showed that addition of B. subtilis in diet improved ($P<0.05$) ADG by 5%, decreased DFI by 4.2% and resulted in 8.7% improved FCR compared to control birds. The overall data (0-42d) depicted that birds fed B. subtilis had higher ($P<0.05$) ADG and DFI but no difference ($P>0.05$) in FCR compared to control birds. The study suggests that B. subtilis strain Correlink™ ABS-747 is a promising new feed additive with growth promoting effects.

Poster 2.98: Effect of dietary selenium source on growth performance and muscle selenium concentration in broiler chickens

B. Vilà[1], C. Torres[2], C. Rapp[2], T. Ward[3]

[1] IRTA / Animal Nutrition, [2] Zinpro Corporation, Boxmeer, Netherlands, [3] Zinpro Corporation, Eden Prairie, USA; borja.vila@irta.cat

Selenium (Se) is an essential trace mineral that has antioxidant properties in animals. Dietary Se historically has been provided from inorganic sources, but several organic sources (which are deposited more efficiently in the body tissues) are also available. The objective of the present study was to compare the effect of various Se sources when added to broiler chicken diets on growth performance and Se concentration in breast muscle. The study had a randomized complete block design (RCB) with 4 Se sources (Na selenite: NaS, Se-yeast: SeY, Zn-L-selenomethionine: ZnSM, and hydroxy-analogue of selenomethionine: HASM) which were added at 0.2 mg Se/kg diet to a basal that contained already 0.2 mg Se/kg from NaS. There was a total of 4 treatments with 10 floor pens of 12 birds (day-of-hatch Ross 308 male broiler chickens) each. A corn-soya based diet was formulated in three phases to meet or exceed NRC (1994) nutrient requirements and was presented in crumble form in the starter phase and pelleted in grower and finisher phases. Feed and water were provided for ad libitum consumption. Growth performance was evaluated up to 42 days post-hatching, and Se in breast muscle from 3 birds per replicate (pooled) at the end of the study. Growth performance was not affected ($P > 0.46$) by dietary treatment. All diets contained adequate Se levels, higher than NRC recommendations. Se content in breast muscle differed ($P < 0.05$) between each of the selenium sources and was 3.3, 3.1 & 2.4-fold that of birds fed Se as NaS for birds fed Se as ZnSM, HASM and SeY, respectively. In conclusion, dietary Se source did not affect broiler chicken growth performance, but organic Se increased the Se concentration in breast muscle, though not all sources were equally effective.

Poster 2.99: Action of Citrobacter braakii phytase on diets formulated with different raw materials

M. Tovborg[1], L. Skov[1], J. W. Wilson[2]

[1] Novozymes A/S, [2] DSM Nutritional Products; MOTJ@novozymes.com

A broiler in vitro model consisting of a crop step (5 min pH 4.6) followed by a gizzard step (5 min pH 3) has been used to evaluate the influence of diet composition on phytase efficiency. 10 diets based on soybean meal (SBM), rape seed meal (RSM), corn and wheat were made to have a variation of phytate (IP6) content and raw materials. The resulting 10 diets were 6 pure diets with increasing phytate-P (0.2 to 0.35) using corn starch as filler and containing 50, 70, 88 % SBM or 30, 42, 53 % RSM, and 4 combination diets consisting of corn/SBM, corn/RSM, wheat/SBM and wheat/RSM with a fixed phytate-P of 0.28. Citrobacter braakii phytase (RONOZYME® HiPhos) efficiency was evaluated by chromatographic separation and quantification of the degradation products (IP6-IP3) after the gizzard step. The phytase readily degraded IP6 in all diets to a comparable level and therefore have no apparent limitations with any of the selected raw materials. During the short in vitro incubation 75 - 90 % of IP6 was converted compared to diets without added phytase. Variation in raw materials and increasing phytate content had no significant effect on efficiency of phytate degradation or level of residual phytate. Wheat diets contained endogenous phytase since no heat treatment was applied. Endogenous phytase contributed to some IP6 to IP5 conversion (mean 12.5 %) but proved far from sufficient in overall P-release.

C. braakii phytase proved to be active even on IP5-(1,3,4,5,6) despite this is not a product typically formed by microbial phytases. IP5-(1,3,4,5,6) is believed to be a product from of endogenous phytase or phytate-ADP-phosphotransferase and was observed in several of the raw materials.

Poster 2.100: Egg yolk pigmentation efficiency of apo-ester compared to a highly concentrated Lutein zeaxanthin product

M. Umar Faruk[1]

[1] DSM Nutritional Products France; murtala.umar-faruk@dsm.com

A 3-week egg yolk pigmentation trial was conducted to compare the pigmenting efficiency of Apo-ester (APE) from Carophyll® Yellow and a highly concentrated Lutein-zeaxanthin (Lut/Zea) tagetes extract from Colortek®. The trial was a 2*4+1 factorial arrangement with 2 carotenoids products (APE vs Lut/Zea). The products were administered at 4 different dosages (2, 3, 4 & 5 mg of pure product per kg feed). Each treatment was randomly allocated to four groups of three hens, housed individually in battery cages. The control group was fed a basal diet low (<5mg/kg) in native xanthophylls. Laying performance was determined per group. Carotenoid content of feed samples and egg yolks were determined by HPLC and carotenoid deposition rate was calculated. Egg yolk color was determined using the YolkFan™. Linear regression analysis was used to establish the pigmenting efficacy equivalence between the two products. Performance (feed intake, egg production & weight) was not affected by treatment. Irrespective of the dose, APE showed a higher ($P<0.001$) deposition rate (53.3%) than Lut/Zea (18.2%). YolkFan™ values were higher ($P<0.0001$) for APE (12) compared to Lut/Zea (9). A significant linear increase in the concentration of egg yolk carotenoids with increasing dose was observed for both APE and Lut/Zea. Comparison of the regression slope (y = 0.0605 APE / y=0.0196 Lut/Zea) showed that 3.1 g of Colortek® is equivalent to 1g of CAROPHYLL® Yellow. In conclusion, APE showed a better egg yolk pigmentation efficacy than the highly concentrated Lut/Zeaxanthin product.

Poster 2.101: In ovo administration of nucleosides improved the performance and digestive enzymes activity in broilers

V. Manojkumar[1], A. K. Verma[2], M. Saravanakumar[2], P. V. Beulah[3], M. Monika[3], J. V. Inay[3], R. Jaydip[3], G. Marappan[3]

[1] PG Scholar, Indian Veterinary Research Institute, [2] Indian Veterinary Research Institute, Izatnagar; [3] Central Avian Research Institute; manobvsc.kumar@gmail.com

Based on our previous experiments with nucleosides in broilers where beneficial effects in terms of performance, the present study was carried out to understand the effect of in ovo administration of nucleosides combination on hatchability and activity of digestive secretions. A total of 600 hatching eggs were collected and incubated for 18 days at standard temperature-humidity conditions. At the end of 18 days, the eggs were candled and injected with nucleosides through yolk sac route. The experimental design included 5 groups (100 eggs each) as; control (Con) without in ovo injection, sham control (SCon) injected with sterile PBS, NI, II and III were injected with nucleosides at 50, 100 and 200 mg/egg, respectively. Injected eggs were hatched out and reared under uniform managemental conditions. The hatch weight (0d) were comparable (P>0.05) among all the treatment groups. However, at 14d of age the birds injected with 100mg/egg significantly (P<0.05) higher body weight when compared to control and sham control. The administration of nucleosides had favorable effect on amylase and lipase activity in broilers. The amylase activity higher (P<0.05) in 100mg/egg group when compared to the sham control, whereas the other three groups exhibited intermediate response at hatch. The similar trend was observed at during 3 and 7d of age but on 14d the sham control exhibited higher (P<0.05) activity. The lipase activity did not vary during the hatch but from 3d post-hatch onwards the supplementary groups exhibited higher lipase activity when compared to both the controls. From the study, it is concluded that, the in ovo administration of nucleosides mixture improved the growth performance and digestive enzymes activity without affecting the hatchability.

Poster 2.102: In-feed resin acids do not accumulate in broiler breast muscle or adipose tissue

E. Valkonen[1], J. Apajalahti[2], H. Kettunen[1], K. Vienola[2], J. Vuorenmaa[1], K. Raatikainen[2]

[1] Hankkija Ltd, Peltokuumolantie 4, FI-05801 Hyvinkää, Finland, [2] Alimetrics Ltd, Koskelontie 19, FI-02920 Espoo, Finland; eija.valkonen@hankkija.fi

Tall oil -based, natural resin acid composition (RAC) of coniferous trees has in previous trials shown positive influence on broiler performance and gut mucosal integrity, reduced the incidence of foot pad lesions, and favoured the ileal presence of lactobacilli. Here we investigated the concentration of resin acids in the gastrointestinal tract and tissues of RAC-fed broilers. Ross 308 broilers were fed with 0 (Control) or 3000 mg/kg of RAC, corresponding to 265 mg/kg of resin acids in feed, and being a 4-fold overdose compared with commercial dosing. Titanium dioxide was added to the diets as a digestibility marker. Faeces were collected on day 34. On day 35, six birds from six pens per diet were sacrificed and sampled for several tissues and intestinal contents. Samples of feeds and birds were analysed by gas chromatography for abietic (AA) and dehydroabietic (DHAA) acids. No resin acids were detected in the control birds' samples. Of the total resin acids introduced to the diets, about 70% was found in jejunal contents, and about 60% in ileum and faeces of RAC-fed birds. The concentration of AA in blood plasma, jejunal tissue and bile of RAC-fed birds was on average 0.31, 2.1 and 44 mg/kg, respectively. The average concentration of AA in breast meat of six birds was 0.02 mg/kg. Resin acids were not detected in adipose tissue. In every sample the concentration of DHAA was lower than that of AA. The ratio of DHAA to AA was relatively constant between the samples. In conclusion, some of the diet-derived resin acids were absorbed in the small intestine and re-introduced into the gut via bile. From the consumer safety perspective it is notable that even a 4-fold overdose of RAC resulted in negligible resin acid levels in muscle and adipose tissues.

Poster 2.103: Hydrolyzed yeast with beneficial effects on performance

E. van Eerden[1], A. Sgherbini[2]

[1] Schothorst Feed Research, [2] Prosol; EvEerden@schothorst.nl

A new additive based on hydrolyzed yeast from Kluyveromyces fragilis (I-Care) was hypothesized to have positive effects on performance due to its immune modulating components betaglucans and mannan oligosaccharides, and due to potential positive effects on villus development and thereby on digestive capacity. This hypothesis was tested in a broiler experiment with 3 dietary treatments and 12 replicate floor pens (20 Ross 308 males/pen). Per treatment a non-supplemented starter (0-14 d), grower (14-28 d) and finisher diet (28-38 d) was formulated (NC). NC was supplemented with 500 mg/kg in starter and grower and 250 mg/kg in finisher (LOW) or with 1500 mg/kg in starter and grower and 750 mg/kg in finisher (HIGH). The feeds contained wheat (40%), maize and soybean meal, but also barley (4-5%) and rye (2-5%) without NSP enzymes or anticoccidials, which was supposed to create a mild intestinal challenge. Water and feed were available ad lib. Body weight gain (BWG), feed intake (FI), FCR, and mortality were analysed by ANOVA. BWG, FI, and mortality were not affected. Compared to NC, LOW improved FCR from 0-14 d (1.161 vs. 1.120; $P = 0.002$) and from 0-38 d (1.501 vs. 1.473; $P = 0.02$) and tended to improve FCR from 14-28 d (1.405 vs. 1.386; $P = 0.09$). Results of HIGH did not differ significantly from NC. This could have been due to unnecessary immune stimulation by betaglucans and mannans when present in higher dose levels or supplied during longer periods of time, especially in conditions with low environmental and/or disease stress. The results indicate that using I-Care at 500 mg/kg in the starter and grower and 250 mg/kg in the finisher can have a positive effect on FCR in broilers under mildly challenging conditions.

Poster 2.104: Effect of dietary phytate and phytase level on precaecal phosphorus digestibility of monocalcium phosphate in broilers

J. van Harn[1], P. Bikker[1], A. Mereu[2], M. van Krimpen[1]

[1] Wageningen Livestock Research, PO Box 338, 6700 AH Wageningen, The Netherlands, [2] Yara International ASA, Drammensveien 131, 0277 Oslo, Norway; jan.vanharn@wur.nl

The precaecal digestible phosphorus (pcdP) content of feed phosphates is usually determined in low-phytate diets without supplementation of microbial phytase. The aim of the current study was to determine the effect of dietary phytate and microbial phytase level on the pcdP content of the phosphate (P) source. Therefore, a digestibility study was conducted in which 500 14-d old Ross 308 broilers were allocated to 50 floor pens (0.75 m^2) with 10 broilers per pen. The experiment was set up as a 2*2*2 factorial design, with P source (no or MCP), phytate level (low or high) and phytase supplementation (no or yes) as explanatory factors. Per treatment 6 replicate pens were used, but the low-P low-phytate basal diet was fed to 8 replicate pens. A semi-synthetic low-phytate basal diet was formulated with 1.7 g/kg P and 2.3 g/kg Ca. The P and Ca contents of the high-phytate basal diet were 2.7 g/kg and 3.5 g/kg, respectively. Both the low- and high-phytate basal diets were split up into 6 batches, and within each basal diet 2 batches were not supplemented with a P-source, 2 with 1.5 g/kg P from MCP, without or with microbial phytase (500 FTU/kg). All diets had a constant Ca:P ratio of 1.3:1. At d23-24, contents of the terminal part of the ileum of all birds were sampled and pooled per pen. PcdP was determined using titanium dioxide as indigestible marker. A high phytate content reduced the PcdP of the P-source, while phytase supplementation increased the pcdP of P-source, both in low- and high-phytate diets.

It can be concluded that both the dietary phytate content as well as phytase supplementation affected the pcdP of the P-source, where a highest pcdP content of the P-source was observed in low-phytate phytase-supplemented conditions.

Poster 2.105: Reducing Salmonella Enteritidis colonization in broilers using a Clostridium butyricum probiotic

W. van der Veken[1], V. Hautekiet[1]

[1] Huvepharma N.V.; wouter.vanderveken@huvepharma.com

Salmonella Enteritidis infection is still a prevalent challenge in modern poultry rearing, as it poses a risk to general food safety. Novel ways of Salmonella reducing methods such as dietary probiotic supplementation can help in mitigating this risk. Butyric acid producing probiotics such as Clostridium butyricum are of special interest, as these have been shown to suppress Salmonella replication.

Miya-Gold® contains viable spores of Clostridium butyricum. A ten day trial was set up using 48 broilers, divided over two groups: a control group fed a basal diet with no Miya-Gold® supplementation and a probiotic group supplemented with 1 kg Miya-Gold®/mton of feed (5 x 10^{11} CFU/mton of feed). Six birds of each group were orally inoculated at five days of age (day 0) with approximately 10^5 CFU Salmonella Enteritidis and put back together with the rest of their respective group. These 'seeder birds' then infected the rest of the group naturally, as would be the case in a commercial broiler house. Ten days post inoculation all birds from both groups were euthanized: samples from the caecum were taken to determine and evaluate the amount of CFU Salmonella/g caecum. Statistical analysis showed a decrease (P<0.1) in amount of Salmonella CFUs when Miya-Gold® was compared to the control: 2.17 vs 3.99, respectively (log 10 values). Additionally, the percentage of Salmonella positive non-seeder birds was 72% in the control, compared to 28% in the Miya-Gold® treatment. Miya-Gold® reduced the colonisation rate of Salmonella Enteritidis and as such should be considered as a novel way of mitigating Salmonella-related food safety risks.

Poster 2.106: Complementary effects between a Bacillus licheniformis probiotic and a chemical anticoccidial in broilers under a coccidiosis challenge

W. van der Veken[1], V. Hautekiet[1]

[1] Huvepharma N.V.; wouter.vanderveken@huvepharma.com

Coccidiosis is a common challenge on-farm, making an anticoccidial program standard practice. The goal of the study was to evaluate if Bacillus licheniformis could be used simultaneously with a chemical coccidiostat, supporting gut health further. 320 one-day-old male Ross 308 broilers were divided over 4 treatments (10 replicates/treatment): a control group fed a basal diet, a probiotic group (500 g B-Act®/mton of feed, or 1.6x10^{12} CFU Bacillus licheniformis DSM 28710/mton of feed), a chemical coccidiostat group (500 g Coxiril®/mton of feed, or 1 ppm diclazuril/mton of feed) and a combination group supplemented with B-Act® and Coxiril® (both at 500 g/mton of feed). The trial lasted 42 days, with a coccidiosis challenge at day one: all birds received a 5x overdose of an attenuated coccidiosis vaccine via oral gavage, containing all relevant coccidiosis species for broilers. Body weights (BWG) and feed consumption were recorded, with feed conversion ratios (FCR) calculated accordingly. Final body weights improved in all treatments compared to the control: birds supplemented with B-Act® weighed on average 49 grams more, whilst Coxiril® yielded 61 grams more. This difference became significant for the combination treatment: BWG improved with 136 grams, compared to the control (P<0.05). FCR improved in all treatments compared to the control. B-Act® improved this parameter significantly (P<0.05), with a value of 1.58 compared to the control's 1.67. Coxiril® improved FCR as well, both in combination with B-Act® as on its own (1.63 for both). Combining probiotics with chemical coccidiostats will result in further improved technical performance of broilers under a coccidiosis challenge, utilising complementary effects to the fullest.

Poster 2.107: Beneficial performance effect of tall oil fatty acids is likely based on the effects of resin acids

J. Vuorenmaa[1], E. Valkonen[1], J. Apajalahti[2], H. Kettunen[1], K. Vienola[2]

[1] Hankkija Ltd, [2] Alimetrics Ltd; juhani.vuorenmaa@hankkija.fi

Tall oil fatty acid -based natural resin acid composition (RAC) originates from coniferous trees and contains free fatty acids (90%) and resin acids (9%). In previous trials RAC has consistently improved animal performance. The mode of action of resin acids on broilers has been investigated, and a direct anti-inflammatory effect on gut epithelium has been suggested. We hypothesized that resin acids are the main bioactive component in RAC, and that concentrated resin acids (CRA) without free fatty acids would have the same effect on broiler performance as RAC. We conducted a 35-day performance trial with 540 male Ross 308 broilers. The birds were housed in 36 pens, 15 birds per pen. The pens were randomly allocated into three dietary treatments to give twelve pen replicates. The commercial-type, wheat-soy -based experimental diets were supplemented either with 750 g/ton of RAC or with 71 g/ton of CRA to yield an equal resin acid content (70 mg/kg feed) for both diets. A non-supplemented diet served as a control treatment (CTR). At 35 days, both experimental resin acid treatments significantly outperformed the control treatment in body weight gain (CTR: 2467 g, RAC: 2610 g, CRA: 2601 g; $P<0.05$). Moreover, the mortality-corrected feed conversion ratio was improved by resin acids until 21 days of age (CTR: 1.42, RAC: 1.37, CRA: 1.39; $P<0.05$). The supplements did not affect feed intake or daily mortality. Thus, similar performance effects were produced by both supplements with the same in-feed resin acid content, whether the resin acids were added as RAC or as CRA. In conclusion, the experiment supported our hypothesis suggesting that resin acids are the most important performance-improving components of RAC.

Poster 2.108: Phytase superdosing increased yolk mineral concentration while decreasing yolk inositol concentration from breeder hens aged 35 or 40 weeks

C. A. Granghelli[1], C. L. Walk[2], L. F. Araujo[1], S. M. Silva[1], M. L. Cuadros[1], Y. G. A. Sartore[1], M. T. Dias[1], C. Brearley[3], M. Smith[3], C. S. S. Araujo[1]

[1] University of Sao Paulo, Pirassununga, Brazil, [2] AB Vista, Marlborough, UK, [3] University of East Anglia, Norwich, UK; carlosgranghelli@usp.br

Previous experiments reported a significant increase in yolk mineral content as phytase dose increased in a diet fed to laying hens. A trial was conducted to determine the influence of increasing doses of phytase on breeder hen production and mineral and inositol concentration in the yolk. Two-hundred and sixteen, 27-week-old breeder hens were obtained from a commercial hatchery and housed at 4 hens/box to 50 weeks of age. Hens were fed one of three nutrient adequate diets, reduced in Ca and P by 0.16 and 0.15%, respectively, and containing 500, 1,500 or 4,500 FTU/kg of phytase. Hen feed intake, gain and egg production were measured every 4 weeks to represent a phase (P1 to P6). Fresh egg yolks were collected from one hen/box at week 35 and 40 and freeze dried. Data were analysed using JMP. The model included diet, replicate cage, and phase as a repeated measure. Hen intake was 169g in P1 and decreased to 162g by P6. Total egg production ($P = 0.06$) and total eggs/hen/week ($P < 0.05$) was greatest in hens fed 1,500 FTU/kg phytase when compared with hens fed 4,500 FTU/kg phytase and decreased ($P < 0.05$) from P2 to P6. Yolk inositol decreased ($P < 0.05$) and yolk glycerol concentration increased ($P < 0.05$) as phytase dose increased from 500 to 4,500 FTU/kg. In general, yolk Ca, Na, and K were greater in 40-week-old breeder hens and influenced by phytase dose × hen age ($P < 0.05$). Phosphorus concentration in the yolk was highest ($P < 0.05$) in hens fed 4,500 or 500 FTU/kg phytase when compared with hens fed 1,500 FTU/kg of phytase, regardless of breeder age. In conclusion, phytase supplementation had an influence on mineral, inositol and glycerol concentration in the yolks of breeder hens and this may have an influence on chick quality, hatchability, and initial growth rate.

Poster 2.109: Phytase dose fed to breeder hens has an influence on yolk inositol concentration, chick quality and hatchability, and early chick growth rate

C. A. Granghelli[1], C. L. Walk[2], L. F. Araujo[1], F. A. Roque[1], B. G. S. Leite[1], P. H. Pelissari[1], C. Brearley[3], M. Smith[3], C. S. S. Araujo[1]

[1] University of Sao Paulo, Pirassununga, Brazil, [2] AB Vista, Marlborough, UK, [3] University of East Anglia, Norwich, UK; carlosgranghelli@usp.br

The objective of this trial was to determine the influence of breeder hen diet on hatchability, chick quality, and yolk nutrient concentration of chicks at day of hatch and subsequent starter diet on chick growth performance to 42-d post-hatch. Breeder hens (n = 216) were fed one of three nutrient adequate diets, with reduced Ca and P by 0.16 and 0.15%, respectively, and 500, 1,500 or 4,500 FTU/kg phytase from 27- to 50-weeks of age. There were 4 hens/cage and 18 cages/diet. At 38-weeks of age, eggs (n = 648) were collected and incubated using standard procedures. At day of hatch, chick quality and hatchability were determined and 18 chicks/diet were sacrificed for yolk sac collection and determination of inositol concentration. The remaining chicks were equally divided into three groups and fed one of three nutrient adequate diets, with reduced Ca and P by 0.16 and 0.15%, respectively, containing 0, 500 or 1,500 FTU/kg of phytase to d 42 post-hatch. Increasing phytase concentration in the breeder hen diet linearly ($P < 0.05$) increased the number of early dead and linearly ($P < 0.05$) decreased the number of late dead and pips. Inositol concentration in the yolk sac at day of hatch increased (quadratic, $P < 0.05$) as phytase dose increased in the breeder hen diet. Body weight of chicks at day of hatch and d 7 increased (linear, $P < 0.05$) as phytase dose increased in the breeder hen diet. There was no effect of breeder hen diet or subsequent broiler chick diet on growth performance at d 42. Inositol concentration in the yolk at day of hatch was positively correlated ($P < 0.05$) with d 7 ($r = 0.32$) and d 21 ($r = 0.30$) chick body weight, indicating inositol may influence the development of newly hatched chicks resulting in an increase in early growth rate.

Poster 2.110: The effect of different selenium sources on growth performance, blood glutathione peroxidase and dietary metabolisable energy when fed to broiler chickens

S. Woods[1], S. P. Rose[2], I. M. Whiting[1], C. Ionescu[3], D. Bravo[3], V. Pirgozliev[1]

[1] NIPH, Harper Adams University, UK, [2] NIPH, Harper Adams University, [3] Pancosma SA, Switzerland; SLWoods@harper-adams.ac.uk

The aim of the study was to evaluate the effect of three sources of dietary Se on growth performance, glutathione peroxidase (GSH-Px) activity in blood, and dietary apparent metabolisable energy (AME) when fed to broiler chickens from 0 to 35 days of age. One hundred and fifty day-old male Ross 308 chickens were allocated to 30 raised-floor pens (5 birds in a pen; 0.36 m^2 each pen). Broilers were fed one of three experimental mash diets: control diet formulated to be nutritionally adequate containing a standard source of Se following breeder's recommendations (sodium selenite protected premix with a potency of 4.50% Se, at 5.55 g/t); diet 2 had the same formulation but was supplemented with 10.35 g/t XSEL3.0 (inorganic source of selenium, Pancosma SE, Switzerland); diet 3 had the same formulation and was supplemented with 136.36 g/t selenized yeast. The results were compared statistically with ANOVA. Duncan's multiple range test was used to determine significant differences between diets.

Birds fed selenized yeast had lower feed intake and weight gain ($P<0.05$) compared to sodium selenite and XSEL fed broilers. There were no differences ($P>0.05$) in feed efficiency, GSH-Px activity in blood, and dietary AME, associated with the diets fed. The results showed that dietary Se provided in different forms may have an impact on birds' performance without influencing GSH-Px levels in blood and dietary AME. However, XSEL can be used in broiler dietary formulations without compromising performance. The mode of action of dietary Se supplements for broilers require further investigation. Knowledge of this type provides the broiler industry with the necessary information to inform the formulation of poultry feeds using different Se sources with confidence.

Poster 2.111: The effect of three selenium sources fed at two levels on the tissue selenium and growth performance of broiler chickens reared at constant high temperature

S. Woods[1], S. P. Rose[1], I. M. Whiting[1], C. Ionescu[2], D. Bravo[2], V. Pirgozliev[1]

[1] NIPH, Harper Adams University, UK, [2] Pancosma SA, Switzerland; SLWoods@harper-adams.ac.uk

The described experiment aimed to compare the effect of three sources of dietary Se fed at two different levels on Se content in breast muscles and liver tissue, and growth performance of broiler chickens reared at constant temperature of 35°C from 14 to 35 days of age. Two hundred and eighty two-weeks-old male Ross 308 chickens were allocated to 56 raised-floor pens (5 birds/pen; 0.36 m^2/pen). Chickens were fed one of seven diets: a control (C) mash diet containing background Se only (no Se supplementation); the C supplemented with Se as sodium selenite at either 0.15 g/t (LSS), or 0.3 g/t (HSS); the C supplemented with Se as Pancosma Se source (Switzerland) at either 0.15 g/t (LBT), or 0.3 g/t (HBT); the C supplemented with Se as selenized yeast at either 0.15 g/t (LSY), or 0.3 g/t (HSY). The results were compared statistically with ANOVA. Duncan's multiple range tests were used to determine significant differences between diets. Birds fed HSY had lower performance ($P<0.05$) compared to most of the diets, but no differences ($P>0.05$) were found between the rest of the diets. Birds fed C had lowest Se breast content ($P<0.001$). HSY improved the Se in breast ($P<0.001$), but there were no differences ($P>0.05$) between LSS, HSS, LBT and HBT. Feeding HSY improved the Se in liver compared to LSY ($P<0.001$), but did not differ ($P>0.05$) from HSS and HBT. There was no difference ($P>0.05$) in liver Se content between birds fed LSS and HSS, and LBT and HBT, suggesting that those Se additives are effective at relatively low level of inclusion. Taking into account the better growth performance when LBT is fed suggests that Pancosma Se source is effective to improve not only tissue Se content but also the growth performance of the birds at relatively low inclusion rate.

Poster 2.112: Growth promoting effects of dietary glycine supplementation in broiler

H. Zhang[1], Y. Ma[1], U. Elahi[1], Q. Feng[1], J. Wang[1], S. Wu[1], G. Qi[1]

[1] Feed Research Institute of Chinese Academy of Agricultural Sciences; fowlfeed@163.com

Glycine is the limiting amino acid after methionine, lysine, and threonine in a corn-soybean meal based diet of broilers. Two broiler feeding trails were conducted to investigate the effects of glycine in normal or low crude protein (CP) diet in broiler chicken. Both trials were conducted with 240 day-old male Arbor Acre broilers. Chicks in normal CP trial was fed a corn-soybean basal diet (22%, 20% CP for starter, grower) or a basal diet supplemented with different levels of glycine (0.1,0.2 or 0.3%). Chicks in low CP trial was fed a basal diet, a low CP diet (18%, 16% CP for starter, grower), a low CP diet supplemented with 1% glycine, or a low CP diet supplemented with 1% glycine and 0.22% L-Cysteine.HCl.H_2O (with less DL-methionine addition to keep constant total sulphur containing amino acid content). The diets were formulated on standardized ileum digestible amino acid basis. Both trials lasted for 42 d. The results of the normal CP diet showed that glycine addition quadratically promoted the growth of broiler, and the 0.2% level resulted in superior feed conversion ratio (1.692 vs 1.737, in 0.2% glycine diet and the basal diet). For the low CP trial, bird fed low CP diet had lower BW and ADG, and inferior feed efficiency. The Glycine addition inhibited the growth depression of low CP diet. In spite of the marginal deficiency of methionine,chicks fed glycine and cysteine fortified low CP diet had comparable growth exhibition to those fed glycine fortified low CP diet. Taken together, glycine could be used as a promising feed additive for broiler chicken fed either a standard or a low CP diet. The growth promoting effect of glycine was not associated with the dietary cyst(e)ine status.

Poster 2.113: Buttiauxella phytase dosed at 3000 FTU/kg improved performance in broilers fed complex diets and under mild challenge

Y. Dersjant-Li[1], R. Hardy[2], E. Esteve[3], L. Marchal[1]

[1] DuPont Animal Nutrition/DuPont IB, Archimedesweg 30 2333CN Leiden The Netherlands; [2] DuPont Animal Nutrition/DuPont IB, Box 777, Marlborough, United Kingdom, [3] Institute of Agriculture and Food Research and Technology, Mas de Bover Ctra. Reus-El Morell km. 3.8, E-43120 Constantí (Tarragona), Spain; Yueming.dersjant-Li@dupont.com

This study evaluated the production benefit of Buttiauxella phytase dosed at 3000 FTU/kg in broilers under mild challenge. Positive control (PC) diets were formulated meeting the requirement of broilers. The negative control (NC) diets had reduced nutrients vs. PC (74 kcal/kg ME, 0.18% available P, 0.19% Ca, 0.04% Na, 0.03-0.04% points in dig AA contributing from 1000 FTU Buttiauxella phytase). A total of 837 Ross 308 male broilers were randomly allocated to 3 treatments with 10 replicates for the PC and NC, and 11 replicates per treatment (27 birds/per pen) for NC+the phytase at 3000 FTU/kg (NC+3000 FTU). Birds were vaccinated with Paracox 5 (10 x recommended dose) on day 5, used litter as bedding materials at the start and with new fresh litter applied on top as the study advanced, to produce a mild challenge that mimics the commercial production conditions. Diets were based on corn/wheat/soybean meal/rapeseed meal and fed ad lib in pellet form in 3 phases: 1-10 d; 11-21 d and 22-42 d. Feed intake and BW were measured on a per pen basis at day 1, 10, 21 and 42. For each phase and over 0-42 d, NC reduced ($P < 0.05$) ADG, ADFI (except 0-10d) and increased FCR vs. PC. NC+3000 FTU improved ($P< 0.05$) ADG and ADFI vs. NC and PC (except for 22-42d ADFI), and maintained FCR compared to PC. NC+ 3000 FTU improved final BW by 17 and 4.7% respectively compared to NC and PC (2650, 2966 and 3105g for NC, PC and NC+3000 FTU respectively, $P< 0.05$). The European Performance Efficiency Factor was 374, 430 and 439 for NC, PC and NC+3000FTU phytase respectively. The data demonstrated that Buttiauxella phytase dosed at 3000 FTU contributed to an extra-phosphoric effect that resulted in improved performance compared to the PC, and lead to production benefit in broilers under mild challenge

Poster 2.114: Effect of age/adaptation time on phytase efficacy measurement based on retainable P and Ca in young broiler chickens

Y. Dersjant-Li[1], L. Marchal[1], C. Kwakernaak[2]

[1] DuPont Animal Nutrition/DuPont IB, Archimedesweg 30 2333CN Leiden The Netherlands, [2] Schothorst Feed Research, P.O. Box 533, 8200 AM Lelystad, The Netherlands; Yueming.dersjant-Li@dupont.com

The study tested the effect of a Buttiauxella phytase dosed at 0 (NC), 250, 500, 750 and 1000 FTU/kg on apparent retention (ret) of P and Ca in broilers at different ages, after a short (2 d), intermediate (8 d), and long adaptation time (13 d). Test diets were fed as pellets to Ross 308 male broilers (6 replicate balance cages/treatment, 20 birds/cage), from 5-20 days of age. Diets were maize SBM based (2900 kcal/kg AMEn, 210 g/kg CP, 4.7 g/kg P, 2.6 g/kg phytate P and 7.5 g/kg Ca, including TiO_2 as marker) fed ad lib. Excreta was collected at d7-8, d13-14 and d18-20. Results were analysed by ANOVA using (adaptation) time and phytase dose as treatment factors. The ret P and Ca were strongly affected by phytase dose, adaptation time and their interaction ($P<0.001$). Across time points, increasing phytase dose from 0 to 1000 FTU/kg improved retention of P (48 to 71%) and Ca (28 to 51%). The ret P and Ca was greater at 20d vs 8d. A strong exponential response ($P < 0.01$) was seen with increasing phytase dose for ret P and Ca, at each sampling point. For NC the ret P increased with longer adaptation time ($P<0.05$: 44% at day 8 vs 54% at day 20), while with phytase at 1000 FTU/kg there was no effect (73% at day 8 vs 70% at day 20). A similar interaction effect was seen for ret Ca. Compared to NC, phytase at 1000 FTU/kg increased ret P by 29, 24 and 15% points, increased ret Ca by 31, 26.7 and 13.3% points, at day 8, 14 and 20 respectively. Effect of time is confounded with age of bird and thus their physiological status. This effect was greater for NC than for the phytase supplemented groups, indicating that adaptation time plays a role and affects the response measured in the phytase efficacy studies

Poster 2.115: Evaluation of the effect of different selenium sources in broiler chickens

D. Kumprechtova[1], V. Marchander[2], R. Raspoet[3], A. Riggi[3]

[1] Institute of Animal Science, Department of Animal Nutrition, Prague, Czech Republic, [2] International Poultry Testing, Ustrasice, Czech Republic, [3] Phileo - Lesaffre Animal Care, France; dana.kumprechtova@gmail.com

Two consecutive trials were conducted in 2017 at the International Poultry Testing Ustrasice (MTD), in Czech Republic, to evaluate the effect of different selenium sources on Se bioavailability, and oxidative stress parameters in broiler chickens. In trial 1, 210 ROSS 308 broilers were allocated in 2 groups of 3 pens of 35 birds each. Group 1 was supplemented with Se-Yeast 1 (Selsaf 3000) at 0,2 ppm of Se in the feed, from 1 day old till 14 days old. Group 2 was supplemented with Se-Yeast 2 at the same concentration of Se. At day 14, samples were done to quantify Se bioavailability and Glutathion peroxydase activity (GPx) in the blood and the muscles (breast and tigh). Se bioavailability in the blood was significantly higher in group 1 than in group 2 (172,9 µg/L vs 140,4). Se concentration in the breast and thigh muscles were also significantly higher in group 1 than in group 2 (respectively 317 and 326 µg/kg vs 242 and 261) ($p<0{,}001$). In trial 2, 140 ROSS 308 broilers were allocated in 2 groups of 2 pens of 35 birds each. Supplementations were the same than in trial 1. The results gave similar results : Se bioavailability in the blood was significantly higher in group 1 than in group 2 (196 µg/L vs 158). Se concentration in the breast and thigh muscles were also significantly higher in group 1 than in group 2 (respectively 302 and 322 µg/kg vs 243 and 260) ($p<0{,}03$). In both trials, the GPx activity in the blood was numerically higher in group 1 than in group 2. No difference for GPx in the muscles was seen.

In conclusion, Se levels were higher in blood, breast and thigh when animals were supplemented with Se-Yeast 1 instead of Se-Yeast 2. Increased levels of Se in breast and thigh muscles are most likely because of higher SeMet presence in proteins.

Poster 2.116: Hydroxychloride trace minerals have a beneficial effect on growth performance, carcass yield and impact the gut microbiota of broiler chickens

S. van Kuijk[1], A. Rodiles[1], A. I. Garcia Ruiz[2], Y. Han[1]

[1] Trouw Nutrition R&D, P.O. Box 299, 3800 AG, Amersfoort, the Netherlands, [2] Trouw Nutrition R&D, Casarrubios del Monte, 45950 (Toledo), Spain; sandra.van.kuijk@trouwnutrition.com

The aim of this study was to compare the effect of hydroxychloride trace minerals (HTM) to inorganic trace minerals (ITM) on growth performance, carcass quality and gut microbiota of broiler chickens. In total 1440 male Ross 308 birds were divided over 12 replicates per treatment (30 birds/pen). Four treatments were tested: 2 with hydroxychloride Zn and 2 with Zn from sulfates that were added to a wheat-soya diet, at low or high level (20 or 80 ppm), with the inclusion of 15 ppm Cu of the same source in each diet. Growth performance was measured after each feeding phase at day 0, 10, 27 and 34. At the end of the study, 5 birds/pen were selected to quantify carcass and breast meat yield, and 1 bird/pen to collect cecal digesta for microbiota analysis (n=12). Results showed a significantly higher body weight in HTM groups compared to the ITM groups at day 27, which continued until day 34 as a trend ($P=0.0542$). Average daily gain and average daily feed intake were also significantly improved in the HTM groups compared to the ITM groups for the overall period, with no difference in feed conversion ratio. Breast meat yield (% of carcass weight) tended to be higher in the birds fed the high level of HTM ($P=0.0764$). Beta diversity results demonstrate that HTM and ITM groups differently impacted the microbial composition of the cecum ($P=0.016$), much more than the levels of trace minerals did ($P=0.067$). In addition, the HTM group showed a potentially higher richness in Shannon diversity ($P=0.066$). Main taxonomy differences at genus level were observed between the groups fed high or low levels of HTM. In conclusion, HTM have a beneficial effect on growth performance, carcass quality and impact the gut microbiota diversity compared to ITM in broiler chickens fed a wheat-soya diet.

Poster 2.117: The effects of dietary lignocellulose on litter quality and broilers performance

L. Makivić[1], D. Perić[2], R. Marković[2], S. Radulović[2], D. Jakić-Dimić[3], D. Šefer[2]

[1] Feed Factory "Rapić" Gradiška, [2] Faculty of Veterinary Medicine University of Belgrade, Department of Animal Nutrition and Botany, [3] Scientific Veterinary Institute of Serbia; makivic.lazar@farmofit.ba

Purified lignocellulose represents a pronutritive substance that affects the viscosity of the intestinal content, increases the absorption of nutrients and reduces the number of pathogenic bacteria in the small intestine. In this experiment the effects of lignocellulose in poultry nutrition was studied. Trial included 384 broilers of Cobb 500 provenance, both male and female, divided into four groups (control group: C and three experimental groups: E-I, E-II and E-III), 96 animals in each. Animals were fed with standard feed mixtures, starter (from 1st to 13th day), grover (from 14th to 28th day) and finisher (from 29th to 42th day), according to the manufacturer's recommendation. A control group (C) diet was without additives. The experimental groups differed in the fact that in the first two mixtures (starter and grover) a commercial preparation of purified lignocellulose (Arbocel® R, J. Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) was added in the amount of 4 g/kg of feed for the E-I group, 6 g/kg of Arbocel® R as an expense of 0.3% soybean meal and 0.3% maize was added for the E-II group and 6 g/kg of Arbocel® R as an expense of 0.6% soybean meal was added for the E-III group. Analyzing the entire period of observation (from 1st to 42th day), adding the lignocellulose in experimental E-II group resulted in the best production indicators (final body weight 2611.00 g, average daily feed intake 96.09 g, average weight gain 2569.29 g and feed to gain ratio 1.67) as well as the best litter quality (moisture content 6.69%). Based on the obtained results, it can be concluded that the use of lignocelluloses in broilers nutrition has its medical, nutritional and economical justification.

Oral presentations

Effects of dietary sulfur amino acid levels on growth performance and intestinal antibody production in broilers challenged with Eimeria spp

Z. Ren[1], D. E. Bütz[2], R. A. K. Whelan[3], V. Naranjo[3], M. K. Arendt[4], M. D. Ramuta[4], X. Yang[1], T. D. Crenshaw[4], M. E. Cook[4]

[1] College of Animal Science and Technology, Northwest A&F University, 22 Xinnong Road, Yangling, Shaanxi 712100, China, [2] Department of Animal Sciences, University of Wisconsin-Madison, 1675 Observatory Drive, Madison, WI 53706, USA, [3] Evonik Nutrition & Care GmbH, 4 Rodenbacher Chaussee, Hanau-Wolfgang 63457, Germany, [4] Department of Animal Sciences, University of Wisconsin-Madison, 1675 Observatory Drive, Madison, WI 53706, US; poultryren@outlook.com

Methionine + cysteine (M+C) requirement may be higher when chickens are infected with Eimeria spp. In a 4 x 2 factorial design, broilers were fed one of four diets containing either 0.6, 0.8, 0.9 or 1.0% SID M+C from 11 to 21 d and gavaged on d 14 with either phosphate-buffered saline (PBS) or a 100x commercial coccidiosis vaccine dose containing live Eimeria acervulina, E. maxima, and E. tenella oocysts. Growth performance was recorded from d 11 to 21. Plasma and intestinal luminal samples were collected on d 14 and d 21. Intestine lesion scoring and fecal oocyst counting were conducted on d 21. When compared to PBS gavaged broilers, Eimeria-challenged broilers had: 1) decreased ($P < 0.05$) body weight gain (BWG), feed intake (FI) and gain-to-feed ratio (G:F); 2) increased ($P < 0.05$) intestine lesion and fecal oocyst counting; 3) increased ($P < 0.05$) plasma anti-Eimeria IgG and intestine-luminal total IgA and anti-Eimeria IgA; and 4) increased ($P < 0.05$) luminal gamma interferon (IFN-γ) in the duodenum, jejunum and cecum and interleukin-10 (IL-10) in the duodenum. Regardless of Eimeria challenge, when compared to 0.6 % SID M+C, broilers fed ≥ 08% M+C had: 1) increased ($P < 0.05$) BWG, FI and G:F; and 2) increased ($P < 0.05$) levels of jejunum luminal total IgA. Eimeria-challenge, broilers fed 0.8 % M+C had increased ($P < 0.05$) levels of jejunum luminal anti-Eimeria IgA compared to broilers fed diets containing 0.6 and 1.0 % M+C. Collectively, the growth suppression caused by Eimeria infection could not be reduced by further increasing dietary M+C ≥ 0.8%, despite an increase in IgA observed in higher doses. Further research should investigate interactions between dietary M+C and other nutrients for support of immune function and growth in pathogen-challenged broilers.

Intestinal proteome changes related to inflammation, endocrine, and metabolism pathways impacted by in ovo administered bacteria in day of hatch chicks

D. Russi-Rodrigues[1], K. M. Wilson[1], M. Trombetta[1], W. Briggs[1], A. F. Duff[1], K. M. Chasser[1], W. G. Bottje[2], L. R. Bielke[1]

[1] Ohio State University, [2] University of Arkansas; bielke.1@osu.edu

Manipulation of GIT microbiota can influence health of poultry. This study compared effects of in ovo inoculation with saline (S), LAB-probiotic (LAB) or different Citrobacter (CF, C2) for changes to inflammation, endocrine and metabolic proteome at day of hatch. Ten chicks for each group were sampled for microbiome and proteome analysis. GIT proteins were identified, compared to S at $p \leq 0.05$, and evaluated for canonical pathways and biological functions, p≤0.05&: z-score ±1.5. Cytokines upregulated by C2 suggested pro-inflammation with up-regulation of acute phase protein signaling (z-score +2.00). LAB indicated decreased inflammation with down regulation of TNFα, IL-4, IL-15 and IFNγ and up-regulation of IL-13 (z-score +2.00). CF created dis-regulated inflammatory state with up-regulation of NFkB, IgG, OSM, TGFβ1. Metabolic changes in LAB included activation of TCA cycle, NAD salvage, tRNA charging, and aspartate degradation. CF resulted in inhibition of oxidative phosphorylation, and purine nucleotide biosynthesis, with C2 activation of sorbitol degradation, citrulline-nitric oxide, urea, and γ-glutamyl cycle and inhibition of adenosine nucleotide, histamine, and purine

nucleotide degradation. Endocrine changes included CF inhibition of insulin and cholesterol regulation, HNF1A and HNF4A (z-scores -1.97 & -2.00). C2 expressed activation of HNF1A (z-score +1.98), progesterone receptor pathways (z-score +1.98) and angiotensinogen (z-score +1.9). LAB showed activation of corticosterone (z-score +1.95) and steroid hormone receptor ERR1 (z-score +1.95) as well as Insulin-1 (z-score +1.49) and inhibition of calcitriol (z-score -1.71) pathways. These changes suggest that pioneer colonizing microbiota an impact on a number of GIT physiological pathways.

Caecal microbiota changes in an intestinal leakage model in broilers

F. de Meyer[1], E. Goossens[1], V. Eeckhaut[1], R. Ducatelle[1], A. Dedeurwaerder[2], M. de Gussem[3], F. van Immerseel[1]

[1] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, [2] Poulpharm bvba, [3] Vetworks; F.DeMeyer@UGent.be

Intestinal health in broilers can be affected by pathogens or nutritional triggers that can act directly (e.g. mycotoxins) or indirectly through the microbiota. The gut microbial community can be influenced by diet composition, antibiotics and host responses, such as mucosal inflammation. To evaluate microbial shifts induced by common intestinal stressors, day old broilers were randomly assigned to two groups, i.e. control and challenge. From day 12, all animals were fed a wheat (57.5%) based diet supplemented with 5% rye. From day 12 to 18, animals from the challenge group were treated with antibiotics. Hereafter, a bacterial cocktail that included opportunistic bacteria among others E. coli and C. perfringens was given daily from day 19 till 21. On day 20, these animals were administered Eimeria (E.) acervulina and E. maxima. On day 26, birds were euthanized. Duodenal tissue was sampled for histological analysis and caecal content collected for DNA extraction following 16S sequencing analysis via amplification of the V3-V4 hypervariable region. Challenged birds had a lower body weight ($p<0.0001$) and higher feed conversion ratio ($p<0.004$). Histological analysis showed shorter villi, deeper crypts and higher immune cell infiltration in the intestinal mucosa ($p<0.0001$). Moreover, challenged animals had a caecal microbial community with lower alpha diversity described by observed OTUs, Chao1 richness and Shannon diversity ($p<0.05$). Based on linear discriminant analysis (LDA) effect size (LEfSe), genera Akkermansia, Eggerthella and Lactobacillus were more abundant in the caecal content of challenged birds. Several genera within the Lachnospiraceae and Ruminococcaceae family were less abundant in caecal content of challenged animals, including the genus Faecalibacterium.

A moist feeding strategy for broiler chickens: effects on performance and intestinal health

E. Frehen-van Calmthout[1], A. Dörper[2], T. Thomas[2], R. Kwakkel[2]

[1] Arkervaart-BramecoZon U.A., Nijkerk, The Netherlands and Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands, [2] Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands; e.frehen@abzdiervoeding.nl

The first days of life are of great importance for the development of the gastrointestinal tract (GIT) in broilers. The GIT is equipped with an intrinsic immune system. The expression of innate immune cells is influenced by nutrients and metabolites from bacterial and dietary origin. This project is aimed to develop a moist based feeding strategy (a mixture of dry feed and water in a ratio of 1:1.7) for broilers. This moist based feed will be used as a novel way to enhance feed intake and potentially modulate the GIT within the to be determined early days of life, which will be considered as the critical window for an accelerated gut development and better nutrient digestibility. This moist feeding strategy should predisposition the GIT towards a better developed GIT, an improved innate immune response, a balanced microbial ecosystem, and hence, more bird welfare and a reduction of the prophylactic use of antibiotics. A total of 382 Ross 308 broilers were divided over 4 different treatments (T1 to T4), with 8 repetitions. T1 birds were fed a commercial dry feed, T2 birds were fed the moist feed for the 1st week of life, T3 birds were fed the moist feed during the first 3 weeks of life and T4 birds were fed the moist feed during the full experimental period of 5 weeks. Performance was recorded on a weekly basis during the experiment. Intestinal health was assessed by cytokine expression, morphological parameters and cecal microbial colonization, in all treatment groups on days 8, 22 and 36. The first results of the immunological, morphological, microbial.

Pioneer colonizing LAB as a driver of gut low-diversity: How does it shape host fitness?

D. Russi-Rodrigues[1], K. Wilson[1], W. Briggs[1], A. Duff[1], K. Chasser[1], J. Bielke[1], L. Bielke[1]

[1] Ohio State University; russirodrigues.1@osu.edu

Low gut diversity may be accounted for by the relative overabundance of few intestinal symbionts or by presence of highly competitive microbial communities and is not always a disadvantage. Adjustment in diversity is tied to changes in host function and disturbances in the microbiota. The aim of this study was to evaluate the impact of chicks exposed, prior to hatch, to lactic acid bacteria (LAB) probiotic or Enterobacteriaceae strains on diversity of ileal mature microbiota. An important outstanding question is whether or not and how these microbes, as first bacterial settlers in the gut, can modulate diversity dynamics. Embryos were inoculated with either saline (S), 102 CFU of Citrobacter freundii (C), Citrobacter sp (C2) or LAB (L) in the amnion. At hatch (DOH) the whole intestine, as well as upper ileum (UI) and lower ileum (LI) at 3d and 10d were collected for microbiome diversity analysis. Shannon diversity was increased in S relative to C, C2 and L at DOH ($P<0.05$). By 3d, in UI, the pattern was the opposite, diversity index was higher in L, C and C2 than S. At 10d, C was more diverse than C2 ($P<0.05$), although it was similar to S and L treatments ($P>0.05$). Comparing diversity shifts over time, L and C2 treatments significantly overlapped in diversity from DOH to 3d in UI and LI. Interestingly, in both GIT sites, there was a reduction ($P<0.05$) of diversity only in L by 10d. Weighted and unweighted UniFrac showed L samples were grouped closer together ($P<0.05$) in LI at a later age. These data suggest that, although L microbiota was ecologically less rich at later age, members of community had phylogenic similar organisms. Importantly, we demonstrate that LAB-probiotic as pioneer colonizers plays a critical role in driving course of microbial diversity up to 10d.

Poster 3.1: Efficacy of a new carvacrol-based product on Campylobacter jejuni in challenge test in vivo and impact on the whole caecal microbiota

M. Allaoua[1], S. Combes[2], V. Noirot[1], P. Etienne[1], J.-F. Gabarrou[1], O. Bouchez[3], M. Treilhou[4], E. Bonnafé[4]

[1] Phodé, [2] INRA, GenPhySE, NED, [3] GeT-PlaGE, Genotoul, INRA, [4] Jean-François Champollion Institute, BTSB laboratory; vnoirot@phode.fr

Campylobacter is the leading cause of foodborne diarrheal disease worldwide. The main source of infection is the meat from poultry origin mostly contaminated during evisceration at slaughterhouse. Oral administration of carvacrol could be a promising method to reduce Campylobacter in digestive tract but this compound is absorbed proximally in the blood system, so it does not get to the sites of Campylobacter growth which are the caeca. A new solid galenic (Phodé, France) has been created to control the release of the carvacrol. In the present study, the efficacy of this formulation (2.5 kg per T of feed) in decreasing the C. jejuni caecal load in broiler chickens artificially inoculated (1.10^8 CFU/chicken) at 21 days is evaluated. The 16S rDNA sequencing allows us to study the impact of both artificial inoculation and addition of the formulation to the feed on the whole caecal microbiota. This study is conducted on 3 groups of chickens, one non-inoculated and untreated, one inoculated and untreated and one inoculated and treated. The formulation significantly decreases the C. jejuni load by 1.4 log in comparison with the inoculated and non-treated control. The sequencing results show that the artificial inoculation impacted the microbiota richness and the relative abundance of major genera. The formulation significantly increased the richness, the diversity and modified the structure of the microbiota in comparison with the control samples (chickens inoculated but non-treated with the formulation). The results show that this formulation of carvacrol modifies the caecal microbiota and limits the growth of C. jejuni under artificial inoculation conditions.

Poster 3.2: Evaluation of a fermentation-derived abiotic on the performance of broiler chickens challenged with subclinical necrotic enteritis caused by Clostridium perfringens

R. Adhikari[1], S. Llamas Moya[1], C. Rasmussen[1], B. Lumpkins[2]

[1] Kerry Inc., Beloit, WI 53511, USA, [2] Southern Poultry Research Inc., Athens (GA), USA; sara.llamasmoya@kerry.com

This study evaluated the efficacy of a fermentation-derived abiotic (UpGrade™ AF) on the performance of broilers reared to 42 days (d) and challenged by induced subclinical necrotic enteritis (NE). A total of 2,000 Ross 708 male day-old chicks (DOC) were placed in one of 40 pens, blocked and randomly allocated to one of 4 treatments (N=10). T1 birds were not challenged with NE, but received bacitracin methylene disalicylate (BMD) at 55 g/MT for the duration of the study. T2, T3 and T4 birds were orally inoculated with a culture broth of Clostridium perfringens (1.0 ml/bird and ~1.0 x 10^{8-9} cfu/ml) on d 19. T2 birds were non-medicated throughout the experimental period, whereas T3 broilers received BMD at 55 g/MT and T4 animals were supplemented in feed with 225 g/MT UpGrade™ AF. Broilers were fed on a typical 3-phase feeding program. Pen weights and feed weight-backs coincided with the change of feeding phase at d 21, 35 and 42 for determination of performance. On d 21, 3 birds/replicate were selected, and sacrificed to assess the severity of NE lesions based on a 0 (normal) to 3 (severe) scale. Results showed no significant effect of treatment on final body weight. Significant differences in NE lesion scores ($P<0.001$) showed normal appearance in T1 broilers, whereas inoculated birds had higher scores, albeit all <1. Feed conversion ratio (FCR) differed between treatments ($P=0.003$), with T2 and T3 being least efficient and statistically poorer than T1 and T4. Simultaneously, T4 was not different to T1 in terms of FCR demonstrating that UpGrade™ AF, a fermentation-derived abiotic, was efficient at lowering the impact on performance of induced NE in broilers.

Poster 3.3: Comparison of the anticoccidial combinations of nicarbazin and ionophores

J. B. Wilson[1], J. Rivers[2], H. Kasab-Bachi[1]

[1] Novometrix Research Inc. 4564 Nassagaweya-Puslinch Townline, Moffat, Ontario, Canada L0P 1J0,
[2] Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada N1G 2W1; jbwilson@novometrix.com

Nicarbazin is demonstrated to have synergistic anticoccidial activity in combination with ionophores. However, there is a general lack of peer-reviewed scientific publications comparing the properties of different ionophore and nicarbazin combinations. In the absence of direct comparative studies and, as nicarbazin is a common component of all these combinations, this review focuses on comparisons of the ionophore component; in the case of Maxiban®, narasin.

Maxiban®, a 1:1 nicarbazin:narasin combination, is used extensively by global broiler producers for prevention of coccidiosis. It has a proven outstanding anticoccidial efficacy profile, and has demonstrated efficacy against Eimeria spp. strains resistant to nicarbazin or ionophores alone.

Considering that several ionophore–nicarbazin anticoccidials are registered globally, why is it that narasin in its combination product Maxiban® has been so successful in comparison? Narasin is well established as an excellent anticoccidial ionophore and has several proven advantages that makes it the ideal ionophore to combine with nicarbazin to provide synergistic anticoccidial effectiveness resulting in optimal growth performance when fed to broilers. Unlike monensin, it is effective at providing a high degree of protection against coccidiosis without depressing weight gain. Narasin is also reported to be the most active among several ionophores against a dual infection with coccidia and Clostridium perfringens, and is the only ionophore reported to reduce the severity of gizzard erosion. Unlike some other ionophores, narasin produces no deleterious side effects when included in the ration of broilers. Further, Maxiban® has no regulatory requirement in some broiler production areas for withdrawal of medication prior to slaughter.

Poster 3.4: In vitro versus in situ measurement of xylan hydrolysis

N. Morgan[1], A. Wallace[1], M. Choct[1]

[1] University of New England; nmorga20@une.edu.au

Xylo-oligosaccharides (XOS) are hydrolytic degradation products of xylan. Selective fermentation of XOS has been shown to instigate positive effects on the composition and activity of gastrointestinal microbiota, thus suggesting XOS can be defined as a prebiotic. In vitro assays provide a rapid and economical tool to evaluate dietary effects, but have limitations. This study examined if a 2-step in vitro digestion assay that simulated the gastric and small intestine (SI) phases of digestion can be used to predict in situ XOS production from xylan in broiler chickens. Male day-old Ross 308 chicks (n=60, 30 birds per treatment) were fed a standard wheat-soybean meal based grower diet supplemented with 2% pure xylan, either with or without 16,000 BXU xylanase (Econase® XT 25, AB Vista Feed Ingredients) from d10-21. Gizzard and ileum samples were collected from 10 birds per treatment on d21. The concentration of xylobiose (X_2), xylotriose (X_3) and xylotetraose (X_4) were analysed in both the in vitro and in situ samples using a Shimadzu LCMS-8050 with a C18 HPLC column by electrospray ionization. The relationship between the in vitro and in situ method was strong for both the gizzard (X_2 r = 0.951, P = 0.049; X_3 r = 0.969, P = 0.031; X_4 r = 0.979, P = 0.021) and the ileum (X_2 r = 0.947, P = 0.053; X_3 r = 0.964, P = 0.036; X_4 r = 0.942, P = 0.058). The X_2, X_3 and X_4 concentration values were consistently numerically higher in the in situ samples compared to the in vitro values, but no significant differences were observed. This suggests that this in vitro assay has potential as a tool to foresee dietary XOS production and xylanase efficacy in the gastrointestinal tract of broiler chickens.

Poster 3.5: Effect of a microbial muramidase supplementation combined with feed enzymes on growth performance, apparent ileal digestibility and jejunal viscosity of broiler chickens

E. Pérez Calvo[1], R. Aureli[1]

[1] DSM Nutritional Products; estefania.perez-calvo@dsm.com

Muramidases are enzymes that hydrolyze peptidoglycans (PGNs) from bacterial cell wall fragments present in the gut. It is hypothesized that the natural microbial cell turnover may be incremented when high concentrations of soluble NSP are included in the diet leading to lower animal performance and nutrient digestibility. The current study evaluated the effects of a novel microbial muramidase (MUR) in combination with feed enzymes on growth performance, apparent ileal digestibility (AID) and intestinal viscosity (IV) in broiler chickens. A total of 432-day-old Cobb 500 were randomly allocated into 24 floor pens of 18 birds per pen. All birds were vaccinated against coccidiosis. A diet based mainly on soybean meal, wheat (45%) and rye (10%) and containing exogenous phytase and protease was distributed into a two-phase feeding program (starter: day 0-21 and grower: day 21-35). Treatments were: Control (C), C + xylanase (XYL), and C + XYL + MUR. Growth performance parameters, AID of protein and energy and IV were determined at day 35. All data were analyzed by ANOVA and means were compared by Newman-Keuls test. The overall bird weight at 35d age was 2526g. Birds fed C + XYL + MUR showed significantly ($p=0.05$) lower FCR (1.54) compared to the control group (1.62). XYL combined with MUR showed the highest AID of energy ($P<0.05$) but AID of protein did not change between treatments ($P>0.05$). However, XYL alone or in combination with MUR showed a decreased IV compared to C. Those results suggest that the muramidase, by hydrolyzing bacterial PGNs in the gut, might provide greater substrate access and with that facilitate the activity of the xylanase resulting in improved AID. However, further investigations are required to understand the mode of action of this novel muramidase.

Poster 3.6: Effect of a microbial muramidase on growth performance and welfare of broiler chickens

E. Esteve-Garcia[1], E. Pérez Calvo[2]

[1] IRTA, Animal Nutrition, [2] DSM Nutritional Products; estefania.perez-calvo@dsm.com

A study was conducted to determine the effect of a microbial muramidase (MUR) on performance, litter moisture and incidence of footpad dermatitis (FPD) in broiler chickens. One thousand one hundred and four day-old male Ross 308 chickens were distributed into 48 pens at 23 per pen and assigned to one of four treatments: 1) control (C); 2) C + 25000 LSU(F)/kg MUR; 3) C + 35000 LSU(F)/kg MUR; 4) C + 45000 LSU(F)/kg MUR. The feeding programme consisted of a wheat, barley, maize and soybean based diet till 21 days (starter), and a wheat, barley and soybean meal based diet till 35 days (grower). Diets contained no supplemental feed enzymes and Clinacox as coccidiostat. Chickens and feed were weighed at 21 and 35 days and performance were determined for each period and for the overall study. The data obtained were analysed with a set of orthogonal polynomials. Average daily gain was linearly improved by MUR in the starter period and in the overall study. FCR was also linearly improved by MUR in the overall study. Dietary supplementation of 45000 LSU(F)/kg of MUR improved body weight by 4.0 % and FCR by 2.8 % compared to the control group. Litter moisture was also reduced with MUR inclusion, and this was reflected in reduction of FPD and in cleaner plumage of the birds at the end of the study. Overall, the data gathered in this study indicate a positive effect of dietary microbial muramidase supplementation on broilers' welfare and performance as suggested by the lower litter moisture, lower incidence of footpad dermatitis and improved FCR.

Poster 3.7: A novel muramidase enzyme effectively depolymerize peptidoglycans from various dead bacteria isolated from the gastrointestinal tract of poultry

M. B. Pedersen[1], M. T. Cohn[1], K. M. Schnorr[1], L. K. Skov[1], S. Buskov[1], E. P. Calvo[2], R. Lopez-Ulibarri[2], M. Klausen[1]

[1] Novozymes A/S, [2] DSM Nutrional Products; mabp@novozymes.com

The gastrointestinal microbiota exists in a complex ecosystem in equilibrium with the host. Microbial cell division and death occur naturally throughout the entire gastrointestinal tract, resulting in a diversified release of defragmented microbial cell components into the gut lumen. The structural polymer peptidoglycan, exclusively found in bacterial cell walls, comprises a significant fraction of the cell wall in all bacterial species present in the gastrointestinal tract. Recent studies reported how in vivo supplementation of a novel fungal muramidase enzyme, capable of depolymerizing peptidoglycan from dead bacteria, significantly improved performance parameters in broiler chickens. To further investigate the efficacy of the muramidase to depolymerize peptidoglycan from various bacterial origins, peptidoglycan from five different isolated chicken gut bacteria was purified and characterized. The muramidase enzyme effectively depolymerized peptidoglycan from all five origins in vitro validated by both reducing ends and mass spectrometry analysis. In addition, a different compositional profile of hydrolysis products was identified by mass spectrometry, indicating different peptidoglycan structures depending on the bacterial origin. Finally, an in vitro peptidoglycan recognition protein assay adapted from silk worm larvae plasma, demonstrated how the muramidase treated peptidoglycan reduced the recognition response compared to the control sample without muramidase supplementation.

Poster 3.8: In vitro enzymatic activity analysis of a novel muramidase for animal feed

J. S. Chimilovski[1], M. B. Pedersen[1], M. Sluis[1], R. N. Maeda[1], L. K. Skov[1], R. Lopez-Ulibarri[2], M. Klausen[1]

[1] Novozymes A/S, [2] DSM Nutriotional Products; mabp@novozymes.com

A novel microbial muramidase that hydrolyzes the peptidoglycan (PGN) polymer of bacterial cell-wall fragments from bacterial debris was recently supplemented in broiler feed to improve performance by hydrolyzing PGN in bacterial cell wall fragments. In this study, we describe the in vitro measurement of the novel muramidase enzymatic activity using a colorimetric dye-release assay. Cells from Micrococcus lysodeikticus ATCC No. 4698 were labeled with the dye Remazol Brilliant Blue (RBB) under alkaline conditions and used as the substrate for the enzymatic hydrolysis. During careful incubation of RBB with the PGN-rich dead cells of M. lysodeikticus under alkaline conditions, the RBB undergoes an elimination reaction and its derivative reacts with glycan hydroxyl groups of the peptidoglycans, producing a dye-labeled substrate for the muramidase. Muramidase cleaves the b-1,4 glycosidic linkages between N-Acetylmuramic acid and N-Acetylglucosamine in PGN, which releases soluble, dyed fragments from the substrate. After the reaction, the insoluble fraction is removed by centrifugation and the soluble hydrolyzed products are measured with a spectrophotometer at 600 nm. The signal was enzyme-dependent and dose-dependent. The colorimetric assay can be used to accurately measure enzymatic activity ranging from 1 to 750 U/mL with % RSD of less than 5%. An additional advantage of the method is that muramidase mediated color release can be visually scored without requirement of spectrophotometers. In vitro enzymatic activity measurements complement the understanding of muramidase and its benefits to gastrointestinal functionality in broilers.

Poster 3.9: A microbial muramidase improves growth performance and feed efficiency of broiler chickens

W. Schliffka[1], K. Kozłowski[2], E. Pérez Calvo[3], R. Lopez-Ulibarri[1], J. Jankowski[2]

[1] DSM Nutritional Products Ltd, Switzerland, [2] Department of Poultry Science, University of Warmia and Mazury in Olsztyn, Poland, [3] DSM Nutritional Products France, France; wolfgang.schliffka@dsm.com

A total of 3930 Ross 308 day-old male broilers were allocated to four studies with three or four treatments to determine the effect of different dosages or batches of a microbial muramidase on growth performance after a 35 days fattening period. Composition of basal diet, similar in all studies, was based on wheat, corn, soybean and rapeseed meal.

Study 1: 990 poults were randomly assigned to one of three treatments: T1 (Control, C); T2 (C + 25000 LSU(F)/kg); T3 (C + 35000 LSU(F)/kg) with 33 replicates per treatment.

Study 2: 990 poults were randomly assigned to one of three treatments: T1 (Control, C); T2 (C + 15000 LSU(F)/kg); T3 (C + 25000 LSU(F)/kg) with 33 replicates per treatment.

Study 3: 990 poults were randomly assigned to one of three treatments: T1 (Control, C); T2 (C + Batch 1, 25000 LSU(F)/kg); T3 (C + Batch 2, 25000 LSU(F)/kg) with 33 replicates per treatment.

Study 4: 960 poults were randomly assigned to one of four treatments: T1 (Control, C); T2 (C + Batch 1, 25000 LSU(F)/kg); T3 (C + Batch 2, 25000 LSU(F)/kg); T4 (C + Batch 3, 25000 LSU(F)/kg) with 16 replicates per treatment. Results of the statistical analyzes of these studies showed that the muramidase treatments significantly improved feed conversion ratio (FCR) in all studies and all dosages. In study 1, 2 and 3, FCR decreased between 1.7 and 2.8% ($p<0.001$). In study 4, FCR decreased between 1.3 and 1.8% ($p=0.04$). In addition, average daily gain (ADG) of birds supplemented with muramidase was significantly improved between 1.9% ($p=0.011$) and 2.6% ($p=0.031$) in studies 3 and 4, respectively.In conclusion, these results show that the microbial muramidase improves ADG and feed efficiency of broiler chickens.

Poster 3.10: Dietary microbial muramidase improves welfare, metabolizable energy and growth of broiler

V. Pirgozliev[1], S. Paul Rose[1], E. Pérez Calvo[2]

[1] The National Institute of Poultry Husbandry, Harper Adams University, UK, [2] DSM Nutritional Products, Research Center for Animal Nutrition and Health Village Neuf, France; vpirgozliev@harper-adams.ac.uk

The study evaluates the effect of graded levels of microbial muramidase (MUR) on feed intake (FI), weigh gain (WG), feed conversion ratio (FCR), footpad dermatitis (FPD), dietary metabolisable energy (ME) and European Performance Index (EPI) when fed to broilers from 0 to 42d age. Nineteen-hundred-twenty day-old male Ross 308 chicks were randomly allocated to 96 floor pens (20 birds each). Broilers were fed wheat-soybean-based diets containing exogenous xylanase and phytase, and Clinacox as coccidiostat. A four-phase dietary program and four experimental pelleted diets were used: a control diet (C, following breeder's recommendations) without MUR supplementation, and three diets based on C supplemented with 25000, 35000 and 45000 LSU (F)/kg of MUR, respectively. Dietary ME was determined at 21d age and FPD was evaluated at the end of the study. The data were analysed by ANOVA. Orthogonal polynomials were used to compare treatment differences for linear and quadratic relationships with increasing MUR activity. In all instances, differences were reported as significant at $P<0.05$. The overall bird weight at 42d age was 2987g. The inclusion of MUR did not change FI ($P>0.05$), but increased WG in linear manner ($P<0.05$) and reduced FCR in a quadratic manner with the optimum being achieved at approximately 35000 LSU (F)/kg. In accordance with the improvement in FCR, 35000 LSU (F)/kg MUR supplementation produced the highest EPI ($P<0.05$). FPD score was linearly decreased with increased addition of MUR ($P<0.05$). Dietary ME responded linearly to the MUR inclusion, as the highest values were obtained with the highest inclusion rate ($P<0.05$). The results suggest that inclusion of MUR on top of feed enzymes improves feed efficiency and footpad dermatitis of birds.

Poster 3.11: Feeding two single strain probiotic bacteria and wheat bran failed to modify the production traits but altered some gut characteristics in broiler chickens

N. Such[1], I. Koltay[1], L. Pál [1], L. Wágner[1], A. Rawash Mohamed[1], Á. Mezőlaki[2], A. Márton[1], A. Molnár[1], K. Dublecz[1]

[1] University of Pannonia, [2] Agrofeed Ltd.; such.nikoletta@georgikon.hu

The effects of a single strain lactic acid producing bacteria (LAB) (Lactobacillus farciminis $5x10^9$ CFU/kg) and a single strain butyric acid producing bacteria (BAB) (Clostridium butyricum $2.5x10^9$ CFU/kg) with or without wheat bran (WB), were investigated on the production traits and several gut characteristics of broiler chickens. In total, 574 male Ross 308 day-old chickens were divided into 24 floor pens and fed a corn-soybean based control diet (C) and five other probiotic or wheat bran supplemented diets (LAB, BAB, LAB+WB, BAB+WB, C+WB) in 4 replicates. The wheat bran content of the starter, grower and finisher diets were 3, 6 and 6%, respectively. During the 37 day long fattening period, the growth rate, feed intake, and feed conversion were measured. At the end of the trial, 8 chickens/treatment were slaughtered and the following parameters investigated: trypsin, lipase and amylase activity of the jejunal chyme, histomorphology of the ileum and caecum, short chain fatty acid (SCFA) content of the caecum, Lactobacillus content of the ileum and caecum, number of coliform bacteria, and pH of the caecal content. None of the treatments resulted significant differences in the production traits, and in the SCFA content of the caecum (P>0.1). BAB supplementation tended to decrease digestive enzyme activity and increase cecal coliform numbers (P=0.077), whereas LAB had a tendency to increase cecal Lactobacillus numbers (P=0.067). Feeding WB in all combination increased crypt depth in both ileum (P=0.002) and caecum (P<0.001), increased the ileal muscle layer thickness (P=0.002) and decreased the villi: crypt ratio (P=0.037) in the ileum.

Poster 3.12: Strains matter

R. Koedijk[1], D. Sandvang[1]

[1] Christian Hansen; nlroko@chr-hansen.com

There is a major difference in the effects that Bacillus sp. generate in poultry. The reason is that the Bacillus Genus consists of 266 species and within that many strains. For this trial a total of 245 Bacillus were selected. A pathogen inhibition assay was performed with E. coli, C. perfringens, S. Typhimurium and S. aureus. Bacillus spp. were spotted and pathogen embedded in agar and incubated. A xylanase and cellulase assay were performed. The inhibitory activity of the Bacillus strains against pathogens was found to be highly species and strain dependent. Most of the B. amyloliquefaciens and B. subtilis strains were variably effective against the poultry pathogens. Production of cellulase and xylanase varied among species tested. Isolates of B. amyloliquefaciens B. mojavensis and B. subtilis produced significant amounts of both enzymes. Highest cellulase activity was determined for three B. amyloliquefaciens and two B. subtilis strains. The B. amyloliquefaciens strains showed high levels of xylanase (50–70 mU/ml) and a minor release of cellulase. Likewise, two strains of B. pumilus exhibited high xylanase activity (more than 120 mU/ml), while they produced poor amount of cellulase (less than 0.2 U/ml). Low enzymatic activities were defined for all B. licheniformis strains (except 15542) and B. megaterium.

Enzyme supplements to animal cereals-based feed is widely known to have a positive effect on feed intake and digestibility. Selection of probiotic strains for specific poultry targets helps in reducing pathogens or increase digestibility. The large quantity and variation between species and strains makes it critical to identify strains that are effective for different purposes in animal nutrition. There is not a single microbe strain that can do it all at once.

Poster 3.13: Bacillus amyloliquefaciens CECT 5940 improve broiler performance under heat stress

J. C. de Paula Dorigam[1], N. Yacoubi[1], K. Doranalli[1]

[1] Evonik Nutrition & Care GmbH; nadia.yacoubi@evonik.com

Environmental conditions and animal characteristics may lead to an imbalance between the animal heat production and its dissipation in the environs, causing heat stress (HS). Although, concentrated feed or additives in the feed and water are used against HS, there is limited information on such strategies. Thus, this study was conducted to delineate the effect of probiotic Bacillus amyloliquefaciens CECT 5940 (Pro) on broiler performance under thermo-neutral (TN) and HS conditions. A 2x2 factorial design was used, where HS condition and the Pro supplementation (10^6 CFU/g feed) were the main factors. Arbor Acres male chicks were allocated to 4 treatments with 14 replicates of 12 birds. Basal diets were formulated to meet the breeder recommendation. The HS group were under continuous high T° from day one (32°C) while the TN group followed the recommendations. Body weight gain (BWG), feed intake (FI), feed conversion (FCR), nitrogen retention (NR), and gut histology were assessed. Blood samples were analysed for immune biomarkers. Data was submitted to ANOVA followed by Duncan test ($\alpha=0.05$). At day 35, HS reduced BWG by 214g, FI by 252g while FCR was increased by 2.7%. On the other hand Pro improved BWG by 3.7%, increased FI by 2.6 % and improved FCR by 1.2%. The NR retention was 1.5% higher in birds fed Pro compared to HS. Pro resulted in longer crypt depth (CD) in jejunum similar to TN, while HS resulted in lower CD in duodenum and jejunum. The HS decreased the CD4 T-cells and immunoglobulin (IgG) in the blood whereas Pro was able to increase both similar to TN. In this study, HS had a negative impact on the immune system of broilers that resulted in decreased performance and health status, but the addition of the probiotic was able to overcome these effects.

Poster 3.14: Combination of dietary nucleosides supplementation augments performance and immunological functions in broilers

G. Marappan[1], J. Jaywant Rokade[2], T. S. Shyamkumar[3], G. Kolluri[2], M. Velusamy[2], T. J. Singh[2]

[1] Central Avian Research Institute. India, [2] Central Avian Research Institute, [3] Indian Veterinary Research Institute; gopsgopi72@gmail.com

Nucleotides are regarded as conditionally essential nutrients particularly during periods of rapid growth and physiological stress. Our earlier studies confirmed that combination of nucleosides had effect in broiler performance. Present experimental was carried out with newly hatched (N = 400) broiler chicks divided into eight groups each consisting of 5 replicates. N1: (Control without antibiotics); N2 (positive control): with antibiotic for 42 days. N3, 4 and 5: combination of nucleosides @ 0.05, 0.1 and 0.15% for first 14 days. N6, 7 & 8: combination of nucleosides @ 0.05, 0.1 and 0.15% for first 21d. The production performance, immunity, intestinal tissue protein content and relative expression of nutrient transporter genes were studied. Highest body weight was observed in N2 (positive control), N5 (1.5g/kg for 21d) and N8 (1.5g/kg for 21d) groups. At 42d group T2, T5 and T8 groups showed higher body weight than other groups. Humoral immunity against NDV and IBD antibody concentration in groups which were supplemented for initial 21d shown significant improvement compare to 14d. NDV HI titre and cellular immunity (absolute and relative) in groups which supplemented for 14d were comparable with groups supplemented for 21d. Non-significant increase in serum bactericidal activity in both antibiotic and nucleosides group was observed. The intestinal protein content was higher in N3 and least in control whereas, the remaining groups exhibited intermediate response. The expression SGLT 1 at jejunum did not show any significant ($P>0.05$) difference among the treatment groups at 7 and 14d. Expression of EAAT3 got upregulated (3d) in antibiotic and nucleosides supplemented groups. The feeding nucleosides @1g/kg feed for 14 days of age improved growth and immunity in broilers.

Poster 3.15: Effect of Eimeria tenella challenge and dietary restriction on growth performance, caecal lesion scores and faecal oocyst counts in broilers

I. Akinci[1], F. Khattak[2], N. Spark[3], O. Hanotte[4]

[1] School of Life Sciences, University of Nottingham, [2] Monogastric Science Research Centre, SRUC, [3] Roslin Institute Building, Scotland's Rural College, [4] International Livestock Research Institute, ILRI / School of Life Sciences, University of Nottingham; ibrahim.akinci@nottingham.ac.uk

Coccidiosis is a protozoan disease, which is caused by different species of Eimeria. It is an important health problem as it impairs the growth and feed utilization of chickens and thus results in economic losses around the world. A study was designed to challenge commercial poultry line with coccidia and investigate the relationship between nutritional status and host immunity. Ninety six 1-d old male broilers (Ross 308) were randomly distributed into 4 treatment groups using a 2 x 2 factorial arrangement. There were 2 types of feeding regimes (ad libitum and restricted feed) with and without coccidian challaenge. Each treatment had 6 replicate pens with 4 birds each. All birds were fed nutritionally adequate but coccidiostat free diets. On day 21, the challenged group were gavaged with 1x104 dosage of the Houghton laboratory strain of Eimeria tenella and non-challenged group were given distilled water. Birds and feed were weighed on d0, 21, 26 and 30. On d26 and d30 two birds/pen were euthanised to examine caeca for lesion scoring and faecal samples were collected for enumeration of faecal oocysts. Data were analysed by ANOVA using GenStat with significance level determined at $P<0.05$. The results showed that 10% of feed restriction (compared to breed target) had a positive effect ($P<0.05$) on the body weight gain (BWG), feed Intake (FI) and FCR during the pre-challenge period (d0-21) but had a neagtive impactt ($P<0.05$) on BWG, FI and FCR during post challenge period (d21-30). Coccicidial challenge model was successful as indicated by the higher ($P<0.05$) cecal lesion scores and faecal occyst counts in challenged birds compared to unchallenged group. However, coccidial infection had no effect ($P>0.05$) on the productivity of the birds at the measured phenotypes.

Poster 3.16: The use of Bacillus subtilis to reduce translocation and invasion of Campylobacter jejuni in poultry

L. Williams[1], N. Sparks[2], L. Thijs[3], I. Somers[3], T. Humphrey[1]

[1] Swansea University, [2] SRUC, [3] Kemin Europa N.V.; Ingrid.Somers@kemin.com

Historically, Campylobacter jejuni was described as a commensal of poultry. However, several publications demonstrate that Campylobacter behaves more as a pathogen, as it can translocate from the gut to infect liver and other edible tissues. This extra-intestinal spread is a public health concern. Damage or inflammation of the intestinal barrier increases the rate at which the bacteria enter the tissues. In this paper, the use of anti-inflammatory molecules is being proposed as an attractive concept to reduce the extra-intestinal spread of C. jejuni.

Ross 308 chickens were given a diet supplemented with Bacillus subtilis PB6 (PB6) or a variant phenotype of PB6 (PR6) showing more anti-inflammatory properties. At day 21 of the trial, all birds were orally gavaged with $8x10^5$ CFU of a known invasive C. jejuni strain M1. Birds were sacrificed at day 35 to sample ileum, liver and caecum.

C. jejuni M1 was found in both caecum and ileum with no differences observed in infection level once infection occurred. However, more Campylobacter negative samples (i.e. caecal and ileal samples) were noticed in the groups given PB6 or PR6 compared to the control i.e. 2 and 4 negative out of 10, respectively, whereas all samples were positive for the infected control. This resulted in significantly fewer Campylobacter-positive livers in the treatment groups compared to the control. This was particularly striking in the PR6 group with only 1 positive liver out of 10.

With this approach, the prevalence of Campylobacter in chicken can be reduced on farm level as shown by a reduction in infected caeca, ileum and liver and thus less infected tissues.

Poster 3.17: Comparison of a butyrate formulation and an AGP for broiler gut development and performance

B. Boomsma[1], O. Pawaskar[2], M. Sagar[2], P. Vervenne[1]

[1] Palital Feed Additives, [2] IP Marketing Services; b.boomsma@palital.com

Introduction: The study was carried out at the R&D complex of Agrivet Consultancy, in India. The goal of the study was to compare the effects of a butyrate formulation and a common AGP on gut development and performance of broilers. The butyrate was Intest-Plus Quattro, which is a 70% matrix encapsulated combination of sodium and calcium butyrate. The AGP was the antibiotic BMD.

Material and methods: The trial consisted of 4 treatments of 10 pens each. Each pen contained 10 healthy cobb 430 broilers. The trial design was a 2x2: a group with the butyrate at 1000 g/T in the starter feed and 500 g/T in the grower + finisher feeds, a group with 500g/T of BMD throughout, a group with neither additive and a group with the combination of both. The trial duration was 35 days. Feed and water were available ad libitum. Data was analysed by ANOVA with pen as experimental unit. Statistical significance was set at P< 0.05.Results: At day 35, BW was not different, FCR was improved upon additive dosing. Individual additives improved EPEF by 20 points, but the combination added another 12 points (n.s.) At day 21 and 35, villus height in the jejunum was increased by all additives. The butyrate increased also the crypt depth at day 35. All additives altered ceacal microflora; in all cases Clostridium and Salmonella counts were decreased. Only BMD did lower Lactobacillus counts. Conclusions: Even though modes of action are different, there is notable overlap in the effects of butyrate and BMD on broiler gut physiology. Notable difference is that butyrate does not suppress ceacal lactobacillus counts, whereas BMD does. A fair comparison of butyrate and BMD as growth promotors should try to use known differences in mechanisms to explain and predict difference in practical outcome.

Poster 3.18: Effects of a blend of glycerol esters of fatty acids on the performance and intestinal ecosystem of broiler chicks in a necrotic enteritis challenge model

V. Tsiouris[1], I. Georgopoulou[1], T. Mantzios[1], K. Kiskinis[1], A. Lauwaerts[2], G. Filiousis[3], G. Papadopoulos[4], P. Fortomaris[4]

[1] Unit of Avian Medicine, Clinic of Farm Animals, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, [2] Proviron Industries NV, G. Gilliotstraat 60, 2620 Hemiksem, Belgium, [3] Laboratory of Microbiology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, [4] Laboratory of Animal Science, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki; biltsiou@vet.auth.gr

The aim of the present study was to investigate the effect of a blend of glycerol esters of fatty acids on the performance and on the pathogenesis of experimental necrotic enteritis in broiler chicks. One hundred and twenty 1-day old broiler chicks were randomly allocated to 4 treatment groups according to the following experimental design: group A, which served as negative control, group B, to which dietary supplementation of a blend of monoglycerides of fatty acids was applied, group C, to which a challenge of the birds with C. perfringens and Eimeria spp. was applied and group D, to which dietary supplementation and challenge was applied. The intestine, gizzard and liver were collected from each bird, and scored for gross lesions. The intestinal digesta were collected for pH and viscosity determination. The statistical analysis and evaluation of the experimental data revealed that the dietary supplementation increased significantly ($P \leq 0.05$) the body weight of birds, while the challenge of birds reduced significantly ($P \leq 0.05$) the body weight of birds. The challenge of birds as well as its combination with a blend of glycerol esters of fatty acids caused significantly ($P \leq 0.05$) more severe coccidiosis lesions, reduced significantly ($P \leq 0.05$) the pH and increased significantly ($P \leq 0.05$) the viscosity of ileum digesta. In addition, the challenge of birds caused significant ($P \leq 0.05$) more severe necrotic enteritis lesions, while the dietary supplementation reduced significantly ($P \leq 0.05$) the severity of necrotic enteritis lesions. The study provides evidence that the dietary supplementation of a blend of glycerol esters of fatty acids can significantly promote the performance and can affect the severity of necrotic enteritis lesions as well as the gut health in broiler chicks.

Poster 3.19: Hydroxy-selenomethionine is used by intestinal Caco-2 cells as a source of selenium and protects against oxidative stress

J. Campo-Sabariz[1], D. Moral-Anter[1], M. T. Brufau[1], M. Briens[2], E. Pinloche[2], R. Ferrer[1], R. Martín-Venegas[1]

[1] Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona. Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona, [2] Adisseo France SAS; joan_campo@hotmail.com

Since selenium (Se) supplementation is required for optimal growth, its incorporation in animal diets is of great importance. Se performs biological functions through its incorporation into selenoproteins in the form of selenocysteine. In the intestine, inadequate dietary Se is considered a risk factor for several chronic diseases associated with oxidative stress and inflammation. The present study investigates the role of the hydroxy-selenomethionine (2-hydroxy-(4-methylseleno)butanoic acid: HMSeBA), a novel organic form of Se, in supporting selenoprotein synthesis and protecting against oxidative stress, in comparison with sodium selenite. The protein and gene expression and activity of different selenoproteins were studied in Se-deprived and Se-supplemented models of Caco-2 cells. GPx activity together with both protein and gene expression of SelP in the Se-deprived model was significantly decreased; while GPx2 gene expression and ROS production significantly increased. In the Se-supplemented model, GPx activity and SelP protein expression were significantly increased irrespective of the selenium source. In contrast, gene expression of SelP and GPx2 were only significantly increased for the organic source. Similarly, decreases in ROS, lipid peroxidation and protein carbonylation after H_2O_2 treatment were only observed for the organic source HMSeBA. Thus, the difference observed between the effects of the Se sources on GPx2 and SelP gene expression may be of importance in terms of protection against oxidative stress. In conclusion, our results indicate that the organic form HMSeBA is used as a Se source by Caco-2 cells and contributes to maintaining intestinal epithelial homeostasis by protecting against oxidative stress.

Poster 3.20: A phytogenic feed additive suppresses inflammatory signals in the gut by controlling the activation of the MAPK pathway and decreasing the level of Enterobacteriaceae in broiler chickens

G. Antonissen[1], F. van Immerseel[2], V. Paraskeuas[3], E. Griela[3], E. Goossens[2], M. Verlinden[2], R. Ducatelle[2], F. Haesebrouck[2], N. Reisinger[4], S. Croubels[5], K. C. Mountzouris[3]

[1] Dept. of Pathology, Bacteriology and Avian Diseases & Dept. of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [2] Dept. of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [3] Dept. of Nutritional Physiology and Feeding, Agricultural University of Athens, Athens, Greece, [4] BIOMIN Research Center, Tulln, Austria, [5] Dept. of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; Gunther.Antonissen@UGent.be

Certain feed ingredients, such as non-starch polysaccharides, may put considerable stress on the digestive system. When passing a certain threshold, even in the absence of any specific pathogens, this may damage the health status of the gastro-intestinal tract, leading to partial loss of function. Therefore, the aim of this study was to investigate the impact of a phytogenic feed additive on the activation of the mitogen-activated protein kinase (MAPK) signaling pathway and intestinal microbiota in an experimental dysbiosis-challenge model. Male broiler chickens (Ross 308) were fed either a dysbiosis challenge diet or a dysbiosis challenge diet supplemented with a phytogenic feed additive (150 g/ton) (5 pens/group, 18 birds/pen). Sampling was performed at day 13, 26 and 39. Intestinal microbiome profiling was performed by next-generation sequencing of 16S ribosomal DNA. mRNA expression of genes involved in the MAPK pathway was evaluated by qRT-PCR. Supplementing the diet with phytogenic feed additive resulted in a significantly decreased abundance of Enterobacteriaceae family and increased abundance of Peptostreptococcaceae in the ileum at the age of 13 days. A significant decreased mRNA expression of the genes encoding c-Jun N-terminal kinase (JNK) 2, P38 MAPK α, β2, and δ, tumor necrosis factor-α, interferon (IFN)γ, and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κβ1) were observed in the caecum of chickens (13 and 26 days old) fed the phytogenic supplemented feed. These findings suggest that supplementation of phytogenics results in a decreased level of lipopolysaccharide containing Enterobacteriaceae and a decreased pro-inflammatory cytokine release, reducing the NF-κβ1/JNK/P38 MAPK pathway activation, decreasing the inflammatory response.

Poster 3.21: Anti - quorum sensing activity of essential oils used as feed additives

I. Čabarkapa[1], J. Bošnjak-Neumüller[2], J. Raj[2], N. Marković[2], M. Vasiljević[2]

[1] University of Novi Sad, Institute of Food Technology, Bul. cara Lazara 1, Novi Sad, Serbia, [2] PATENT CO. DOO., Vlade Ćetkovića 1A, 24 211 Mišićevo, Serbia; ivana.cabarkapa@fins.uns.ac.rs

For many poultry pathogens, the expression of the virulence depends on the ability to communicate with other members of the population by means of quorum sensing (QS). QS is a bacterial intercellular communication mechanism, which depends on bacterial cell population density and controls the pathogenesis of many organisms by regulating gene expression, including virulence determinants. QS has become an attractive target for the development of novel anti - infective agents that do not rely on the use of antibiotics. Anti-QS compounds are known to have the ability to inhibit bacterial pathogenicity.

Owing to the importance of quorum sensing during bacterial pathogenesis, this research has been focused on evaluation of anti - QS properties of six essential oils (EOs) (thyme, coriander, mint, lavender, eucalyptus and oregano) using strain of Chromobacterium violaceum CV026. The anti-QS potential of the EOs was assayed in range of concentration 250 - 3.9 µl/ml using disc diffusion method. EOs of coriander, mint, lavender, eucalyptus exhibited anti-QS activity as it was indicated by non-pigmented ring with dilution-dependent manner on Petri plates. EOs of thyme and oregano displayed different active principles, i.e., antimicrobial activity indicated by inner clear ring and anti-QS activity indicated by outer non-pigmented ring, in a concentration-dependent manner. The lowest dilution of EOs of coriander and mint in which they exhibited visually detectable inhibition of violacein synthesis were 7.8 µl/ml.

Preliminary results suggest that QS-inhibitory EOs may inspire the formulation of new generation of antimicrobial agents to control gut health.

Poster 3.22: Comparative performance of polyherbal mixture and synthetic anticoccidian on broiler performance challenged with coccidiosis

A. Atkinson[1], S. Olivera[2], J. F. Le Roux[1], H. Borin[2], C. Alleno[3]

[1] Nuproxa France, [2] Nuproxa Switzerland Ltd, [3] Zootests; angela.atkinson@nuproxa.ch

In a 1200m2 Colorado type house 26400 broilers ROSS PM3 were raised from which 1600 males were divided into 40 cages. 4 batches of 10 repeats of 40 males were performed. Diet was supplemented without coccidiostat, group T1; with a synthetic anticoccidian (Maxiban G160), group T2; with a polyherbal mixture (Peptasan) at 1.0kg/ton, group T3 and at 0.5kg/ton, group T4. Average body weight at day 15, before coccidiosis challenge was significantly higher ($p<0.001$) for groups T2 (553g) T3 (548g) and T4 (556g) vs group T1 (528g). Coccidiosis challenge at day 15 affects significantly ($p<0.001$) animal growth during the following 10 days. Average weight at day 25 of Group T2 (1381g) was higher than groups T3 (1336g) and T4 (1323g) that were higher than negative control group T1 (1250g). Coccidiosis challenge affects significantly ($p<0.001$) daily feed consumption between day 15 and day 25. Feed consumption of group T2 (114.6g) and group T3 (144.8g) were higher than group T4 (112.3g) that was also higher than group T1 (108.1g). From day 15 to 25, period following coccidiosis challenge, feed conversion ratio was significantly lower ($p<0.001$) for group T2 (1.38) compared to groups T3 (1.46) and T4 (1.47) that were lower than group T1 (1.50). On the total period, from day 0 to day 35 feed conversion ratio was significantly lower ($p<0.001$) for group T2 (1.58) compared to group T3 (1.61) and T4 (1.62) that were lower than group T1 (1.64). There was a significant difference ($p<0.001$) on litter scoring with a better quality for groups T2 (2.5) and T3 (2.7) than groups T4 (3.4) and T1 (3.4). In conclusion, in a coccidiosis challenge, Maxiban and Peptasan have been able to show significant improvements on zootechnical parameters.

Poster 3.23: Algal polysaccharides to improve gut health

M. Garcia[1], M. Rodriguez[1], M. Berri[2], C. Rosseau[3], P. Nyvall-Collen[1]

[1] Olmix SA, [2] INRA, [3] Intestinal Biotech Development; animalcare.pm@olmix.com

The cell wall of marine algae is mainly composed of water soluble sulfated polysaccharides with several biological activities such as modulation of the immune response and reinforcement of gut barrier function. A research project in collaboration with INRA (France) led to the demonstration of the ability of a specific extract from green algal Ulva sp to upregulate the expression of immune mediators and the molecular mechanisms underlying this immunomodulatory activity by identifying the cell receptor and the signaling pathways involved (Berri et al., 2016 & 2017). The potential enhancement properties of a red algal extract from Solieria Chordalis on intestinal integrity were assessed using in vitro cell models HT-29 MTX and Caco-2 cells, under physiological and inflammatory conditions (Intestinal Biotech Development, 2017). Ulva sp extract has the capacity to upregulate the expression of immune mediators: TNFα, CCL20, IL-1α, TGF-β that are involved in cell differentiation and proliferation, recruitment of immune cells and anti-inflammatory activities. Ulva sp extract interacts with TLR4 and TLR2 and leads to rapid activation of transcription factors PI3K and NF-κB. The red algal extract was shown to upregulate the expression of different target genes related to claudin-2, ZO-1 and ZO-2. The red algal extract upregulates the expression of mucin targeted genes: MUC4, MUC2, MUC5B and MUC5AC. The combination of both algal extracts can reinforce gut health targeting barrier function which is the first line of mucosal defense and via modulation of local innate and adaptive immune responses and induction of anti-inflammatory activities. The use of macroalgal sulfated polysaccharides can play an important role within the reduction of antibiotics in farms.

Poster 3.24: Effect of selected yeast fraction on the growth of clostridium perfringens: quantitative determination of growth inhibition and adsorption capacity

E. Santovito[1], D. Greco[1], V. D'Ascanio[1], G. Avantaggiato[1], V. Marquis[2], R. Raspoet[2], A. Riggi[2]

[1] CNR-ISPA, Bari, Italy, [2] Phileo-Lesaffre Animal Care, France; a.riggi@phileo.lesaffre.com

YCW fractions have been proven effective in reducing the incidence of necrotic enteritis induced by Clostridium perfringens. This inhibition mechanism seems to be limited to some specific Gram-negative enteropathogens (Salmonella and E. coli), although several in vivo studies report their effect also on Gram positive pathogens like clostridia (Santovito et al., 2018).To provide in vitro evidences, the effectiveness of YCW fractions in inhibiting the growth of several C. perfringens strains was quantitatively determined. The bacterium was grown in the presence of different YCW fractions at different concentration levels. The effect of YCW fractions on the growth parameters was analysed. The selected product, at an optimal dosage of 1.25 mg/mL, increased the lag phase duration, and reduced the maximum growth rate and the final cell count in a significant manner with respect to the control. The adsorption of the pathogen to YCW was studied using the isotherm adsorption approach. The effect of YCW dosage, incubation time, and bacterial concentration on the adsorption was evaluated. The study proved that the product adsorbed C. perfringens cells in a dose and time dependent manner. Equilibrium isotherms showed that the cell adsorption onto the product was fast, stable over the time, and occurred with high affinity and capacity. The selected product sequestered up to ca. 10^4 cells of C. perfringens per mg. To the best of our knowledge, this is the first report showing the in vitro efficacy of yeast fraction products to inhibit the growth of C. perfringens, and to reduce the culturable cells by an adsorption process. The in vitro approach proposed herein is as a powerful tool to study the adsorption of aerobic or anaerobic pathogens by eubiotics.

Poster 3.25: Performance of broilers fed diets supplemented with an experimental direct-fed microbial in the presence of a moderate necrotic enteritis challenge

T. Gaydos[1], T. Kiros[2], A. Riggi[2], R. Raspoet[2], E. Auclair[2], D. Hooge[3], M. Sims[4]

[1] Phileo Lesaffre Animal Care, Milwaukee, WI, USA, [2] Phileo Lesaffre Animal Care, Marcq, France, [3] Consulting Poultry Nutritionist, Pleasant Grove, UT, USA, [4] Virginia Diversified Research Corporation, Harrisonburg, VA; tj.gaydos@phileo.lesaffre.com

A novel direct-fed microbial (DFM) was evaluated in a dose-response trial to determine broiler live performance to 42 d with a commercially available coccidiosis vaccine administered at placement (d0), used litter on d4, and toxin-producing Clostridium perfringens (Cp) challenges on d17-18. There were 8 dietary treatment groups with 10 replicate pens of 30 chicks each per treatment (2,400 Cobb 500 straight-run chicks total).Other treatments were: non-supplemented non-challenged (NS-NC); yeast cell wall 250 ppm (YCW); or DFM at Log 4, Log 4.5, Log 5, Log 5.5 or Log 6 cfu/g feed.The NE scores were higher for NS-C (2.17) than for NS-NC (0.59) (P=0.028) with YCW and DFM groups intermediate (1.17 DFM Log 4 to 1.47 YCW). Feed conversion ratio (FCR) 0-28 d was higher (P<0.01) for NS-C group than YCW and DFM supplemented diets (statistically equivalent to NS-NC). The 42-d BW were approaching significance (P=0.059) with NS-C (1.580 kg) vs. NS-NC (1.850 kg) with other groups intermediate (1.681 to 1.745 kg). The FCR and mortality-adjusted FCR (MAFCR) were higher for NS-C than NS-NC (P<0.01), and DFM Log 4 and DFM Log 5 to Log 6 improved FCR, and YCW, DFM Log 4 and DFM Log 5 to Log 6 improved MAFCR vs. NS-C. Mortality % from 0-28 d was not different (P=0.40) but from 0-42 d (P=0.013), NS-C was higher than NS-NC (18.06 vs. 4.44%) and improved with DFM Log 4.5 (8.00%) or DFM Log 5 (7.67%) with other groups intermediate (8.67 to 10.00%). In conclusion, under Cp challenge, YCW diets improved 0-28 d FCR and 0-42 d MAFCR vs. NS-C, and DFM Log 4 to Log 6 diets improved 0-28 d FCR, and DFM Log 4 and Log 5 to Log 6 diets improved 0-42 d MAFCR vs. NS-C showing effectiveness. The DFM Log 4.5 and Log 5 diets lowered 0-42 d mortality % vs. NS-C indicating efficacy.

Poster 3.26: Effect of benzoic acid on growth performance of broiler chickens under coccidiosis vaccine challenge

H. Liu[1], J. Zhang[1]

[1] DSM China Animal Nutrition Research Center Co., Ltd.; winnie.liu@dsm.com

Coccidiosis caused by Eimeria spec. is an intestinal infection disease associated with metabolic and structural changes in the intestinal mucosa lesions, leading overall to malnutrition and lowered performance. The objective of the present study was to evaluate the effect of benzoic acid (VevoVitall®) on growth performance of broiler chickens under coccidiosis vaccine challenge. A total of six hundred day-old male broiler chickens (Arbor Acres Plus) were allocated to 3 dietary treatments with 8 replicates and 25 birds per pen. The treatments were, a non-challenge group (PC), a challenge group (NC) and NC supplemented with 1000 mg/kg benzoic acid. The diets were formulated based on wheat, corn and soybean meal and were fed to birds during the starter (d 0-21) and grower (d 21-35) period. At 4 days of age, the birds in the challenge groups were received a coccidiosis vaccine (Coccivac B®) at a dosage equivalent to 2-fold of the recommended dose by gavage. The results showed that birds in NC had impaired weight gain (P = 0.109) and feed intake (P < 0.05) in the starter period compared to those in PC. The supplementation of the NC with benzoic acid increased (P < 0.05) weight gain and feed intake and values obtained reached a similar level to that of the PC. Coccidiosis vaccine challenge resulted in growth depression of broiler chickens, while the supplementation of benzoic acid (VevoVitall®) could compensate this negative effect.

Poster 3.27: Effect of different enzyme combinations on growth performance of broiler chickens

H. Liu[1], J. Zhang[1]

[1] DSM China Animal Nutrition Research Center Co., Ltd. winnie.liu@dsm.com

The present study was to evaluate the effect of different enzyme combinations on growth performance of broiler chickens fed diets based on corn and soybean meal. One thousand and two hundred day-old male broiler chickens (Arbor Acres Plus) were allocated to 5 dietary treatments: corn-soybean meal based diet as control group (8 replicates of 25 birds each), and control group supplemented with different enzyme preparations A, B, C and D (10 replicates of 25 birds each). The trial lasted for five weeks. The results showed that, enzyme A (RONOZYME® SN111 250, a cocktail of α-amylase, xylanase, β-glucanase and pectinase) significantly increased body weight at d 21 and weight gain of d 0-21 compared to the control and the other enzyme-supplemented groups. Feed intake, weight gain of d 21-35 and 0-35, and body weight at d 35 were significantly increased by enzyme A (RONOZYME® SN111 250) compared to enzyme D. In the overall period, the FCR in percentage relative to NC was 98.0%, 97.5%, 98.1% and 98.7% for enzyme A, B, C and D, respectively. The effect of enzyme A (RONOZYME® SN111 250) on growth performance was highest for all the enzyme products and gave benefit of each bird about 0.044 USD relative to the control.

Poster 3.28: Effect of soybean acid oil and palm fatty acid distillate on epithelial barrier function in intestinal Caco-2 cells

R. Martín-Venegas[1], J. Campo-Sabariz[1], D. Moral-Anter[1], R. Ferrer[1], B. Jimenez-Moya[2], R. Sala[2], F. Guardiola[3], E. Varona[3], A. Tres[3]

[1] Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona. Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona, [2] Animal Nutrition and Welfare Service. Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, [3] Departament de Nutrició, Ciències de l'Alimentació i Gastronomia. Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona. Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona; raquelmartin@ub.edu

Acid oils from chemical refining (AO) and fatty acid distillates from physical refining (FAD) are fat by-products rich in fatty acids that can be valorized as animal feed fat ingredients. However, this use is nowadays limited by the great variability in composition and growth performance. The objective of the study is to investigate the effect of soybean AO (SAO) and palm FAD (PFAD) on intestinal barrier function. Differentiated intestinal Caco-2 cells have been incubated with SAO or PFAD, as well as with the corresponding refined and crude oils (at 1, 1.5 and 2 mg/mL). Epithelial barrier function has been assessed from transepithelial electrical resistance (TER) after 5 and 72 h of incubation. Neither SAO nor the corresponding refined and crude oil modify TER values at any concentration tested. However, PFAD induced a disruption of epithelial barrier function at higher concentrations, effect that is not observed for the corresponding refined and crude oil. Further studies will be done in order to try to elucidate the components responsible for this activity. This would allow establishing recommendations of use for these products to be used as ingredients in feed to ensure suitable health and an improvement in growth performance.

Poster 3.29: Impact of Encapsulated butyrate upon intestinal microbiota, pH and digestive function of broilers suffering from dysbacteriosis

M. Naghizadeh[1], T. Sørensen Dalgaard[1], R. Margarete Engberg[1]

[1] Department of Animal Science, Aarhus University, Blichers Allé 20, P.O. Box 50, DK-8830 Tjele, Denmark; mn_naghizadeh@anis.au.dk

An imbalance in gut bacteria composition (dysbacteriosis) results in partial loss of gastrointestinal (GI) function of broilers. In the present study, the effect of an encapsulated dietary butyrate supplement (EBS; Ultraguard Duo®, Devenish) on the GI bacterial composition, PH and performance of broilers was investigated applying an experimental model for dysbacteriosis. A total of 672 day-old male broiler (Ross 308) were randomly assigned to 3 experimental groups (8 replicates/group): Gr.1, no dietary supplement (negative control); Gr.2, EBS (0.5g/kg); Gr.3, salinomycin (69 mg/kg). The dysbacteriosis model included the feeding of a grower feed providing 5% rye from day 10, and an oral challenge with a 10x overdose of a coccidiosis live vaccines (Hipracox®, Hipra) on day 17. On days, 21, 23, 29 and 35 four birds from each pen were sacrificed to evaluate pH, lactic acid bacteria (LAB), Enterococci, E. coli, and C. perfringens counts of the jejunum, ileum, and cecum contents, and total numbers of aerobic bacteria in the livers. Feed conversion ratio (FCR) and drinking water/feed intake ratio (WFR) were measured by pen. No differences between the EBS group and the control group was observed on bacterial composition and PH of intestinal contents ($P>0.05$), while salinomycin decreased PH (caecum) and number of C. perfringens (ileum, caecum), L. salivarius, LAB, E. coli, (caecum), and increased E. coli (jejunum), lactose-negative bacteria (ileum and jejunum) ($P<0.05$). FCR and WFR were decreased in the group with salinomycin supplementation ($P<0.001$) as compared to the other groups. Moreover, salinomycin decreased numbers of aerobic bacteria at day 21 of age ($P<0.05$). More work is needed in order to establish a potential effect of EBS on broilers suffering from dysbacteriosis.

Poster 3.30: HoloFood – a new holo'omic framework to improve animal husbandry performance

D. Sandvang[1], M. Abel-Kistrup[1], J. Tarradas[2], A. Estonba[3], J. Zentek[4], D. Józefiak[5], M. T. Limborg[6], E. Johansen[1], S. Marcos[7], A. Alberdi[6], T. P. Gilbert[6]

[1] Chr. Hansen A/S, [2] IRTA,[3] Genetika, UPV/EHU, [4] Freie Universitaet Berlin,[5] Uniwersytet Przyrodniczy w Poznaniu,[6] University of Copenhagen,[7] University of the Basque Country; dkdhsa@chr-hansen.com

HoloFood is a European H2020 Action that showcases a holistic approach to improve the efficiency of food production systems by deciphering the biochemical interactions between animals and their associated microorganisms. Many feed additives have not acknowledged the specific means of action of the additives on the microbiome and host organisms, and consequently their performance is inconsistent across animals with different genetic background and when grown under different environments.We will implement our newly developed holo'omic framework to understand the biochemical interactions between broiler chickens and their intestinal microorganisms through the analysis of whole animal genomes, deep intestinal transcriptomes, microbial metagenomes, microbial metatranscriptomes and intestinal metabolomes, all of them in relation to key performance indices. A total of 1300 chickens will be biochemically, physiologically and phenotypically characterized through the analysis of over 15000 samples. The knowledge generated will be used to optimise feed additive administration strategies of already implemented products by tailoring them to the genetic background and developmental stage of the animals as well as production environment, to improve the quantity, quality and safety of the produced food, as well as sustainability of food production and animal welfare. HoloFood will also serve to raise awareness about the importance of microbiomes in food production, and to establish bridges between companies and academia to foster science-based strategies. HoloFood is an Innovation Action comprised of 10 partners from 6 European countries and will be running until 2022.

Poster 3.31: Effect of released-targeted blend of organic acids and essential oils on broilers challenged with a necrotic enteritis reused litter

N. Abdelli[1], E. Vilarrasa[2], J. F. Pérez[1], D. Solà-Oriol[1]

[1] Animal Nutrition and Welfare Service. Department of Animal and Food Science. Universitat Autònoma de Barcelona, Bellaterra 08193, Spain, [2] FARMFAES- TECNOVIT, 43365 Alforja, Spain, nedra.abdelli@uab.cat

Increasing broilers resilience against intestinal infections is a key to ensure production performance under challenge conditions. Blends of organic acids and phytogenics have been reported to reduce intestinal microbiome pathogenicity and improve gut integrity. A 41-d pen trial was conducted to test the efficacy of an encapsulated blend of organic acids and essential oils (ACITEC-MC) using 240 male Ross chicks distributed into 2 treatments, Negative control (NC) and a dose of 2 kg of ACITEC-MC with 10 pens / treatment, and 12 chicks / pen. Chicks were challenged by a 90 % reused commercial litter from d 1 previously selected to contain high counts of Clostridium perfringens (>10^5 UFC/g) and given a corn-wheat diet to accentuate the challenge. Body weight gain and feed intake were measured at d 0, 10, 28 and 41, and feed efficiency was calculated considering the mortality effect. On d 42, a bird per pen was euthanized and samples were taken from ileum for histology and from jejunum for gene expression analysis. Data was analyzed by one-way ANOVA using SAS and means were compared using the multiple comparisons Tukey and significance was based on $P \leq 0.05$. Results showed that ACITEC-MC significantly improved (p=0.001) the average daily gain (52.8 vs 44.8), feed conversion rate (1.82 vs 2.07) and the villi height (1054.6 vs 828.5 µm; p=0.0001) while reduced crypt depth (182.5 vs 219.6 µm; p=0.0004) compared to NC. No treatment effect was observed on the expression of genes involved in barrier function, immune response, nutrient transport, metabolism and oxidation. In conclusion, supplementing a targeted-released ACITEC-MC blend exhibits some benefits on performance and intestine histology which may suggest a reduction of negative impact of necrotic enteritis on broilers.

Poster 3.32: Ulvan activates chicken heterophils and monocytes through Toll-Like Receptor 2 and Toll-Like Receptor 4

N. Guriec[1], F. Bussy[2], C. Gouin[2], O. Mathiaud[3], B. Quero[3], M. Le Goff[2], P. Nyvall-Collen[2]

[1] Brest university and medical school, France, [2] Amadeite SAS, France, [3] R&D Breizh, France; nathalie.guriec@univ-brest.fr

Responsiveness to invasive pathogens, clearance via the inflammatory response, and activation of appropriate acquired responses are all coordinated by innate host defenses. Toll-like receptor (TLR) ligands are potent immune-modulators with profound effects on the generation of adaptive immune responses. This property is being exploited in TLR-based vaccines and therapeutic agents in chickens. However, for administering the TLR agonist, all previous studies used in ovo, intra-muscular or intra-venous routes that cannot be performed in usual farming conditions, thus highlighting the need for TLR ligands that display systemic immune effects when given orally (per os). Here we have demonstrated that an ulvan extract of Ulva armoricana is able to activate avian heterophils and monocytes in vitro. Using specific inhibitors, we have evidenced that ulvan may be a new ligand for TLR2 and TLR4; and that they regulate heterophil activation in slightly different manner. Moreover, activation of heterophils as well as of monocytes leads to release pro-inflammatory cytokines, including interleukin1-β, interferon α and interferon γ, through pathways that we partly identified. Finally, when given per os to animals ulvan induces heterophils and monocytes to be activated in vivo thus leading to a transient release of pro-inflammatory cytokines with plasma concentrations returning towards baseline levels at day 3.

Poster 3.33: Combining short-chain organic acids and phytogenics to improve performance parameters in broilers with necrotic enteritis

J. Estévez[1], B. Vilà[2], J. Tarradas[2], S. Carné[1]

[1] Industrial Técnica Pecuaria S.A. (ITPSA), [2] Institut de Recerca i Tecnologia Agroalimentàries (IRTA); jestevez@itpsa.com

Necrotic enteritis (NE) is one of the most important pathologies in broilers, with high economic impact for producers. Phytogenics (Phy) combined with organic acids (OA) induce beneficial effects on gut health, acting against bacterial pathogens, improving epithelial integrity and nutrient digestibility, thereby promoting growth and feed efficiency (Bozkurt et al., 2012). The aim of this study was to assess the effects of Phy (ajowan extract + phytogenic substances)-short chain OA combinations on performance parameters in broilers with NE. 624 day-of-hatch male chicks (Ross 308) were divided in 5 groups [10-11 pens/group; 12 birds/pen]. 4 groups were inoculated at day 9 with attenuated oocysts from 5 species of Eimeria spp. and infected at days 15, 16 and 17 with 107-108 CFU of C. perfringens/bird/day. Broilers were fed a wheat, barley, and soya basal diet; 3 groups were supplemented (2.5 kg/t) with combinations with different OA:Phy proportions: blend 1 (1.8:1, with acetic acid), blend 2 (3:1, with formic acid) and blend 3 (1.8:1, with formic acid). Performance and mortality were assessed weekly for 35d. Inoculated animals showed an impairment ($P<0.05$) in bodyweight (BW), feed intake, feed conversion ratio (FCR) and mortality vs. negative control. Whereas blend 1 restored performance parameters and mortality partially, blend 2 normalized the assessed parameters totally. Blend 3 showed the best results, improving ($P<0.05$) BW (13%) and FCR (5.4%) vs. inoculated control group, and even improved ($P<0.05$) vs. non-inoculated birds (3.8% BW). In-feed inclusion of OA-Phy combinations improve performance and are an effective strategy against necrotic enteritis in broilers, being more effective those combinations containing formic acid and a higher proportion of Phy.

Poster 3.34: Effect of dietary zinc sources and necrotic enteritis challenge on the expression of tight junction and zinc trafficking genes in broiler chickens

C. Bortoluzzi[1], B. Lumpkins[2], G. Mathis[2], W. D. King[3], D. Graudnard[3], K. Dawson[3], T. Applegate[1]

[1] Department of Poultry Science, The University of Georgia, Athens, GA, USA, [2] Southern Poultry Research, Inc., Athens, GA, USA, [3] Center for Animal Nutritgenomics and Applied Animal Nutritional Research, Alltech Inc., Nicholasville, KY, USA; bortoluzzi.c@gmail.com

Zinc is an essential micromineral required for growth of animals and influence intestinal homeostasis during and after infections. The objective of this study was to compare the effects of two sources of Zn (inorganic and proteinate) on the expression of tight junction (TJ) and Zn trafficking genes in broiler chickens induced to necrotic enteritis (NE). One-day-old male Cobb 500 broiler chicks were fed dietary treatments in a 3x2 factorial design (8 cages/treat; 8 birds/pen) with 3 dietary formulations (0 or 90 mg/kg supplemental Zn from $ZnSO_4$ or proteinate Zn (Bioplex® Zn, Alltech Inc.)), with or without challenge. On d 14, challenged birds were orally gavaged with ~5,000 Eimeria maxima sporulated oocysts, and on d 19- 21 were given a broth culture of Clostridium perfringens (10^8 cfu/d). There was no interaction between Zn source and challenge. The challenge upregulated ($P=0.002$) the expression of Claudin-1 and downregulated the expression of occludin ($P=0.02$) and ZO-1 ($P=0.05$) in the jejunum of broilers. On d 21, the challenge reduced ($P=0.001$) Zn concentration in the serum and increased ($P=0.001$) its concentration in the liver vs. non-challenged birds. On d 27, $ZnSO_4$ increased ($P=0.02$) Zn concentration in the serum vs. non-supplemented birds. Zn supplementation downregulated the expression of ZnT5 ($P=0.04$), regardless of the source, and the challenge upregulated ZIP13 ($P=0.04$) and downregulated ZnT7 ($P=0.03$) in the jejunum and downregulated the expression of all Zn transporters in the cecal tonsils ($P<0.01$) vs. non-challenged birds. Zinc supplementation and challenge increased the movement of Zn into the cytoplasm which may allow Zn to be incorporated into intracellular proteins, which may cause the enterocyte to have insufficient Zn supply during a challenge.

Poster 3.35: Post gizzard active compounds evaluated in the absence of antibiotics both with and without water acidification

M. Bekker[1], E. Magtagnob[2], A. Sultan[3], K. De[4]

[1] Novus International Oceania, [2] Novus International Phillipines, [3] Novus International Pakistan, [4] Novus International India; matthew.bekker@novusint.com

Resistance to antibiotic compounds by bacterial populations is increasing. There are many feed ingredients that have been tested as alternatives to antibiotics in isolation. This study was designed to look at the effectiveness of four separate hind gut active compounds in a single study without antibiotics. The compounds were tested both with and without an effective foregut acidifier. Arbor Acres Plus male broilers (1,512) were randomly assigned to one of nine treatments with 8 replications per treatment. Diets were based on corn and soy bean meal. Treatment one contained a traditional antibiotic growth promoter as control. Treatment two as negative control containing no antibiotics or additives. Treatments three and four included a combination of calcium formate, silicic acid and benzoic acid contained within a vegetable fat matrix. Treatments five and six included a combination of carvacrol and thymol presented in a matrix protected form. Treatments seven and eight included copper in the form of copper MHA chelate at 30ppm. In treatments four, six, eight and nine, water acidification was achieved using a blend of liquid MHA, formic and propionic acid. At ten days a difference was seen in both feed intake P=0.002 and FCR P=0.0094 for the combination fore and rear gut active organic acid treatment four. Carcase integrity markers showed numerical deviation with tibia head lesion (THL) score in the combination acid, treatment four presenting half the rate seen in the antibiotic fed group. The negative control group THL score was 39% greater than the antibiotic treatment. Copper MHA treatment alone, and the combination of fore and hind gut active organic acids had the only numerical advantage over the antibiotic fed birds in the incidence of clean footpads.

Poster 3.36: Different strains of Clostridium perfringens cause different effects on expression of genes encoding intestinal nutrient transporters in broilers under necrotic enteritis infection

K. Gharib-Naseri[1], S. Kheravii[2], M. Choct[3], R. Swick[3], N. Morgan[4], S. Wu[5]

[1] University of New England/ Phd Student, [2] University of New England/ Researcher, [3] University of New England/ Professor, [4] University of New England/Researcher, [5] University of New England/ A. Professor; kgharibn@myune.edu.au

Necrotic enteritis (NE), mainly caused by the gram-positive anaerobic bacterium Clostridium perfringens (Cp), is an enteric disease with a substantial negative economic impact on broiler production. It has been reported that the Cp strains play a critical role in the occurrence and severity level of this disease. This study investigated the impact of two different strain of Cp (NE18 and NE 36) on broilers fed with diets supplemented with antibiotics. A 3 × 2 factorial experiment was designed with factors: Antibiotics (yes or no), and NE challenge (no, NE18 and NE36). A total of 468 d-old Ross 308 chickens were assigned to the experimental treatments each with 6 replicates for 35 days. To induce NE in the challenged groups, oral administrations of Eimeria species (d9) and according to their challenge groups, Cp stains (d14 and 15) were used. On day 16, jejunum tissue samples were taken from two birds per replicate. After total RNA extraction, quantitative PCR was performed. Both groups of challenged birds showed downregulation of APN ($P < 0.001$), BoAT ($P < 0.001$), PepT2 ($P < 0.01$), GLUT2 ($P < 0.001$) and ATP1A1 ($P < 0.001$) but significantly upregulated LAT1 ($P < 0.001$). The NE36 strain significantly downregulated GLUT2 ($P < 0.001$) compared to NE18 strain. An interaction between antibiotic treatment and challenge was observed on the expression ASCT1 gene ($P < 0.05$), where antibiotics increased the expression of this gene in challenged birds but lowered the expression in the unchallenged birds. These results indicate that NE challenge alters the expression of genes encoding proteins involved in nutrient uptake and that different strains of Cp may cause different up- or down regulations of these genes, thus dictating the severity of the disease in broiler chickens.

Poster 3.37: Effect of Saccharomyces cerevisiae yeast products in reducing direct colonization and horizontal transmission of Salmonella Heidelberg in broilers

T. Kiros[1], T. J. Gaydos[1], J. Corley[1], R. Raspoet[2], A. Riggi[2], C. Hofacre[3], R. Berghaus[4]

[1] Phileo - Lesaffre Animal Care, Milwaukee, WI, USA, [2] Phileo - Lesaffre Animal Care, Marcq-en-Baroeul, France, [3] Southern Poultry Research Group, Athens, Georgia, USA, [4] Department of population and health, College of Veterinary Medicine, Univ. Georgia, Athens, Georgia, USA; tj.gaydos@phileo.lesaffre.com

This study was conducted to evaluate the effectiveness of different levels of yeast cell wall (YCW) or yeast culture (YC) in reducing the direct colonization and horizontal transmission of Salmonella Heidelberg in broilers. At day of hatch, 2000 Ross male broiler chicks were randomly assigned into 5 treatment groups with 8 replicate pens per treatment and 50 birds per pen. T1, no treatment, T2 to T4, YCW at 125, 250 and 500 ppm respectively and T5, YC at 1250 ppm. 25 birds per pen were tagged and challenged with nalidixic acid-resistant Salmonella Heidelberg by direct inoculation of 10^7 CFU orally (seeders) and the remainder by horizontal transmission through inoculated penmates (contacts). At 42 days, ceca were aseptically collected from 10 horizontal contact and 5 seeder birds per group and submitted to determine the prevalence and enumeration of S. Heidelberg. YCW at 500 ppm decreased Salmonella prevalence of the total infected birds (seeders and contacts) to 41.7% as compared to 54.2% in the untreated birds. The effect of YCW in reducing prevalence of positive birds was even greater when we considered the contact birds only; 32.5% in contact birds treated with 500 ppm as compared to 57.5% in the untreated group (p=0.09). Furthermore, enumeration of Salmonella Heidelberg colonization level in the cecum using the most probable number (MPN) method showed that YCW at 500 ppm reduced the bacterial load in the cecum of positive birds to 1.7 MPN/g as compared to 2.7 MPN/g in the control group. The reduction was statistically significant (p=0.04) in the contact birds. The same effect was not seen in the YC treatment. From the overall data, it was summarized that YCW at 500 ppm had better effects in reducing Salmonella contamination than the YC treatment.

Poster 3.38: Inclusion of 3-strains of Bacillus amyloliquefaciens in-feed reduces non-beneficial E.coli in the intestine and increases performance in commercial broilers flocks

J. Villatoro-Hernandez[1], S. Gilani[1], A. M. Debicki-Garnier[1]

[1] DuPont Animal Nutrition; julio.villatoro-hernandez@dupont.com

Colibacillosis is an economically relevant poultry disease caused by avian pathogenic Escherichia coli (APEC). We identified presence of virulence-associated genes (VAG) in the genome of potential pathogenic E. coli by molecular methods (multiplex PCR). The E. coli isolates showed specific genomic sequences (1) in different European commercial farms. Additionally, we have seen increasing numbers of beneficial bacteria in the gut of broilers when they are supplemented with the Bacillus strains in the feed (2). Here we identified intestinal APEC before and after including the 3-strain probiotic (Enviva PRO, Dupont Animal Nutrition) in broilers on European commercial farms (n= 900,000). The gut samples from the farms were analyzed for VAG by multiplex PCR as a baseline to identify the potential pathogenic E. coli sub-population (VAG's in the genome) and the non-pathogenic E. coli population (lacking VAG's). The probiotic was included for three consecutive cycles at a concentration of $1{,}5x10^5$ CFU / g of feed. The analysis of gut samples before and after feeding 3-strain Bacillus amyloliquefaciens, showed 24% reduction in APEC, 53% reduction in VAG of APEC. The addition of the 3-strains of Bacillus, induced a reduction of the potentially pathogenic E.coli at the intestinal level and resulted in 3 points of improvement in FCR at the end of the 3rd cycle.1. Kemmett, K. et al 2013. A longitudinal study simultaneously exploring the carriage of APEC virulence associated genes and the molecular epidemiology of faecal and systemic E. coli in commercial broiler chickens. 2. Lei, X. et al 2015. Effect of Bacillus amyloliquefaciens-based Direct-fed Microbial on Performance, Nutrient Utilization, Intestinal Morphology and Cecal Microflora in Broiler Chickens

Poster 3.39: Improving the intestinal health of broiler chickens under the influence of willow bark extract powder

T. Gavris[1], T. Panaite[1], A. Untea[1], C. Tabuc[1], M. Saracila[1], C. Soica[1]

[1] National Research-Development Institute for Animal Biology and Nutrition; teogavris@gmail.com

The objective of this study was to evaluate the influence of a willow bark extract powder (WBEP) on the productive performances and intestinal microflora health of chicken broiler. The experiment was conducted on 80 Cobb 500 broiler chicks (14 days), as hatched, assigned in two experimental groups (40 chickens/group). The broilers were reared in an environmental controlled hall (indoor system) at a stocking density of 10 birds/m^2. The light regimen was 23 hours with light and 1 hour of darkness. Compared to the control diet (C), the experimental diet (E) included 0.01% (WBEP). The metabolic energy of the compound feed used in the growing period was 3140 kcal / kg and 22% protein content. For the finishing period the metabolic energy was 3250kcal / kg and 20% protein content. Growth performances were monitored through the entire experimental period (14-42 days). At 35 and 42 days of age 6 chickens form each group were slaughtered and samples of caecal content were collected. The WBEP did not affected the daily feed intake, feed conversion rate and body weight. The weight of the thigh chickens from group E was higher ($p<0.05$) than of C group at 35 days. Concerning the microbial load of caecum, the group supplemented with WBEP had a significantly ($p<0.05$) lower concentration of bacteria from genus Staphylococcus spp. and E.coli for chicken slaughtered at 35 days. The number of Lactobacillus spp. colonies was higher for chickens from group E compared to group C, this being a significant ($p<0.05$) difference for both periods (35 and 42 days of age). Diet supplementation with willow bark extract powder supplementation has maintained the balance of caecal microflora of broiler chickens.

Poster 3.40: Effect of dissolved oligosaccharide organic acid as drinking water supplement on prevalence and antibiotic resistance of Salmonella sp. and gut microbiota in Broiler

Y. Theapparat[1], N. Roekngam[1], K. Soisuwan[2], D. Faroongsarng[1]

[1] Drug Delivery System Excellent Center, Department of Pharmaceutical Science, Faculty of Pharmaceutical Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand, [2] Department of Animal Science, Faculty of Agriculture, Rajamagala University of Technology Srivijaya, Thung Yai, Nakhon Sri Thammarat 80240, Thailand; yongyuth.theap@gmail.com

The effect of yeast cell wall oligosaccharide dissolved in lactic acid used as drinking water supplement on pathogen mitigation and antibiotic resistance of Salmonella sp. from environmental infection in broilers was investigated. 810 one-day-old male broiler chicks (Ross 308) were allocated into 3 treatment groups including (1) negative control (fresh drinking water, no supplemented oligosaccharide or antibiotic) (2) positive control (drinking water continuously supplemented with the antibiotic Colistin sulphate; at the level of 0.50 ml per L of water), and (3) active treatment with oligosaccharide supplemented in drinking water in the levels of 1.60 ml per L water. Each treatment was divided into 9 replicates of 30 chicks each (30 males). At 42 day of age, all chickens were sacrificed and counted total Salmonella sp. colonies in selective media. It was demonstrated that the active treatment highly and significantly improved ($p<0.05$) pathogen mitigation in term of reduced Salmonella sp. prevalence compared with negative control. The minimal inhibiting concentration (MIC) of antibiotic colistin for Salmonella sp isolated from ceca in control group was significantly higher than that of active treatment one($p<0.05$), while colistin resistance of all Salmonella sp colonies isolated from both groups was susceptible in the antibiotic resistance test. The microbiota in the ceca of chicken previously treated with oligosaccharide was dominated by the bacterial phyla of Firmicutes and Bacteroidetesat 46.59 and 43.60%, respectively, with a significantly lower percentage of proteobacteria (3.95%) and all other (2.98%). It was concluded that yeast cell wall oligosaccharide dissolved in lactic acid may be a potential alternative antibiotic in Broilers.

Poster 3.41: Influence of a detoxifying agent on the toxic effects of T-2 toxin in broiler chickens

M. Hinrichs[1], E. Haas[1], A. Schlagheck[1]

[1] Biochem mbH; Hinrichs@biochem.net

T-2 toxin (T-2) is one of the most critical mycotoxins for poultry. To evaluate the effectiveness of the detoxifying agent BTF (B.I.O.Tox® Farm) in T-2 toxicosis, a trial over 21 days was conducted. 360 one-day-old male broiler chicks (Cobb 500) with an average initial-weight of 39.1 g were randomly and equally divided into 6 treatment groups (6 replicates per group): positive control group (PCG) with no BTF or T-2 added, a negative control group (NCG) with 2 ppm T-2, a BTF-0 group with added 0.4% BTF, a BTF-1 group with 0.1% BTF plus 2 ppm T-2, a BTF-2 group with 0.2% BTF plus 2 ppm T-2 and a BTF-4 group with 0.4% BTF plus 2.0 ppm T-2. Performance, jejunal morphology and serum biochemistry were surveyed in all groups. On day 21, body weight (BW), body weight gain (BWG) and feed intake (FI) were significantly decreased in the NCG compared to the PCG (9.7%, 12.0% and 6.3% respectively; $P<0.05$). Independently of the used BTF quantity, negative effects of dietary T-2 on performance parameters were compensated. Total plasma protein (TPP), albumin (ALB), both mycotoxin-specific parameters, and serum phosphorus of NCG were significantly lower in comparison to the PCG ($P\leq0.05$). With increasing inclusion of BTF in the diet, TPP and ALB parameters showed numerical increased levels, indicating liver function resuming closer to NCG. The villus:crypt ratio was significantly decreased in the group which was fed T-2 alone compared to the PCG ($P\leq0.05$). The addition of BTF influenced this ratio positively about all BTF-groups. The relative weight of liver and the feed conversion ratio (FCR) did not differ between treatments. This study confirms a liver protective function of BFT due to improved TPP and ALB serum levels. BTF prevented the negative effect of 2ppm T-2 on performance.

Poster 3.42: Impact of direct fed microbial on gut health, and intestinal worm populations from free-range hens in the late cycle

R. D. Malheiros[1], K. Cupo[2], R. B. Beckstead[2], K. E. Anderson[2]

[1] Prestage Poultry Sci. Dep. NC State University, 2711 Founders Drive, Raleigh, NC, USA, 27695. [2] Prestage Poultry Sci. Dep. NC State University, Raleigh, NC, USA, 27695; rdmalhei@ncsu.edu

With only diatomaceous earth or essential oils for natural endo-parasite control, one alternative could be use of probiotics. Better intestinal health and morphology is correlated with better animal performance and health. The objective of this study was to evaluate intestinal health, egg quality, egg production, and intestinal worm numbers on later stage in Free-Range System (FRS) hens provided a Direct Fed Microbial (PrimaLac®, Star-Labs Inc, Clarksdale, MO, USA), (DFM). The study was conducted using FRS at the NCDA Station, Salisbury, NC, USA. Late cycle commercial egg hens, Hy-Line Silver Brown (400 hens), from 89-109 wks, were divided into 2 groups with 4 rep. of 50 hens each, on control feed and 4 rep provided (DFM) at 3 lbs/ton. Egg quality and mortality were collected by a period. At 109 wks, 20 hens/rep were euthanized and intestinal worm counts performed and samples collected. The add of DFM has no significative differences with the control ($P>0.05$) in production parameters (HD%, HH%, Egg/hen, gEgg/gFeed, Egg Mass, Egg weight, and Mortality). All eggs quality measurements (shell strength, shell elasticity, vitelline membrane (VM) strength, Haugh unit) were not significantly different ($P>0.05$) during the period. The DFM has a positive impact on the VM elasticity ($P=0.0349$), and on egg yolk color ($P=0.0071$). The histomorphology was significant ($P=0.331$) for crypt depth, with 161 vs 137 um, for Ct and DFM. By counting the worms indicated no change in tapeworms, a great reduction in roundworms (1.50 to 0.48 worms/hen) and a significant reduction in ceca worms (38 to 19 worms/hen, $P=0.0486$) with the DFM. These results showed that use of DFM in FRS hens, in the late cycle, has a beneficial effect on the VMS, gut health, and reduction of ceca parasitic infection.

Poster 3.43: Influence of a detoxifying agent on the toxic effects of T-2 toxin in broiler chickens

M. Hinrichs[1], E. Haas[1], A. Schlagheck[1]

[1] R&D Department, Biochem Zusatzstoffe GmbH, Küstermeyerstr. 16, 49393 Lohne, Germany; Hinrichs@biochem.net

T-2 toxin (T-2) is one of the most critical mycotoxins for poultry. To evaluate the effectiveness of the detoxifying agent BTF (B.I.O.Tox® Farm, Biochem GmbH, Germany) in T-2 toxicosis, a trial over 21 days was conducted. 360 one-day-old male broiler chicks (Cobb 500) with an average initial-weight of 39.1 g were randomly and equally divided into 6 treatment groups (6 replicates per group): positive control group (PCG) with no BTF or T-2 added, a negative control group (NCG) with 2 ppm T-2, a BTF-0 group with added 0.4% BTF, a BTF-1 group with 0.1% BTF plus 2 ppm T-2, a BTF-2 group with 0.2% BTF plus 2 ppm T-2 and a BTF-4 group with 0.4% BTF plus 2.0 ppm T-2. Performance, jejunal morphology and serum biochemistry were surveyed in all groups. On day 21, BW, BWG and FI were significantly decreased in the NCG compared to the PCG (9.7%, 12.0% and 6.3% respectively; $P<0.05$). Independently of the used BTF quantity, negative effects of dietary T-2 on performance parameters were compensated. Total plasma protein (TPP), albumin (ALB), both mycotoxin-specific parameters, and serum phosphorus of NCG were significantly lower in comparison to the PCG ($P\leq0.05$). With increasing inclusion of BTF in the diet, TPP and ALB parameters showed numerical increased levels, indicating liver function resuming closer to NCG. The villus:crypt ratio was significantly decreased in the group which was fed T-2 alone compared to the PCG ($P\leq0.05$). The addition of BTF influenced this ratio positively about all BTF-groups. The relative weight of liver and the feed conversion ratio (FCR) did not differ between treatments. This study confirms a liver protective function of BFT due to improved TPP and ALB serum levels. BTF prevented the negative effect of 2ppm T-2 on performance.

Poster 3.44: A specific composition of 1-Monoglycerides of Short- and Medium Chain Fatty Acids prevented Campylobacter jejuni colonization in experimentally challenged broiler chickens

M. Parini[1], A. Paoli[1], G. Tosi[2], P. Massi[2], L. Fiorentini[2]

[1] SILO International, [2] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna; manuela.parini@silospa.com

A specific composition of 1-Monoglycerides of Short- and Medium Chain fatty acids showed antibacterial efficacy against C. jejuni in vitro, at pH 6-7, that is at the pH values of the gut of chickens. The minimum inhibitory concentration (MIC) of the composition against the C. jejuni was 0.01%, while the beneficial Lactobacillus plantarum and Lactobacillus acidophilus were not inhibited by the composition. A trial with broiler chickens was carried out to assess the efficacy of the composition in reducing C. jejuni counts in the caeca after experimental infection. Sixty female one-day old Ross 308 broiler chicks were randomly housed in isolators and allotted to two treatments, three replicates. The control group received a standard commercial feed, while the 1-Monoglycerides group received the same feed supplemented with 0.25% of the composition during the first 10 days of life and with 0.02% of the composition from day 11 to day 37. At 12 days of age all the birds were challenged via endoesophageal inoculation with 1mL of saline solution containing 1×10^7 CFU/bird of C. jejuni. At day 17, 25 and 37 ten chickens from each group were sacrificed; samples from caeca were analyzed for C. jejuni counting. Results showed that in the 1-Monoglycerides group there was a 5-log reduction of the CFU counts compared to the control group ($p = 0.02$. Two-way ANOVA). The 1-Monoglycerides composition prevented C. jejuni colonization in the caeca. The in vitro trial showed a selective antibacterial effect of the 1-Monoglycerides composition against C. jejuni without inhibiting the beneficial Lactobacillus. Specific compositions of Short- and Medium-Chain 1-Monoglycerides may represent a valid alternative to enteric antibiotics in broiler chickens to improve gut health and meat safety.

Poster 3.45: The effect of Deoxynivalenol on the relative organ weight, morphology and histology of the small intestine of broiler chickens

I. Riahi[1], A. M. Pérez-Vendrell[1], V. Marquis[2], A. Javier Ramos[3], M. Nofrarias[4], J. Brufau[1]

[1] IRTA, Animal Nutrition, Ctra. Reus-El Morell, Km 3.8, 43120 Constanti, Spain, [2] Phileo-Lesaffre Animal Care, 2580 Chemin de Vallesvilles, 31600 Seysses (France), [3] Food Technology Department, University of Lleida. Avda Rovira Roure, 191. 25198 Lleida, Spain, [4] Centre de Recerca en Sanitat Animal (CReSA08193), Bellaterra, Barcelona, Spain; insaf.riahi@irta.cat

A feeding trial was conducted to evaluate the effect of deoxynivalenol (DON) at different levels (5 or 15 mg/kg feed) in the diet of broiler chickens on relative weight of organs, morphology and histology of the small intestine. Forty-five broiler 1-day-old chickens (Ross 308 males) were randomly assigned to 3 different experimental groups; 1) control non-contaminated, 2) contaminated diet with 5 mg DON/kg of feed and 3) contaminated with 15 mg DON/kg of feed. Five replicated cages of three birds were used for each treatment in a randomized complete block design for 45 days. The results revealed that the dietary inclusion of DON at both levels (5 and 15 mg/kg) over the whole experiment decreased the relative weight of small intestine and colon ($p<0.1$) and increased the relative weight of gizzard and thymus ($P<0.05$). Feeding of the contaminated diets (5 and 15 mg/kg) increased significantly the length of the small intestine and decreased its density ($P<0.05$). Consumption of DON at both levels (5 and 15 mg/kg) did not alter significantly the villus height, crypt depth and villus to crypt ratio. Feeding DON altered the relative organ weight, the small intestinal morphology but the changes were not dose related.

Poster 3.46: Effect of modified insect oil on growth performance, carcass traits, gut morphology, histological features and fecal microbiota in broiler chicken diets

S. Dabbou[1], A. Lauwaerts[2], I. Biasato[3], I. Ferrocino[3], E. Colombino[1], C. Garcés Narro[4], M. Gariglio[1], L. Gasco[3], M. Capucchio[1], L. S. Cocolin[3], A. Schiavone[1]

[1] Department of Veterinary Science, University of Turin, largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy, [2] Proviron Industries nv, G. Gilliotstraat 60, 2620 Hemiksem, Belgium, [3] Department of Agricultural, Forest and Food Sciences, University of Turin, largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy, [4] Faculty of Veterinary Medicine, Universidad CEU Cardenal Herrera. CEU Universities. E-46115 Alfara de Patriarca, Valencia, Spain; sihem.dabbou@unito.it

The aim of the present trial was to evaluate the effect of modified Hermetia illucens L. fat on broiler chicken diets. A total of 200 male broiler chickens (Ross 308) were assigned to 4 dietary treatments (5 pens/treatment; 10 birds/pen). The experimental diets were provided ad libitum from 0 to 11d (starter period; SP), and from 11d to 33d (grower-finisher period; GFP). A basal diet containing soybean oil (SO) as added fat was used as control group (C), tested against three experimental diets where the SO (4.63% and 5.93% in SP and GFP, respectively) was partially substituted by insect oil (0.29%, and 0.16% in SP and GFP, respectively) or one of two types of modified insect oil (MIO1; 0.80% and 0.40% in SP and GFP, respectively) and MIO2 (0.35% and 0.18% in SP and GFP, respectively). MIO1 and MIO2 had a high and low ratio of monobutyrin to monoglycerides of medium chain fatty acid, respectively. Fecal content was sampled and fecal microbiota assessed by means of 16S rRNA amplicon-based sequencing. The birds were slaughtered at 34d of age and carcass traits, gut morphometric indices and histopathological alterations were evaluated. Data were analysed by one way ANOVA. For fecal microbiota, not-normally distributed variables were assessed by Wilcoxon matched pairs test or Mann-Whitney test as appropriate. Diet did not influence the growth and slaughter performances, gut morphometric indices and the histopathological alterations in all the organs. As far as fecal microbiota is concerned, MIO1 and MOI2 reduced the presence of Clostridium and Corynebacterium, which can frequently cause infections in poultry. In conclusion, MIO may positively modulate the fecal microbiota of broiler chickens without influencing the growth performance and the intestinal morphology.

Poster 3.47: Effects of whole white sorghum supplemented with phytase on growth performance and carcass yield, gut development and health, net protein utilization and apparent ileal digestibility of protein and phosphorus in broilers

S. N. Qaisrani[1], M. M. Anwar[1], G. Mustafa[1], F. Bibi[2], S. Naveed[1], T. N. Pasha[1]

[1] Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan,
[2] Department of Livestock and Poultry Production, Faculty of Veterinary Sciences, Bahauddin Zakriya University, Multan; shafqat.qaisrani@uvas.edu.pk

In total, 240 straight run (Ross 308) day-old broilers were used to test the hypothesis that whole white sorghum (WWS), supplemented with phytase, may replace corn in broilers diets without compromising their growth performance. Ten (5 × 2) experimental diets, (with five graded levels of WWS (0, 25, 50, 75 and 100%) and two levels (1000 and 1500 FTU/kg) of phytase), with four replicates each, were tested in a completely randomized block design. Increasing the levels of WWS did not influence ($P > 0.05$) feed intake (FI), whereas body weight gain (BWG) and feed conversion ratio (FCR) were improved ($P < 0.05$). Greater levels of WWS resulted in an improved ($P < 0.05$) gut health, carcass yield and digestibility of protein and phosphorus. Broilers fed 100, 75 and 50% WWS had, on average, 38, 32 and 19% higher net protein utilization, respectively, compared with those fed 0% WWS. Broilers fed 100, 75, 50 and 25% WWS based diets had, on average, 11, 7 and 5% heavier gizzard, respectively, compared with those fed 0% WWS. Broilers fed diets containing higher levels (1500 FTU/kg) of phytase showed a better ($P < 0.05$) FI, BWG and FCR compared with those fed 1000 FTU/kg phytase. Broilers fed 1500 FTU/kg phytase supplemented diets had, on average, 6% longer villi, 5% shorter crypts and 5% greater VCR compared with those fed 1000 FTU/kg supplemented diets. Greater levels of phytase, similarly, positively influenced ($P < 0.05$) NPU by 11%. In conclusion, feeding phytase supplemented whole sorghum resulted in a better growth performance, enhanced gut development specifically the gizzard weight, villus height and crypt depth that improved the digestibility of protein and phosphorus in broilers.

Poster 3.48: Feeding (Bacillus amyloliquefaciens CECT 5940) improves performance of broiler chickens

A. Ortiz[1], A. Ion[1], L. Stef[2]

[1] Evonik Nutrition & Care GmbH, Rodenbacher Chausse 4, Hanau, Germany, [2] University of Agricultural Sciences and Veterinary Medicine of Banat, C. Aradului 119,Timisoara, Romania; alvaro.ortiz@evonik.com

Based on the potential of probiotic to improve intestinal microbial balance, it was hypothesized that Bacillus amyloliquefaciens CECT 5940 (Ecobiol®) can improve the performance of broilers when added in the feed. The objective of the trial was to determine the efficacy of feeding Ecobiol® on broiler performance under European feeding conditions. 300 Ross 308 male day-old chicks with an initial body weight (BW) of 42±3 g were randomly distributed in two dietary treatments, each with 25 replicates and 6 birds per pen. Dietary treatments included a basal control diet and a basal diet supplemented with 1.0×10^6 cfu of B. amyloliquefaciens CECT 5940 per g of feed. Diets were formulated according to Ross 308 guidelines and fed in mash form. Due to the extremely controlled sanitary conditions in the trial facility, diets did not contain coccidiostats, no vaccination was conducted in the flock and no medication was administered. The ceca from probiotic-supplemented group had a 68% lower coliform count (4.25×10^8 cfu vs. 1.37×10^8 cfu) and a 79% lower E. coli count (3.6×10^8 vs 7.61×10^7) than in control group. Final BW at day 35 was numerically higher in the probiotic group (2.114 g) compared to the control group (2.086 g). Feed conversion ratio (FCR) was significantly lower in the probiotic group at 20 days of age (1.344 vs. 1.316) and at 35 days of age (1.533 vs. 1.508). Larger difference in the FCR at day 20 implies that feeding Ecobiol® improves performance when the gut-challenges are higher during the grower phase. Overall, results of this study demonstrate that B. amyloliquefaciens CECT 5940 is able to improve broiler performances even when the performances of the animals are close or equal to genetic potential of the breed.

Poster 3.49: Exploring the effects of organic acids supplementation on growth performance, carcass characteristics, caecal microbiota and gut morphology in broilers

S. Naveed[1], K. Saleem[1], A. R. Sial[2], T. N. Pasha[1], A. Mahmud[3]

[1] Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan, [2] Department of Animal Sciences, University of Veterinary and Animal Sciences, College of Veterinary and Animal Sciences, Jhang, Punjab, Pakistan, [3] Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan; saimamahad@uvas.edu.pk

Antibiotics use as growth promoters in poultry feed is a major predisposing factor of drug resistance in humans. Efforts are being made to search for alternatives to antibiotics. Organic acids have exhibited a great potential as an alternative and have been used as feed additives. To explore more about organic acids, a total of 900 day old broiler chicks (Cobb-500) were procured from local hatchery and distributed into 9 treatment groups having 5 replicates of 20 birds each. The duration of biological trial was of 35 days. Group T1 served as control group without any dietary supplementation. Groups T2 and T3 were supplemented with different levels (100g/ton and 250g/ton) of enramycin (antibiotic) and T4, T5 and T6 were supplemented with different levels (2kg/ton, 3kg/ton and 4kg/ton) of ammonium formate and propionate, whereas T7, T8 and T9 were supplemented with different levels (2kg/ton, 3kg/ton and 4kg/ton) of calcium formate and propionate. The results revealed significant improvement ($P<0.05$) in body weight gain and FCR in groups T3, T5 and T9, whereas feed intake remained unaffected. Carcass evaluation resulted in a significant increase ($P<0.05$) in dressed and eviscerated weight along with carcass yield in groups T5, T7, T8 and T9. Significantly lower ($P<0.05$) total bacterial count was observed in groups T3, T5, T8 and T9. Significantly improved ($P<0.05$) villi length was seen in groups T5, T6 and T9. Total protein, globulin, HDL and LDL levels remained non-significant ($P>0.05$) among different organic acids treatments. In conclusion, organic acids can be utilized as a better replacement of antibiotics. Supplementation of organic acids at a dose rate of 3kg/ton and 4kg/ton is recommended for efficient performance of broilers.

Poster 3.50: Use of a novel feed additive preparation in broiler chicken: effects on performance and gut health

M. Castillo[1], S. Budnik[1], S. Peris[1], F. Yan[2]

[1] Novus Europe S.A./N.V.- Edifici CEPID, Avda. Cambra del Comerç, 42,43204, Reus, Spain, [2] Novus International, Inc., 20 Research Park Drive, Missouri Research Park, St. Charles, Missouri, 63304; marisol.castillo@novusint.com

The use of CIBENZA®EP 150 has demonstrated an improvement of the digestibility of protein in poultry while maintaining production performance (Wedekind and Escobar 2016). It is a feed additive which combines a heat stable protease (EC 3.4.21.19) with the spores of its production organism Bacillus licheniformis (ATCC 53757). A trial was designed to study the effect of this additive on chicken's gut health. A total of 792 1-day old chickens were divided into three groups (22 birds/pen, 12 replicates per group). All the animals received corn-soybean meal diet (Negative Control; CON), supplemented with the protease at 500 ppm (Protease; PRO) or with bacitracin methylene disalicyclate (BMD) at 500 ppm as a positive control. The performance was checked at d 14, d 21 and d 29 and inflammatory cytokines and enterotoxin levels (ET) were measured at d 30. The animals from PRO and BMD groups showed the highest body weight at d 29 (1.70 kg and 1.72 kg respectively for PRO and BMD) versus CON (1.62 kg; $P=0.04$) with a tendency to improve feed conversion ratio (FCR): 1.46 and 1.45 respectively for PRO and BMD, versus CON (1.49; $P=0.07$). Mortality was numerically lower for PRO compared to BMD and CON (7.5% versus 10.9% and 12.8% for PRO, BMD and CON respectively, $P=0.09$). Cytokine IL10 in serum was significantly lower for PRO and BMD (0.49 pg/ml and 0.38 pg/ml for PRO and BMD respectively) compared to CON (1.08 pg/ml, $P=0.0004$). ET concentration tended to decrease in PRO and BMD (408.9 pg/ml and 411.6 pg/ml for PRO and BMD respectively) versus CON (589.2 pg/ml, $P=0.086$). The use of the combination of a protease with its producing microorganism Bacillus licheniformis improved performance and decreased pro-inflammatory cytokines confirming its beneficial effects on broiler gut health.

Oral presentations

Low protein supplementary feed – a tool to control the growth of slow growing broilers

J. Søholm Petersen[1], T. Munk Schou[1]

[1] SEGES, Organic Innovation, Agro Food Park 15, 8200 Aarhus N, Denmark; tomu@seges.dk

In Europe, due to animal welfare issue, the demand for slow growing broilers is increasing. When raising slow growing broilers, farmers have to comply with the strong regulation for the individual production types. Although the birds genetically have a reduced potential for growth and feed intake, the farmers need to control that the growth stays within the regulation for daily growth or slaughter age. The purpose of the current study was to obtain new practical knowledge about how farmers can control the growth of slow growing broilers during ad libitum feeding. Three trials were conducted with 624 male ColorYield broilers from Hubbard on a commercial organic broiler farm. In each trial, 52 broilers were placed in 12 pens (N=624) placed in an organic broiler house. Trial 1 and 3 had 3 treatments and 4 replicates, and trial 2 had 2x2 treatments and 3 replicates. In each of the three trials we compared different feed types and feeding strategies differing in protein content. The results showed that standard feed diluted with low-protein supplementary feed, reduced the growth of the broilers. Especially low protein content in starter-feed (18.6 % vs. 22.6 %, Trial 2) reduced the broiler's daily growth from 40.0 g/day to 37.5 g/day ($P<0.05$). While low protein content in grower feed (14.5 % vs. 17.1 %) did not significantly decrease the broiler's growth (from 40.0 g/day to 39.1 g/day; $P>0.05$).

It was concluded, that using a protein adjusted feeding strategy, allows the farmer to control the broiler's growth. The reduction in growth could be achieved without feed restriction. Lowering the protein content from 17.2 % to 16.3 % in Trial 1, also had a positive impact on the broiler's welfare, as the score for food pad dermatitis was reduced from 60 to 8 ($P<0.001$).

Performance, carcass and meat quality of slow-growing broilers fed different energy and crude protein levels

E. Delezie[1], S. Leleu[2], M. Lourenço[2]

[1] Flanders Research Institute for Agriculture and Fisheries, Animal Sciences Unit, [2] ILVO; evelyne.delezie@ilvo.vlaanderen.be

Little information is available regarding the requirements of slow-growing broilers. Some have suggested that their crude protein (CP) requirements are lower than that of fast-growing broilers. Others suggested that the lysine requirement is the same. However, these results refer to older genetic lines. As there is a relative lack of data concerning the needs of slow-growing broilers, this study focused on their responses to different energy/CP. More specifically, to assess the effects of energy and CP/amino acid (AA) levels (normal/reduced) on performance, carcass yield, meat quality and litter quality. Four dietary treatments were used in a 2x2 design. Each treatment was repeated 8 times (88 male Sasso broilers/treatment). A starter was fed until d18, a grower to d34 and a finisher to d69. For the control treatment energy and CP values were 12.10MJ/kg and 21% (starter), 12.4MJ/kg and 20% (grower) and 12.7MJ/kg and 19% (finisher). For the reduced diets, energy was reduced by about 1MJ/kg and CP by 1%. Feeding the broilers a standard fast growing diet during the starter period significantly reduced their feed intake compared with broilers fed the nutrient reduced diets. Reducing only the energy or CP level resulted in the highest feed intake. Lowest growth rate and highest feed conversion were obtained if both energy and CP level were reduced. During the grower and finisher period there was no correlation between performance and nutrient levels in slow-growing broilers. Also no significant effect on carcass characteristics, meat quality and litter conditions were observed. Therefore, the composition of a slow-growing broiler diet is less critical than that of a fast-growing. Downwards adjustments to the energy and CP/AA contents are possible without negative effects.

Novel feedstuffs for poultry: the in vivo nutrient digestibility of silage and silage residue of the seaweed Saccharina latissima in broilers

L. Stokvis[1], M. M. van Krimpen[1], P. G. van Wikselaar[1], P. Bikker[1]

[1] Wageningen University and Research; lotte.stokvis@wur.nl

To feed the growing world population living a life of increasing welfare, novel sources of feed, for the production of meat, milk and eggs, need to be found that are not competing for resources currently in use. Seaweed could be such a novel source. In broilers, a digestibility experiment was conducted using a corn-soybean meal based basal diet with 10% of the seaweed Saccharina latissima added as silage (S) or silage residue (SR; leftover material after aqueous extraction). Diets were formulated to meet or exceed all requirements for broilers. The digestibility study was conducted with 160 14-day old Ross 308 broilers in 16 floor pens (0.96 m^2) with 10 birds per pen. From day 14 to 22, 5 pens received the S-diet, 5 pens the SR-diet and 6 pens the basal diet. Bodyweight (BW) was monitored during the trial, faeces were qualitatively collected at day 20, 21 and 22, and at day 22 ileal content was collected. All data were pooled per pen. Chickens fed the S-diet performed worse compared to both chickens fed the control or SR-diet, considering almost all response parameters. The SR-diet was more promising, with a BW not differing from BW of chickens fed the control diet, although feed intake and thus FCR were higher ($P<0.001$). Ileal protein digestibility of the seaweeds was low for both S (65%) and SR (69%, versus 93.6% for the basal diet), whereas ileal amino acid (AA) digestibility of SR was higher for all AAs compared to S, except for lysine. The cause of the higher ileal AA digestibility in SR requires further investigation. The studied seaweed products showed a low protein digestibility, whereas seaweed is potentially high in protein (up to 40%). Thus, it is relevant to investigate the possibilities of protein extraction from seaweed, for inclusion in broiler diets.

Effect of limestone source and particle size on calcium digestibility and on phosphorus digestibility

R. Angel[1], H. Enting[2], M. Hruby[3], P. Plumstead[4]

[1] University of Maryland, College Park, MD, USA, [2] Cargill Animal Nutrition, Velddriel, The Netherlands, [3] DuPont, Danisco Animal Nutrition, Woodbury, MN, USA, [4] Chemunique PTY LTD, Randburg, South Africa; rangel@umd.edu

A study was conducted to determine effects of 6 sources of limestone (LM), obtained from different regions (US, Indonesia, Poland, Brazil), on calcium (Ca) and phosphorus (P) digestibility in broilers. All 6 LM were ground to 0.8 mm GMD and 2 were also ground further to a 0.15 mm GMD to test effects of particle size. LMs were analyzed for 9 minerals, particle size and solubility in 2 different acid solutions (0.2N HCL and 3 pH HCL solution buffered with 3 molar glycine) at 3 time points. A basal diet was mixed (corn and SBM containing 2.3 g/kg phytate P and 0.05% Ca). The basal was subdivided into 8 batches and used at 98.4% in the LM treatment (TRT) diets. LM were added to achieve 0.65% Ca in the final diets. A commercial phytase (PHY) from Buttiauxella spp. was used in a factorial arrangement of 8 LM (6 sources but 2 LM at 2 GMD) and 2 PHY concentrations (0 and 1000 U.kg). There were 19 TRT fed (16 from the factorial arrangement) plus basal diets without LM added and with or without PHY and a purified diet for endogenous loss determination. Each TRT was replicated 7 times (n = 7 cages with 4 broilers each). Diets were fed for 36h after which broilers were euthanized and distal ileal digesta samples were taken for Ca, P, and titanium analysis. There were main effects of LM and PHY as well as interactions ($P<0.01$) as well as a particle size effect on both Ca and P standardized Ileal digestibility (SID). SID Ca for LM of 0.8 mm GMD ranged between 0.33 to 0.64 g/kg in the absence of PHY and between 0.43 to 0.69 g/kg in diets with PHY. SID P ranged from 0.22 to 0.48 g/kg in diets without PHY and 0.60 to 0.93 g/kg in diets with PHY. Results indicate there is a clear effect of limestone source and particle size on Ca and P digestibility.

Modelling dietary phosphorus utilisation by broilers

B. Meda[1], C. Couture[2], A. Narcy[1], M.-P. Létourneau-Montminy[2]

[1] UMR BOA, INRA - Université de Tours, [2] Université Laval - Département des sciences animals; bertrand.meda@inra.fr

The optimisation of phosphorus (P) utilisation by broilers remains crucial for a sustainable production i.e to avoid P overfeeding and limit P excretion. The objective of the present work was to develop a model which simultaneously predicts P and Ca absorption, deposition and excretion in response to dietary P, Ca and microbial phytase levels. Two sub-modules are included representing the growth and the metabolic fate of the minerals respectively. An equation derived from a meta-analysis was considered to predict the digestive absorption which takes into account the different forms of P, dietary Ca and microbial phytase levels and their interactions. For growth, weight gain is calculated as the sum of daily depositions of protein, water, fat and ash. The daily protein deposition (feathers and body) is estimated from the weight of the animal and then modulated according to the daily intake of lysine. Daily fat deposition is then quantified by the difference between energy intake and heat loss and protein deposition. The second submodule predicts the deposition of ash, P and Ca into bone, soft tissues and feathers. A priority for P deposition into soft tissues and feather is applied; when dietary P level decreases, its deposition in soft tissues and feathers remains stable while it is reduced in the skeleton. Decreasing dietary Ca level from 1.0 to 0.5% slightly increases P absorption but P retained is reduced and urinary losses enhanced (more than 20-fold) due to a lack of Ca. The negative impact of high Ca concentration (>1.0%) on feed intake and P absorption can result in reduced P deposition although P is not supposed to be limiting. This model will be useful to define new feeding strategies facing environmental, economic or animal welfare challenges.

Poster 4.1: A meta-analysis of whole grain feeding and the possible mechanisms driving responses other than heavier gizzard weights

A. Moss[1], H. Truong[2], S. Liu[1], P. Selle[1]

[1] Poultry Research Foundation within The University of Sydney, NSW, Australia., [2] CSIRO Integrated Sustainable Aquaculture Production, QLD, Australia; amy.moss@sydney.edu.au

Whole grain feeding (WGF) is widely adopted in countries where wheat is the dominant feed grain to reduce feed manufacturing costs, improve gut integrity, feed conversion ratio (FCR), apparent metabolisable energy (AME) and litter quality. However, responses of broilers to WGF is variable due to the range of methodologies adopted in WGF studies. Therefore, a meta-analysis was conducted to determine the effect of pre- and post-pellet WGF on relative gizzard weights and contents, FCR and AME of broiler chickens. The database consisted of 42 papers after applying selection criteria. Data were analysed by General Linear Model procedures using JMP Pro 13 and experiment identifiers included in models as a co-variate. Post-pellet WGF increased gizzard weights by 32% (16.78 versus 12.67 g/bird; $P < 0.001$) and gizzard contents by 31% (7.17 versus 5.48 g/bird; $P < 0.001$) compared to the control. Overall, relative gizzard weights were not related to FCR. However, FCR was linearly related to gizzard contents, and was more indicative of performance than gizzard weights. Pre- and post-pellet WGF tended to generate more efficient FCR (1.671 and 1.672, respectively) compared to the control (1.695). Additionally, the highest AME was achieved by birds offered pre-pellet WGF, significantly increasing AME by 0.41 MJ (13.63 versus 13.22 MJ/kg DM) compared to the control. Thus, pre-pellet WGF increased energy utilisation and tended to improve FCR without a robust gizzard response. These performance responses may be driven by increases in slowly digestible starch (Moss et al., 2017). Therefore, generating performance responses under WGF regimes is more complex than simply heavier gizzard weights, and is likely influenced by starch and protein digestive dynamics.

Poster 4.2: Effects of dietary crude protein level on litter and manure composition and ammonia emission in broiler breeders

R. van Emous[1], A. Aarnink[1], C. de la Cruz[2], V. Naranjo[2]

[1] Wageningen Livestock Research, [2] Evonik Nutrition & Care GmbH; rick.vanemous@wur.nl

The purpose of this study was to evaluate the effects of two dietary crude protein (CP) levels (High = CPh and Low = CPl) on litter and manure composition and ammonia emission in broiler breeders. The high CP diets were based on the recommendations of the breeder company. Diets were formulated to be iso-caloric and calculated CP content of the low CP diets was 15 g/kg lower (phase 1: 135 vs. 150, phase 2: 125 vs. 140, and phase 3: 115 vs. 130 g/kg) during all phases (phase 1: 23 to 34, phase 2: 35 to 46 wk, phase 3: 47 to 60 wk of age). The experimental diets were supplemented with free amino acids to meet the minimum essential AA levels recommended by the breeder company. A total of 480 female and 64 male Ross 308 20 wk of age broiler breeders were used. The breeders were housed in 16 floor pens with 30 females and 4 males per pen at the start of the laying period at 20 weeks of age. The pens contained an elevated floor (25% of the total floor surface) with plastic slats. Litter (floor) and manure (below slatted floor) composition and ammonia concentration were measured at 34, 44 and 54 weeks of age. Ammonia emission was measured using a flux chamber on top of the litter or manure under the slatted floor. Dietary protein levels did not affect dry matter (DM) content of the litter and manure. Combined results of litter and manure samples showed that birds fed the CPl diets had 8.3 and 11.4% lower total-N and ammonia-N (NH_4^+) content, respectively. The lower content of total-N and ammonia-N in the litter and manure resulted in an average 9% lower ammonia emission across the different ages. In conclusion, reducing dietary CP level by 15 g/kg compared to current breeder standards reduces nitrogen excretion in the litter and ammonia emission to the environment by 9%.

Poster 4.3: Broiler performance, gut health and litter quality as affected by crude protein level and protease supplementation

M. van den Brink[1], A. Vandeweghe[2], W. Merckx[3], S. Massart[3], V. Verlhac Trichet[4]

[1] Twilmij B.V., [2] DSM Nutritional Products N.V., [3] Z.T.C. KU Leuven, [4] DSM Nutritional Products France; madri.brink@ilvo.vlaanderen.be

The effect of decreased crude protein (CP) supply either or not combined with a protease was evaluated in a performance trial. Additional blood analyses were performed to support the data.

A total of 450 male broilers (Ross 308) were divided in 3 treatments with 10 replicates (floor pens) each: (1) control diet, representative for Benelux practice; (2) control with 1% lower CP levels and (3) as (2) supplemented 0.02% protease (Ronozyme® ProAct, DSM). Metabolizable energy and digestible essential amino acid levels were equal in all treatments. The broilers were fed a 3 phase feeding program, with wheat-soybean meal-corn based diets and nutrient levels according to CVB recommendations. Birds were housed at commercial density and had ad libitum access to feed and water. Average pen weight and feed intake were recorded at 1, 11, 30 and 38 days of age. Feed conversion ratio (FCR), average daily growth (ADG) and average daily feed intake (ADFI) were calculated for each period and the total period. Litter quality and foot pad lesions were scored visually at day 22, 30 and 35. At day 28, one broiler per pen was sacrificed for determination of blood parameters. Decreasing dietary CP levels either or not supplemented with protease numerically improved FCR and ADG. Broilers which received low CP diets showed dryer litter and less foot pad lesions ($P<0.05$). These effects were more pronounced when protease was added. Considering the blood parameters, AGP-1 and d-ROM were significantly lower in broilers fed low CP diets including protease. These findings indicate there is a beneficial effect of decreasing CP levels and use of a protease on broiler performance and gut health.

Poster 4.4 : Impact of broiler breeder nutrition, body composition and weight on chick quality

J. Heijmans[1], M. Duijster[2], R. P. Kwakkel[3], H. van den Brand[4]

[1] De Heus Animal Nutrition, Ede, The Netherlands; Adaptation Physiology Group, Wageningen University, Wageningen, The Netherlands; Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands, [2] De Heus Animal Nutrition, Ede, The Netherlands, [3] Animal Nutrition Group, Wageningen University, Wageningen, The Netherlands, [4] Adaptations Physiology Group, Wageningen University, Wageningen, The Netherlands; jheijmans@deheus.com

It has been demonstrated in chickens that maternal nutrition can have an effect on later life development, welfare and health. The mechanism behind this phenomenon has yet to be elucidated. It is hypothesized that the energy to protein ratio in the breeder diet and her body composition can affect chick quality. It is speculated that, mainly in young breeders, competition for nutrients (energy and protein) between tissue accretion (mainly fat mass growth at the end of rearing) and egg production occurs. The need for fat mass growth may lead to less nutrients deposited in the eggs of young breeders and this may consequently have effects on chick quality. A higher energy intake during rearing will result in breeder hens with a higher fat mass in the body at the end of rearing. As these breeder hens have a higher fat mass, they might have a lower strive for fat accretion at start of production. More nutrients can be partitioned towards the egg, which may benefit the chick and result in a better chick quality. In a 2 x 4 experimental design, impact of two rearing growth curves (standard vs. 15% higher) of the broiler breeder hen and four linear energy to protein ratios (-4%, 0%, +4% and +8% compared to the standard) in the diet of the broiler breeder hen on her body composition and reproductive performance will be investigated. Hatching eggs of these broiler breeder hens will be incubated at three different maternal ages (28, 36 and 44 wk of age) and chick quality will be assessed at hatch and during the grow-out period. Preliminary data of the first ten weeks of rearing of the broiler breeder hens will be presented at the symposium.

Poster 4.5: Sustainable 100 weeks age layer hen in a cage free systems

X. Arbe Ugalde[1]

[1] H&N International; xarbe@hn-int.com

Nowadays the genetic potential of the layers is targeting an extended age of production of 100 weeks making it more sustainable. Europe is moving towards a cage free production and for achieving the extended production in the cage free we need to focus on: 1.Body weight of the pullet during the first 5-6 week of life: there is a clear correlation between flocks that didn't achieve the body weight at 5-6th week and the early cull in production. 2.The feed intake capacity : at the start of the production there are high needs and the feed intake is far from the average feed intake of a mature layer hen. We need to use certain amount of fiber in the pullet feeds to overcome the challenge. 3.Energy and amino acid needs are different: there will be an increase of the maintenance need due to the activity of the birds and the temperature at the farm. The amino acids need will change and the ideal protein ration should be reviewed as the oxidative stress will increase as the birds will produce more free radicals as consequence of high metaboic rate, diseases and temperature variations. 4.Calcium and phosphorus: the phosphorus is important during the growth of the bird after the peak of production, the needs are low. How calcium is supplied to birds is very important, big particle size (>3mm) is needed to keep a good egg shell quality. 5. Feed structure: birds are able to select where and what to eat, so making a homogenous particle is key to avoid choice feeding. The mixing, milling and selected raw materials will have an impact on the final structure of the feed. In summary there are several key points to change if we want to achieve a 100 week old layer in cage free systems.

Poster 4.6: Evaluation of a magnetic nanoparticle attached phosphorus compound as a novel phosphorus source for broilers

J. Wang[1], *J. Xu*[2], *W. Kim*[1]

[1] University of Georgia, [2] Georgia Institute of Technology; jjw26921@uga.edu

Magnetite (Fe_3O_4) based magnetic nanoparticle (MNP) is a sorbent used to remove phosphorus (P) species in poultry processing wastewaters. Approximate 13% (wt.) P was attached on the spent MNP. We hypothesized P in spent MNP is a potential P source for broilers. A study was conducted to evaluate the potential of using the spent MNP as a P source for broilers. A total of 240 Cobb 500 male broilers were randomly allocated to 4 dietary treatments with 6 replicates and 10 birds each. Treatment consisted of 1) a positive control (PC) with 0.90% Ca and 0.45% nonphytate-P (nPP); 2) a negative control (NC) with a reduction of 0.15% nPP; 3) NC plus 0.075% of P from MNP; 4) NC plus 0.15% of P from MNP. Body weight, feed intake, feed conversion ratio was recorded at day 7, 14, and 18. At d 18, 3 birds per cage were randomly selected for DXA scanning. Liver, heart, and spleen were collected for Al and Fe residue analysis. Ileal digesta were collected from the rest of the birds for P digestibility. Data were subjected to SAS using one-way ANOVA following GLM procedure. The significance level was set at $P<0.05$. Means were separated using Duncan's Multiple Range Test. The addition of 0.075 and 0.15% P from MNP increased ($P<0.05$) the body weight gain at d 7 to 14. NC showed a significant lower ($P<0.05$) body mineral density and body mineral content compared to PC. Diets with additional 0.075 and 0.15% P from MNP increased ($P<0.05$) body mineral density and body mineral content compared to NC, and it reached to the same level as PC. No significant difference was found between the treatments for Al and Fe residue in liver, heart and spleen. The ileal P digestibility was not influenced by MNP. In conclusion, tested P in MNP could be efficiently and safely used by broilers as a P source.

Poster 4.7: Meta-analysis of prececal digestibility of phosphorus in growing broilers: effects of dietary phosphorus, calcium and phytase supply

C. Couture[1], A. Narcy[2], R. Chiasson[1], M.-P. Létourneau-Montminy[1]

[1] Université Laval - Département des sciences animals; [2] UMR BOA, INRA - Université de Tours; marie-pierre.letourneau-montminy.1@ulaval.ca

The development of new feeding strategies for phosphorus (P) requires the improved prediction of P absorption by broilers taking into account the main variables: dietary P sources, calcium (Ca) concentrations, exogenous phytase addition. Given the large number of publications, a meta-analysis was performed to quantify the impact of dietary P (Non-phytic P (NPP) and Phytic P (PP)), calcium (Ca) and microbial phytase (PhytM) on apparent prececal digestibility of P (pcdP) in broilers. A database of 480 treatments from 95 experiments in 59 published articles was used. A linear multiple regression model of digestible P (total P x pcdP, g / kg) with the experiment as random effect ($R^2 = 0.94$) showed that in the absence of phytase, pcdP is 70% for NPP from monocalcium phosphate, 69% for animal NPP, 66% for plant NPP, and 55% for NPP from dicalcium phosphate. The prececal disappearance of PP depends on the level of Ca (Ca x PP, $P < 0.001$) with a value of 23% for 10 g Ca / kg and 45% for 6 g Ca / kg. In the global database with phytase, when NPP is expressed as the sum of plant, mineral and animal sources, a linear effect is observed on digestible P ($P < 0.001$). Dietary Ca reduces the digestibility of NPP and PP (Ca x NPP and Ca x PP, $P < 0.001$). The response in terms of digestible P with the addition of PhytM is quadratic and depends on the amount of substrate (PP x PhytM, $P = 0.002$). This effect is influenced by the intake of Ca (Ca x PP x PhytM, $P < 0.001$) and shows that the response in terms of digestible P to PhytM addition increases with high PP and Ca diet. This meta-analysis provides a better knowledge on the modulation factors of the utilisation of dietary P and will be useful to define new feeding strategies.

Poster 4.8: Meta-analysis of the impact of dietary phosphorus, calcium and microbial phytase on growth performance of broilers

A. Hedhli[1], M. Hamdi[1], A. Narcy[2], M.-P. Létourneau-Montminy[1]

[1] Université Laval, département des Sciences Animales; [2] UMR BOA, INRA - Université de Tours; marie-pierre.letourneau-montminy.1@ulaval.ca

This study is part of an ongoing program developing a robust mechanistic model predicting P and calcium (Ca) utilization by broilers in a large range of dietary situations. In that model, the impact of the main modulating factors of P utilization, mainly dietary P, Ca and microbial phytase (PhytM) on absorption and retention of Ca and P are considered. However, these factors are also reported to modify growth performance. The objective of the current work was thus to quantify the impact of dietary P, Ca and PhytM on growth performance of broilers. A database built from 58 publications, including 137 experiments with 367 treatments was used to predict average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in boilers from 0 to 21 days of age. The random effect of the experiment has been included in the models. The response of the 3 criteria to non-phytate P (NPP) is curvilinear (NPP and NPP x NPP, $P < 0.001$). Dietary Ca also modified growth performance; increasing dietary Ca reduces growth performance, an effect that is alleviated in high NPP diet for ADG and FCR (Ca x NPP, $P < 0.01$). The response of growth performance to PhytM is also curvilinear (PhytM x PhytM, $P < 0.001$) and depends on NPP for the 3 criteria (PhytM x NPP, $P < 0.05$) and on Ca for ADG and ADFI (PhytM x Ca, ADG: $P < 0.05$). This interaction showed that the more the P deficiency is acute, i.e. in high Ca and low NPP diet, the more the response of growth performance to NPP and PhytM is high. Different interactions between NPP, Ca and microbial phytase can be quantified based on literature data and must be considered when simulating the metabolic response of growing broilers to P.

Poster 4.9: Effect of an increased supply of protein, lysine and energy on zootechnical performance in meat quail

M. Bournazel[1], A. Mathiaud[1], S. Toussaint[1], N. Bernard[1], S. Klein[1]

[1] MIXSCIENCE; marion.bournazel@mixscience.eu

The performance of Japanese quails has been improved during recent years due to genetic selection. Therefore, there is a need of updating optimal nutritional requirements, in particular concerning energy and protein supply. To do so, a 28-day-trial was performed in a French experimental farm. A total of 5616 quails (males and females) were allotted in 24 pens. Four crumbles diets in three phases (starter: 0-10 d, grower: 10-21 d and finisher: 21-28 d) were formulated: a control (C), C+dL with a higher digestible Lysine (dL) content, C+dL+CP, with a higher crude protein (CP) and a higher dL content and C+dL+CP+ME, with a higher metabolisable energy (ME) and higher CP and dL content. C+dL did not affect growth performance nor carcass yield. C+dL+CP tended to increase body weight (BW) and average daily gain (ADG) compared to C on overall period. In particular, it seems of interest to consider this diet during the starter period, as the BW, ADG and feed conversion ratio (FCR) were improved compared with C (+5% BW and ADG, and -5% FCR, $P<0.05$). For all phases, C+dL+CP+ME allowed significant increase of the final BW, ADG and a significant decrease of feed intake and FCR compared with the other diets (-5% on average, $P<0.05$). On overall period, water consumption was higher (+5%; $P<0.05$) in C+dL+CP compared to other groups. Concerning water to feed consumption ratio, C+dL+CP and C+dL+CP+ME presented higher values than the two other groups (2.07 vs 1.99 on average; $P<0.05$). Regarding breast meat yield, C+dL+CP increased it (+0.5%) and C+dL+CP+ME decreased it (-0.6%), linked to the ingested protein and amino acid. Consequently, this trial showed a response to the increase of ME content on growth performance, whereas carcass yield seems to be more affected by CP and dL supply.

Poster 4.10: Comparative gastrointestinal, tibia and plasma attributes in 48-day-old fast and slow-growing broiler strains subjected to similar management regimen

M. Mohammadigheisar[1], V. Shouldice[1], S. Torrey[1], T. Widowski[1], E. Kiarie[1]

[1] University of Guelph; mmohamm@uoguelph.ca

Advances in genetics has certainly produced broilers chickens of greater growth, meat yield and feed efficiency. However, emerging market differentiation for broiler meat from slow and fast strains is necessitating comparative research on various production aspects of these strains. Samples of 48-day old straight run broilers were obtained from fast-and slow-growing flocks maintained under the similar management regimen. Eight birds were randomly selected from a fast (FG[ST1], representative of modern commercial strains) and each of 4 slow-growing strains (SG). The strains differed by time to 2.2 kg bodyweight corresponding to 36, 50, 42, 44 and 50 days for FG, SG1, SG2, SG3 and SG4, respectively. Birds were weighed, bled and necropsied for comparative study of empty gastrointestinal (GIT) weight (gizzard and small intestine), ceca digesta short chain fatty acids (SCFA), left tibia ash content and plasma grouping of analytes (proteins, enzymes, metabolites and electrolytes). Gizzard was heavier ($P<0.01$) for SG1, 2 and 3 compared with FG and SG4 whereas small intestine was lighter ($P<0.01$) for FG, SG1 and SG2 compared with SG3 and SG4. There were no ($P>0.05$) strain differences on SCFA, jejunal villi height and crypt depth, plasma proteins and electrolytes. SG1, 2 and 3 exhibited higher ($P=0.01$) tibia ash concentration compared with the FG, however, SG4 was intermediate and not different from any strain. FG birds showed higher ($P<0.01$) plasma concentration of aspartate transaminase, creatine kinase, lactate dehydrogenase and creatinine compared with SG strains. In conclusion, although FG had lighter GIT suggesting more efficiency, low tibia and higher circulating plasma enzymes is an indication of impaired internal tissue or organs particularly liver.

Poster 4.11: Humic substances improve productive performance and eggshell characteristics

R. A. Sanmiguel[1], D. Andrey Sierra[1], J. D. López[1]

[1] Universidad Cooperativa de Colombia; rosa.sanmiguel@campusucc.edu.co

Humic Substances supplementation (HS) in animal production diets are considered an alternative to improve zootechnical, physiological and immunological parameters. The aim of this paper was to evaluate the effect of HS and Calcium carbonate ($CaCO_3$) in 144 Hy Line Brown laying hens at 54 weeks old in a completely randomized design in the Universidad Cooperativa de Colombia´s experimental farm. Birds was distributed in 4 groups with 10 replicates each of 3 hens per replicate, and they were subjected to fifteen days of adaptation. Along 8 weeks, group 1 did not receive any supplementation, group 2 was supplemented with 2 g $CaCO_3$/bird/day. Group 3 received in the diet 0,2% of HS and group 4 was supplemented with 0,2% of HS + 2 g $CaCO_3$/bird/day. Productive performance (productive percentage, food intake and egg weight) and egg quality (eggshell percentage, thickness and weight, albumin and yolk height, Haugh Units and eggshell and yolk color) were evaluated. Prior confirmation of statistical assumptions, a hypothesis test was conducted with 95% of confidence in SPSS software. Results showed that group 3 had a significantly lower feed intake and a highest productivity than other groups ($p<0,05$). Between egg quality characteristics evaluated, eggshell weight, percentage and width pole thickness were significantly highest in HS supplemented groups ($p<0,05$) without difference between group 3 and 4. The other variables analyzed did not show significant differences between treatments. Based on the obtained results is inferred that HS optimize calcium metabolism in eggshell making.

Poster 4.12: Intrinsic antibiotic resistance in the genomic era – what to conclude?

A. von Wright[1], P. Halimaa[1]

[1] Biosafe - Biological Safety Solutions Ltd; atte.vonwright@biosafe.fi

Intrinsic antibiotic resistance in the genomic era – what to conclude? In the EU, special attention has been paid to the possibility of microorganisms intentionally added into food chain to act as a reservoir of resistances against clinically relevant antibiotics. The first guidance on the topic was adopted already in 2001. The latest version of the regularly updated guidelines is included in the “Guidance on the characterisation of microorganisms used as feed additives or as production organisms” published by the European Food Safety Authority (EFSA) in 2018. A conceptual difference has always been made between intrinsic resistances that are present in “all the strains of the species”, and acquired resistances that arise either mutationally or via some transmissible genetic element. The actual transmissible genes have been the cause of concern. The EFSA 2018 guidance introduced the requirement for Whole Genome Sequences (WGS) of bacteria and yeasts that are intended for feed additives to provide both an unequivocal taxonomic identification of the strain and a further guarantee of safety. The genomic approach, while certainly helpful in the safety evaluation, has made the once clear distinction between acquired and intrinsic resistance somewhat elusive, and the not always quite clear wording of the guidance further complicates the interpretation. In the presentation some practical examples of the phenotypic and genotypic antimicrobial resistances, their interpretation and their safety implications are discussed.

Poster 4.13: Relationship between feed efficiency and functionality of the digestive tract in broilers: a transcriptomic analysis

A. Juanchich[1], C. Hennequet-Antier[1], C. Cabau[2], E. Le Bihan-Duval[1], M. Duclos[1], S. Mignon-Grasteau[1], A. Narcy[1]

[1] UMR BOA, INRA - Université de Tours, [2] SIGENAE - GenPhySE, INRA; agnes.narcy@inra.fr

Feed efficiency and adaptability to various feedstuffs are keys for improving the sustainability of poultry production. The different parts of the digestive tract must operate in a complementary way to optimize feed digestion. RNAs from the gizzard and the gastro-duodenal junction of 20 individuals selected for their digestive efficiency on a wheat based-diet (AMEn+; AMEn-), within an F2 cross between two divergent lines, were sequenced. The lists of genes differentially expressed between the two tissues for each line were compared in order to study the relationship between functions of the different segments and feed efficiency. In total, 8390 and 8402 genes were differentially expressed between gizzard and gastro-duodenal junction in AMEn+ and AMEn- individuals, respectively. Among these genes, 1337 were specific to the former and 1349 to the later. Functional analysis using Biological Process category of Gene Ontology showed a significant enrichment of the genes involved in the differentiation of neuronal support cells, fatty acid metabolism and DNA catabolism in AMEn+ individuals. In AMEn-, four functional groups were identified: microtubule organisation, protein metabolism, regulation of gene expression and response to extern stimuli. Interestingly, the term “Response to Food” is significantly enriched in these birds which are known to be more sensitive to feed quality. This analysis provides an overview of the relationships between feed efficiency and the functionality of the digestive tract. In the future, this knowledge will be useful to define sustainable strategies of co-adaptation of the feed and the animal.

Poster 4.14: Evaluation of a specific vectorization of curcumin to reduce inflammatory indicators and improve broiler’s growth performances

V. Noirot[1], M. Allaoua[1], A. Ezanic[1], J.-F. Gabarrou[1]

[1] Phodé; vnoirot@phode.fr

Intensive system of production increases oxidative stress of animals. Curcumin is well known for its antioxidant and anti-inflammatory properties. The main goal of this experiment was to evaluate the effect of a formulation of a curcumin potentiated by a technology of delayed release provided by Phodé (France). Eighty-four 10-days-old Ross-308 were randomly distributed in individual cages into 6 groups (n=21). The first group received a standard diet (without any plant extract). The second one received the same control diet supplemented by 6.66ppm of a commercial curcumin feed additive. The 2 other diets contained respectively 3.33ppm and 2.20ppm of vectorised curcumin. IL-6, IL-10 and TNF-α blood interleukins level were evaluated at 35 days on 10 birds per groups. Feed intake (FI), live body weight (LBW) and feed conversion ratio (FCR) were evaluated at 35 days (n=21). Statistical analysis was carried out using the General Linear Model procedure of SPSS®. No significant difference was observed between groups for IL-6 and TNF-α indicators. Whatever the dosage and/or the kind of curcumin, curcumin increased significantly IL-10, suggesting that anti-inflammatory effect of curcumin is mediated through anti-inflammatory cytokines’. The control group presented significant lower performances compared to groups supplemented with curcumin, suggesting that the anti-inflammatory effect of curcumin enable a better growth performance. On the other hand, no significant difference was observed between 3.33ppm of vectorized curcumin and 6.66ppm of standard respectively for FI: 126.8 vs. 127.9 g/day; LBW: 2227 vs. 2263 g and FCR: 1.629 vs. 1.612. Vectorization of the curcumin permitted animals to exhibit the same level of performance with half of the commercial curcumin feed additive.

Poster 4.15: Evaluation of a specific blend of spice oleoresins and essential oils to replace an antibiotic growth promoter program on broilers performance

V. Noirot[1], M. Allaoua[2], G. Derousseaux[2], M. D. Sims[3], D. M. Hooge[4], J.-F. Gabarrou[2]

[1] Phode, France, [2] Phodé, France, [3] Virginia Diversified Research Corp, [4] Consulting Poultry Nutritionist, Pleasant Grove; vnoirot@phode.fr

Plant extracts are usually used to improve bird's performance. A big issue in Europe is also to reduce N and P wastes. The main goal of this experiment was to evaluate the effects of a specific blend of essential oils and spice oleoresins on broilers performance between 7 and 42 days compared to usual conditions of production. A total of 1,200 newly hatched chicks (Ross-308) were randomly distributed (30/pen) into 3 groups with 12 replicates for control and 2x14 replicates in 2 others treatments. Control group received only non-medicated feed. Birds from AGP group received 55 ppm of Bacitracin Methylene Disalicylate (BMD®) from d 8 to d 28 shuttled to 22 ppm of Stafac® from 29 to 42 d. In the tested group (OLEO), AGP were substituted by a feed additive (Oleobiotec, PHODE, France) at 100 g/MT. N and P emissions were estimated by the difference between N and P intake and N and P body composition. Statistical analysis was carried out using the General Linear Model procedure of SPSS Software. $P<0.05$ was considered as significant. The control group presented significant lower performances from 0-42 d compared to OLEO group (1.906 vs. 2.061 kg BW; 1.933 vs. 1.775 feed conversion ratio or FCR). OLEO also decrease losses significantly compared to control (28.2% vs. 31.8% for N and 41.1 vs. 44.1% for P). Both groups, OLEO and AGP, presented statistically equivalent performance on mortality rate, live body weight and feed efficiency. The feed additive Oleobiotec, appeared to be effective to increase bird's performance and reduce Nitrogen and Phosphorus losses.. Further studies are warranted to investigate whether this product improves digestibility of energy and nitrogen to explain those results.

Poster 4.16: Effects of functional sensory molecules on the stress level and broiler's growth performance under two different stocking density

V. Noirot[1], M. Allaoua[1], D. Martinez[2], A. Auvray[1], J.-F. Gabarrou[1]

[1] Phodé, France, [2] University of La Molina, Perù; vnoirot@phode.fr

High stocking density reduces broiler performance by negatively impacting feed intake and growth. The objective of this study was to evaluate the effect of functional sensory molecules (FSM) based on sweet orange (VeO, PHODÉ, France) effect on broilers subjected to density stress. One day old Cobb chicks (n=272) were dispatched into 8 pens X 3 treatments (24 pens). Treatment 1 (T1) was the negative control with low stocking density ($10/m^2$) and treatment 2 and 3 (T2 and T3) were group with high stocking density ($12/m^2$). T1 and T2 did not receive FSM, T3 received FSM in the feed (250 g/T) from day 1 to day 42 days old. Stress level was evaluated by underwent an immobility test (Gordon&Galup1974) at 42 days (n=16), cortisol was also measured in blood samples (n=8). Feed intake (FI), live BW and feed conversion ratio (FCR) were determined at day 42. Statistical analysis was carried out using the General Linear Model procedure of SPSS. $P<0.05$ was considered as significant. The frequency of animals categorized as stressed (immobility test) was higher for T2 birds (62.5%) compare to T1 (18.8%) and T3 ones (17.3%). Blood cortisol for T2 groups was also higher (21.1±2.9ng/mL) than T1 (17.3±2.1ng/mL) and T3 ones (17.3±1.9ng/mL). Total FI was also negatively impacted for T2 birds with 5290±17g against 5537±29g for T1 and 5353±22g for T3 birds. Similarly, T2 birds were lighter (3115±22g) than T1 (3308±12g) and T3 birds (3190±35g). Therefore T2 birds presented a higher FCR (1.721±0.012) compared to T1 (1.697±0.007) and T3 birds (1.701±0.017). Results showed that stocking density was a stressor for birds and indicated FSM benefits to mitigate environmental stress consequences on growth parameters. Additional research is warranted to examine the benefits of FSM on other stressors.

Poster 4.17: Evaluation of the effect of dietary crude protein content and feed form on ammonia emissions of broilers

M. Brink[1], E. Delezie[1], P. Demeyer[2], O. Bagci[2], J. Buyse[3]

[1] Institute for Agricultural and Fisheries Research, Animal Sciences Unit, Scheldeweg 68, 9090 Melle, Belgium, [2] Institute for Agricultural and Fisheries Research, Agricultural Engineering Unit, Burg. Van Gansberghelaan 115, 9820 Merelbeke, Belgium, [3] Laboratory of Livestock Physiology, Department of Biosystems, KU Leuven, Kasteelpark Arenberg 30, 2456 Heverlee, Belgium; marien.van-den-brink@dsm.com

This study will investigate the effect of a reduction in dietary crude protein (CP) content on nitrogen excretion and ammonia emissions. Dietary treatments with a standard and a reduced (approximately 10 and 20% reduction) CP content will be compared. Feed form (mash vs. pellets) influences the development of the gastrointestinal tract as well as feed and water intake (and also the nitrogen and moisture level of the excreta). Therefore, each dietary treatment will be fed in mash and pellet form, resulting in a 3 (CP level) × 2 (feed form) experimental design with six treatments, each with six replications. A total of 2232 birds will be placed in 36 pens. An available technique involving containers constructed as a dynamic flux chamber will be implemented to measure ammonia emissions accurately on litter level around the age of 32 and 40 days. The effectiveness of the treatments investigated will further be evaluated on performance, the presence and severity of foot and hock lesions and meat quality.

To separate the effect of treatment on the nitrogen and moisture level (at excreta level) from the litter applied, a digestibility study will be performed during which excreta samples (without litter) will be collected at slaughter age. The study forms part of a project of which the general aim is to assist the poultry sector towards a socially acceptable low-emission poultry farming system. The goal is to explore techniques to reduce ammonia emissions by implementing efficient feed, management and ventilation strategies. This benefits not only the environment, but also the farmer and animals by optimising the indoor climate. The project will initially focus on the broiler sector.

Poster 4.18: Combination of enzymes and fermentable oligosaccharides improve sustainability of broiler production

G. Gomes[1], R. Ten Doeschate[1], O. Kinsey[1], C. Young[2], C. Kwakernaak[3]

[1] AB Vista, [2] AB Sustain, [3] Schothorst Feed Research; gilson.gomes@abvista.com

The objective was to study the effect of supplementation of an additive combination to a diet with reduced nutrient contents on broiler performance and carbon footprint (CO_2e). Two diets with 3 phases (0-13, 14-24 & 25-34 days of age) were fed to Ross 308 sexed broilers housed in 8 replicate floor pens per sex (24 birds/pen). The wheat-based (49-68%) control diets (PC) were formulated according to AB Vista's matrix recommendations with 500 FTU/kg phytase. The nutrient reduced diets (RED) were lowered by an extra (g/kg) 0.7 avP, 0.8 Ca, 0.2 Na, 0.33 dLys, 0.1 dM+C, 0.17 dThr, 0.17 dVal and 0.42 MJ/kg AME, and were supplemented with 1500 FTU/kg phytase (Quantum Blue), and a combination of 9600 BXU/kg xylanase and fermentable xylo-oligosaccharides (Signis). Feed (pellets) and water were freely available. BWG, FI and FCR were determined from 0-34 d. At day 34, carcass and cuts yield were determined on 4 birds/pen. CO_2e was calculated using an accredited model (AB Sustain, 2017) and expressed as kg of CO_2 per kg carcass. Data was submitted to two-way ANOVA. Liveability was not affected by diet. Compared to males, females had lower FI (11%; $P<0.001$) and BWG (12%, $P<0.001$) but similar FCR. Females tended to have higher breast yield than males ($P=0.06$). Compared to PC, performance and carcass traits were similar with RED ($P>0.05$), and CO_2e was reduced (4%, $P<0.001$). There was a tendency ($P=0.10$) that this CO_2e effect was more pronounced in females. It was concluded that using high doses of phytase, combined with xylanase and fermentable xylo-oligosaccharides, can allow a reduction in dietary nutrients while maintaining broiler performance, reducing CO_2e and potentially lowering production costs.

Poster 4.19: Marathon versus 100 metres – what are the hallmarks of persistency in late lay?

C. O'Shea[1]

[1] University of Nottingham; sbzco@nottingham.ac.uk

Extending the length of the lay cycle in laying hen flocks is of interest to the industry in terms of greater utilisation of resources and profitability. The concept of 500 eggs at 100 weeks of age is now a key target of breeding companies, producers and associated disciplines. However the obstacles to longer time in lay are a general decline and greater variability in bird productivity, health and welfare, and egg quality as flocks progress from the mid to late lay cycle. Applied research in terms of nutrition and management has justifiably focused on addressing the problems which are manifest in late lay including bone status, egg quality and persistency in lay. While the genetic potential of the contemporary laying hybrid is remarkable, myriad factors prior to and post-hatch, during rearing and early lay may have implications for actual field outcomes for both individual animals and more generally for flocks. There is great need and potential to join up various scientific disciplines to produce and support resilient animals capable of optimal performance up to and exceeding 100 weeks. An area where greater knowledge is required is to understand the extent of variation for multiple traits between individual hens and how these change within individuals over time. Under experimental conditions, marked and persistent variation between individuals has been observed where some laying hens capable of producing in excess of 500 eggs have been recorded. Greater profiling of the general attributes of such animals and likewise individual animals with poor persistency will contribute to strategies for improvement and foster readiness for the advances in genetic potential that are underway.

Poster 4.20: Appraisal of heavy metals contamination in controlled environment broiler facilities

R. Yasmeen[1]

[1] Lahore Garrison University; raheelasattar44@gmail.com

The present study was conducted to determine the metal concentration in feed, litter and air in controlled environment poultry farms with differing feed. Air samples along with litter and feed were collected from ten poultry farms, differing in feed, in Punjab, Pakistan grouped into three categories: Group I (Feed A), Group II (Feed B) and Group III (Feed C). These samples were analyzed by Inductively Coupled Plasma Atomic Mass Spectroscopy (ICP-MS) for trace metals such as Silver (Ag), Aluminium (Al), Arsenic (As), Barium (Ba), Beryllium (Be), Cobalt (Co), Lithium (Li), Molybdenum (Mo), Antimony (Sb), Selenium (Se), Tin (Sn), Strontium (Sr), Thallium (TI) and Vandium (V). The results were reported in mg/kg. In general, the concentration of all trace metals found to be highest in feed followed by litter and in air samples. However, the concentration of all metals was lower in outdoor air samples compared to indoor samples except, Aluminium (Al), Cobalt (Co) and Tin (Sn). It was noticed the concentrations of trace metals found in feed were lower as compared to the maximum acceptable limit in the feed apart from Al and Sn. There is need to ensure intervention strategies and management practices in intensive poultry farming facilities are in place to keep trace metal levels at a minimum to reduce their bioaccumulation in food chains.

Poster 4.21: Usage of a mixed enzyme (xylanase, amylase, and protease) in phytase-supplemented diets increased energy digestibility, growth performance and feed efficiency of broilers

A. Bello[1], D. Tang[2], Y. Dersjant-Li[1], W. Li[1], L. Marchal[1]

[1] DuPont Animal Nutrition, DuPont Industrial Biosciences, [2] College of Animal Science And Technology, Gansu Agricultural University, Lanzhou, China; abiodun.bello@dupont.com

The efficacy of a mixed enzyme (xylanase at 2,000 XU, amylase at 200 AU, and protease at 4,000 PU per kg of feed; XAP) was evaluated on 21 d apparent ileal digestibility of energy (AID E) and protein (AID CP) and 1-42 d growth performance and feed efficiency of broilers. Four diets, assigned to 1,000 Cobb 500 male broilers (10 floor pen replications × 25 birds) using complete randomized design, were a nutrient adequate (PC), PC reduced in ME by 100 kcal/kg and in dig AA by 2% (NC) and each supplemented with XAP (PC+XAP and NC+XAP, respectively). Diets were based on corn, SBM, cottonseed meal, rapeseed meal, and DDGs supplemented with phytase (1000 FTU/kg) and fed in crumble (0-10d) and pellet forms (11-21, 22-35, and 35-42d) ad libitum. Data were analyzed with Proc. GLM of JMP 14.1. While there was no diet effect on AID CP, PC+XAP also did not increase AID E (3,069 vs 3,086 kcal/kg), however, NC+XAP increased ($P<0.001$) AID E (2,785 vs 2,873 kcal/kg). Also, PC+XAP did not significantly improved 1-42 d BWG (2,618 vs 2,629 g) and FCR (1.731 vs 1.716). Likewise, NC+XAP also improved ($P<0.001$) 1-42 d BWG (2,453 vs 2,541 g) and FCR (1.871 vs 1.782). The results indicated that supplementation of XAP in diets allow for significant reduction in ME and dig AA of diets fed to broilers to enable increased feed efficiency, nutrient utilization, and reduce feed cost. While XAP offered limited improvement on nutrient digestibility and growth performance when used in nutrient adequate broiler phytase-supplemented diet, the benefits of the mixed enzymes are most profoundly observed in broilers fed phytase-supplemented diet reduced in ME- and dig AA. Hence, XAP usage in commercial diet offers chance to reduce feed cost and improve feed efficiency.

Poster 4.22: Phytogenic feed additive on top of 1,000 FTU/kg phytase increased crude protein digestibility and showed potential to improve feed efficiency of broilers through 42 days of age

A. Bello[1], S. Bai[2], Y. Dersjant-Li[1], W. Li[1], L. Marchal[1]

[1] DuPont Animal Nutrition, DuPont Industrial Biosciences, [2] Institute of Animal Nutrition, Sichuan Agricultural University, Sichuan, China; abiodun.bello@dupont.com

The effects of dietary supplementation of a phytogenic additive (Envivo® EO; minimum of 4.5 % cinnamaldehyde and 13.5 % thymol) was evaluated on ME and crude protein digestibility at 21 d and growth performance, feed efficiency and livability through 42 d in broilers. Diets were corn-SBM based containing cottonseed meal, rapeseed meal, and DDGs and were fed ad libitum. Two dietary treatments were each assigned to 250 male Ross 308 broilers (10 floor pen replications × 25 birds; n=500) in a completely randomized design and fed in 4 dietary phases as crumble (0-10d) and pellet (11-21, 22-35, and 35-42d). Treatments were antibiotics growth promoter-free nutrient adequate basal diet with 1,000 FTU/kg phytase (BD) and BD supplemented with EO at 100 g/ton (BD+EO). There was no difference ($P>0.05$) between ME digestibility of the BD+EO (2,878 kcal/kg) and BD (2,789 kcal/kg), however, crude protein digestibility was increased ($P=0.05$) by the BD+EO (76.9%) compared to BD (73.9%). There was no difference ($P>0.05$) between the 1-42 d BWG of the BD+EO (2,613 g) and BD (2,594 g) birds and between the 1-42 d feed intake of BD+EO (4,264 g) and BD (4,288 g) birds. Similarly, no effect was observed in percentage of mortality and culled birds. However, the 1-42 d mortality corrected-FCR tended to be lowered ($P=0.065$) by the BD+EO (1.632) compared to BD (1.653). The results imply that supplementation of the phytogenic feed additive increases crude protein digestibility and potentially supports better feed utilization in broiler under non-experimentally challenged conditions.

Poster 4.23: Effects of inclusion of Bacillus subtilis (DSM17299) to energy- and protein-reduced diet on growth performance, nutrient digestibility, and meat quality and gas emission in broiler chickens

S. D. Upadhaya[1], R. Koedijk[2], I. H. Kim[1]

[1] Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam 330-714, Korea, [2] Christian Hansen; frcbos@chr-hansen.com

816 day-old Ross 308 broiler chicks were used. Birds were allotted to 1 of 6 dietary treatments (8 replicate pens per treatment / 17 birds per pen) in a 3 × 2 factorial design with 3 levels of metabolizable energy (ME) and crude protein (CP) with constant amino acid (100% ME and CP(1+2), 98.8% ME and (CP2+3), and 97.6% ME and CP (4+5)) supplemented with or without 500 g/ton of GalliPro (treatments 2,4,6). As a result, a significant reduction ($P < 0.05$) in BW and an increment in feed conversion rate (FCR) were observed with the reduction in diet density. During the growing and finishing periods the birds fed probiotics were heavier ($P<0.05$) than those fed diets without. FCR improved ($P< 0.05$) in the probiotic groups, but feed intake (FI) remained unaffected compared to birds without probiotics (P=0.6 (density) and P=0,73 (probiotics) (3642g>FI<3674g total feed intake). Total ME intake 1=11498, 2=11528, 3=11463, 4=11411, 5=11283 and 6=11260 kcal. Total CP intake was 1=766, 2=768, 3=764, 4=760, 5=751 and 6=750 g. The apparent total tract digestibility of dry matter (DM) tended ($P = 0.051$) to be lower in ME- and CP-reduced diets. In addition, the ATTD of nitrogen (N) and energy was significantly lower ($P < 0.05$) in ME and CP-reduced diets than the control diet. A trend in the reduction ($P = 0.059$) of NH3 gas emission from the excreta was observed for birds fed ME- and CP-reduced diets. In addition, birds fed probiotic supplemented diets also showed reduced ($P < 0.05$) NH3 emission compared to those fed diet without probiotic. In conclusion, inclusion of probiotic to ME- and CP-reduced diet improved performance, had comparable effect on meat quality, and reduced ammonia emission from the excreta. No interactive effects of diet types and probiotic were observed.

Session 5: Other Species

Oral presentations

Efficacy and tolerance of supplemented Guanidinoacetic acid (GAA) in diets for male turkey chickens

U. Braun[1], M. Rademacher-Heilshorn[2], J. Hoffmann[3], S. Richert[2], O. Müller[1], K. Männer[4]

[1] AlzChem Trostberg GmbH, [2] Evonik Nutrition & Care GmbH, [3] Agroproduct Qualitätssicherungs-GmbH, [4] Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin; ulrike.braun@alzchem.com

Guanidinoacetic acid (GAA) is the precursor of creatine, which plays an important role in energy metabolism of animals. 192 male BUT6 turkeys were assigned to 4 treatments with 6 replicates each of 8 turkeys per pen. The experiment was conducted to evaluate the efficacy and tolerance of GAA supplemented to diets for male turkeys from day 1 to 42. A corn-soybean meal diet (control, no GAA added) was supplemented with 3 levels of GAA at 0.8, 1.6 and 2.4 g/kg, respectively. Feed intake was increased (p=0.001) when 1.6 and 2.4 g/kg GAA was supplemented to the feed. In comparison to the control diet, GAA supplementation increased (p<0.001) body weight gain and improved feed conversion ratio (p=0.001) up to the highest supplemented level of 2.4 g/kg. Body weight gain increased up to 11.9% (2567 g vs 2873g) and feed conversion ratio improved up to 6% (1.655 vs 1.559) compared with the control group. Blood clinical chemistry and hematology data were within the reference range in healthy turkeys. Gross pathological examination at the end of the study showed no signs of adverse effects. Mortality was overall very low and no negative effect of GAA was noted. GAA supplementation elevated (p<0.001) GAA levels in blood with increasing supplementation from 0.8 to 2.4 g/kg whilst blood creatine was increased (p<0.001) to a similar level at all supplementation levels. Creatine in breast muscle was increased (p<0.001) in a dose dependent manner from 0.8 to 2.4 g/kg GAA supplementation. In summary, GAA supplementation to turkey diets from d 1 to d 42 was efficient in improving body weight gain and feed conversion as well as creatine concentrations in blood and breast muscle. No adverse effects up to a level of 2.4 g/kg to feed were noted.

The effect of different dietary ratios of arginine, methionine and lysine on the immune and antioxidant status of turkeys

J. Jankowski[1], D. Mikulski[1], K. Ognik[2], P. Konieczka[1], M. Krauze[2], Z. Zdunczyk[3]

[1] Department of Poultry Science, University of Warmia and Mazury in Olsztyn, 10-719, Olsztyn, Poland,
[2] Department of Biochemistry and Toxicology, University of Life Sciences, 20-950, Lublin, Poland,
[3] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland; p.konieczka@ifzz.pl

The aim of this study was to determine the effect of different Arg, Met and Lys ratios in diets whose Lys content was consistent with NRC (1994) recommendations on the immune and antioxidant status of turkeys.The experiment, which lasted 16 weeks, was performed on 864 day-old female Hybrid Converter turkeys. The turkeys were randomly assigned to 6 groups, with 8 replicates per group and 18 birds per replicate. Six feeding programs, with 3 dietary Arg levels (90, 100 and 110%) and 2 dietary Met levels (30 and 45%) relative to Lys content, were compared in the study. Different dietary inclusion levels of Arg, relative to Lys content, did not induce significant changes in the levels of TNF-α, IgA, IgY, IL- 2, Casp 8, TAS and GSH or PGx and CAT activity. A decrease in Arg content to 90% of Lys content reduced BWG (10.62 vs. 10.44, P=0.023), increased the concentration of IL-6 (1.311 vs. 1.762, P=0.028) and SOD activity (507 vs. 961.4, P<0.001), and decreased globulin concentration (25.78 vs. 19.24, P<0.001) in the blood of turkeys. An increase in Arg content from 100% to 110% of Lys content did not increase BWG, but stimulated the antioxidant system including an increase in SOD activity (507.6 vs. 837.7, P<0.001) and Cp (0.891 vs. 1.276, P<0.001) and Casp 3 (0.41 vs. 0.57, P<0.001) levels in the blood of turkeys. An increase in Met content from 30% to 45% of Lys content increased body weight gains (P<0.001) and the plasma levels of ALB (P=0.016) and Cp (P<0.001). It can be concluded that decreasing Arg content to 90% of Lys content compromised body weight gains and the immune status of turkeys, whereas increasing Arg content to 110% of Lys content stimulated the antioxidant system of birds. This work was supported by the National Science Centre, Grant No. 2017/27/B/NZ9/01007

Maternal diet deficient in riboflavin induces embryonic death associated with alterations in the hepatic proteome of duck embryos

J. Tang[1], S. Hou[1]

[1] Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China; tangjing198601@163.com

Background: Maternal riboflavin deficiency (RD) induces embryonic death in poultry. We investigated embryonic hepatic proteome changes induced by maternal RD to explain embryonic death.

Methods: A total of 80 45-week-old breeding female ducks were divided into two groups of 40 birds each, and all birds were raised individually for 8 weeks. All the female ducks received either a RD or a riboflavin adequate (control, CON) diet, which supplemented the basal diet with 0 or 10 mg riboflavin /kg of diet respectively. Results: The riboflavin concentrations of maternal plasma and egg yolk, as well as egg hatchability declined markedly in the RD group compared to those in the CON group after 2 weeks, and declined further over time. The hepatic proteome of E13 viable embryos from 8-week fertile eggs showed that 223 proteins were upregulated and 366 proteins were downregulated (> 1.5-fold change) in the RD group compared to those in the CON group. Pathway analysis showed that differentially expressed proteins were mainly enriched in the fatty acid beta-oxidation, electron transport chain (ETC), and tricarboxylic acid (TCA) cycle. Specifically, all the proteins involved in the fatty acid beta-oxidation and ETC, as well as six out of seven proteins involved in the TCA cycle, were diminished in the RD group, indicating that these processes could be impaired by RD. Conclusion: Maternal RD leads to embryonic death of offspring and is associated with impaired energy generation processes, indicated by a number of downregulated proteins involved in the fatty acid beta-oxidation, ETC, and TCA cycle in the hepatic of duck embryos. These findings contribute to our understanding of the mechanisms of liver metabolic disorders due to maternal RD.

Replacement of soybean oil by Hermetia illucens fat in young turkey nutrition

J. Sypniewski[1], B. Kierończyk[2], M. Rawski[2], A. Benzertiha[2], D. Józefiak[2]

[1] PIAST GROUP Research and Development Center, [2] Poznań University of Life Sciences; bartosz.kieronczyk@up.poznan.pl

Hermetia illucens (black soldier fly, BSF) is one of the most examined insect species which is currently considered as a novel poultry feed ingredient, BSF contains a high concentration of crude protein (up to 58%) and fat ~38% (DM). The available literature is mostly focused on the substitution of soybean meal in broilers nutrition, however, there is a lack of data in terms of replacement of commonly used energy sources by insect origin fat. The usage of super-critical CO_2 extracted oil from BSF as a soybean oil total replacement resulted in similar growth performance (BWG, FI, FCR; unpublished data) of broiler chickens. Whereas, there is no information about the application of insect origin fat in young turkey nutrition. Therefore, the aim of the study was to investigate the effect of partial or total soybean oil replacement by BSF fat obtained using super-critical extraction with CO_2 on the growth performance of young turkeys. 216 seven-day-old female B.U.T. 6 young turkeys were randomly allocated to 3 dietary treatments contained 6 replication (12 birds each). The birds were kept in floor pens from day 7 till 35 d of age. The following treatments were applied: SO – 100 % of soybean oil, HI50 – 50% of BSF fat and 50% of SO, and HI100 – 100% of BSF fat. The obtained results of soybean oil replacement by BSF fat confirmed previous experiments carried out on broiler chickens. The partial or total replacement by BSF oil have shown similar growth performance results to the SO treatment in the 7-35d period. Moreover, the addition of BSF fat to the turkey's diet did not affect ($P > 0.05$) selected internal organ weights, as well as the gastrointestinal tract length. In conclusion, the BSF fat may be an alternative source of energy used in young turkey nutrition.

Digestibility of Muscovy duck fed with black soldier fly partially defatted meal

M. Gariglio[1], S. Dabbou[1], L. Gasco[2], I. Biasato[2], J. M. Sanchez[3], S. Martinez[3], F. Gai[4], C. Caimi[2], A. Schiavone[1]

[1] Department of Veterinary Sciences, University of Turin, largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy, [2] Department of Agricultural, Forest and Food Sciences, University of Turin, largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy, [3] Department of Animal Production, University of Murcia, Campus de Espinardo, 30071 Murcia, Spain, [4] Institute of Science of Food Production, National Research Council, largo Paolo Braccini 2, 10095 Grugliasco, Turin, Italy; marta.gariglio@unito.it

The apparent digestibility of Muscovy duck (Cairina moschata domestica) fed a partially defatted Hermetia illucens (HI) larva meal was evaluated. A total of 192 3d old female broiler ducks (average live weight (LW): 71.3±2.9) were randomly housed in 24 pens (6 replicates/treatment, 8 birds/replicate) and reared until 50d. Four isonitrogenous and isoenergetic diets were formulated, with increasing substitution level of corn gluten meal with HI (0, 3, 6 and 9%; HI0, HI3, HI6 and HI9, respectively), divided in 3 feeding phases: starter (3-17d), grower (17-38d) and finisher (38-50d). Titanium dioxide was used as inert marker (0.3%). At the end of each feeding phases, excreta were collected, lyophilized and stored at 4°C. The dry matter (DM) and the nitrogen retention ((nitrogen retention=total excreta nitrogen-excreta uric acid nitrogen)×6.25) was evaluated as well as the apparent total tract digestibility coefficient (ATTDC) for the ether extract (EE). Data were analysed by one-way ANOVA evaluating the effect of dietary HI inclusion level by polynomial contrasts (significance at P<0.05). The DM retention was not affected by dietary treatments throughout the trial, as well as the nitrogen retention in grower and finisher periods. The nitrogen retention decreased linearly in the starter period with a minimum corresponding to HI9 group (P<0.05). The EE ATTDC increased linearly in all the 3 feeding phases, with a maximum corresponding to HI9 group (0.962, 0.977 and 0.983 in starter, grower and finisher periods, respectively, P<0.05). Dietary inclusion of increasing levels of HI larvae partially defatted meal do not have negative effect on apparent digestibility, with any effect on final LW of the birds (average: 2515.7±98.6), resulting a valuable ingredient in duck nutrition.

Poster 5.1: Effects of fermented cassava leaf meal and palm kernel cake mixture on egg characteristics and egg-yolk fat content of ducks

Y. Rizal[1], A. Yuniza[1], T. D. Nova[1], S. Y. Sari[1], A. Amalluffiah[1]

[1] Faculty of Animal Science, Universitas Andalas; yrizal@ansci.unand.ac.id

The purposes of this experiment were to study the effects of fermented cassava leaf meal (CLM) and palm kernel cake (PKC) mixture (80:20%) on the egg characteristics and egg-yolk fat content, and to obtain appropriate level of this fermented CLM and PKC mixture included in the diet of Kamang ducks. One hundred sixty Kamang laying-ducks at the age of 14 months and at 65% production stage were applied in this experiment. This experiment was performed in a completely randomized design with 4 treatments and 4 replicates. The treatments were levels (0, 6, 12 and 18%) of fermented CLM and PKC mixture in the diets. The CLM and PKC mixture was fermented with Bacillus amyloliquefaciens at the dose of 8% (19.2 x 10^{10} cfu/g) for 8 days. Diets were formulated at iso-protein (18%) and iso-energy (2650 kcal/kg). Drinking water and diet were provided ad-libitum. Measured variables were egg characteristics (egg index, egg-shell thickness, egg-shell strength and egg-yolk color index), and egg-yolk fat content. Results of experiment indicated that egg index, egg-shell thickness, egg-shell strength and egg-yolk color index were not affected ($P>0.05$) by different levels of fermented CLM and PKC mixture in the diets. On the other hand, crude fat content was significantly influenced ($P<0.05$) by different levels of fermented CLM and PKC mixture in the diets. The 6% fermented CLM and PKC mixture in the diet increased the crude fat content of egg-yolk of ducks, but when the levels of fermented CLM and PKC mixture were added to 12 and 18% in the diets, the crude fat content declined. In this experiment the fermented CLM and PKC mixture could be included up to 18% in duck's diet without having adverse effect on egg characteristics.

Poster 5.2: Growth performance of Muscovy and Pekin ducks fed a blend of insect and shellfish based-diet

J.-M. Larivière[1]

[1] Institut de technologie agro-alimentaire; jean-marc.lariviere@mapaq.gouv.qc.ca

Use of alternative proteins is expected to increase to support sustainable poultry production and as a response to the increase of poultry egg and meat consumption. The purpose of this trial was to investigate whether including low levels of a blend of insects (mealworms) and shellfish (shrimps and gammarus) in diet influences growing performance of ducks. The experiment used 113 Muscovy (R71L) and 113 Pekin (Star 53 medium) male ducklings (Grimaud & Frères), allocated to two feed treatment groups: an insect and shellfish based supplement added to a formulated pelleted feed at 2% of the diet, and a control diet. A total number of 8 pens with two replicates per feed treatment and 28 ducklings of each strain per pen, were randomly distributed. Ducklings were fed ad libitum with free-access to water and body weight was recorded on a weekly basis from day-old to 9 weeks of age. Significant differences were recorded for body weight in Muscovy and Pekin ducks fed the different diets. Feed conversion ratio was increased in ducks fed the supplemented diet compared with the control diet. Livability did not differ significantly. Substitution of valuable protein by those provided by insects and shellfish may offer benefits in waterfowl production as to offer an interesting alternative protein source in poultry nutrition.

Poster 5.3: Effect of dietary sodium and chloride on growth performance, hematology and serum biochemistry of geese

Y. Chen[1], Z. Wang[1]

[1] Yangzhou University; 402962700@qq.com

A total of 702 1-day-old male Jiangnan white geese and a total of 432 29-day-old male Jiangnan white geese were used in the two experiments, study the effect of altered Na^+ and Cl^- levels on its growth performance, hematology, and serum biochemistry. In the two experiments, geese were randomly allotted to nine treatment in a 3×3 factorial arrangement with three contents of Na^+ (0.10%, 0.15%, 0.20%) and three contents of Cl^- (0.15%, 0.20%, 0.25%) with six pens per treatment. The purpose of this experiment was to explore the response of the geese to Na^+ and Cl^- levels, further clarify the appropriate levels of Na^+ and Cl^- to provide a reference for the scientific preparation of geese feed and the healthy breeding of geese. The current results are as follows: (1)The different levels of Na^+ and Cl^- in the diet significantly affected the body weight(BW), average daily gain (ADG) and feed/gain ratio (F/G) of the 28-day-old geese ($P < 0.05$), the effect of low Na^+ and Cl^- is most significant.(2) $Na^+ \times Cl^-$ has a significant impact on blood hemoglobin (HGB) and hematocrit (HCT) in 70-day-old geese ($P < 0.05$). HGB increased linearly with the increasing level of Na^+, HGB and HCT increased first and then decreased with the increase in Cl^- level. (3) Serum urea of the geese increased linearly with the increase of Na^+ content in 28-day-old geese ($P < 0.05$). Serum creatinine (CR) and uric acid (UA) of the geese increased linearly with the increase of Cl^- content ($P < 0.05$). The experimental results showed that within the scope of this experiment, low levels of Na^+ and Cl^- could have significant adverse effects on the growth of 1-28 day old geese, rather than the 29-70 day old geese. Combine two experiments, it is timely to add 0.20% Cl^- and not less than 0.20% Na^+ for geese.

Poster 5.4: Effect of dietary copper on growth performance, slaughter performance and nutrient content of fecal in growing Goslings from 28 to 70 days of age

Z. Yang[1], Y.-J. Chen[2], Z.-Y. Wang[3], H. Yang[3]

[1] Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, [2] College of Animal Science and Technology, Yangzhou University, [3]College of Animal Science and Technology, Yangzhou University; zhiyang@yzu.edu.cn

A dose-response experiment with four dietary copper concentrations (4.17, 8.17, 12.17 and 16.17 mg/kg) was conducted to estimate the growth performance, slaughter performance, nutrient content of fecal and liver copper concentrations of growing Goslings from 28 to 70 d of age. Two hundred healthy male Yangzhou geese with similar body weight were randomized to four groups with five replicates per treatment and ten geese per replicate. Average daily feed intake, average daily gain and the feed conversion ratio of geese for each pen were measured from 28 to 70 d of age. At 70 d of age, two geese were selected randomly from each pen and slaughtered to evaluate carcass quality. Metabolism experiment was conducted with five male geese from each group (one goose per pen) which body weight was close to the mean weight of the group from 64 to 70 d of age. Significant effects of dietary copper were found on body weight, feed conversion ratio, carcass yield, fecal copper concentrations, and liver copper concentrations. Body weight, feed conversion ratio, and carcass yield showed a significant quadratic response to increasing dietary copper concentration, while fecal copper concentration and liver copper concentration showed a significant linear response. The result showed that dietary Cu addition can improve growth by increasing the use of the feeding stuff and improving carcass yield in growing goslings. Furthermore, taking into consideration, the optimal level of gosling dietary copper was between 8.77 and 11.6 mg/kg from 28 to 70 days of age.

Poster 5.5: The impact of expansion process on nutritional quality of rapeseed cake for turkey nutrition

F. G. Boroojeni[1], K. Kozłowski[2], A. Drażbo[2], D. Boros[3], M. Senz[4], J. Zentek[1]

[1] Institute of Animal Nutrition, Department of Veterinary Medicine, Freie Universität Berlin, Königin-Luise-Str. 49, 14195 Berlin, Germany, [2] Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland, [3] Laboratory of Quality Evaluation of Plant Materials, Institute of Plant Breeding and Acclimatization – National Research Institute, 05-870 Radzikow, Blonie, Poland, [4] Research and Teaching Institute for Brewing in Berlin, Institute of Biotechnology and Water, Department Bioprocess Engineering and Applied Microbiology, Seestrasse 13, 13353 Berlin, Germany; Farshad.Goodarzi@fu-berlin.de

This study aimed to evaluate the impact of expansion on nutritional value of rapeseed cake (RC) for turkey nutrition. Expansion altered crude protein (CP), fat and fiber content of RC from 347.8, 139.3 and 121.8 to 361.2, 126.0 and 122.2 g/kg, respectively. It also slightly increased amino acids (AA) content of RC (for instance, 17.02 vs. 17.57 g/kg for Lys, 15.78 vs. 16.35 g/kg for Arg and 247.5 vs. 261.3 g/kg for total AA). Expanded RC (ERC) had slightly lower glucosinolates compared with RC (18.3 vs 19.4 µmol/g). However, ERC tended to have lower in vitro CP digestibility compared with RC (60.73% vs. 63.21%, P=0.082), which might be due to the protein denaturation caused by expansion leading to a reduction in protein solubility. An in vivo digestibility experiment was conducted using 120 three-week-old Hybrid Converter female turkeys. Birds were randomly assigned to 24 cages and received 3 different diets (8 replicates and 5 birds/replicate) including RC, ERC and a N-free diet, all supplemented with vitamins and minerals. The experiment lasted 7 days and at the end, the ileal content of birds in each cage was collected, pooled and analyzed for CP and gross energy content. Apparent and standardized ileal CP digestibility coefficients (AIDC and SIDC, respectively) as well as nitrogen-corrected apparent metabolizable energy (AME_n) of RC were numerically (P>0.05) increased by expansion (59.1% vs. 58.4%, 71.2% vs. 70.5% and 11.46 MJ vs. 11.32 MJ). Given the impact of expansion on in vitro CP digestibility of RC, it can be speculated that the slight increases in AIDC, SIDC and AME_n of ERC were due to the reduction of anti-nutrients (ANFs). In conclusion, expansion might be able to slightly improve nutritional value of RC for turkey nutrition by reduction in ANFs.

Poster 5.6: Effects of a fat coated betaine on breast meat yield, liver weight and oxidative stress in turkeys

S. Toussaint[1], S. Klein[1], M. Bournazel[1], C. Messant[1]

[1] MIXSCIENCE; solene.toussaint@mixscience.eu

Betaine, the glycine trimethyl derivative, has osmoprotective properties and may improve growth performance and breast meat yield (BMY) in poultry. However, this product can be difficult to manipulate in feed process due to its high hygroscopy. Fat coating allows reducing such caking issues and improves betaine valorization in poultry. Our aim was to evaluate the capacity of a specific fat-coated betaine product (BeTane; slow-release technology) to improve antioxidant capacity and BMY in turkeys.

In our study, 416 day old male B.U.T. Premium turkeys allocated to a randomized complete block design in 32 pens, received one of the following feeds: NC (negative control, no betaine), B1000 (NC+1000 mg/kg non-coated betaine), CB500 (NC+500 mg/kg coated betaine) and CB1000 (NC+1000 mg/kg coated betaine), each replicated 8 times. Liver weight was recorded on 16 turkeys per group at 56 days. 32 birds per group were sacrificed at 105 days and meat yields were measured. Blood was sampled on 2 birds per pen for glutathion peroxydase (GSH-pxp) dosage at the same age. There was no significant effect of betaine addition on growth performance. Turkeys fed with BeTane CB500 and CB1000 diets tended to have a higher BMY than the NC and B1000 groups (+0.35 and +0.49 pts, respectively). Relative liver weight tended to be less important for CB500 and CB1000 than in group NC (-8pts for each), that suggests a minor fatty storage in the liver and a better insulin activity. GSH-pxp rate tented to increase for CB500 compared to NC group (+7.3%), that evokes a better cells protection against free radicals.

Overall, our results suggest that BeTane can improve BMY in turkeys and improve liver activity, with a protective effect against cellular damages induced by potential environmental stress.

Poster 5.7: Dietary use of lysolecitin is a profitable tool in growing turkeys

J. Aka[1], M. di Benedetto[1], A. L. Wealleans[1], H. Glawatz[2]

[1] Kemin Animal Nutrition and Health, [2] Moorgut Kartzfehn; alexandra.wealleans@kemin.com

Lysolecithin has been shown to improve nutrient absorption in many species, but little data is available in turkeys. This trial aimed to assess the effect of lysolecithin (LYSOFORTE® EXTEND, Kemin Animal Nutrition and Health; LEX) on performance in BUT 6 turkey toms fed an energy-reduced basal diet (wheat, corn, SBM, rapeseed meal). For P1/P2 feed phases, LEX at 500 g/t was applied on top of the basal diet. For P3-P6, LEX was applied in reformulation, removing 0.291 MJ ME/kg from the diet. At d145, in both groups similar body weights were achieved (19.76 control vs 19.46 kg LEX; P=0.15). FCR was numerically lower in supplemented toms (2.82 control vs 2.72 lysolecithin; P=0.27), demonstrating maintained performance, although differences were reduced after correction for high levels of mortality and sickness in control toms. Given the nutrient reduction, approximate savings over the course of the entire trial were 2€/t feed, including the cost of LEX. Accounting for differences in feed cost, bird weight/corresponding meat price, and feed efficiency, this equates to a net value of 0.45€/bird reared. In this study, despite reductions in nutrient density, there were no significant performance differences. At the same time, mortality and removal of birds to sick pens were substantially lower in birds fed diets supplemented with LEX. Use of lysolecithin in turkeys was shown to be profitable due to feed cost savings and reductions in FCR over the whole trial.

Poster 5.8: Optimal arginine supply for performance in the starter period of turkeys

D. Siebert[1], D. R. Khan[1], A. Tischler[2], J. Tossenberger[2]

[1] CJ Europe GmbH; [2] Kaposvár University; dr.khan@cj.net

Introduction: In practical poultry diets L-arginine (Arg) can be regarded as 5th limiting amino acid. However, less information is available about the optimal Arg:Lys ratio for turkeys, especially in the critical starter period. Aim of this study was to determine the Arg requirements of turkeys in the starter period through performance parameters. Animals, materials and methods: A total of 624 BUT 6 hybrid female turkeys received in the starter period (day 1 – 21) one of six dietary treatments (calculated values): T1) basal diet without Arg supplementation (8.7 g/kg dArg); T2) Arg content 33 % below the recommendation (11.9 g/kg dArg); T3) Arg content 16.7 % below the recommendations (14.8 g/kg dArg); T4) meeting Arg recommendations (Aviagen Turkeys) (17.7 g/kg dArg); T5) Arg content 16.7 % above the recommendations (20.7 g/kg dArg) and T6) Arg content 33 % above the recommendations (23.5 g/kg dArg). CP level of all diets was 270 g/kg, while lysine (Lys) content was fixed at 17.3 g/kg dLys. Diets consisted of corn and soybean meal and were provided ad libitum. Results and discussion: Supplementation of L-Arg to a deficient basal diet improved the turkey's performance in a dose-response manner. The birds' live weight on the 21th day of the trial was significantly higher (by 14.8%) in T5, compared to birds of treatment T1 (T1: 465 g; T5: 546 g). Moreover, FCR was significantly lower in treatment T5 compared to T1 (T1: 1.41; T5: 1.33). Conclusion: Optimal performance results have been observed in treatment T5 (20.7 g/kg dArg), thus modern turkeys may benefit from a higher dietary Arg content in the starter phase. References: Aviagen Turkeys: Feeding Guidelines for Nicholas and B.U.T. Heavy Lines. NU06 Version 1: 14.

Poster 5.9: Effect of a Bacillus licheniformis probiotic in turkeys

W. van der Veken[1], V. Hautekiet[1]

[1] Huvepharma N.V.; wouter.vanderveken@huvepharma.com

B-Act® is a probiotic feed additive containing viable spores of Bacillus licheniformis (DSM 28710). The goal of the study was to evaluate the effect of the probiotic on gut health and technical performance of turkeys.1300 one-day-old female Hybrid Converter turkeys were divided over 2 treatments (13 replicates/treatment): a control group fed a basal diet and a probiotic group supplemented with 0.5 kg B-Act®/mton of feed ($1.6x10^{12}$ CFU Bacillus licheniformis DSM 28710/mton of feed). The basal diet was wheat/barley/rye/triticale/soybean/rapeseed meal based and fed in a three-phase feeding system (starter, grower, finisher). The trial lasted 84 days. Body weights (BWG) and feed intake were recorded, with feed conversion ratios (FCR) and average daily gain (ADG) calculated accordingly.cFor the whole study period FCR and ADG improved in the B-Act® treatment group, compared to the control: 2.38 vs. 2.33 (FCR) and 94 vs. 95.9 grams (ADG), respectively. As a result the final body weight (BW) was higher in the B-Act® treatment group, with an average final body weight of 8.12 kg compared to the control's 7.95 kg. This was achieved without a significantly increased feed intake, highlighting B-Act®'s positive effect on feed efficiency. Supplementing fattening turkeys with 0.5 kg B-Act®/mton of feed had a positive effect on feed conversion ratio, final body weight and average daily gain, without relying on increased feed intakes to achieve this. As a result the final economic calculation was positively influenced, with feed being used more efficiently.

Poster 5.10: The effect of feeding Diamond V fermentation metabolites on performance and processing yield of male turkeys reared under commercial conditions in Europe

W. Abdelrahman[1], D. Smith[1], H. Pavlidis[1], D. McIntyre[1]

[1] Diamond V; wabdelrahman@diamondv.com

The objective of this study was to determine the effects of feeding the fermentation metabolites of Diamond V Original XPC_{LS} (FM) on performance and processing yield of commercial male turkeys. A total of 568 day-old BUT Big 6 turkeys were raised to 145d in 8 floor pens in a commercial trial facility in Europe. Birds were allocated to 2 feeding treatments with 4 replicate pens per treatment. Birds were fed either a typical commercial turkey control diet (CON) or a diet supplemented with FM at an inclusion rate of 1.25 kg/MT (week 0 to 5) then 0.625 kg/MT (week 5 to 21). Data were analyzed in SAS with feeding treatment as the main effect and significance considered at $P < 0.05$. Overall, feeding FM significantly increased BW at 145d compared to CON, 21.42 kg vs. 20.82 kg, respectively. Average daily gains were also significantly increased when feeding FM compared to CON, 149 g vs. 145 g, respectively. No significant differences were observed for mortality (8.68% vs. 9.47% for FM and CON, respectively) or adjusted FCR (2.65 for each treatment). At 146d, feeding FM significantly increased carcass weight and yield compared to CON (16.62 kg and 76.82% vs. 15.71 kg and 75.20% for FM and CON, respectively). Breast meat weight and yield at 146d was also significantly increased in FM fed birds compared to CON (5.193 kg and 31.25% vs. 4.640 kg and 29.51% for FM and CON, respectively). Footpad lesions were also subjectively scored (0-4 scale) at 146d in the processing plant. Feeding FM resulted in an observed improved (lower) footpad lesion scores compared to CON. Overall these data suggest that the addition of FM to the diets of commercial male turkeys can have positive effects on improving key economically important commercial performance and processing yield traits.

Poster session 6: Other

Poster 6.1: The combined use of spray dried porcine plasma and low Ca strategies in the starter phase could lead to better broiler performance

R. Franco-Rosselló[1], A. I. García-Ruiz[1], A. Pérez-Bonilla[2]

[1] Trouw Nutrition R&D, [2] Trouw Nutrition; r.franco@trouwnutrition.com

During the incubation period, the yolk is the main source of P for the chicken embryo. After hatching (from 0 to 10 days), the remaining P in the yolk is minimal and could compromise bone mineralization, especially when relatively high dietary Ca levels are offered (> 9 g/kg of Ca) due to the interference of Ca in the absorption of P. Recent works suggested that lowering Ca in the first 4 days of age leads to greater voluntary feed intake and growth without impairing bone mineralization. In addition, the use of spray dried porcine plasma (SDPP) could have benefits boosting gut development, immunity and overall growth. The current study aimed to investigate whether enriching the low Ca diets with SDPP could have a synergistic effect on early growth with carry over effects up to market age (37 days). A total of 750 male day-old Ross 308 broilers were allocated to 30 experimental units and randomly assigned to 3 experimental treatments, from placement up to 4th day, consisting in 1) standard Ca diet, 2) low Ca diet and 3) low Ca diet+SDPP; after which all birds received common starter, grower and finisher diets. Chicks fed low Ca+SDPP had greater body weight at 4 days of age compared to chicks fed standard diet (P=0.002) but were similar to low Ca strategy alone. The final body weight was not influenced by adding SDPP but an improvement on FCR in the global period relative to standard diet (P=0.006) was observed. The highest mortality incidence occurred in the standard diet (12%) whereas low Ca+SDPP had the lowest (4.8%). Based on these results, the combined used of low Ca strategies with SDPP could result in better feed efficiency and lower mortality in the global period.

Poster 6.2: Comparison the ability of different types of birds in deriving energy from corn and soybean meal

N. Zahroojian[1], H. Moravej[2], M. Zaghari[2], S. Aminzadeh[3]

[1] PhD student of Poultry Science, Department of Animal Science, University of Tehran, Iran; [2] Professors of Poultry Science, Department of Animal Science, University of Tehran, Iran, [3] Associate Professor of Biochemistry, National institute of genetic Engineering and Biotechnology; mzaghari@ut.ac.ir

This study was conducted to evaluate the ability of different types of birds in deriving energy from corn and soybean meal (SBM). Total of 36 broiler breeder hens (62 weeks old), 72 male broiler chickens (35 days old) and 36 commercial layer hens (40 weeks old) were used. The AMEn and apparent ileal digestible energy (AIDE) of corn and SBM were evaluated. Celite® (10 g/kg) was used as a marker in the three experiments. The intestinal digestive enzymes activity (amylase, lipase and aminopeptidase) and intestinal morphology in the birds were measured and compared. The AMEn value of SBM for broiler breeder hens (10.57 MJ/kg DM) was significantly higher than broiler chickens (9.27 MJ/Kg DM) and commercial layer hens (9.66 MJ/Kg DM; P < 0.05). But corn AMEn for broiler breeder hens (13.08 MJ/Kg DM) was lower than broiler chickens (14.15 MJ/Kg DM) and laying hens (13.83 MJ/Kg DM; P < 0.05). There were not significant difference among the birds for AIDE values of each ingredients (P > 0.05). Similarly, the difference between AMEn and AIDE of both corn and SBM was not significant in each experiment (P > 0.05). The results showed that the villus length, villus surface area and villus length to crypt depth ratio in broiler breeder hens and broiler chickens were higher than commercial layer hens (P < 0.05). Digestive enzymes activity (excluding lipase) were significantly different among birds (P < 0.05). In conclusion the results demonstrated that there are significant difference among broiler breeder hens vs. broiler chickens and layer hens in ability of deriving energy from corn and SBM. Therefore, it seems that using the same energy value for corn or soybean meal for diet formulation in different types of birds cause to energy imbalance diets.

Poster 6.3: Effect of an association of plant extracts on white-striping and wooden breast appearance in chicken breast

K. Bebin[1], *D. Gardan-Salmon*[1], *M. Panheleux-Lebastard*[1]

[1] CCPA GROUP; kbebin@ccpa.com

Chicken production accounts for about 90% of world poultry production. The majority of this meat is consumed as cut or processed products, mainly from standard production. In recent years, production has faced the appearance of degenerative defects in the breast: white-striping, wooden breast, spaghetti meat. These defects have multiple origins: growth, genetic, vascularization of the muscle, oxidative stress and inflammation, environmental conditions. In this study, we measured the effects of an association of plant extracts combined with vitamin E in the feed, on the prevalence of white-striping and wooden breast defects on chicken breast. 216 Ross 308 male chickens were divided into 3 groups : G1 = control feed with 80 ppm of vitamin E from 0 to 42d; G2 = feed with plant extracts (36.8 mg expressed as gallic acid equivalent/Kg of feed) + 200 ppm Vitamin E from 0 to 42d; G3 = same feed as G2 from 0 to 30d only. The growth performance, the breast weight and ultimate pH were the same (NS). The prevalence of white-striping (moderate and severe notes) was significantly reduced by the plant extracts and vitamin E (72.6%, 45.5% and 44.4% respectively for G1, G2, and G3; P < 0.01). Similarly, the prevalence of wooden breast was lower with plant extracts and vitamin E (75.8%, 68.2% and 61.9% presence respectively for G1, G2 and G3, P<0.1; moderate and severe notes). In conclusion, the association of selected plant extracts for having a role on cell protection, coupled with an antioxidant (vitamin E), fed from the early stage of growth improved the quality of chicken breast by decreasing the structural defects such as white-striping and wooden breast.

Poster 6.4: Endogenous antinutritive substances in cereals intended for use in poultry feeds

M. Gzowska[1], *D. Boros*[1], *A. Fraś*[1], *P. Dopierała*[2], *T. Schwarz*[3]

[1] Institute of Plant Breeding and Acclimatization – NRI, [2] KWS Polska Sp. z o.o., [3] UR Kraków;
d.boros@ihar.edu.pl

Four varieties of rye, two hybrid and two population genotypes, and a variety of wheat, triticale and barley, planted at 6 various locations in Poland, were analysed for the content of antinutritive substances. Among rye genotypes was a sample of unknown origin sown by the farmers for many years. All cereals originated from the 2017 harvest. nAs expected, a significant variation was shown in amount of endogenous antinutritional components in the grains of various cereal species, with a highly significant environmental impact. Content of dietary fibre was the highest in hulled barley (20.2%), due to the large amount of insoluble nonstarch polysaccharides and lignin. Rye had 25% less fibre than barley, however, significantly more than wheat and triticale. Rye varieties had similar dietary fibre contents, in the range of 14.8% to 15.1%. However, they were characterized by almost twice higher the content of water-extractable arabinoxylan fraction and four times greater viscosity of grain aqueous extract than other cereals. Rye varieties showed a significantly higher amount of alkylresorcinols and trypsin inhibitor, whereas barley had significantly higher content of phenolic compounds and tannins. Rye of unknown genetic origin in comparison to cultivated varieties differed only in lignin content, which could have been affected by lower kernel weight. Research was financed by the NCRD, within the project "ENERGYFEED".

Poster 6.5: Effect of diet protein level on carcass value and meat quality in fast-, medium- and slow-growing chickens

D. Chodová[1], E. Tůmová[1], V. Machander[2]

[1] Department of Animal Husbandry, Czech University of Life Sciences Prague, 165 00 Prague, Czech Republic, [2] International Testing Station Ústrašice, 390 02 Tábor, Czech Republic; chodova@af.czu.cz

The aim of this study was to evaluate the effect of two protein diet levels on carcass value and physical meat characteristics in fast- (Ross 308), medium- (JA757) and slow-growing (ISA Dual) chickens. The chickens were fed using diets containing 2 different crude protein levels. Experimental group had 6% lower crude protein compared to control group. The housing and microclimate conditions were the same for all groups. For carcass value and meat quality, 10 chickens per each group were slaughtered at live weight 2 kg. The samples of Pectoralis major (PM) for meat quality analysis (pH, colour, drip loss) were taken 24 hours post mortem. The Ross 308 reached 2 kg live weight at 35 days, JA757 at 42 days and ISA Dual at 70 days of age. There were not detected interactions of genotype and diet on any of evaluated carcass characteristics. JA757 and ISA Dual chickens had lower (-2.0%, -9.8%, respectively) dressing out percentage than Ross. ISA Dual chickens had higher thigh ($P<0.001$) and thigh meat percentage ($P<0.002$) but lower breast percentage ($P<0.001$) than JA757 and Ross. From meat characteristics, pH measured in PM was affected ($P<0.001$) by genotype with the lowest value in ISA Dual. Colour of PM was lighter ($P<0.001$) and less intensive ($P<0.001$) in ISA Dual compared to other genotypes. The chickens fed by low protein diet had darker meat colour ($P<0.001$) and more intensive colour parameters ($P<0.001$) than control group. The low protein diet increased drip loss ($P=0.005$) and meat tenderness values than in control group. In conclusion, low protein diet increased meat colour characteristics but had negative effect on drip loss and meat tenderness. The ISA Dual had worse meat quality parameters than Ross or JA757.

Poster 6.6: Analysis of eggshell mineral composition for tracing the origin of table eggs

T. H. Dao[1], R. A. Swick[1], V. T. Nguyen[2], P. W. Hunt[3], B. Hine[3], I. Ruhnke[1]

[1] School of Environmental and Rural Science, Faculty of Science, Agriculture, Business and Law, University of New England, New South Wales, Australia, [2] Faculty of Animal Science and Veterinary Medicine, Tay Nguyen University, 567 Le Duan, Buon Ma Thuot, Dak Lak, Vietnam, [3] CSIRO Agriculture & Food, F.D. McMaster Laboratory, Armidale, New South Wales, Australia; tdao@myune.edu.au

In Australia and many other countries, free-range eggs can be sold at significantly higher prices than cage eggs. The mislabeling of cage eggs as free-range eggs and vice versa has been documented, and has a significant impact on consumer confidence and therefore egg consumption. The development of methods to distinguish eggs produced from different production systems is necessary to satisfy consumer demand. The objective of this study was to determine if eggshell mineral composition could be used as a way to differentiate eggs originating from each production system. Our hypothesis was that hens having access to soil would have higher levels of trace minerals in their egg shells than would caged hens. Egg samples were randomly collected from six commercial caged and six commercial free-range flocks in Australia. Twelve eggshell samples from each flock were analysed for mineral composition (calcium, phosphorus, magnesium, sodium, aluminium, boron, copper, manganese, iron, potassium, sulfur and zinc) using inductively coupled plasma optical emission spectrometry (ICP-OES). The results showed that eggshells from free-range hens contained significantly higher macro-minerals (P, Mg and Na) but lower micro-minerals (Cu, Fe, K, S and Mn) than eggshells from caged hens ($P < 0.05$). Mean differences in mineral content of eggshells were observed between the two production systems with 1025 μg Na /g eggshell for free range versus 917 μg/g for cage and 3.38 μg Fe /g eggshell for free range versus 4.64 μg/g for cage. However, variable levels of eggshell minerals (including Na and Fe) were noted within and between production systems. Thus, it is concluded that analysis of eggshell mineral composition is not effective to differentiate the origin of eggs.

Poster 6.7: Breast meat quality of chickens reared on pasture affected by fat source

M. Englmaierová[1], M. Skřivan[1], J. Vlčková[1]

[1] Institute of Animal Science; englmaierova.michaela@vuzv.cz

Pasture herbage is a rich source of PUFAs with n-6/n-3 ratio 0.3. Thus the meat in the quality of a functional food can be obtained by fattening of chickens on pasture. But this fact is affected by the fat source in the diet. Therefore, the aim of the study was to evaluate the effect of the fat source (rapeseed oil, palm kernel oil and a combination of both) in diet on the cholesterol content, FA composition and oxidative stability of meat in pastured slow-growing cockerels Hubbard JA757 (n=90). The chickens were housed in the mobile boxes from 28 to 56 days of age. Pasture intake at 50th day of fattening ranged from 5.24 to 5.39 g of dry matter. The cholesterol content in the blood, liver and breast muscle was not influenced by the source of fat in the diet. The highest ($P<0.001$) ratio of n-6/n-3 PUFAs had the meat of cockerels fed palm kernel oil (7.2), while no difference was found between rapeseed oil (5.0) and combination of both fats (5.0) in diet. A similar trend was observed in atherogenic ($P<0.001$) and thrombogenic index ($P<0.001$). The higher ($P<0.001$) values of the ratio between hypocholesterolemic and hypercholesterolemic FAs were achieved in meat of cockerels fed rapeseed oil (3.22) and a mixture of fats (3.33) in diet compared to palm kernel oil (2.50). The rapeseed oil decreased (0.378 mg/kg of MDA) oxidative stability of breast muscle tissue stored 5 days at 4 °C. The highest oxidative stability of fats in meat was recorded in the treatment with a mixture of both fats in diet (0.327 mg/kg MDA). In conclusion, the addition of oil rich in PUFAs into feed mixture for chickens with higher content of SFAs in combination with the possibility of grazing can wipe the negative effect of SFAs source in diet on meat quality evaluated in terms of human health. Research was supported by the Ministry of Agriculture of the Czech Republic (Project No. QK1910387).

Poster 6.8: High level of Fumonisin B1 detected in corn samples received from Serbia during August to November 2018

J. Raj[1], H. Farkaš[1], R. Čepela[1], I. Pol[1], J. Bošnjak-Neumüller[1], M. Vasiljević[1]

[1] PATENT CO, DOO., Vlade Ćetkovića 1A, 24 211, Mišićevo, Serbia; jog.raj@patent-co.com

Mycotoxins are the biggest challenge for animal feed producers and therefore, regular monitoring of mycotoxins is necessary. Fumonisin B1 (FB1), is a common contaminant of maize-based poultry feeds. FB1 entails the risk of mycotoxicosis and other major diseases in farm animals such as poultry (nephrotoxic and immunosuppressing effects), pigs and cattle. The aim of the present study was to screen corn samples for mycotoxins, received from different regions of Serbia between August to November 2018. The samples were analysed by LC-MS/MS based multi-mycotoxin method for quantitation of all mycotoxins (Aflatoxin B1, B2, G1 and G2, Ochratoxin A, Zearalenone, Deoxynivalenol, FB1&B2, T-2 & HT-2 toxins) regulated in EU in feed by EU Directive 2002/32/EC, 2006/576/EC and 2013/165/EU.A total of 41 samples were received for analysis and out of these, 76% samples were found contaminated with one or other mycotoxins. Fumonisin B1 (FB1), Fumonisin B2 (FB2) and T-2/HT-2, OTA & DON and ZON were detected in 76%, 41%, 12 %, 22%, and 7% corn samples, respectively. FB1 ranging from 42 to 19128 ppb, FB2 from 43 to 3892 ppb, T-2/HT-2 from 13 ppb to 148 ppb, DON from 84 to 1000 ppb, OTA ranging from 7 to 148 ppb and Zearalenone ranging from 33 to 47 ppb, were detected in corn samples. Therefore, this survey concludes that the corn harvested in Serbia in 2018 has high levels of Fumonisin B1.

Poster 6.9: Effect of dietary soybean lecithin on broiler chickens performance, ileal fatty acid digestibility and adipose saturation degree

A. Viñado[1], L. Castillejos[1], A. C. Barroeta[1]

[1] Animal Nutrition and Welfare Service, Department of Animal and Food Science, Facultat de Veterinària, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra 080193, Spain; Alberto.Vinado@uab.cat

A total of 1440 newly hatched female Ross-308 were distributed in an environmentally controlled farm with 60 pens (24 chickens per pen) with the aim to evaluate soybean lecithin (L) as dietary energy source and its effects on performance, ileal fatty acid (FA) digestibility and the saturation degree of the abdominal fat pad (AFP). Animals were fed with five experimental treatments (12 replicates) during grower (from 15 to 28 d) and finisher (from 29 to 46 d) phases. The control diets were supplemented with soybean oil (S) at 2.00% (T1) and different levels of L were included replacing S in grower (T2: 0.25%; T3: 0.50%; T4: 0.75%; T5: 1.00%) and finisher (T2: 0.50%; T3: 1.00%; T4: 1.50%; T5: 2.00%) diets. Titanium dioxide was added to finisher diets at 0.5%. At d 46, AFP and ileal digesta samples were collected and pooled per pen. No negative effects were associated to L inclusion on performance parameters in any phase or during the overall trial ($P > 0.05$). Furthermore, total FA digestibility resulted unaffected by L inclusion ($P > 0.05$), however, a reduction on polyunsaturated FA (PUFA) digestibility was observed for T3, T4 and T5 ($P = 0.017$). Higher levels of L inclusion (T4 and T5) reduced PUFA concentration of the AFP ($P = 0.002$), and thus, its unsaturated-to-saturated FA ratio ($P = 0.005$). The present experiment demonstrates that soybean lecithin represents an alternative for soybean oil in grower and finisher broiler diets without impairing performance and total FA digestibility. However, an inclusion of soybean lecithin equal or higher than a 1% reduced PUFA digestibility and modified carcass saturation degree.

Poster 6.10: The effect of soybean oil replacement by Hermetia illucens oil on broilers growth performance and nutrients digestibility

B. Kierończyk[1], M. Rawski[2], Z. Mikołajczak[1], M. Noak[1], A. Barc[1], D. Józefiak[1]

[1] Department of Animal Nutrition, Poznań University of Life Sciences, [2] Division of Inland Fisheries and Aquaculture, Institute of Zoology, Poznań University of Life Sciences; bartosz.kieronczyk@up.poznan.pl

The aim of the study was to investigate the effect of partial or full replacement of soybean oil by Hermetia illucens oil (HI) obtained via super-critical CO_2 extraction on the growth performance and nutrients digestibility of broilers. In an experiment lasting 35 d, 1 day-old male broilers (Ross 308) were used. The birds were assigned to 5 groups per 10 replicates (16 birds per rep). Food and water were supplied ad libitum. The following design of the experiment was used, SO - control treatment with 100% soybean oil; HI25, 50, 75, 100 - experimental treatments with partial, i.e., 25, 50, 75 or full 100 replacement of soybean oil by HI oil. TiO_2 (0.3%) as an internal marker was used. The growth performance parameters, i.e., BWG, FI, and FCR were measured at 14, 21, 28, and 35 d, as well as coefficients of apparent ileal digestibility of nutrients, were calculated at 28 and 35 d of age. In general, there were no statistically significant differences between control SO and experimental groups in terms of BWG, FI, as well as FCR. Only in the first two weeks (1-14 d) HI oil decreased FI ($P<0.001$), and FCR ($P=0.031$) values in comparison to the SO control group. However, in the case of FCR, the most efficient were groups with 75% or 100% of HI oil in the broiler diets. The usage of HI oil as a replacement for soybean oil negatively affected the crude protein ($P<0.001$) and AME_N ($P<0.001$) ileal digestibility at 28 d of birds age. However, the results of digestibility calculation at 35 d were not affected by HI oil, in terms of crude protein ($P=0.854$), ether extract ($P=0.203$), as well as AME_N ($P=0.810$). Present data suggest that soybean oil may be replaced by H. illucens without negative effects on the growth performance during 35 d rearing. This study was performed in the frame of the IN OIL project: An innovative method for bio-conversion of by-products from food processing industry that was financed by the National Centre for Research and Development within the Lider VII Programme No. 0148/L-7/2015.

Poster 6.11: Effect of short-term fasting on the circulating spexin level and expression in broiler peripheral tissues

P. A. Kolodziejski[1], E. Pruszyńska-Oszmałek[1], M. Sassek[1], M. Kubiś[2], H. Marcin[3], K. W. Nowak[1], D. Fiksa[1], J. Bien[1], B. Szczepaniak[1], P. Mackowiak[1], S. A. Kaczmarek[2]

[1] Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, Poznan, Poland, [2] Department of Animal Nutrition, Poznan University of Life Sciences, Poznań, Poland, [3] Department of Animal Breeding and Product Quality Assessment, Poznań University of Life Sciences, Poland; pawelbigi@o2.pl

Spexin (SPX) is a 14 aa peptide discovered in 2007 using bioinformatics methods. Previously, it was shown that SPX is involved in regulation of energy metabolism, food intake, insulin secretion, puberty and reproduction in fish and mammals. Despite the high similarities in the structure of this protein in many animal species, knowledge about the role of SXP in birds is very limited. Our previous research has shown that SPX and SPX receptors (GALR2 and GALR3) are expressed in many different chicken tissues (Kolodziejski et al. 2018). Therefore, we decided to investigate the effect of short time fasting (2, 4, and 8 h) on SPX concentration in blood serum and expression of SPX gene and its receptors in liver, muscle and fat tissue. Moreover, we also correlated SPX concentration and other determinants of metabolic status in chickens (like: triglycerides, insulin, glucose, cholesterol etc). The experiment was conducted using blood and tissues from ROSS 308 male broiler chickens (n-8 per group). Total RNA was isolated from tissues using Tripure Isolation Reagent according to the manufacturer's instructions and cDNA was generated from 1 µg total RNA. Real-time PCR was performed using gene-specific primers. We found that level of blood serum SPX increases during fasting ($p<0.05$). Moreover, our research has shown that SPX correlates with other metabolic markers like e.g. concentration of triglycerides and glucose ($p<0.05$; $p<0.01$ respectively). We also noted statistically significant changes in mRNA level of SPX gene and its receptors in liver, muscle and fat tissue ($P<0.01$). Obtained results suggest that SPX might be involved in the regulation of metabolic processes in chickens. The research was supported by NCN grants no: 2015/19/D/NZ9/03580 and 2015/19/N/NZ4/00572.

Poster 6.12: Analysis of more than 380 mycotoxins in feed and raw material samples in 2018

A. Marchioro[1], U. Hofstetter[1], I. Taschl[1], A. Mueller[1], M. Sulyok[2]

[1] BIOMIN Holding GmbH, [2] University of Natural Resources and Life Sciences Vienna; alexandro.marchioro@biomin.net

More than 89.000 feed and raw material samples were analyzed within the Biomin Mycotoxin Survey since 2004. The aim of this study was to obtain information on the occurrence and contamination level of multiple mycotoxins in feed and feed raw material from samples sourced worldwide in 2018. In total 649 samples were collected and screened for the presence of multiple mycotoxins and other secondary metabolites. The samples were analyzed by using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) screening for more than 380 mycotoxins and other secondary metabolites. For practical relevance a cut-off level for all mycotoxins was established at >1 ppb (except aflatoxin at > 0.5 ppb). On average, 34 different metabolites were detected per sample. Only 2% of all analyzed samples contained less than 10 mycotoxins. 88% of samples tested positive for moniliformin, 85% of samples tested positive for aurofusarin, 82% of samples tested positive for culmorin, 75% of samples tested positive for deoxynivalenol, 72% of samples tested positive for Enniatin B_1, 69% of samples tested positive for beauvericin, 59% of samples tested positive for enniatin A_1 (average of positives 105 µg/kg, 719 µg/kg, 678 µg/kg, 783 µg/kg, 71 µg/kg, 21 µg/kg and µg/kg; maximum found 1.696 µg/kg, 25.872 µg/kg, 19.792 µg/kg, 22.984 µg/kg, 1.846 µg/kg, 343 µg/kg, and 549 µg/kg respectively). The sensitivity of mycotoxin analysis and especially of LC-MS/MS increased by 200-fold in the last 10 years leading to the fact that more mycotoxins are found. Performing multi-mycotoxin analysis definitely elucidates the occurrence of mycotoxins but more research is needed to evaluate the practical impact of most of these "new" mycotoxins on animals and humans.

Poster 6.13: Multi-level assessment of metabolizable energy of feedstuffs: influence of cereal grain type and length of adaptation to experimental diets

O. Olukosi[1]

[1] University of Georgia

The aim of the current experiment is to investigate whether the substitution level of a test cereal grain in a test diet impacts the measured metabolizable energy (AME) of the cereal grain and if this effect is dependent on length of adaptation of birds to the test diet. In this experiment, 252 broilers were used to study the influence of length of adaptation to experimental diets based on 2 cereal grains, fed for different periods, and assessed at multiple substitution levels. At 14 days old, the birds were allocated to 14 treatments with 6 replicates each. The diets were fed for either 7 or 4 days of adaptation and excreta were collected at 21 days of age. Within each adaptation period, 7 diets comprising one basal and 6 test diets in which each of the two test cereal grains (corn or barley) substituted the basal diet at rates of 150, 300 or 450 g/kg were fed. The AME of the cereal grains was calculated using the difference method and data were analyzed as 2×3×2 factorial for the influence of adaptation length (2), substitution level (3) and cereal grain type (2). There was a trend for cereal grain × adaptation length interaction ($P = 0.09$). The AME values for corn were 15.07 vs. 14.98 MJ/kg compared with barley 9.68 vs. 8.01 MJ/kg determined after 7 vs. 4 days of adaptation, respectively. Determined AME was lower ($P < 0.01$) for barley than corn. In addition, AME determined after 4 days of adaptation tended ($P = 0.06$) to be lower than the values determined after 7 days of adaptation. It was concluded that the influence adaptation length on AME of cereal grains with different fibers is primarily influenced by cereal grain type not the substitution level. Consequently, there is need to consider these factors when comparing different assays for AME of feedstuffs.

Poster 6.14: Impact of different prestarter feeding strategies on zootechnical performance and meat quality of ROSS 308 broilers

M. Panheleux-Lebastard[1], *K. Bebin*[1], *N. Hubert*[1], *M. Mireaux*[1]

[1] CCPA group; mpanheleux@groupe-ccpa.com

Early feeding can influence the growth of broilers and performance at slaughter. The objective of this trial (4 groups of 8 repetitions of 39 broilers) was to compare 3 different strategies of prestarter feeding to a control group (G1) on the growth of broilers and white stripping scoring at slaughter. Three different parameters were modified: raw materials sourcing, duration of the distribution and nutrient concentration. The broilers from the second group (G2) received a prestarter feed concentrated in crude protein and amino-acids (+14% compared to G1) and manufactured with conventional raw materials during 7 days. A third group (G3) received, during 7 days, a feed with the same nutritional levels than G2 but produced with "more digestible" feedstuffs. In the 4th group (G4), the birds were fed with a more concentrated prestarter feed (+20% of crude protein and amino-acids, +3.5% of metabolizable energy compared to the control) during a shorter period (4 days). The broilers from the 4 groups were weighted at 4, 7, 10, 21 and 35 days of age. The feed intake was measured at the same ages. Feed conversion ratio and average daily gain were calculated at each stage. The early nutrition impacted the weight of the birds which were significantly different between groups from 4 days of age until 35 days. The different strategies did not impact the performance in the same way. The G3 group (prestarter strategy with "more digestible raw materials") got the highest growth. The weights at 35 days were respectively: 2070 g (G1), 2243g (G2), 2257g (G3) and 2174g (G4). The prevalence of white stripping was statistically influenced by prestarter strategies: 12.5% (G1), 40.6% (G2), 18.7% (G3) and 28.1 % (G4) indicating that early nutrition strategies can also influence meat quality.

Poster 6.15: Efficacy of disinfectants and plant extracts for the control of poultry red mite (Dernayssus gallinae)

K. Park[1], S. Kim[1], H. Kang[1], H. Kim[1], J. Jeon[1], J. Son[1], E. Hong[1], C. Kim[1]

[1] Poultry research institute/National institute of Animal science/Rural Development administration; wwf4ever@korea.kr

Poultry red mite, D. gallnae, is the major external parasite that directly affect chickens through blood-sucking, causing serious damage worldwide. Currently, not only in South Korea but also around the world, measures against D. gallnae are inadequate, and food safety such as eggs is threatening by using inappropriate acaricides. Although some disinfectants and plant extracts are known to be effective for the control of D. gallnae, there are urgent need for scientific verification and development of technology that can be utilized in poultry farms. Therefore, we have evaluated the effectiveness against D. gallnae on these disinfectants and plant extracts. Disinfectants which are widely used for the purpose of inhibiting harmful bacteria and viruses in the environment of the poultry house, and plant extracts which are known to have a acaricidal effect on the D. gallnae were selected by reviewing articles, and farm survey. D. gallnae were collected from six layer farms in South Korea, and used in experiments within 10 days of harvesting. the acaricidal effect was examined by using and filter paper based bioassay. A total of 30 disinfectants and plant extracts were evaluated at various concentrations. Among them, 9 substances with acaricidal effect against D. gallnae were selected. The selected substances were peracetic acid disinfectants, calcium oxide, calcium hydroxide, ethanol, ethanol gel, clove extracts, mixture of clove, cinnamon and saponin extracts, and salt solution. These substances showed an average of more than 80% acaricidal activity against D. gallnae. In addition, the acaricidal effect was measured by mixing calcium hydroxide and alcohol. As a result, 100% acaricidal activity was observed in a mixture of 10% aqueous calcium hydroxide and 30% ethanol.

Poster 6.16: Effect of limestone particle size and phytase source on phosphorus digestibility in broilers

S. Taylor[1], M. Sinclair-Black[1], C. Jansen van Rensburg[2], A. M. Debicki-Garnier[3], P. W. Plumstead[1]

[1] Chemunique PTY LTD, [2] University of Pretoria, [3] DuPont Animal Nutrition; stuart@chemunique.co.za

A study determined if limestone (LM) with different particle sizes (PS) and in-vitro solubility characteristics can alter apparent ileal digestibility (AID) of P and phytase (Phy) efficacy in broilers. Three LM (A,B,C) were sourced in Germany with respective PS, calculated as Geometric Mean Diameter (GMD), of 123.2 um, 174.1 um and 733.4 um. The respective LM solubility at 5 min using the method of Kim et al., (2019) was 95.9%, 63.3%, 26.3%. The experimental design was a 3 x 3 factorial arrangement of treatments with three LM and 3 Phy treatments of 0 FTU/kg or 1000 FTU/kg from either Buttiauxella spp. or E.coli phytase. A basal diet composed of corn, soybean meal, sunflower meal, rapeseed meal and chromic oxide marker with no added LM, or inorganic P was mixed, subdivided into 9 batches and LM added to achieve 0.8% Ca in the final 9 test diets. Each treatment was replicated 8 times, in battery cages with a cage of 6 Ross 308 male broilers as the experimental unit. Diets were fed for 34h after which broilers were euthanized and distal ileal digesta removed and lyophilized. Data were analysed as a two-way ANOVA using a mixed model in SAS (SAS, 9.4) at a significance level of $P<0.05$. There were significant main effects of LM and Phy and interactions of LM and Phy on apparent Ileal digestibility (AID) of P. AID P increased from 46.14%, 50.69%, and 59%.79% as LM particle size increased and initial LS solubility at 5min decreased. Diets containing Phy from Buttiauxella spp. had greater AID P vs. E.coli phytase, with greater differences when diets contained fine PS LM. The greatest AID P was achieved in diets containing LM C with the highest PS and lowest solubility at 5min. Results indicate that LM particle size can alter P digestibility and phytase efficacy in broilers.

Poster 6.17: Effect of different light intensities on growth performance, serum biochemistry and behavior of broiler chickens

C. Ik Lim[1], M. Rana[1], K. Seon Ryu[1]

[1] Chonbuk National University; dlacjsdlr@naver.com

This study was conducted to compare the effect of different light intensities on growth performance, serum biochemical variables and behavior of broilers. Nine hundred and seventy two newly hatched Cobb 500 broiler chicks were randomly assigned to six light intensity treatment with six replicate in 36 floor pens. In a completely randomized design, the following treatments 5, 10, 20 and 30 lux were fixed among of six in whole study period and other two light intensities treatment were 30, 20, 10 and 20, 10, 5 lux in that case, it was gradually altered after ending of each period. Birds were fed a common pre-starter, starter and grower diet from 0 to 11d, 12 to 25 d and 26 to 42 day of age, respectively. Body weight and feed intake were measured at the end of each period, FCR was calculated and serum biochemical characteristics were determined after termination of study at 42 days of age. Behavior of broilers under different light intensity was recorded during every phase for a 24 hours period and two pens each treatment was considered. The results showed that body weight, weight gain, feed intake and FCR were unaffected by light intensity in the duration of each phase and whole period while biochemical concentrations of serum did not alter. When the behavioral expressions were assessed and evaluated during different age period, birds spent time in an inactive behavior (sitting, standing) more and less time in an active behavior (pecking, walking) under low intensity group of 5 and 10 lux. However, the results obtained in the current study suggested that growth performance was statistically similar among different light intensities but birds exhibited less calm and relaxed under intensity of 20 and 30 lux as those of other groups.

Poster 6.18: Effect of phytase supplementation on production performance, egg quality and serum biochemical activities of Hy-Line brown laying hens fed different level of phosphorus

M. Rana[1], C. Ik Lim[2], K. Seon Ryu[2]

[1] Chonbuk National University, [2] Chonbuk National University; mdmasudrana2002@gmail.com

A 12-week feeding trial was conducted to examine the effects of phytase supplementation on performance and egg quality of laying hens fed diets containing different levels of available phosphorus from 60 to 72 weeks of age. Five hundred and forty Hy-line brown laying hens were randomly divided into six dietary treatments. Each treatment was replicated six times with fifteen hens per replication in a completely randomized design. Three isonitrogenous and isocaloric corn-soya based diets were formulated to contain 2.0, 2.5 and 3.0g AP/kg with constant level of 45g Ca/kg and supplementation of phytase at 30ppm per kg diet. Parameters were evaluated including egg production, egg weight, daily eggmass, feed intake, feed conversion and egg quality in terms of egg shell thickness, albumen height, haugh unit, yolk color and serum quality. Results showed that egg production till 72 weeks of age was affected by different dietary treatments. There was no statistical difference seen in egg production rate for AP concentration diets 2.0 and 2.5g/kg or 2.5 and 3.0g AP/kg diet as well the variation of AP with phytase groups. However, among the treatments numerically highest egg production was observed in 2.5g AP/kg with phytase and it also shown that phytase supplementation to 2.5g AP/kg diet increased egg production by 1.14% than without phytase group. Egg weight, daily eggmass, feed intake, FCR and egg quality characteristics were not influenced by dietary variation of AP and phytase enzyme as well serum biochemical concentrations but only glucose level tended to be reduced in phytase addition groups. Thus, supplementation of phytase in aged hens diet may be improved phosphorus utilization resulting that hens consuming 2.5g AP/kg plus phytase performed better egg production.

Poster 6.19: Effects of drying at low temperatures on inositol phosphates concentrations in excreta of caecectomised laying hens and broilers

W. Siegert[1], P. Hofmann[1], M. Rodehutscord[1]

[1] University of Hohenheim; w.siegert@uni-hohenheim.de

The chemical composition of excreta can change after voiding due to microbial activity or exposition to the environment. The extent of change in chemical composition after excreta drying at low temperatures and the impact on research projects is largely unknown. The aim of this study was to investigate the effect of low temperature drying on inositol phosphate (InsP) concentrations in excreta of caecectomised laying hens and broilers.Excreta were frozen immediately after voiding, or dried at one of two temperatures: 19 or 29°C and 23 or 33°C in a caecectomised laying hen experiment (E1) and broiler experiment (E2), respectively. Excreta were collected from 7 and 8 replicate cages, each housing 2 and 3 birds, for 72 and 96 continuous hours in E1 and E2, respectively. Immediately after voiding, droppings were mixed and assigned to the treatments. After drying for 6h in a temperature-controlled oven, excreta were immediately frozen. Dry matter content of the excreta increased from 19 to 40% and from 16 to 47% in E1 and E2, respectively. Phosphorus concentrations in excreta dry matter were unaffected by treatment ($P \geq 0.29$) indicating no loss of organic matter during drying. Concentrations of $InsP_6$ and lower InsP isomers were not significantly affected by drying in E1 ($P \geq 0.15$). In E2, no differences were determined for $InsP_6$ and most measured lower InsP isomers ($P \geq 0.06$) except for $Ins(1,2,3,4)P_4$. The $Ins(1,2,3,4)P_4$ concentration was 0.6 µmol/g dry matter when excreta were immediately frozen or dried at 29°C and to 0.7 µmol/g dry matter ($P = 0.03$) when dried at 19°C. These results give no evidence that drying at low temperatures affects InsP isomer concentrations in excreta to a relevant extent for nutritional studies.

Poster 6.20: The effect of hatching eggs storage on broilers performance

M. Skoupa[1], M. Foltyn[1], M. Lichovnikova[1]

[1] Mendel university in Brno; xskoupa2@mendelu.cz

The aim of the study was to evaluate the effect of hatching egg storage on broilers performance. The age of Ross 308 parent stock was 36 weeks when eggs were stored for 18 days, at 14 °C, humidity 60%, turned each hour (stored eggs, S). Two weeks later second batch of hatching eggs was stored for 4 days (fresh eggs, F), the eggs came from the same parent stock 38 weeks of age. Both batches were incubated under the same conditions at the same time. Hatchability of the fertile stored eggs was 90.1% and of the fertile fresh eggs significantly higher 94.1% ($P<0.05$). Two hundred seventy chickens from each batch were used for the experiment. Live body weight of one day old chickens was almost the same in both groups; 41.0g F, 40.8g S. The uniformity was 78.1% in F and 88.4% in S ($P<0.05$). Chickens were housed into 12 boxes (2x6) with litter material, water and feed intakes were at libitum. Temperature and light regime were regulated according to the Ross 308 recommendation and EU legislation. At eight day of age 15 chickens from each group were killed to measure trypsin activity and to measure villi height. There was no significant difference in trypsin activity anyway at 10, 17, 24, 31 and 35 days of age chickens F were significantly heavier ($P<0.05$) than S chickens. The live body weight was 2.08kg in S and 2.13kg in F chickens at 35 days of age. There was no significant difference in FCR (0-35) 1.44 F and 1.47 S. There was no significant difference between the groups in FCR at 10, 17, 24 and 31 days of age too. At the end of the experiment uniformity in F was 57.1% and 58.1% in S ($P>0.05$). Hatching egg storage for 18 days at parent stock age 36 weeks significantly affected growth of broilers.

Poster 6.21: Influence of freezing method on determined phytate concentration in gizzard and ileum samples of broiler chickens

S. Künzel[1], V. Sommerfeld[1], M. Schollenberger[1], I. Kühn[2], M. Rodehutscord[1]

[1] University of Hohenheim, Institute of Animal Science, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany, [2] AB Vista, Feldbergstr. 78, 64293 Darmstadt, Germany; susanne.kuenzel@uni-hohenheim.de

When sampling for analysis of inositol phosphates (InsP), digesta is usually taken with a spatula (gizzard) or rinsed with water (ileum), pooled per pen, and then frozen. Endogenous or added phytases might continue to function in the period between sacrificing the bird and freezing the sample to the core with unknown effects for analyzed InsP. The objective was to study whether such effects exist. Broilers were fed P and Ca reduced diets with or without phytase addition. Content from the gizzard and ileum was removed with a spatula, pooled per pen, and split: one portion was immediately shock frozen in liquid nitrogen (LN), another put in a -20 °C freezer immediately (F) or after a 30 min period of holding at room temperature (RT, gizzard) or mixed with double distilled water to mimic flushing before placing in the freezer immediately (W, ileum).Without added phytase, $InsP_6$ concentration in the gizzard (n=7 samples) was significantly lower in RT than LN (p=0.007). With added phytase, $InsP_6$ and $Ins(1,2,5,6)P_4$ were lower in F and RT than in LN, while $InsP_3$ was only lower in RT (p<0.05). In both diets, method F was in between RT and LN. In the ileum (n=6 samples), $InsP_6$ concentrations were not different when phytase was added, but significantly lower in W when phytase was not added (p=0.015). In both diets, concentrations of some InsP isomers were minimally lower in W than in other procedures (p<0.05).

We concluded that fast freezing can prevent or reduce post-slaughter activity of phytase in the upper digestive tract. Ileal samples seem to be less influenced by sampling procedure, possibly due to the high level of $InsP_6$ disappearance up to this point and the reduced phytase activity at ileal pH.

Poster 6.22: Urease activity (Delta Method) versus urease activity (European Method) determined on 45 commercial soybean meal samples and correlation with trypsin inhibitor content

N. Ruiz[1], F. de Belalcázar[2], J. Castillo[3]

[1] Nelson Ruiz Nutrition, LLC, [2] Nutrianálisis Ltda., [3] Castillo Consulting, Inc; nelsonruiz@nelsonruiznutrition.com

A total of 45 commercial soybean meal (SBM) samples were analyzed for urease activity (UA) by both the AOCS Method (Official Method Ba 9-58, 2011) and the European Method (Journal officiel des Communautés européennes, n° L 155, 1971). Also, each sample was analyzed for trypsin inhibitor (TI) contents by the ISO Method (Animal feeding stuffs: determination of trypsin inhibitor activity of soya products. ISO 14902:2001). The 45 SBM samples were selected from a set of samples previously analyzed by the AOCS Method (Delta pH) and ranged from 0.000 to 2.051 pH units. Re-analysis yielded a range from 0.000 to 1.822 pH units with an r-square of 0.80 (P<0.001) for the correlation between previous analysis and the re-analysis. Analysis of the 45 samples by the European Method (EM) yielded a range from 0.000 to 1.440 mg N/g/min. The correlation between the Delta pH Method and the EM was 0.80 (P<0.001). TI contents ranged from 1.34 to 20.68 mg/g SBM. The correlation between Delta pH and TI contents was 0.81 (P<0.001). However, the correlation between the EM and TI contents was only 0.52 (P<0.001). Nevertheless, the regression equation for each method vs. TI contents provide similar estimates of TI values around the range of adequacy (0.000-0.050 urease activity). These data indicate that there is a statistical correlation between the trypsin inhibitor concentration in SBM and the urease activity as determined by the Delta pH Method and the EM and both methods are highly correlated.

Poster 6.23: Evaluation of the ratio digestible Lys/ME on performance of broilers chickens

H. D. Gil Arenas[1], J. D. Fernandez Correa[1], C. A. Giraldo Vargas[1]

[1] Premex – Alura; hernan.gil@premex.co

An experiment was conducted to evaluate the ratio of digestible (d) Lys to ME on the performance of Ross 308 AP broilers. Corn-SBM diets were formulated to contain 1.35% dLys with 12.26, 12.55, 12.84 or 13.4 MJ of ME/kg in the prestarter (PreSt, 0 to 7 d); 1.22% dLys with 12.68, 12.97, 13.26 or 13.56 MJ of ME/kg in the starter (St, 8-21 d); 1.09% dLys with 13.10, 13.39, 13.68 or 13.97 MJ of ME/kg in the grower (Gr, 22 to 35 d) and 1.03% dLys with 13.10, 13.39, 13.68 or 13.97 MJ of ME / kg in the finisher (Fn, 36 to 42 d). The dM+C levels were 1.0% in the PreSt, 0.92% in the St, 0.85% in the Gr and 0.78% in the Fn. Data were analyzed as a randomized complete block design (n=160). In the PreSt there was no effect of dLys to ME ratio on performance. However, high ratios (96.21, 94.06 mg dLys/MJ) generate a higher FI and a higher FCR than low ratios (92, 89.97 mg dLys /MJ) (P <0.05) in the St. In the Gr phase, high ratios (83.21, 81.40 mg dLys/MJ) resulted on a higher FI than low ratios (79.68, 78.02 mg dLys/kcal) (P <0.05). BWG in the Gr phase was improved when broilers were fed the 81.40 mg dLys/MJ ratio as compared to that of birds fed the low ratio (78.02 mg dLys/MJ) (P <0.05). In this same phase, FCR of birds fed the low ratios (79.68, 78.02 mg dLys/MJ) resulted in better FCR that of broilers fed the high ratio of 83.21 mg dLys/MJ (P <0.05). Feeding the high ratios (78.63, 76.92 mg dLys/MJ) in the finisher phase resulted in higher FI and FCR than those obtained when the higher ratios (75.29, 73.72 mg dLys/MJ) (P <0.05) were fed.In conclusion, the results showed that the optimal ratios for a better FCR were 92 mg dLys/MJ in the St. phase, 79.68 mg dLys/MJ in the Gr. phase and 75.29 mg dLys/MJ in the finishing phase.

Poster 6.24: Economic modelling of broiler performance under different diet densities, feeding programs, and challenge conditions using a factorial design

L.-M. Gomez-Osorio[1], N. Tillman[2], O. Restrepo[3], C. Mesa[3], O.-D. Munera[3], R. Angel[4]

[1] Research and Development Unit, Solla S.A. Company, Colombia/Grupo Nutec, Mexico, [2] Nutritional Statistics LLC, Georgia, USA; [3] Research and Development Unit, Solla S.A. Company, Colombia, [4] Department of Animal and Avian Sciences, University of Maryland, College Park, MD; lmgomez@gponutec.com

This study was conducted to evaluate broiler performance under two different feeding programs, two diet densities and two types of challenges. A total of 5,760 day-old male Ross 308 broilers were randomly allocated to 32 pens of 60 birds/pen across 3 houses, with a total of 12 reps/Trt (4 reps/Trt/house). Birds were fed a pelleted Prestater (Pst) from 0-10d, Starter (St) from 11 to 24d and Grower (Gr) from 25 to 42d. A total of 8 Trts based on a 2x2x2 factorial arrangement: 2 feeding programs (ad libitum and restricted), 2 diet densities (high =1.11% digestible lysine (dLys) in Pst and 0.9% dLys in the St and Gr, and low =0.8% dLys in Pst and 0.64% dLys in the St and Gr) and 2 challenges (unchallenged vs challenged (no feed for 24 h at day 18)). Pen BW was recorded every 2 days while pen FI was measured daily. Statistical analysis using factorial design was used to analyze BW and FI by feeding period. BW data by day was used to generate growth models using Gompertz, Logistic, Mechanistic Growth and Quadratic models. Gompertz model had the lowest AICc, BIC, and SSE. Thus, a total of 8 different Gompertz models were obtained for each Trt scenario. The best commercial decision should consider profitability, thus the FI model was generated as a function of days and 8 different commercial scenarios. Our results showed that at the same selling price, the most profitable scenario at low feed cost was higher density, ad libitum and unchallenged. When feed cost increased by 25%, the most profitable scenario was higher density, restricted feeding and unchallenged. The least profitable scenario was low density, restricted and challenged. This study suggested that when feed cost is high, feed restriction can be considered to increase profitability for a production system

Poster 6.25: Effect of dietary saturation and acidity level on fatty acid absorption along the gastrointestinal tract in young broiler chickens

B. Jimenez-Moya[1], A. C. Barroeta[1], D. Solà-Oriol[1], F. Guardiola[2], R. Sala[1]

[1] Animal Nutrition and Welfare Service, Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra 08193, Spain, [2] Nutrition, Food Science and Gastronomy Department–XaRTA-INSA, University of Barcelona, Barcelona, Spain; beatriz.jimenez@uab.cat

The effect of dietary saturation degree and free fatty acid (FFA) level on the apparent digestibility coefficients (ADC) of fatty acids (FA) was studied along the gastrointestinal tract (GIT) in young broiler chickens. A total of 576 broilers were randomly allocated in 8 dietary treatments (6 cages/treatment) from 0 to 11d. A basal diet was supplemented at 6% with different fats [saturated: palm oil (P) and palm fatty acid distillate (PFAD); unsaturated: soybean (S) and soybean acid oil (SA) and blends of P-SA and S-PFAD] to obtain four levels of saturation (15, 22, 30, 38%) and FFA (11, 29, 46, 67%). At 11d, digesta from lower jejunum, upper and lower ileum and excreta were collected. TiO_2 (5g/kg) was used to calculate the ADC. Total FA (TFA) absorption, calculated as a proportion of total digestibility in excreta, mainly took place at jejunum level (>65%). As the saturation degree increased, FA absorption rates decreased ($P<0.001$) in all GIT segments. The increase in saturation degree from 15% to 38% caused a decrease in the ADC of TFA from 0.71 to 0.37 in the jejunum and from 0.78 to 0.52 in the excreta. The increase in the ADC of TFA from the jejunum to the excreta was due to the absorption of the monounsaturated and polyunsaturated FA at ileum level. Saturated FA (SFA) showed the lowest ADC in all segments and saturation degrees (0.22 to 0.54). Increasing levels of FFA decreased the ADC of SFA (jejunum, ileum and excreta, $P< 0.05$), but not of the other FA. When the level of FFA increased from 11% to 67% a decrease of SFA absorption was observed of 22% in the jejunum and 16% in the excreta. The results suggest that the ADC of FA was much more influenced by the dietary FA profile than by the FFA levels.

Poster 6.26: The effects of feed ingredients on the exocrine pancreatic function, nutrient digestibility and blood biochemical indices in Hisex white chicken

A. Grozina[1], V. Vertiprakhov[1], V. Fisinin[1]

[1] Federal Scientific Center "All-Russian Research and Technological Institute of Poultry" of Russian Academy of Sciences; alena_fisinina@mail.ru

The trial was performed on 10 Hisex White chickens with chronic pancreatic fistulae inserted at 100-120 days of age. Main pancreatic duct was implanted into an isolated duodenal section serving as an external anastomosis allowing sampling of pancreatic juice and redirection of the juice into the duodenum between the samplings. The chicken were allotted to two treatments (Trt); control Trt1 was fed a wheat-soy diet, experimental Trt2 was fed diet with content of sunflower cake 4.2 times higher, content of full-fat soybeans lower by 54.8% in compare to control diet. Both diets had similar nutritive characteristics. Pancreatic juice was sampled during 10 days, from starved birds and during 3 hr after the feeding; blood was sampled from the axillary vein. Five replicates for each Trt were made. It was found that the activities of pancreatic lipase and proteases in Trt2 were significantly higher compared to control (by 33.8 and 28.1%, respectively, $P<0.05$) while concentrations of total protein, Ca, and K were similar. Digestibility of fiber, DM, protein, and methionine in Trt2 was lower compared to control by 12.3; 1.5; 1.4 and 1.4%, respectively, while digestibility of fat higher by 3.5%. Tryptic activity in blood serum in Trt2 was lower (by 31.8%, $P<0.05$) while concentration of total protein, glucose, and triglycerides higher by 64.2; 46.4 and 55.3%, respectively. These data evidenced the explicit reaction of the pancreas to the change in diet ingredients, even if the nutritive characteristics of the two diets are similar. The study was financed by the subprogram "Studying the mechanisms of adaptation of the digestive system of mammalian animals and poultry to rations with different ingredient composition of feed" (Decree of the Presidium of RAS, No. 132, July 5, 2017).

Poster 6.27: Hermetia illucens oil as a potential ingredient in poultry nutrition

B. Kierończyk[1], M. Rawski[2], Z. Mikołajczak[1], M. Noak[1], A. Barc[1], D. Józefiak[1]

[1] Department of Animal Nutrition, Poznań University of Life Sciences, [2] Division of Inland Fisheries and Aquaculture, Institute of Zoology, Poznań University of Life Sciences; bartosz.kieronczyk@up.poznan.pl

Hermetia illucens larvae (Black Soldier Fly), are considered as a novel ingredient rich in crude protein and dietary fat, which could be commonly used in poultry nutrition. Nowadays in the available literature, the H. illucens meal is indicated as an alternative to soybean meal, as well as the fish meal, in animal nutrition. Moreover, extracted insect oils may also substitute soybean, palm, as well as fish oil. However, the nutritive value of insects is directly dependent on biotic factors (rearing medium). The aim of the study was to evaluate the effect of selected food waste used as a rearing medium on H. illucens chemical composition. The following substrates were used: T1 – wheat bran, T2 – carrots, T3 – cabbage, T4 – potatoes, T5 – a mixture of all above-mentioned materials. Each group contained 10 replications with 2 grams of larvae. The trial lasted 13 days. In the insect larvae, the following parameters were determined, i.e., the dry matter, ether extract, as well as fatty acid profile. The usage of carrots, cabbage, as well as a mixture of all selected ingredients, increased the fat content. The dominant fatty acids were C12:0, C14:0, C16:0, C18:0, C18:1 n9, C18:2 n6. The lauric acid content varied between the groups, and the highest value (42,1 g/100 g of fat) in the mixture of food wastes (T5) was noticed, contrary to the wheat bran substrate (26 g/100 g of fat). The current study suggests that the nutritive value of H. illucens is strictly dependent on the insects' feed (food waste) and should be taken into consideration during invertebrates production to obtain the most valid novel ingredient which could be used in poultry diets.

Poster 6.28: Effects of early feeding on growth, organ development, and blood biochemical profile in broiler chickens

H. K. Kang[1], S. B. Park[1], C. H. Kim[1], J. J. Jeon[1], H. S. Kim[1], H. C. Hong[1], K. T. Park[1], J. S. Son[1], S. H. Kim[1]

[1] Poultry Research Institute, National Institute of Animal Science; magic100@korea.kr

This experiment was conducted to investigate the effect of early feeding time on performance, organ weight, blood biochemical and leukocyte profile in post-hatch broilers for 35 day of age. A total of 200 one-day broiler chickens were randomly assigned to 5 treatments with 4 replications and 10 broilers each. Five treatments include: feeding within 3, 12, 24, 36 and 48 h after hatch, From 21 d, early feeding within 3 h after hatch had a significant ($P<0.01$) effect on body weight gain, whereas, at 14 d, the feeding 3 and 12 h after hatch was significant heavier ($P<0.01$) than the feeding 24, 36 and 48 h after hatch. The feed intake was significantly higher ($P<0.01$) the feeding within 3 and 12 h after hatch when compared with the feeding at 24, 36, and 48 h for 7 day of age. In the feed intake for 21 d of age, the feeding within 3 h after the hatch was significant higher ($P<0.01$) feed intake than the feeding 12, 24, 36, and 48 h. No significant differences were found for feed conversion ratio. At 3 d, gizzard weight was significant heavier ($P<0.05$) the feeding at 24, 36 and 48 h after hatch when compared to 3 and 12 h, whereas, the small intestinal weight was significantly higher ($P<0.05$) In blood biochemical profile, the concentration of alanine aminotransferase (ALT) was significant higher ($P<0.01$) the feeding at 3, 12 and 36 h after hatch than 24 and 48 h. Total protein content was higher ($P<0.01$) in the feeding at 48 h after hatch, and ALT content was higher ($P<0.01$) in the feeding at 3 and 24 h for 21 day of age. At 35 day of age, the concentration of alanine aminotransferase (ALT) was higher ($P<0.01$) when the feeding at 36 and 48 h after hatch.

Poster 6.29: Effects of supplementation of oil-enriched diets with antioxidants on the broiler productive performance and fatty acid composition of breast muscle

M. Đ. Stojčić[1], L. Perić[1], D. Žikić[1], J. Leskovec[2], A. Levart[2], J. Salobir[2], V. Rezar[2]

[1] University of Novi Sad Faculty of Agriculture Department of Animal Science, Serbia, [2] Department of animal science, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia; djukic@polj.uns.ac.rs

The aim of this work was to investigate whether, and if so to what extent, the combined supplementation of vitamin E, vitamin C, and selenium was superior to their individual supplementation concerning the broilers productive performance and fatty acid composition of breast muscle. Four hundred 21-day-old Ross 308 male broilers were allocated to 5 experimental groups and fed the following linseed oil (5%)-enriched finisher diets: Cont (no supplement), +E (200 IU vitamin E/kg feed), +C (250 mg vitamin C/kg feed), +Se (0.2 mg selenium/kg feed), or +ECSe (concentrations as in the sole supplementation, combined). Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were studied. In breast muscle, the analyses of fatty acid (FA) composition were carried out.No differences in the BW were observed among groups. Also, the ADG was not different among groups except from the 21st to the 40th day, when group +ECSe had a lower ADG (for 6.9%) ($p < 0.05$) than Cont. The ADFI was lower in the +ECSe group than in the Cont group from the 21st to the 40th d and from the 1st to the 40th d (5.4% and 4.3%, respectively), as well as from the 1st to the 40th d in group +E (4.2%). No differences among groups were detected concerning the FCR. Furthermore, no differences in the FA composition of breast muscle were observed among animals fed the different experimental diets. In conclusion, results indicated that broilers that were fed combination of the used antioxidants showed reduced ADG and ADFI compared to control group. However, the effect of antioxidants on FA composition of breast muscle was not observed.

Poster 6.30: The effect of genotype and crude protein on chicken meat nutritional value

E. Tůmová[1], D. Chodová[1], V. Machander[2], M. Ketta[1]

[1] Department of Animal Science, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague-Suchdol, Czech Republic, [2] International Poultry Testing Station Ústrašice, 390 02 Tábor, Czech Republic; tumova@af.czu.cz

The aim of the study was to compare the effect of two levels of crude protein on chicken breast meat composition in three different genotypes (Ross 308, JA757, ISA Dual). Chickens of both sexes were fattened till slaughter weight 2 kg. Chickens were split into 6 groups, with 2 replicates of 135 chickens per group. In control groups, chicken received mixtures with following crude protein content: 21.5 % in starter, 19.7 % in grower and 18.1 % in finisher. Experimental feed mixtures had 6 % lower crude protein content. Ross chicken reached slaughter weight 2 kg at the age 35 days, JA 757 at 42 days and ISA Dual at 70 days of age. At the slaughter age, 10 males and 10 females per group were selected for meat composition assessment. Regarding genotype, ISA Dual chicken showed the significantly highest dry matter and crude protein in breast meat and the lowest ether extract content. Lower crude protein decreased only dry matter ($P \leq 0.05$) and Energetic value ($P \leq 0.05$) of the meat. Cholesterol content was affected by interaction of genotype and feed crude protein ($P \leq 0.001$). The highest cholesterol content was in control group Ross 308 and the lowest in JA 757 fed feed mixtures with lower crude protein. Results of the study showed greater differences in meat composition regarding to genotype than to feed crude protein.

Poster 6.31: Dietary arabinoxylan digestibility in broilers

N. Morgan[1], S. Wu[1], M. Choct[1]

[1] University of New England; natalie.morgan@une.edu.au

This study determined the impact of soluble and insoluble AX on ileal and total tract dry matter (DM) digestibility. Male day-old Ross 308 chicks (n=210) were housed in 42 cages with excreta trays, 5 birds per cage, from d21-28 post-hatch. They were fed one of 7 commercial broiler diets ad libitum, 6 cages per treatment, composed of varying levels of wheat, corn, soybean-meal, sorghum and canola-meal. The soluble AX content of the diets varied from 2.08-11.68g/kg, and the insoluble AX from 29.36-38.84g/kg. Excreta was collected daily per cage from d25 to d28, and ileal digesta was collected from two birds per cage on d28. Titanium dioxide content was quantified in the diets, digesta and excreta samples by UV-spectroscopy, and soluble and insoluble AX was analysed by an enzymatic-chemical method. DM was determined by oven-drying. The data revealed that soluble AX was almost completely digested (average 96.1%), even in the absence of enzymes, whereas as little as 8% of the insoluble NSP was digested. Interestingly, soluble AX had no impact on dry matter digestibility. Digestibility of insoluble AX and dry matter were lowest ($P<0.001$ for both) in the diet containing the most canola meal, irrespective of AX content. There was a strong correlation between total and ileal insoluble AX and ileal DM digestibility ($r=0.537$, $P=0.004$ and $r=0.704$, $P<0.001$, respectively). Dietary insoluble AX content also strongly correlated with both ileal and total tract DM digestibility ($r=0.615$, $P<0.001$ and $r=0.618$, $P<0.001$, respectively). Generally there has been more industry wide interest in soluble AX in diets based on viscous grains, such as wheat, but in commercial diets based on two or more viscous and non-viscous cereals, the impact of the insoluble AX fraction becomes more significant.

Poster 6.32: Dose response to digestible methionine in the feed for two brown and two white strains of laying hens

B. Renouf[1], N. Bernard[1]

[1] EURONUTRITION, Domaine expérimental Le Pavillon, 72240 SAINT-SYMPHORIEN, France; nolwenn.bernard@mixscience.eu

In France, brown strains are mostly used for the shell eggs market. However, an increase of white strains for egg products is currently observed. In this context, two experiments were designed to evaluate the effect of digestible methionine on laying performances of two brown (A and B) and two white strains (C and D), in a French experimental farm (Euronutrition, Saint-Symphorien). At 33 weeks of age, 480 hens of each strain were allocated into 6 treatments (4 or 5 replicates of 20 birds), consisting in increasing levels of digestible methionine (dMet), from 0.22% to 0.47% with steps of 0.05% between each group. Both trials lasted for 12 weeks. Performance and egg quality were recorded weekly. Strain A showed a lower average egg weight, and a higher feed intake, laying rate, feed intake per egg and per egg mass than strain B ($P<0.05$). Irrespective of the strain, significant lower feed consumption and lower egg weight were observed with the groups having the lowest level of dMet (0.22). In terms of egg quality, strain B had more downgraded eggs (mostly broken eggs), regardless of the dMet level. Strain A and B showed a decrease in eggshell strength with the increase of dMet, but the egg vitelline membrane was stronger. Strain D consumed more feed, and had a higher egg weight that strain C ($p<0.05$), with no difference regarding feed efficiency. There were more downgraded eggs with strain D but no significant impact of the dMet level was observed. The eggshell strength decreased between 33 and 45 weeks, regardless of the dMet supplementation. These trials showed the combined effects of laying strains and nutrition. Other nutrients were also tested in dose response trials, which allow the proposition of specific nutritional recommendations depending on the laying strain.

Poster 6.33: The effect of rye, wheat, narrow-leaved lupin grinding degree on broiler chickens performance

M. Hejdysz[1], S. A. Kaczmarek[1], M. Kubiś[1], K. Perz[1], A. Rutkowski[1]

[1] Poznan University of Life Sciences; marcin.hejdysz@up.poznan.pl

A 4-week feeding trial was conducted to examine the effects of rye, wheat, narrow-leaved lupin grinding degree on broilers chickens performance. Four hundred and sixty one day old male ROSS 308 broiler chickens were randomly divided into six dietary treatments. Each treatment was replicated ten times with eight birds per replication in a completely randomized design. Six isonitrogenous and isocaloric corn-soybean meal based diets (mash form) were formulated to contain 30% of studied component (rye or wheat or narrow-leaved lupin) in two different grinding degree (0.1 mm or 1.5mm). Parameters were evaluated including body weight gain, feed intake and feed conversion ratio in three different periods (0-14 d; 15-28 d; 0-28 d). Results showed that no effect of grinding degree in all experimental groups for determined parameters. Birds fed diets with wheat were characterized by the best production results in all determined parameters, whereas the worst have been confirmed in groups with rye. Conclusion, grinding degree of rye, wheat as well as narrow-leaved lupin did not affect broiler chickens performance.

Poster 6.34: Insect oil as an alternative of palm oil in broiler chicken nutrition

A. Benzertiha[1], B. Kierończyk[1], M. Rawski[2], Z. Mikołajczak[1], M. Noak[1], A. Józefiak[3], D. Józefiak[1]

[1] Poznań University of Life Sciences, Department of Animal Nutrition, [2] Poznań University of Life Sciences, Division of Inland Fisheries and Aquaculture, [3] Poznań University of Life Sciences, Department of Preclinical Sciences and Infectious Diseases; basset@up.poznan.pl

The study was conducted to evaluate the effect of total replacement of palm oil and poultry fat with Tenebrio molitor oil (TM oil) in broiler chicken diet on the growth performance and lipid fatty acid composition of liver and breast muscle tissues. A total of 72 seven-day-old female Ross 308 were used. The birds were randomly distributed to 3 different groups, 12 replicates per group and 2 birds per replicate. The experiment was 30 days in metabolic cages. The basal diet was formulated on maize and soybean meal basis. 5% of palm oil, poultry fat or TM oil was added to the diet. The growth performance parameters (BWG, FI, and FCR) were measured during day 7, 14, 21, and 30. The fatty acid profile results of TM oil and PF used in this experiment were dominated by oleic and linoleic. However PO had a high values of palmitic and oleic. BWG, FI or FCR were not affected by dietary treatments. The liver tissue of the chickens fed a diet supplemented with TM oil showed the lowest value of SFA ($P = 0.004$) and the highest of UFA ($P = 0.004$). In addition, TM oil significantly decreased MUFA ($P < 0.001$) and increased PUFA ($P < 0.001$) in comparison to PF and PO. Furthermore, SFA and UFA profile of the breast tissue was not affected by any of the dietary fat sources. However, TM oil reduced MUFA content ($P < 0.001$) and increased PUFA ($P < 0.001$). Moreover, n-3 and n-6 fatty acids were significantly increased in case of TM oil supplementation ($P = 0.006$; $P < 0.001$ respectively). In conclusion, the use of TM oil in broiler chicken diet did not show any effect on the growth performance. Moreover, TM oil supplementation improved the fatty acids profile of the liver tissue and the breast muscle.

Poster 6.35: Feeding status of free-range scavenging chickens in different agro-climatic region of India

B. Prakash[1], S. K. Verma[2], S. V. Rama Rao[1], M. V. L. N. Raju[1], S. S. Paul[1], A. Kannan[1]

[1] ICAR-Directorate of Poultry Research, [2] ICAR-Central Institute for Research on Cattle, Meerut; drbhukyaprakash@gmail.com

The aim of the study was to determine the feeding status of the backyard chickens (BC) of four different agro-climatic conditions (Semi-arid, warm-humid, arid and cool-temperate) of India during different seasons and to compare their feeding status with those reared under confined feeding system (CFS). The dry matter content of crop was higher (P<0.01) by 30.56% and gizzard by 40.12% in chickens reared under backyard conditions compared to reared under CFS. The higher (P<0.01) crop and gizzard contents were recorded during winter compared to the rainy season. The proportion of grains in crop during summer and winter season was higher compared to rainy season. The proportion of insects was higher by 93.54% during rainy and winter compared to summer season. Whereas, the proportion of insects was higher in chickens reared in warm-humid and cool-temperate compared to arid and semi-arid conditions. The CP, Ca, P and gross energy of the crop content was higher in chickens reared under CFS compared to those reared in the backyard system. The CP content was lower in BC reared in Warm-Humid and Semi-Arid regions compared to other studied areas. Similarly, significantly lower (P<0.01) Ca in ASR, and P and gross energy in Telangana compared to the chickens reared in the backyard in other regions. The energy and protein content were critically deficient in the semi-arid region. Therefore, evolving location specific supplementary diets using locally available feed resources to meet the nutrient requirement of BC is essentially required for optimizing the production potential.

Author's Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Diamond Sponsors

Biomin®

DSM BRIGHT SCIENCE. BRIGHTER LIVING.

NOVUS® SOLUTIONS SERVICE SUSTAINABILITY™

PATENT CO.®

Platinum Sponsors

EVONIK POWER TO CREATE

zoetis

CHR HANSEN Improving food & health

J. RETTENMAIER & SÖHNE GMBH + CO KG JRS Fibers designed by Nature

Gold Sponsors

ABVista

ADISSEO A Bluestar Company

BASF We create chemistry

CJ BIO

DUPONT®

Elanco™

ew nutrition

HAMLET PROTEIN

Perstorp

PHYTOBIOTICS Be curious. Be brave. Be genius.

Silver Sponsors

agromed natural effects

CCPA GROUP Our expertise, your efficiency

Diamond V® The Trusted Experts In Nutrition & Health®

Delacon performing nature

KEMIN®

nuscience member of the royal agrifirm group

LALLEMAND LALLEMAND ANIMAL NUTRITION

Impextraco® Optimizing feed ingredients

Phileo LESAFFRE ANIMAL CARE

SILVATEAM

olmix for a better life

EASTMAN

mixscience Innovate for Life

trouw nutrition a Nutreco company

Partners

HUVEPHARMA® We add performance to your business

NOACK GROUP OF COMPANIES

Media Patronage

POULTRY WORLD

Media Partners

International Hatchery Practice

veterinary sciences

WATT GLOBAL MEDIA

International Poultry Production