UNIVERSITY OF NEW ENGLAND

IMPLEMENTATION OF A NET ENERGY SYSTEM FOR MEAT CHICKEN FEED FORMULATION

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ABSTRACT

The benefit of formulating broiler diets using net energy (NE) versus ME is under debate for a long time. Formulated during this study, were a series of diets to determine if NE or AME was a better system for broiler feed formulation. Diets offered to broilers housed in closed-circuit calorimetry chambers were used to determine the energy values.

The findings showed that both AME and NE could be used to formulate broiler diets. Broilers offered diets formulated using NE system performed equally well as broilers offered AME diets. Using NE versus AME to formulate broiler diets impacted the procurement strategy for raw materials.

Protein ideally balanced with NE leads to high weight gain and low FCR. It is the ratio of NE to protein or amino acid that dictates the performance, live weight and FCR, and not AME content of the diet. Hence, while both NE and AME systems are appropriate for broiler feed formulation, NE is a better performance predictor, for FCR and weight gain, than AME.

The supremacy of the NE system is higher in lower energy diets and when formulating diets during the early age of the broilers.

CERTIFICATION OF DISSERTATION

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that to the best of my knowledge, any help received in preparing this thesis and all sources used have been duly acknowledged.

07/11/2019

Moreen Afroza Ali Date

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SUMMARY

When formulating broiler diets, the benefit of using NE vs AME is an ongoing debate. The objective of this study was to determine the impact of the implementation of a NE system for broiler feed formulation in Australia.

During the first study (chapter 2) the effect of feed nutrient density on weight gain, feed intake, FCR, and carcass yield (breast, thigh and fat pad) was determined. Flock variability was measured to establish if there was a difference for diets formulated using AMEn versus NE. Two diets formulated, using AMEn and NE, had different nutrient densities, but the same AMEn to amino acid ratio. Results showed that even with differences in nutrient density, the birds offered these diets had similar weight gains throughout the study. There was a significant difference in the FCR during the early age of d 10 to 25. Birds offered high-density diet had lower FCR during d 10 to 25. The feed intake differences, however, were observed in older birds during d 25 to 34. The results showed that the bird's response to nutrients differs for the different ages.

Both AMEn and NE could be used to formulate broiler diets to meet the production targets. Birds fed both formulations, NE and AMEn, had similar flock variability.

AMEn and NE diets created in the first study were offered to broilers to measure the HP and RQ using the closed-circuit indirect calorimetric (CIC) chamber (chapter 3). The results showed that the feed intake for both diets was comparable with no significant differences. The nitrogen intake and AMEn intake between the two groups were significantly different due to the differences in the nutrient density of the feed. The HP and RQ of the birds offered the two diets were also significantly different. The birds offered low-density NE diet had lower HP and higher RQ compared to birds offered high-density AMEn diets. However, the differences in NE measurements were not significant.

When formulating NE versus AMEn diets in the first and second study, the raw material composition of the diets changed. Investigated during the subsequent trial (chapter 4) was the effect of raw material composition on AMEn and NE.

AMEn and NE diets created using a range of raw materials had different raw material composition but similar nutritional analysis. The results showed that even with differences in the raw material composition of the diet, formulated with AMEn and NE system, the performance of birds, FCR and live weight, met production targets. It seems that the accuracy of the raw material nutritional analysis has precedence over the choice of using AMEn or NE for feed formulation.

Formulating diets with significantly different NE levels seemed difficult unless different raw material options were available. When only a limited number of raw materials are available, formulating for both AMEn and NE likely ensue in the same diet.

When formulating using NE system, the age of birds and nutrient density of diet dictated the effect NE had on the performance of birds. Broilers offered high-density diet during the early growth period, had a lower FCR. There was a correlation between NE and FCR with R^2 =0.86 for diets at 12.55 MJ/kg for all ages. However, for birds offered 13.39 MJ/kg AME diet, the correlation between NE and FCR was only during the first three weeks of age (R^2 = 0.85). Older birds offered 13.39 MJ/kg diet did not show a correlation between NE and FCR (R^2 = 0.44) and had a lower FCR ($P < 0.001$). This lack of response to changing NE may be due to birds performing to their optimum level with sufficient NE in the diet; thus, any further changes to NE showed no effect.

Diets formulated during the earlier studies failed to cause a change in the live weight gain in birds. The subsequent study (chapter 5) was to investigate if dietary NE or amino acid levels affected the weight gain in broilers. Four diets formulated at two different levels of NE and amino acids, to investigate the interaction between NE, amino acid and the performance of birds. The AME level for all diets was the same. Increased amino acids in diets reduced the FCR in the birds at all ages and caused greater weight gain in broilers during an early age. Broilers offered low NE diet had higher feed intake. A balanced protein to NE level in the diet decreased FCR and increased weight gain in broilers.

The earlier trial (chapter 4) concluded, raw material differences are insignificant when formulating diets using the NE system. However, research shows that feed

additives change the AME of diets. The next two studies were conducted to investigate the effect feed additives, zinc bacitracin and phytase, had on NE of the diet.

Birds were offered diets with and without zinc bacitracin to study its effect on NE. Using CIC chamber, RQ, HP and NE of diets were measured (chapter 6). The results showed that adding zinc bacitracin to feed lowered the HP in birds. The addition of zinc bacitracin failed to cause changes in the RQ, HI nor the NE of the diet.

Similar to the above experiment, the effect a combination of phytase and xylanase in a wheat-based diet on RQ, HP and HI of the birds were measured using a CIC chamber (chapter 7). Results indicated that adding phytase to a wheat-based diet containing xylanase did not affect RQ, HP, HI or NE of the diet. However, the addition of phytase significantly reduced nitrogen in the excreta.

Studies conducted so far had confirmed the impact of nutrition and raw materials on NE of the diet. This study investigated the effect physical feed characteristics had on the NE of the diet. Using a CIC chamber, RQ, HP, HI and NE of a pelleted diet and a diet with finer particles (chapter 8) was measured.

While the feed particle size reduction did not cause a difference in AME, the diets had lower measured NE levels. The NE of pelleted diet, 11.20 MJ/kg, was higher than the NE of the fines, 10.21 MJ/kg. This observation is consistent with lower HP in birds offered a pelleted diet (0.73 MJ/kg) compared to HP in birds fed fines (0.79 MJ/kg). Birds offered pelleted diets had significantly higher weight gains as compared to those offered fines at d 25 (1.864 kg and 1.472 kg respectively).

Diets created during the final experiment (chapter 9) were at three different AME, NE and protein levels. Diet A had high AMEn of 13.85 MJ/kg, diet E had low AMEn of 13.29 MJ/kg, while diet B, C and D were the same AMEn level of 13.62 MJ/kg. Diets A, B and E had similar protein levels between 19.0% to 19.6%. Diet C had a low protein of 18.4%, and diet D had a high protein of 21.9%. The NE content of diets A and B, and diets C and D were the same (11.04 MJ/kg and 10.79 MJ/kg respectively).

The results showed that protein balanced with NE leads to higher weight gain and FCR. It is the ratio of NE to protein (amino acid) that dictates live weight and FCR, and not AME.

Feed intake in birds seems to be dictated by the most limiting nutrient. Birds are, to some extent, self-regulating their nutrient intake by increasing feeding when nutrients are limiting in the diet. Protein, NE, followed by AME, seem to be the priority order for the birds feed intake response. However, feed intake may or may not eventuate in greater live weight and lower FCR of the broilers. During this study, feed intake had a greater correlation with NE than AME.

Regression analysis indicated that feed intake is more difficult to predict than the FCR and weight gain of the birds. The response for feed intake varied with the nutrient content of the diet and the age of the birds.

Birds offered diets with low protein digestibility had larger livers compared to birds offered high protein diets. There was a negative correlation between thigh yield and liver size. The birds with higher thigh yield had small livers.

Both AME and NE can be used to formulate broiler diets. When formulating using different energy system using the least cost formulation, different raw materials were selected. Using AME versus NE impacts the procurement strategy for raw materials.

The inclusion of zinc bacitracin and phytase combined with xylanase in a wheat-based diet did not cause gain in the NE of the diet. Diet particle size had a significant impact on NE. Diets with higher levels of fines resulted in lower NE than did pelleted diets.

Protein ideally balanced with NE leads to high weight gain and low FCR. It is the ratio of NE to protein or amino acid that dictates the performance, live weight and FCR, and not AME. Net energy predicts FCR with greater accuracy than AME.

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Abbreviations

Apparent digestible energy **ADE**

Apparent metabolisable energy **AME**

Closed-circuit indirect calorimetric chamber **CIC**

Comparative slaughter method **CSM**

Digestible Energy **DE**

Dry matter **DM**

Effective energy **EE**

Endogenous energy loss **EEL**

Fasting heat of production **FHP**

Feed conversion ratio **FCR**

Feed intake **FI**

Gross energy **GE**

Heat increment **HI**

Heat production **HP**

Indirect calorimetry method **ICM**

Metabolisable Energy **ME**

Net energy **NE**

Nitrogen-corrected apparent digestible energy **ADEn**

Nitrogen-corrected apparent metabolisable energy **AMEn**

Nitrogen-corrected true ME **TMEn**

Nitrogen retention **NR**

Productive energy **PE**

Respiratory quotient **RQ**

Retained energy **RE**

Starch equivalents **SE**

Scandinavian feed units **SFU**

Total digestible nutrient **TDN**

True ME **TME**

Volatile fatty acids **VFA**

INTRODUCTION

A metabolisable energy system is currently used for broiler feed formulation by the Australian Poultry Industry. The ME system used for feed formulation fails to predict the performance, FCR, of the birds accurately (Scott et al., 1999; Black et al., 2005). One of the factors contributing to the lack of correlation between ME and FCR, hence inability to predict FCR, is the variability in the prediction of ME.

Research shows that ME level of the diet may vary due to:

- ingredient factors including variety and geographical location of grains (Osbaldiston, 1966; Olson et al., 1972; Miller, 1974), the raw material composition of feed (Rajaguru and Ravindran 1985; Choct et al., 1996a) and use of feed additives, e.g. enzymes (Huyghebaert and De Groote, 1997; Tukei, 1998; Hughes et al., 2000).
- animal factors including bird age (Zelenka, 1968; Guirguis, 1976; Peterson et al., 1976; Kussaibati et al., 1982; Sibbald, 1982), strain and species (Begin, 1969; Proudman et al., 1970; Pym and Farrell, 1977) and the level of feeding (McDonald et al., 1995).
- nutrition and processing including variation in the chemical composition of feed (NRC, 1994), feed processing and testing (Cave et al., 1965; Bayley et al., 1968)

Some of the sources of variation listed above are natural and hence inherent of the ME system. Further to this ME measures the loss of energy between ingestion and excretion and ignores the metabolic fate of absorbed nutrients. Metabolisable energy fails to give a measure of energy available for growth, maintenance and production.

Net energy, however, is the energy available for growth, maintenance and production. The debate of whether a NE system is needed has been ongoing for many decades and, consequently, there have been studies have to examine the benefit of NE system over AME. While De Groote (1974) showed the benefit of using a NE system over AME, Fraps and Carlyle (1939), Carré et al. (2002) and

Van Der Klis (2010) failed to show benefits of using NE system in their independent studies.

One of the most significant drawbacks, limiting the use of the NE system, was the inability to measure the HI accurately. HI can be determined by, calculating the difference between ME intake and energy gain as body tissue, using comparative slaughter technique or by calorimetry (Liu et al., 2017). Calorimetry can be direct or indirect. The indirect calorimetry can be an open or closed system (Farrell, 1971; Fuller et al., 1983; Fedde, 1993; Arch et al., 2006; Choct, 2012).

The calorimetric techniques have improved with new designs now in place to measure NE (Swick et al., 2013; Liu et al., 2017; Hilton et al., 2019). The improvement in the measuring techniques of HI allows reassessing of the NE system.

In 2009, Noblet et al. successfully used indirect calorimetry to formulate pig diets. Pigs diets formulated using NE could predict performance where prior diets formulated using DE and ME failed. As a result, pig diets formulated using NE system had higher efficiency.

Formulating broiler diets using the NE system may lead to similar accuracy in predicting performance. The feeding efficiency of the diet may improve, leading to increased broiler performance. With the benefits that a NE system could bring and the improvements in the testing techniques, it seems worthwhile to reassess a NE system to formulate meat chicken diets.

Chapter 1: LITERATURE REVIEW

1.0 INTRODUCTION

In 2018, a feed survey assessed the production of 30,000 feed mills in 144 countries Alltech, 2018). The findings showed that the broiler and layer feed made up a substantial portion of global feed produced (450 million metric tons of the 1.103 billion metric tons of total feed production) annually. Alltech estimated that these numbers would continue to grow (Alltech, 2018).

With 60 to 70% of the diet composition being grains and about 20% made of soybean meal, broiler feed production has a global impact on resources like crops, land and carbon footprint. Any efficiency improvement in FCR (feed: gain) improves the utilisation of feed resources and have a global economic impact.

The feed is also the major contributor to the cost of poultry production. One of the goals when formulating poultry feed is to find a balance of ingredients to meet the nutrient requirement of the birds best. A wide range of raw materials used in poultry feed formulation contains diverse amounts of various classes of nutrients.

The commercial broilers rely solely on a diet to deliver all the nutrients required for growth. The critical role of providing nutrients via the diet is known for a long time. Protein, fat, carbohydrate and even fibre have been identified by various research as essential macronutrients for broiler growth. These also contribute to the chemical energy of the diet. The efficiency of dietary chemical energy utilisation determines the extent to which animals convert feed into performance targets (Bickel, 1988; Close, 1990).

Energy and protein are essential for growth and maintenance and hence determine the production performance of the birds. They are also the main contributing factor towards feed cost. Getting the right balance in feed cost and bird requirement is imperative for high financial returns.

Earlier feed standards printed in the 1950s used nutrients; proteins, carbohydrates and fats, for feed formulation (NRC, 1954) but not energy. While this technique of feed formulation addressed the challenge to meet the nutritional requirement, it overlooked that the birds' physiological control mechanisms may not differentiate and perceive all these nutrients as an energy source.

The current feed standards use energy and not carbohydrate. There are many different types of energy systems used for feed formulation. There were numerous studies conducted to determine which energy system is most useful to decide on the energy value of the feed, feed ingredients and the energy requirement of the bird (Fraps and Carlyle, 1939; Titus, 1956; Davidson et al., 1957; Hill and Anderson, 1958; Richardson et al., 1960; Ivy et al., 1968; De Groote, 1974; Koh and MacLeod, 1999; Carré et al.,2002; Lopez and Leeson, 2008; Van Der Klis, 2010). The energy systems to be used for formulation and the accuracy of techniques used to measure energy has been the ongoing controversy in the industry for decades.

Figure 1 below shows the various types of energy and its partitioning among different functions in broilers.

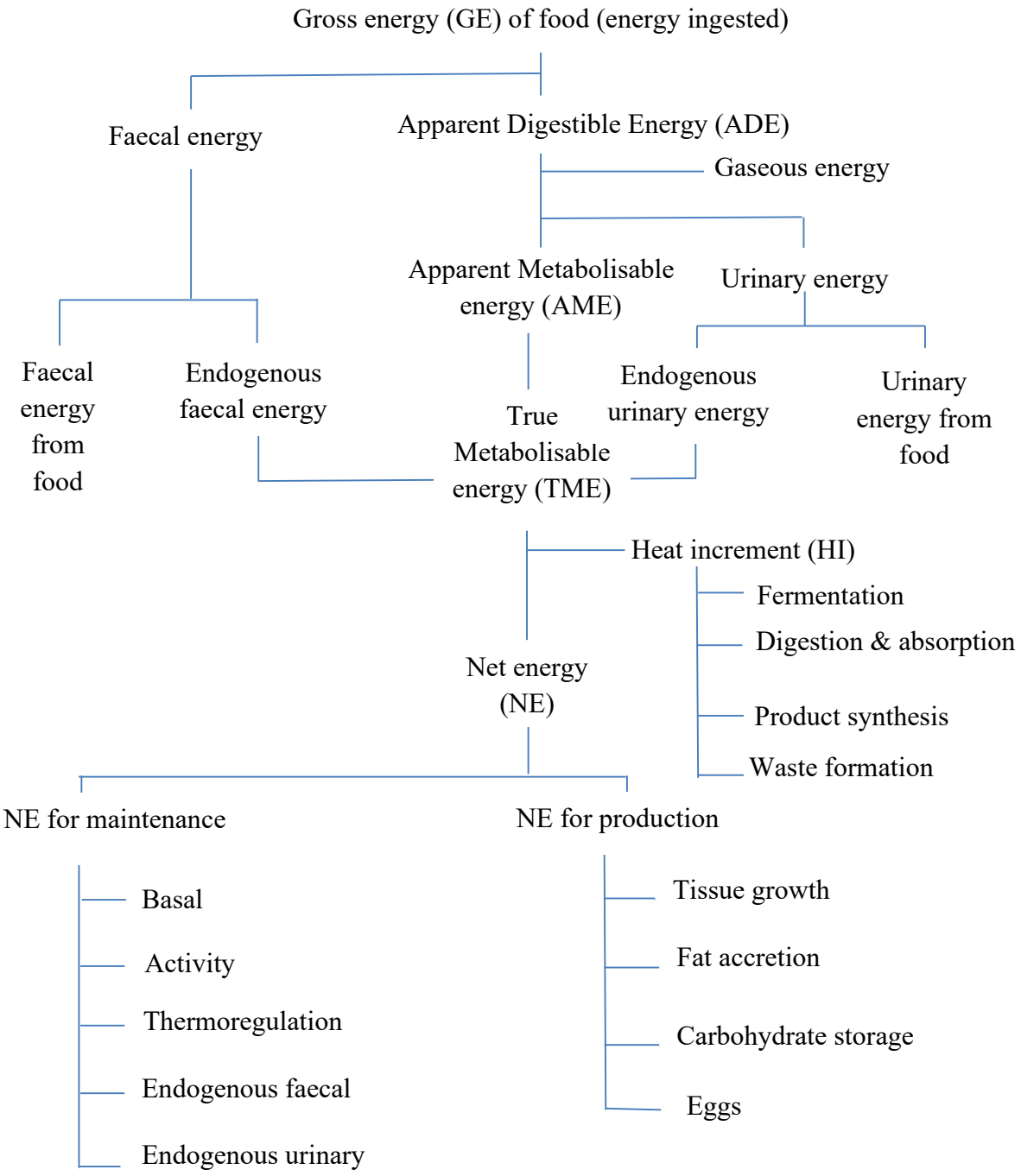


Figure 1 The partition of energy in broiler chickens.

Adapted from Sibbald (1982). © Canadian Science Publishing

Figure 2 illustrates the proportion of ingested energy disposition in various forms of energy in layers.

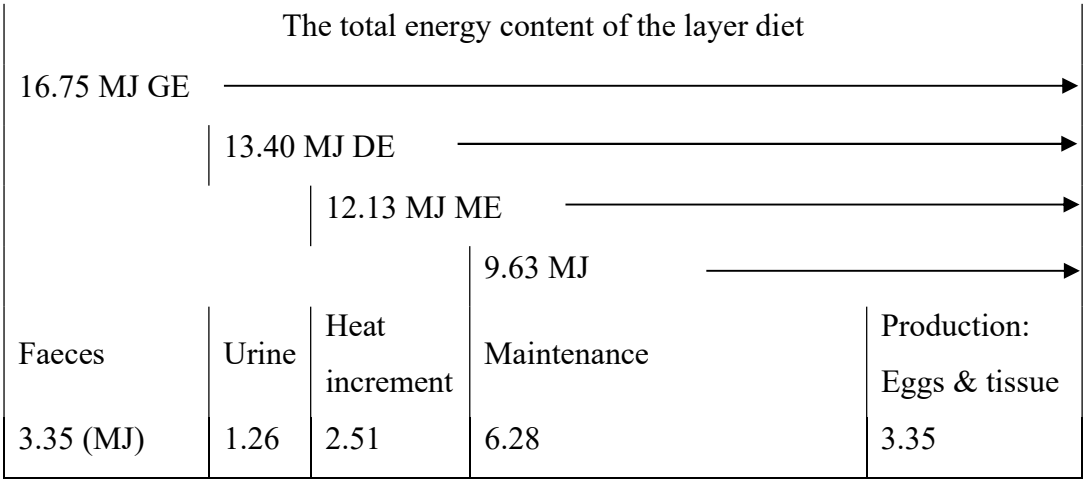


Figure 2 Energy partitioning of a typical laying hen diet.

Adapted from NRC 1984.

Metabolisable energy is currently used for poultry feed formulation, however, from the partitioning and comparative charts shown above it can be seen that net energy and not the ME is the key driver of maintenance and production. Formulating diets for net energy may also ensue in an adequate diet for broiler production.

2.0 TYPES OF ENERGY

2.1 Gross energy

Gross energy, measured as the heat of combustion, is the chemical energy stored in the food determined by burning the food in a bomb calorimeter. Food burnt in oxygen, in a closed chamber, raises the temperature of the surrounding water. The energy in food calculated using the weight of food, the weight of water and the temperature increase of the water. Published by various authors is the gross energy values of ingredients (McDonald et al., 2011).

Another way to calculate gross energy is via chemical composition of ingredient. McDonald et al. (2011) defined GE as a measure of the degree of oxidation of food and expressed it as a ratio of carbon plus hydrogen to oxygen. Since chemical structures do not change, McDonald et al. (2011) used the gross energy of 17.5 MJ/kg on dry matter basis (DM) for carbohydrates and 39.0 MJ/kg DM for triglycerides or fats, to calculate gross energy of food.

By deducting the physiological energy losses in the transformation processes from gross energy, the DE and ME in the feed is calculated (de Boer and Bickel, 1988).

2.2 Digestible Energy

Following ingestion of feed, the digestion process begins. Feed not digested is excreted from the body as faeces. The energy of the digested food absorbed by the animal is the digestible energy (DE). The gross energy in the faeces is subtracted from the gross energy in the food to calculate DE. Digestible energy calculated in this manner is true DE. To determine DE, energy in both components; food and faeces, are determined using bomb calorimeter on collected samples. The flaw in this technique is that not everything in the faeces is undigested food. Gut secretions, enzymes, body cells and gut linings may also be part of the faeces. Excess minerals are also sometimes excreted in the faeces. These may lead to a false (low) value of true DE, and therefore the measure is usually referred to as apparent digestible energy, ADE (Farrel, 1981; Sibbald, 1989; McNab, 1990).

Since it is difficult to differentiate which components of faeces relate to undigested feed in animals, apparent digestible energy is more likely to be used than true DE.

2.3 Metabolisable Energy

As the animal uses the DE by oxidising the digested food, it produces metabolites, excreted through urine, and fermentation by-products, released as methane. Both by-products have energy that is no longer available to the animal. Subtracting these metabolic by-products from the DE gives ME (Farrel, 1981; Sibbald, 1989; McNab, 1990).

Excreta energy subtracted from DE gives the ME of diet. Excreta energy and DE of feed are determined calorimetrically. However, the undigested food, faeces, and the metabolic by-product, urine, both are voided together in poultry, making it difficult to measure the DE without surgically altering the digestive tract of the chicken.

Faeces must be collected separately from urine to determine DE and ME of feed. To separated urine from faeces, the broilers need to undergo a surgical procedure called a colonoscopy (Richardson et al., 1960; Ivy et al., 1968), performed with or without a cannula. This surgical procedure, however, has caused difficulties. For example, Richardson et al. (1960) failed to secure healing between the mucosa of the intestine and the skin. The surgical procedures also cause severe health and welfare issues when high fibre diets dried in the cannula, causing it to clog up, resulting in its expulsion.

As an alternative to surgical modification, Vogt and Zoiopoulos (1988) analysed the uric acid quantity in the excreta to calculate the total urinary nitrogen. The high cost and the inaccuracy of the technique limited its use.

Due to the issues faced above, ME is used more commonly in poultry than DE. When endogenous losses not considered, it gives the apparent metabolisable energy, AME (Sibbald, 1989; McNab, 1990; North and Bell, 1990). By subtracting energy due to endogenous losses, true metabolisable energy (TME) is determined.

Endogenous energy loss (EEL) defined as that arising from the bird or its metabolic processes. The contributors to endogenous energy losses include; excretory products of nitrogen metabolism, sloughed-off gut lining, bile excretions, and unabsorbed enzymes (Sibbald, 1975a; 1976; McNab, 1990).

A rapid method for measuring TME, which directly determined EEL was developed by Sibbald (1976) who argued that TME should be used to express the energy of feedstuffs. TME for poultry as agreed by Vogt and Zoiopoulos (1988), is the GE of the feed minus the GE of the excreta of feed origin hence a correction had to the applied to EEL which made it complicated. Further to this, Hartel (1986) noted that Sibbald had used fasted birds fed small amounts of feed which cause misleading coefficients in the regression equations used to calculate energy excretion from feed intake. An additional concern was that the TME system had used adult roosters to produce values to predict energy in growing meat chickens. Vogt and Zoiopoulos (1988) reported that due to these difficulties, the European countries did not adopt the TME method.

The AME system is currently the preferred method of energy measurement for meat chickens. In full-fed birds, endogenous losses are considered small in contrast to the TME assay with reduced feeding. To accurately calculate AME, energy losses in the form of methane and volatile fatty acids (VFAs) is determined (McDonald et al., 1988, 1995). While some animals, like ruminants, produce large amounts of fermentation by-products, the production of methane in poultry occurs only in the hindgut and is negligible (Vogt and Zoiopoulos, 1988). Annison et al. (1968); McDonald et al. (1995) and Choct et al. (1996a), all reported variable amounts of VFAs produced in the various gut segments (i.e. proventriculus and gizzard, duodenum, jejunum, ileum, caeca, and colon) of the digestive tract of birds with the caeca being the major site.

Although birds do not digest the cellulose from the cereal grains, some hemicellulose digestion occurs in the caeca (McDonald et al., 1995), producing VFAs. These are mainly acetate, propionate, and butyrate (Annison et al., 1968; Choct, 1995; McDonald et al., 1995) that acts as an energy source when absorbed into the blood system. However, the energy contribution due to the quantity of VFAs produced may be negligible (McDonald et al., 1995); hence energy losses due to methane and VFAs had been negligible for broilers. Due to this, fibre is

viewed as a nonessential nutrient when discussing energy. However, recent studies have shown that while fibre may not contribute to the energy content of the diet, small to moderate addition of fibre to the diets improves the gastrointestinal development, function, and health, thereby enhancing nutrient digestibility and growth performance (De Vries, 2015).

The ME content of feeds is influenced more by the type of digestive system an animal has rather than the species, i.e. the amount of ME available to a monogastric is significantly different from ruminants and not so different between the different ruminant groups. For example, in monogastric animals such as broilers, higher feed intake leads to higher ME intake. In contrast, in ruminants, high intake may lead to low retention time in the rumen and hence high faecal energy loss. The energy intake of the animal depends on the amount of energy in feed and the feed intake (Iskander and Pym, 1987).

The gross energies of the faeces and urine and the gross energy of the food consumed, used to calculate ME, are determined using a calorimeter. Hence with the advancement in calorimetric research, ME become more desirable when evaluating the energy of poultry feed ingredients and the energy requirements of the birds (De Boer and Bickel, 1988).

ME calculated by the equation below:

ME = GE of feed - (GE of faeces +GE of urine + GE of fermentation gases)

The ME value expressed as:

- the apparent metabolisable energy (AME),
- true metabolisable energy, (TME),
- nitrogen corrected apparent metabolisable energy, (AMEn)
- nitrogen corrected true metabolisable energy (TMEn) as described by Farrel (1981); Sibbald (1989), and McNab (1990).

Over the years, several systems used to assess the energy value of raw materials and diets have become obsolete (these include total digestible nutrients (TDN), starch equivalents (SE) and Scandinavian feed units (SFU) (Blaxter, 1956.)

Numerous studies conducted to measure the energy of feed for broilers; however, when results pooled, it becomes challenging to identify the measure of energy used, making the data collaboration a challenge.

As seen earlier, depending on the assay procedures used, the type of ME varies, and often research studies did not detail the ME tested (Sibbald, 1989 and Jiang, 2004). Many research papers further fail to identify if expressed results were as dry matter or as is, adding to the challenge.

Apart from techniques not being adequately identified, there is also an inherent variation in the ME of raw materials. The ME values used for ingredients vary globally. Wheat AME values reported by authors from various locations summarised in table 1.

Table 1 AME (MJ/kg) of wheat in various locations.

Author	Wheat source	AME (MJ/kg)
Sibbald and Slinger (1962)	Canada	12.31 – 16.56
Schumaier and McGinnis (1967)	United States	12.04 – 13.46
Wiseman and Inborr (1990)	United Kingdom	13.00 – 15.23
Mollah et al. (1983); Rogel et al. (1987)	Australia	10.49 – 15.89

Apart from the variation in wheat from geographical location, the differences above may due to the testing techniques used. In 2005, Black et al. published a range of AME values on selected cereal grains highlighting AME differences between broilers and layers, shown in table 2.

Table 2 AME values (MJ/kg DM) of cereal grains in laying hens and broiler chickens.

Cereal	Layers	Broilers
Wheat	12.20 – 15.60	11.90 – 15.90
Barley	11.40 – 14.20	10.90 – 13.60
Oats	12.80 – 16.10	12.10 – 14.90
Triticale	11.80 – 14.30	12.10 – 14.50
Sorghum	14.80 – 16.03	15.30 – 16.70
Rice	13.00 – 14.80	17.60 – 17.80

Adapted from Black et al. (2005).

The table above shows the variation in AME of cereals due to bird type and cereal variety.

In addition to cereal variety and geographical location (Osbaldiston, 1966; Olson et al., 1972; Miller, 1974), other causes of variation in ME were:

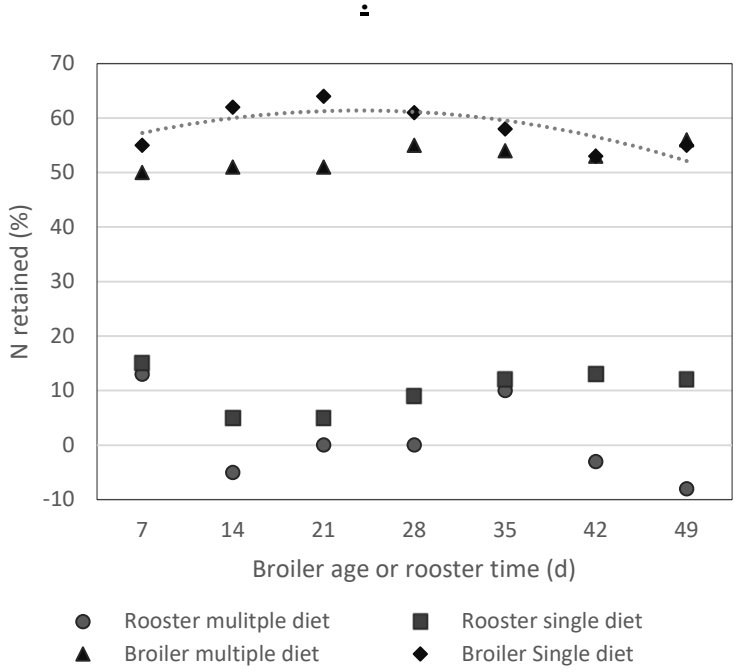
- bird age (Zelenka, 1968; Guirguis, 1976; Peterson et al., 1976; Kussaibati et al., 1982; Sibbald, 1982)
- strain and species (Begin, 1969; Proudman et al., 1970; Pym and Farrell, 1977)
- variation in the chemical composition of feed (NRC, 1994)
- methodology and laboratories (Reid et al., 1980; Rajaguru and Ravindran, 1985; Standing Committee on Agriculture (SCA), 1987)
- feed processing (Cave et al., 1965; Bayley et al., 1968)
- the raw material composition of feed (Rajaguru and Ravindran 1985; Choct et al., 1996a)
- level of feeding (McDonald et al., 1995)

- nitrogen-retention (Sibbald and Slinger, 1963; Davidson et al.,1964; Miller, 1974; Sibbald, 1989)
- feed additives, e.g. enzymes (Huyghebaert and De Groote, 1997; Tukei, 1998; Hughes et al., 2000).

The variation in ingredients influence the digestibility and hence the ME of the diet (Janssen and Cane, 1989; Classen and Bedford, 1991; McDonald et al., 1995; Annison et al., 1997). Fibre has been recognised as one the feed components that affect the digestibility of broiler diets (Rajaguru and Ravindran, 1985; Classen and Bedford, 1991; Choct and Annison, 1992; McDonald et al., 1995; Choct et al., 1996a). NSPs from the fibre may hinder digestion by increasing the gut viscosity (Classen and Bedford, 1991; Choct et al., 1996a). Thus, NSP degrading exogenous enzymes increase the AME of diets containing arabinoxylan and other NSPs (Tukei, 1998; Hughes et al., 2000). The quality of protein and starch used also plays a role in the AME of the diets.

Studies conducted to measure the ME of feed, and feed ingredients show that the variation in bird age (Zelenka, 1968; Guirguis, 1976; Peterson et al., 1976; Kussaibati et al., 1982; Sibbald, 1982), strain and species (Begin, 1969; Proudman et al., 1970; Pym and Farrell, 1977) cause variation in ME. The inherent nitrogen retention differences may contribute towards this. Nitrogen corrected AME (AMEn) converts data to a nitrogen equilibrium basis, which is useful for comparative purposes. Nitrogen correction eliminates differences in AME due to variability in growth and body protein accretion among birds.

Lopez and Leeson (2007) reported nitrogen retention in broilers and rooster for different feeding regimes (Graph 1).



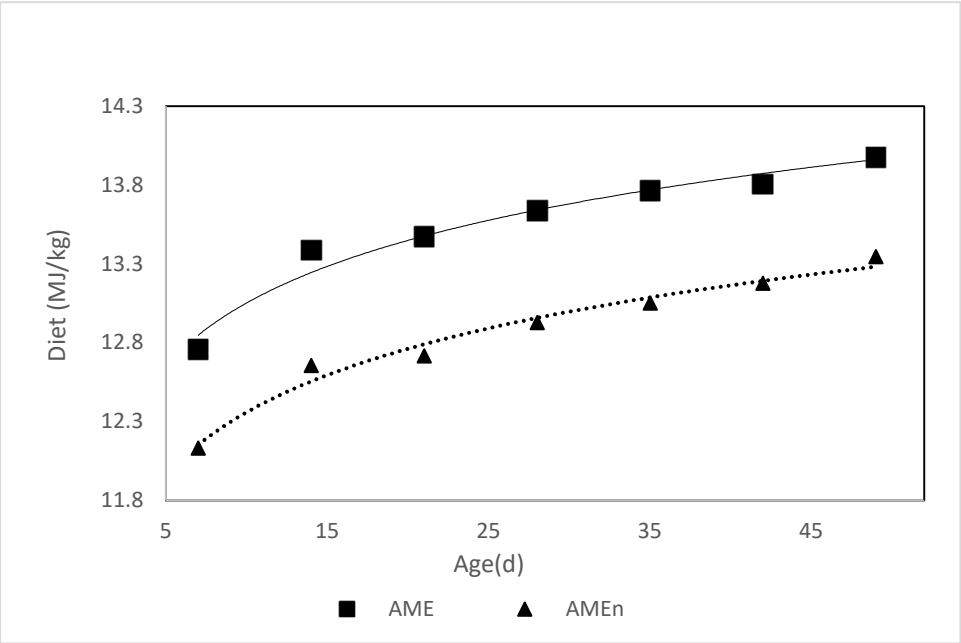
Graph 1 Nitrogen retained for male broilers and Leghorn roosters using different feeding programs.

(adapted from Lopez and Leeson, 2007).

The graphs show that nitrogen retention varies between broiler and rooster and with the diet regime. Nitrogen retention (NR) seems to be affected by age and diet.

Studies conducted by Lippens et al. (2002) showed NR in Ross 208, 308 and 508 were in the range of 42.7% to 59.9% of the total nitrogen intake. Unlike Lopez and Leeson (2007), Lippens et al. (2002) had failed to see a trend between percentage NR and age nor strain. It is important to note that Lippens et al. (2002) also reported that this study might have been affected by chick quality.

Nitrogen not retained is removed as uric acid by the birds. The nitrogen retention correction assumes that the oxidation of protein tissue yields uric acid, which has a GE per gram of N of 8.22 kcal (Lopez and Leeson, 2007). Sibbald (1982) reported an alternate value of 8.73 kcal/g of nitrogen adjustment, which was closer to the combustion energy of endogenous nitrogenous constituents of chicken urine. The correction value is added to the excreta energy for each gram of N retained, reducing the AME of the diet. Graph 2 below shows the AME and AMEn for male broilers in a study carried out by Lopez and Leeson (2007).



Graph 2 AME and AMEn for male broilers fed during different ages.

(adapted from Lopez and Leeson, 2007).

In the above study, there is minimal variation in protein accretion for the broilers with age. If the NR varies as little as seen in the study above, AME values may be a more appropriate measure of energy for commercial broiler nutritionist (Lopez and Leeson, 2007).

The NR adjustment in energy penalises high protein diets and ingredients. Lopez and Leeson (2008) showed that nitrogen correction for soybean meal had a higher penalty of 7 to 12% while maize was 3 to 5%. Their study also showed that formulating AME or AMEn both showed comparable performance; however, formulating using AME significantly lowered feed cost. Nitrogen correction is a tool to compare ME of ingredients at different protein levels (Leeson et al., 1977). However, it penalises the protein meals, which are generally higher cost and hence impact the feed cost. Further research is needed to determine the benefits of nitrogen correction.

2.4 Net energy

As animals use energy, to produce body heat and that energy lost. An AME system is not capable of accounting for losses of these chemical energies

(Pirgozliev and Rose, 1999). This heat loss, when subtracted from the ME, gives net energy which is the energy available for growth, maintenance and production.

Debated for many decades was the need to develop a NE system. Numerous studies conducted examined the benefit of NE system over AME. The results of studies testing equivalent systems to NE are conflicting, making it difficult to come to a unified approach to the best energy system to use for broiler feed formulation. AMEn is prefered to other energy forms for broiler diet formulation today.

In 2004, Daskiran et al. conducted a study on xylanase in maize-soy based diets, suggesting that the enzyme did not affect AME. However, carbohydrase improved NE without any change in AME. Studies like this highlight the limitation of the AME system and the need to further investigate the NE system.

2.4.1 Heat increment

Following ingestion, loss of energy as heat beyond the amount used to keep the body warm is referred to as the heat increment (HI) and expressed as MJ/kg DM. Heat increment substantiates the extent to which the bird utilises the ME of the feed, and hence the NE (Farrell, 1974; Reid et al., 1980; McDonald et al., 1995).

Koh and MacLeod (1999) studied the effect of feed intake, and the ambient temperature had on HI. Their results showed that HI increased with increasing feed intake and decreasing body temperature. HI is useful for the broilers when exposed to environmental temperature below thermo-neutral zone; however, is counterproductive during hot environmental conditions. Diet composition can also affect the HI of the bird. Heat increment is higher for diets using protein as an energy source than when carbohydrate or fat used as an energy source (Musharaf and Latshaw, 1999).

In 1978, a HI study conducted by Smith et al. showed that different organisms have different heat increments for fat, protein and carbohydrates. The study compared the HI for protein in fish with birds and mammals. The results showed that fish had lower HI hence higher NE for protein, making them more energy-efficient than birds and mammals when offered high protein diets. Heat increment may vary between organisms, nutrients and ingredients.

In 1939 Fraps and Carlyle recognised the importance of energy in feeding outcomes. They set up an experiment to measure productive energy (PE) which was the sum of calories in the gain and the calories used for maintenance. The energy ratios for PE/ME in their experiment range from 0.61 to 0.76. This result was not significant. The lack of statistical significance may be due to the small population size of the study as only a total of 60 chicks were used in the experiment as a 0.61 to 0.76 range in energy ratio is a vast range. The broad energy ratio range reported above by Fraps and Carlyle (1939) is not replicated since (Carré et al., 2002).

Fraps and Carlyle (1939) also showed that while ME of feed influenced the feed intake, the higher intake did not equate to higher weight gain. They had not exploited this observation. For this study, calculating the cost per weight gain might have been a total game-changer. The one thing that this study did highlight is the variability in the flock and the need for more robust mechanisms to measure net energy.

In 1974, De Groote conducted a study to compare NE and ME systems using the least-cost diet formulation to compare broiler performance and economic efficiency. The findings were in favour of NE diets with their greater economic returns.

Unlike De Groote (1974); Fraps and Carlyle (1939), Carré et al. (2002) and Van Der Klis (2010), conducted independent studies on a range of diets to compare the efficiency of performance using the metabolisable and net energy systems and they all failed to show benefits of using NE system.

Reduced HI improves the NE of the diet. Guo et al. (2011) conducted a study to reduce HI using the enzyme xylanase. In their study, xylanase addition led to reduced weight and relative proportion of active organs, including the gastrointestinal tract. This reduced heat increment, and the total cost of maintenance, improving the NE of diet.

Calculate total heat production using the Brouwer equation (Brouwer, 1965) and the respiratory exchange of the bird by indirect calorimetry and respiration chambers (McLean and Tobin, 1987; McDonald et al., 1995). The details of both

these procedures are discussed further in section '3.0 Methods of energy evaluation in the poultry industry today'.

When formulating to NE, with lower HI, it may minimise waste of nutrients, reduce feed cost, have more consistent carcass growth and reduce ammonia in the excreta (reducing carbon footprint) through the reduction of waste heat production. However, even though some poultry nutritionists may agree that NE is a better measure, they do not formulate to NE due to lack of accurate data on NE value of feed ingredients. More data is available on ME due to the ease of measurement. Even with the variation seen in ME (Black et al., 2005), it is often less variable and more consistent than NE (Farrell, 1999). The industry has since opted to use ME for broiler feed formulation.

2.4.2 Basal metabolic energy

All organisms consume feed to gain energy to support life functions including cell activity, blood circulation, respiration and all other functions required to survive. When an animal is in its thermo-neutral environment, awake and not under any stress, its energy requirement is the basal metabolic energy. Maintenance energy of an animal in a thermo-neutral, unstressed environment is equivalent to its basal metabolic energy requirement. The fulfilment of basal energy is imperative before spending energy on production goals. Energy spent in maintenance leaves the body as heat. Digestion and muscle activity in the body also produces heat (Farrell, 1974; Sibbald, 1982).

One of the challenges of the net energy system is to determine the basal energy requirement of the bird. One of the ways to determine basal energy would be to measure fasting heat production. If food is not passing through the gut, all heat generated at optimum environmental conditions correlates to the basal energy of the bird. However, the metabolic pathway for fasted birds is likely different from a well-fed bird (Sturkie, 1986; Spratt et al.,1990).

A study conducted by Spratt et al. (1990) showed that diet did not influence oxygen consumption in tissues however energy expenditure as a percentage of the total energy intake was 26% and 30% in fed and fasted hens respectively. Sturkie (1986) reported that during fasting, birds immediately mobilise hepatic carbohydrates to support normal blood glucose level. This observation indicates

that a change in metabolism occurs as a result of fasting, with fasted birds expending more energy than those fed. Spratt et al. (1990) showed that if fasted birds were used to measure the basal energy, it might give an overestimated value.

The other way to estimate the basal energy would be to calculate the mathematical asymptotes. Mathematical asymptotes were used by Labussière et al. (2011) when calculating the net energy in pigs. The asymptotic value of heat of products was determined to calculate the basal energy, which was referred to as the heat production at zero activity or fasting heat production (Labussière et al., 2011).

Buyse et al. (1998) reported fasting heat production for two strains of broilers to range from 646 to 724 kJ/kg$^{0.60}$ while the estimate by Romero's et al. (2011) was lower at 528 kJ ME/kg$^{0.60}$. Romero et al. (2011) suggested that the relationship between maintenance requirements and relative ME intake may not be linear beyond the values reported in their study and hence a need for caution when making any further comparison.

Hoffmann et al. (1991) showed that when the environment temperature decreased from 35ºC to 15ºC the maintenance energy increased from 433 kJ/kg LW $^{0.75}$/d to 693 kJ/kg LW $^{0.75}$/d, however, maintenance energy was not affected by the protein content of the diet. The relationship between environmental temperature and maintenance energy was parabolic in agreement to the observations of Romero et al. (2011) above. When maintenance energy requirement is similar to the basal energy, more energy is available to the bird for production.

More recently, Noblet et al. (2015), reported that there was a linear correlation between fasting heat of production (FHP) to the metabolic bodyweight of broilers. FHP was asymptotically determined per kg of BW$^{0.70}$ and ranged between 410 and 460 kJ/day. While Noblet et al. 2015 noted that the traditional value for body weight was 0.75, and stated that comparisons to the historical data were difficult due to change is measurement conditions and testing techniques.

2.4.3 Metabolic pathways

Broilers metabolise carbohydrates, fats and proteins to fulfil energy requirements. Glucose and monosaccharides are the products of break down of carbohydrates, glycerol, and fatty acids are products of fats (triglycerides), and amino acids are the products of protein. These digested products produce acetyl coenzyme A

during various stages of the metabolic pathways (glycolysis, citric acid cycle or the Kreb's cycle and electron transport chain). Acetyl coenzyme A produces adenosine triphosphate (ATP), energy currency for the body (Whitney and Rolfes, 2011). Figure 3 below summarises the metabolic pathway nutrients carbohydrate, fat and protein.

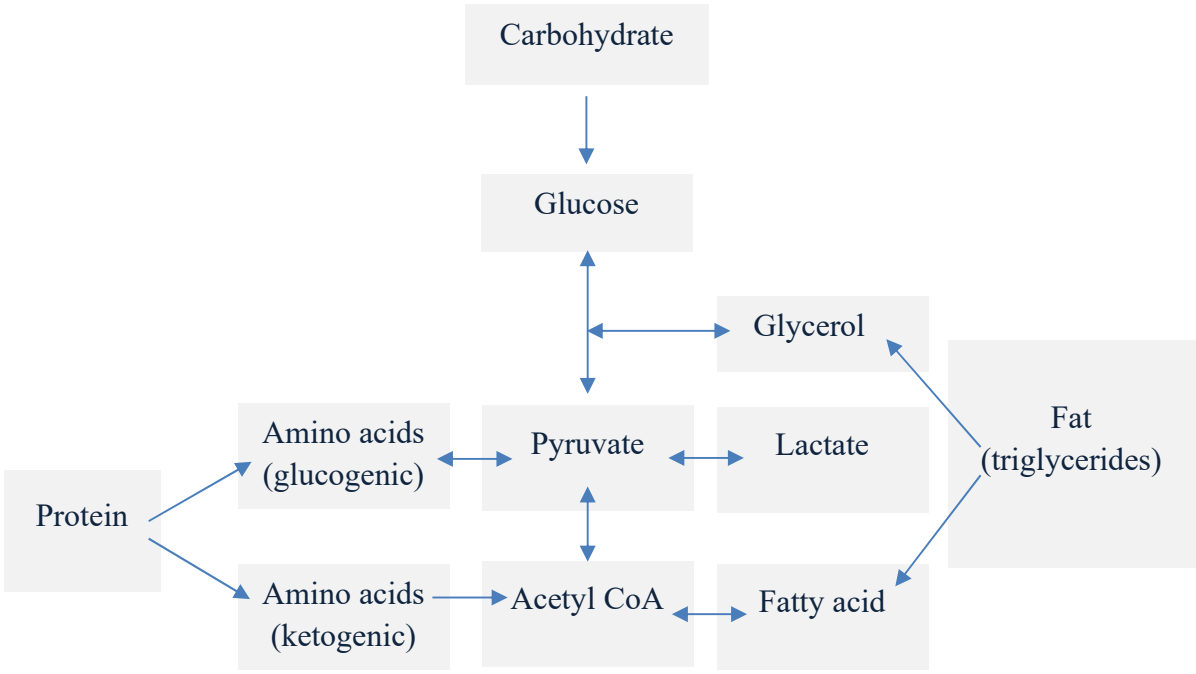


Figure 3 The metabolic pathway of nutrients.

Adapted from Whitney and Rolfes, 2011; Understanding Nutrition 12E. © 2011 Brooks/Cole, a part of Cengage, Inc. Reproduced by permission www.cengage.com/permissions.

Most of the reactions in the metabolic pathway are reversible reactions, and there is more than one way to get to any intermediate product in the path. The paths link the nutrients carbohydrate, fat and protein, creating a web with reversible reactions in the metabolic pathway (Whitney and Rolfes, 2011) suggesting that one could replace another in the energy chain.

Proteins, fats and carbohydrates can all be used in place of each other and as energy sources. These nutrients are not independent of each other. Capturing how different nutrients are changing in addition to the nutrient under investigation is imperative during a nutrition study.

The metabolic pathways are complicated with common end products which makes it difficult to trace which part of the path is changed. It may be beneficial to pinpoint performance to the specific path, to achieve the perfect balance of nutrients and hence cost-effective diets.

3.0 METHODS OF ENERGY EVALUATION IN THE POULTRY INDUSTRY TODAY

Apparent metabolisable energy as used in poultry formulation varies tremendously between ingredients and hence in formulated diets. Apparent metabolisable energy used to formulate poultry diet and yet the assay technique to test is not standardised.

Some of the commonly used techniques to determine AME are; bioassays (Hill and Anderson, 1958; Sibbald, 1980; Hartel, 1986), prediction via chemical composition (Fisher, 1982; Bourdillon et al. 1990; Carré, 1990), in vitro analysis (Valdes and Leeson, 1992a), near-infrared reflectance spectroscopy (Valdes and Leeson, 1992b; Black et al., 2010), and mathematical models (Emmans, 1994; Gous, 2010). However, to analyse a nutrient so variable, affected by variable interaction with an animal, is a challenge. In vitro analysis removes the animal interaction and endogenous losses but includes the variability due to ingredients. NIR predictions and equations do not capture the variability in ingredients. Using mathematical models, we get a step closer to the true picture as it considers the gut changes and the change in the ability of the chicken to use the energy more efficiently as it ages. However, the accuracy of these predictions is dependent on the accuracy of the database of AME values. Bioassays take a snapshot of the measurement at a given time.

Using the total collection method to determine AME is one of the commonly used technique by the industry. The technique assumes that the energy lost as a by-product of digestion is insignificant and is ignored (Sibbald, 1989). The equation represents apparent metabolisable energy:

$$\text{AME / g of feed} = [\ (Fi \times GE\ f) - (E \times GE\ e)\ ] / Fi$$

Where Fi is the feed intake (g);

E is the excreta output (g)

GE f represents the GE/g of feed

GE e is the GE/g of excreta, on a DM basis.

At the beginning and the end to mark the start and stop of data collection points the birds are starved, or an inert digestibility marker is included in the feed to identify the bioassay period (Miller, 1974; Sibbald, 1982). This technique is labour intensive.

The indicator method is an alternative to the above technique. The advantage of using the indicator method is that the total excreta output does not need to be measured (Miller, 1974; Sibbald, 1982; Fisher and McNab, 1989). Quantitative analysis of indicators in the feed and excreta gives a measure of the amount ingested. Chromic oxide (Cr_2O_3), titanium dioxide (TiO_2) and the acid insoluble ash (AIA) are some of the indicators commonly used. Chain alkanes, such as hexatriacontane ($C_{36}H_{74}$), has also been suggested by Choct and Hughes (1996).

Rapid methods of bioassays use adult cockerels for a comparative bioassay of the feeds. This method is more reproducible and less expensive than conventional methods which used young chickens (Farrell, 1978). The feed needs to be pelleted to ensure adequate feed intake by cockerels. However, the pelleting technique can introduce variability (Sibbald, 1985). Also, when using this technique, birds need to be trained for the specific feeding pattern, which can be laborious and time consuming (Schang and Hamilton, 1982).

Productive energy is a measure of NE for growth and not maintenance (McDonald et al., 1988, 1995), determined by a carcass analysis or comparative slaughter technique using growing chicks (Hill and Anderson, 1958; Farrell, 1974b; McDonald et al., 1988, 1995).

Maintenance energy requirement and thus, the PE values of the diet were derived using the equation below:

$$WM + G = FX$$

Where X is the production energy/unit weight of the feed

W represents the average chick weight

M is the maintenance energy requirement per unit of body weight

G is the carcass energy gain

F is the feed intake. (Hill and Anderson, 1958; Farrell, 1974b)

In the above estimation, the assumption made was that maintenance energy is constant for the range of body weights of interest. Since the maintenance energy requirement of growing chickens was not proportional to body weight, this assumption has been widely criticised (Hill and Anderson, 1958; Sibbald, 1982).

Effective energy (EE) was introduced by Emmans (1994), which considered the energy costs required to process a diet and hence accounts for the heat increment of feeding. It uses the digestible crude protein and the amount of carcass lipid synthesised from dietary lipids, both of which are a challenge to determine.

Heat increment of the feed measured using animal calorimetry is a direct calorimetry technique. It measures the heat given off during feeding. The other way to measure heat increment is indirect calorimetry where the oxygen consumed and carbon dioxide expired are used to calculate the heat increment for a given time frame in a set environment (Farrell, 1971; Fuller et al., 1983).

Indirect calorimetry can be an open system or a closed system. In an open system, to determine oxygen inhaled and carbon dioxide exhaled, the gas flow rate and the concentration of both gases at the inlet and outlet are measured. Differences between the inlet and outlet oxygen and carbon dioxide concentrations and flow rate used to calculate oxygen consumption and carbon dioxide generation. Some systems even trap the carbon dioxide at the inlet. Fuller et al. (1983) had used an open-circuit calorimeter to determine NE and concluded that results were accurate and supported the data generated using a closed-circuit system by Farrell (1971). The study concluded that heat production could be successfully measured using the gas exchange.

The drawback of an open system is that the amount of air expired by the bird is generally minimal when compared with the volume of the chambers, e.g. Fedde (1993) measured that a 1.6 kg bird expired a total volume of 759 mL per minute. Freeman (1984) calculated that chickens breath contained 5% carbon dioxide. Choct (2012) calculated that for a 100 cm x 70 cm x 65 cm net energy chamber, the expired air would be 0.167% of the chamber volume. Hence carbon dioxide would occupy 0.00835% of chamber volume provided the air in the chamber is homogenous. The sensitivity of the testing technique (Arch et al., 2006) may

present a limitation if a snapshot of the air composition is measured. Data should be collected over a long period, while ensuring that the gases equilibrium within the chamber remains unchanged, to reduce the analytical limitation.

A closed system is not open to the outside air. Oxygen flows into the system and is measured gravimetrically. Carbon dioxide is trapped and removed as the air is drawn out of the chamber activating a pressure switch which feeds more pure oxygen into the chamber. Apart from getting the pneumatics working, making the chamber airtight is the major challenge for this system. Farrell (1971) had designed a closed-circuit calorimeter constructed of Perspex. The estimation of heat production by carbon and nitrogen agreed. The fat and nitrogen content of tissues were also in agreement with numbers predicted using the chamber. The chamber was effective in measuring heat production.

The systems used to measure NE discussed above all have a size limitation. To build a laboratory unit is an economical option however with the current knowledge of bird variation (Hughes and Choct, 1997; Mignon-Grasteau et al. 2004; Lopez and Leeson, 2007) it would be a concern if the small population size would give a correct picture of the NE values. The interference caused by bird variation may play a more significant role in the study, as seen in a similar study carried out by Carré et al. (2002). More replicate runs need to be considered for laboratory units to overcome the bird variation.

Another challenge may occur when formulating nutritionally equivalent diets. In 1998, Tukei formulated two diets to compare heat production in maize versus barley. When formulating Tukei simply substituted maize with barley, while the remaining ingredients were kept unchanged. The nutritional difference between the two diets due to this change of ingredients not considered. Table 3 below shows the raw material composition of the maize and barley diets formulated by Tukei.

Table 3 Maize and barley diets formulated for measurement of HP.

Ingredient (g/kg)	Diet 1	Diet 2
Maize	800	
Barley		800
Dicalcium phosphate	20	20
Limestone	11	11
Salt	5	5
Choline chloride	2	2
Premix*	5	5
Casein (dried)	150	150
D, L methionine	7	7

* The active ingredients contained in each kg of the vitamin-mineral premix were as follows: retinol 3.03 mg, cholecaliferol 0.09 mg, all-racalpha-tocopherol acetate 20 mg, menadione 6.3 mg, riboflavin 8 mg, pyridoxine hydrochloride 5 mg, biotin 0.01 mg, niacin 30 mg, Fe 20 mg, Cu 5 mg, I 1 mg, Co 0.3 mg, Se 0.5 mg, Mn 0.16 mg, cyanocobalamin 0.15 mg.

Adapted from Tukei (1998)

Tukei (1998) had a simple model to compare maize versus barley. The results showed that the birds offered the barley-based diets were inefficient and lost energy as volatile fatty acid in the excreta while maize had a higher NE. However, replacing maize at 8.6% crude protein and high in Arabinoxylan with barley at 11.5% crude protein and high beta-glucans makes both diets nutritionally different. That being the case, it is debatable if the difference observed in the study was due to the ingredient differences or nutritional differences.

Determining NE of raw material is challenging. A single ingredient if offered to chickens would not be nutritionally balanced, and hence metabolic functions of

the bird would be affected. Diets are nutritionally balanced using a combination of raw materials. However, using a combination of raw materials presents a challenge to determine the NE contribution due to any one ingredient. When using purified nutrients to substitute raw materials, the ingredient interactions are lost. Hence as seen in the above study by Tukei (1998), despite appearing to be simple, measuring NE of individual ingredients is not.

Net energy can be determined using equations and chemical analysis of the diets. Some of the equations published to determine the NE of raw materials are below:

$$NE = 13.4\ digCP + 35.3\ digEE + 13.0\ digNFE$$

Where digCP is digestible crude protein

digEE is digestible ether extract

digNFE is the digestible nitrogen-free extract (De Groot, 1974)

$$NE = 10.8 dig\ CP + 33.5\ digEE + 13.4\ (digNFE + digCF)$$

Where digCP is digestible crude protein

digEE is digestible ether extract

dig is digestible nitrogen free extract

digestible crude fiber (Hoffmann and Scheimann, 1980)

$$EE\ (kJ/g) = 1.17ME - 4.2\ crude\ protein - 2.44$$

Where EE is effective energy kJ/g

ME metabolisable energy kJ/g (Emmans, 1994)

$$NE{:}ME = 0.9151 - 0.0053\ crude\ protein\ (\%DM) + 0.00295\ fat\ (\%DM) - 0.0069 \times insoluble\ NSP\ (\%DM)$$ (Swick et al., 2013)

NE = 239 × (0.164 × 0.760 × crude protein (%) + 0.310 × 0.862 lipids (%) + 0.162 × 0.797 starch (%) + 0.079 × 0.633 sugars (%) (Carré et al., 2014)

Hilton et al. (2019) used a respiratory chamber and a dual-energy X-ray absorptiometry to determine NE.

$$\text{Ark NE (kcal)} = \text{NEg} + \text{NEm},$$

Where NEm (net energy maintenance) = HP – HI.

$$\text{NEg (net energy of gain)} = \text{protein grams} \times 5.66 + \text{fat gain grams} \times 9.35$$

$$\text{Classic NE (kcal)} = \text{ME} - \text{HI}$$

Where HI = HP - FHP

Ark NE, compared against the classic NE system, supported protein-calorie gain compared to fat calorie deposition supported by classic NE system (Hilton et al., 2019).

While methods various methods are used to determine AME and NE, there is yet no standardised method globally accepted for the determination of these.

In addition to that, energy systems used to formulate feed, work on the assumption that the energy content of the raw materials added equates to the energy of the diet. However, the total energy in the feed may or may not be the sum of energies of the ingredients. The anti-nutritional factors in some ingredients may change the nutrient availability in other ingredients. Further to this, ME and NE interact with the animal, environment and all the ingredients in the feed. The variance that exists within the population of chickens is also well known. The linear formulation techniques do not consider these factors; hence the chemical equations and the current formulation strategy used may need to be challenged.

4.0 HISTORY OF GLOBAL FEED FORMULATION

The domestication of animals occurred 12000 years ago, while domestication for food did not occur until 9000 -7000 BC (Gascoigne, 2001). With domestication came the responsibility to manage the feeding of the animal and gradually as animal husbandry changed from passive to more profit oriented, nutrition study became scientific.

Albrecht Daniel Thaer developed one of the earliest feed standards identified in 1810 (Kleiber, 1940). Thaer used a nutritive equivalent technique, assigning feedstuffs an equivalent value to hay to achieve comparative performance. The nutritive equivalence was determined using feeding trial and no knowledge on the nutrient content of the feedstuff. The farmers used this nutritive equivalence to get greater financial returns during the period, which reported a shortage of hay.

The feedstuff nutrient profiles became available with the advancement in the chemical analysis, which occurred in the mid-1800, led by German scientists. This advancement in chemical testing trigged initiation of feed formulation based on proximate analysis data. Dr E Wolff (1864) printed the first feed standards using the digestible nutrients, protein, fibre, carbohydrates and fat. This standard was gradually accepted and used by the Americans, and other printed materials were published.

The change occurred in 1915 when W. A. Henry and F. B. Morrison wrote about net energy in their book ‘Feeds and Feeding’. They described net energy as useful energy which remains after masticating, digesting and assimilating food and used by the animal for their organ functions, body maintenance, growth, fat, milk and wool. Henry and Morrison published net energy data from the work of Kellner, working with respiration chamber, and Armsby, using respiration calorimeter. At that stage, studies on net energy had only just begun, and data was limited. They printed net energy of nutrients; fat (peanut oil), protein (wheat gluten) and starch, and feedstuffs; cornmeal, hay and wheat straw for ox (Henry and Morrison, 1915).

Requirements for vitamins and minerals which was lacking in the feeding publications attracted attention in the following years hence initiating studies in these areas. In 1942 Committee on Animal Nutrition of the National Research Council (NRC) in the US, summarised nutrient requirements for farm and

laboratory animals in tables. These printed as the NRC standard in 1944 was used as a global standard for feed formulation for animals, including poultry. Later the suppliers of the broiler strains started to publish standard nutrient requirements for the best performance of their breeds. Some companies nowadays are maintaining an up to date database on ingredients for feed formulation, which may not be up for sale.

The earliest energy systems used in broiler diets was as PE proposed by Fraps (1946). Mraz et al. (1957) formulated diets using Fraps PE data and determined that 0.75 cal/cm^3 to 0.79 cal/cm^3 of diet was adequate for the maintenance requirement of a growing broiler. Studies published by Titus (1956), Davidson et al. (1957) and Hill and Anderson (1958), showed that ME was more precise than productive energy. Hill and Anderson (1958), did not see an effect on the feed intake at various levels of ME and PE. They established that PE was 75% of ME (cal) and used the equation below for ME:

$$\text{ME/g of dry matter} = \text{E diet} - \text{E excreta} - 8.22\ \text{N}$$

N (nitrogen retention/g of diet dry matter) =

$$\text{Nitrogen / g of diet} - \text{nitrogen /g of excreta} \times \frac{Cr_2O_3\ \text{/g diet}}{Cr_2O_3\ \text{/g excreta}}$$

Titus et al. (1959) used the following equation to determine ME:

$$ME = Hd - \left[\frac{He \times Cd}{Ce} + 0.0873^{\#}\ \{Nd - \frac{Ne \times Cd}{Ce}\}\right]$$

Where ME is metabolisable energy/g

He is the heat of combustion of 1 g of excreta

Hd is the heat of combustion of 1 g of diet

Cd is % chromic oxide in the diet

Ce is % chromic oxide in excreta

Nd is % nitrogen in the diet

Ne is % nitrogen in excreta

[#] 8.73 is the average heat of combustion (kcal) of urinary nitrogen compounds per gram of urinary nitrogen.

A nitrogen correction factor of 8.73 kcal/g (Titus, 1956) or 8.22 kcal/g (Hill and Anderson, 1958), as shown above, brings AME to a basis of zero-nitrogen retention. Nitrogen retention of a bird is assumed to be 20% of body weight gain or loss, divided by nitrogen factor of 6.25.

AME (kcal/kg) as is =
[(GE feed x feed consumed) – (GE excreta x excreta)]/feed consumed

AMEn (kcal/kg) as is =
[(GE feed x feed consumed) – (GE excreta x excreta) – (NR x K)]/feed consumed

NR (nitrogen retention) is 20% of body weight gain/loss divided by 6.25

K 8.73 kcal/g (Titus, 1956) or 8.22 kcal/g (Hill and Anderson, 1958)

In 1966, Harris introduced the concept of TME, which was supported by further work conducted by Guillaume and Summers (1970). Sibbald (1976) later developed practical bioassay technique for TME.

Jansman et al. (2004) used a new technique based on ATP yield of carbohydrates, amino acids, glycerol, fatty acids, and volatile fatty acids to derive a NE formula. The NE_{ATP} calculation is:

NE_{ATP} kJ/g = 9.7 x dig. True protein + 26.1 x dig. Crude fat + 11.7 x dig. Starch + 10.6 x dig. Sugars + 8.2 x dig. Nitrogen-free residue

Further development of the above equation, using comparative slaughter technique and regression analysis, to determine NE_{ATP} requirement in kJ/d for male and female broilers, is shown below:

NE_{ATP} (req) = 278 kJ $NE_{ATP}/BW^{0.75}$/d x $BW^{0.75}$ + 3.058 x energy in protein deposition (kJ NE_{ATP}/day) +1.053 x energy in fat deposition (kJ NE_{ATP}/day)

During the validation study of restricted feeding of broilers, Jansman and Van Diepen (2008), did not find any benefits of NE_{ATP} over AME.

In 2006 Gous released mechanistic modelling in pig and poultry to use performance prediction equations to formulate the diets.

The debate on NE versus ME continued. For ad libitum feed intake, Carré and Juin (2015) reported NE: ME as 76.4% while Yang et al. (2008) had reported it as 70.5%. Diet composition, breed, sex and age were some of the factors which contribute towards this difference.

After formulated more efficient NE diets for pigs in 2009, Noblet et al. formulated poultry diets for NE. Later, like observations made by Carré et al. (2002), Noblet et al. (2010) reported that there was no benefit of using NE over ME for poultry. There was no interaction seen between NE and crude protein (Noblet et al., 2007) nor NE and fat (Noblet, 2009). These findings contradicted the observations made by Wu et al. (2019), who reported correlations between NE and AME, crude protein and fat. Wu et al. (2019) determined NE of diets in a closed-circuit indirect calorimetric chamber and using linear regression derived an equation to determine the NE of the diets.

Wu et al. (2019) and Swick et al. (2013) used a closed-circuit indirect calorimetric chamber to determine the net energy of diets and derive an equation for predicting NE of raw materials. Liu et al. (2017) determined the net energy of maintenance by the indirect calorimetry method (ICM) and the comparative slaughter method (CSM) and reported both techniques as reliable.

While ICM and CSM are both accepted techniques to determine NE, the difference in values reported between these techniques, and the variance within the technique limited the use of NE to formulate poultry diets.

Hilton et al. (2019) combined the use of dual-energy X-ray absorptiometry and respiratory chambers to measure ARK NE, targeting to formulate for protein-calorie gain and hence performance. ARK NE seems to be closest to predicting broiler performance.

5.0 NET ENERGY FOR PIGS VERSUS POULTRY

Feed and ingredient energy evaluation in pigs is on their DE or ME value (NRC, 1988; INRA, 1989; Noblet and Perez, 1993). Further studies with pigs have shown that the utilisation of DE or ME is not constant and is affected by dietary chemical characteristics, age and environmental conditions (Schiemann et al., 1972; Just, 1982 and Hoffmann et al., 1990). Since NE is the 'true' energy value, it emphasises the need to formulate pig diets based on NE. Net energy prediction equations for pigs proposed for various stages of production uses the chemical composition of diets (Schiemann et al. (1972), Just (1982) and Noblet et al. (1993, 2013). In a report by Noblet (2007), different energy systems when compared showed that formulating using NE changed the hierarchy of ingredients and resulted in higher return in performance.

Noblet et al. (2003a) studied the difference in energy utilisation between pigs and broilers to show that pigs and broilers responded differently to diet composition. Warpechowski et al. (2004) drastically made starch and fat contents of the diets different to see a significant change and yet observed a minimal difference in the utilisation of ME for the broilers. A study reported by Wiseman et al. (1998) further showed that the DE values for pigs were consistent for all ages, which were comparable to adult poultry while these were different for young chicken. Pig diets formulated using NE could predict performance with greater accuracy (Noblet, 2010). The studies above show that while there was a benefit of using NE to formulate pig diets, there was no benefit when NE was used to formulate broiler diets. In 2010, Noblet et al. summarised the benefits of NE of pig diet formulation while necessitating further investigation required for poultry to explain the differences observed in the broiler response to NE.

6.0 CONCLUSION

While numerous studies have measured energy at the various stages of energy partitioning, there is no internationally accepted standard for the testing techniques of AME and NE. In addition to this, the difference in the laboratory testing values and variation in the bird's population makes it more difficult to achieve comparable data across various research.

Net energy may be a closer measure to the production targets of broiler chickens, but this needs further research to be confirmed.

Lack of data and variation seen in the NE reports is causing a challenge to accept this for formulating broiler diets. ME does not consider the heat increment, which is an essential factor to consider when targeting performance.

A lower dietary HI improves the energetic performance of birds.

Chapter 2: PERFORMANCE COMPARISON BETWEEN DIETS FORMULATED USING AME AND NE

ABSTRACT

Diets were formulated using AME and NE values and then fed to broilers to determine if there would be differences in live weight or FCR. Two diets formulated had the same amino acid and AME ratio but had different nutrient density. A mixture of these two diets, used to create two more diets, had varying nutrient densities. The hypothesis was, birds fed high-density diets based on AME formulation would have higher weight gain, lower FCR and lower feed intake when compared to birds fed a low-density diet based on NE formulation. A common standard starter diet was fed to 610 Ross 308 day old male chickens reared in floor pens. On d 10, 12 pens, each with 12 chicks, were assigned to each of the four diets. The layout was a completely randomised design.

The results showed that live weight of birds was similar when offered diets of different nutrient density. At the early age of the grow-out, higher density AME diet had lower FCR while there were no significant differences in feed intake of the broilers offered various diets. However, birds fed high density AME diet had higher feed intake at a later age, d 25 to 35.

Both NE and AME could be used to formulate broiler diets. The energy system used for feed formulation did not affect the carcass (thigh, breast and fat pad) yield or flock variation.

INTRODUCTION

The need to provide broiler chicken with adequate energy diets for a gain in performance has long been recognised (Batterham, 1990) and continued researched in current times. While consensus is on the need for adequate energy, there are differences in opinion on the energy system to be used for feed formulation in poultry.

Net energy and AME are the two energy systems usually debated when formulating broiler diets. The difference between AME and NE is the heat increment (HI) which, when removed from AME gives the NE available for production.

There are many studies conducted to show the benefits of one energy system over the other. In 1974 De Groote had formulated series of diets with AME ranging from 12.55 MJ/kg to 14.23 MJ/kg and net energy range of 9.04 MJ/kg to 10.71 MJ/kg to study the difference between the two energy systems. To create the diets, De Groote used a whole range of ingredients and was aware that this had introduced an additional variable in the study. In the study, De Groote successfully formulated an optimum AME and an optimum NE diet. During the study, both energy systems were successful in feed formulation, and the formulation predictions resulted in different diets for the two systems. De Groote reported that broilers offered NE diet had significantly higher weight gains of 20 to 30 g, at 4 and 6 weeks of age, giving a higher financial return when compared to birds offered AME diets. In this study, De Groote did not measure the energies of diets and used calculated values.

Years later, Noblet et al. (2009) formulated diets with a marked difference in fat levels (2.7 and 9.6%) in AME diets and measured AME and NE of the diets using respiratory chambers. Noblet et al. (2009) observed no difference in the NE/ME ratio of the diets and concluded that there was no superiority of NE over the AME system for diet formulation. Later Noblet et al. (2010) reported that they were unable to detect significant NE/ME differences for diets that had markedly different chemical composition. By this time Noblet's team had successfully shown the benefit of using NE system when formulating pig diets. While the nutrient ranking between the two species, pigs and poultry, were the same, the difference seen between the nutrients was smaller in poultry. Hence Noblet et al. 2010 did not observe the benefit of using NE for broiler feed formulations. Carré et al. (2002) had also faced similar struggle and failed to show differences in NE of the diets predicted to be different. In their study, they also failed to see significant differences in NE/ME ratio. The variation in the assay was contributing towards this lack of differentiation.

This current study replicates some of the work done by De Groote (1974). Using AME and NE systems created least-cost diets. Measured the performance of broilers offered these diets to determine the differences between formulating using AME and NE.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England, approved this experiment conducted at the animal house (W002) at the University of New England, Armidale.

Two grower diets formulated to meet Ross 308 nutrient specifications (Aviagen, 2007) were pelleted. The first diet formulated used AMEn as per the Aviagen nutrient specification, control diet, while in the second diet, NE replaced AMEn, test diet. The control grower diet, GME, (treatment 1), formulated for 13.15 MJ/kg AMEn, was a high nutrient density feed. The test NE grower diet, GNE, (treatment 4), formulated for 10.26 MJ/kg NE, was a low nutrient density feed. Diets of varying nutritional density were created by mixing these two diets. Two new diets were created by combining different ratios of the two original diets; treatment 2; 60% GME and 40% GNE and treatment 3; 40% GME and 60% GNE.

The equation below was used to calculate NE.

NE = 0.808 AMEn MJ/kg DM - 0.017 crude protein % DM + 0.031 crude fat % DM (Wu et al. 2019)

The ratio of amino acids to AMEn and NE to AMEn were the same for both diets. The same ingredients and prices were offered to both diets when formulating.

Similarly, a control finisher diet, FME, was formulated to 13.39 MJ/kg AME and a test finisher, FNE, formulated to 10.45 MJ/kg NE. Two new finisher diets were created by mixing different ratios of the two original diets; treatment 2; 60% FME and 40% FNE and treatment 3; 40% FME and 60% FNE. Table 4 shows the costs of the major ingredients.

A common standard starter diet (shown in table 5) was fed to 610 Ross 308 day old male chickens until d 10. Twelve pens, each with 12 chicks, were assigned to each of the four diets. The arrangement of treatments was a completely randomised design.

On day (d) 10 the birds were switched to one of the four grower diets, each with 12 replicates. On d 24 the grower diets were switched to the corresponding finisher diets. Table 5 shows the composition of the main grower and finisher diets.

On d 10 and d 34 each bird was weighed to determine the flock variation. On d 25 and d 34 live weight and feed intake were measured. FCR calculated as:

FCR = Kg of feed consumed / kg live weight.

On d 35 for every pen, five randomly sampled, euthanised birds, were used to weigh the breast meat, thigh and fat pad.

Statistical analysis

PROC GLM was used to determine the significance of diets. Analysed data for one-way ANOVA.

Table 4 The cost of major ingredients used for feed formulation.

Raw materials	$AUD/t	AME (kJ/kg)	NE (kJ/kg)
Maize	280	13.8	11.7
Wheat	260	13.0	10.9
Canola seed	635	21.8	30.8
Soybean meal	650	10.0	7.9
Meat meal	655	10.3	10.8
Canola oil	1190	37.2	30.9
Limestone	250		

Table 5 Raw material composition of the diets and their nutritional profile.

Ingredients (%)	Diets				
	Starter	Grower GME	Grower GNE	Finisher FME	Finisher FNE
Wheat	61.9	66.8	59.1	72.4	63.9
Maize			13.4		15.0
Soybean meal	22.5	16.1	16.5	10.7	10.4
Canola seed	4.8	6.0		6.0	
Meat and bone meal	8.0	7.6	7.8	7.5	7.7
Canola oil	1.0	1.8	1.6	2.0	1.5
Limestone	0.47	0.35	0.34	0.35	0.34
Xylanase	0.005	0.005	0.005	0.005	0.005
Salt	0.108	0.048	0.037	0.047	0.036
Sodium bicarbonate	0.104	0.200	0.200	0.200	0.200
L-lysine HCl	0.381	0.344	0.347	0.315	0.393
D, L-methionine	0.357	0.357	0.304	0.222	0.232
L-threonine	0.210	0.180	0.181	0.141	0.144
Sacox® 120****	0.050	0.050	0.050	0.050	0.050
Premix***	0.130	0.130	0.130	0.130	0.130
Calculated Nutrient composition					
AMEn MJ/kg	12.66	13.15	12.99	13.36	13.17
NE MJ/kg*	8.98	10.55	10.40	10.78	10.58
Crude protein %	23.9	22.0	22.2	20.1	20.3
Dig** lysine %	1.27	1.1	1.06	0.96	0.98
Dig methionine & cysteine %	0.94	0.84	0.81	0.73	0.70
Dig arginine %	1.34	1.14	1.10	0.99	0.95
Dig threonine %	0.83	0.73	0.70	0.63	0.61
Dig isoleucine %	0.85	0.75	0.72	0.67	0.64
Dig valine %	0.99	0.87	0.84	0.78	0.75
Dig tryptophan %	0.24	0.21	0.20	0.18	0.17
Calcium %	1.05	0.84	0.83	0.82	0.81
Available phosphorous %	0.50	0.42	0.41	0.41	0.41
Sodium %	0.16	0.16	0.15	0.16	0.15
Chloride %	0.23	0.18	0.19	0.18	0.19
Crude protein analysed %	25.3	23.7	23.1	21.6	21.4
Crude fat analysed %	4.5	8.7	8.8	9.4	9.1

*Calculated using the equation of Wu et al. 2019. ** Dig digestible

*** Vitamins and mineral mix supplied the following amounts per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg

****Sacox® 120 - 120 g/kg sodium salinomycin

RESULTS

Shown in table 6 are results on feed intake, live weight, FCR and carcass yield.

The feed intake was significantly higher for the high density AME diet from 25 to 34 days of age ($P < 0.05$); however, there was no significant difference in feed intake at an earlier age.

Broilers offered diets formulated for AME and NE both had higher weight gain than the Ross 2009 target. The Ross target live weight was 2.147 kg while birds weighed in the range of 2.158 kg to 2.203 kg on d 35.

The Aviagen target FCR was 1.546, which was higher than the observed range of 1.487 to 1.509 during the study. During the early age between 10 to 25 days, the FCR of the birds offered high-density AME diet (treatment 1) was significantly lower than birds offered the low-density NE diets ($P < 0.01$). The diets did not cause a difference in FCR at the later age of broilers.

The flock variation with the birds offered AME diet was the same as birds offered NE diet ($P > 0.05$). The breast yield and thigh yield for all treatments were also similar and had similar variation (C.V. for the pool were not significantly different). The fat pad was significantly different ($P < 0.05$) with treatment 3 (which was a mix of 40% AME: 60% NE diet) having the largest fat pad.

Table 6 The effect of dietary treatment on broiler performance parameters.

Item	Trt 1*	Trt 2*	Trt 3*	Trt 4*	P-value	SEM
Feed intake (g/bird)						
10 to 25 days	1485	1527	1544	1535	*NS*	8.58
25 to 34 days	1786[b]	1760[b]	1757[b]	1694[a]	0.016	10.83
10 to 34 days	3272	3287	3301	3229	*NS*	14.49
Weight gain (g/bird)						
10 to 25 days	1110	1128	1113	1103	*NS*	4.78
25 to 34 days	1090	1075	1075	1056	*NS*	5.58
10 to 34 days	2201	2203	2188	2158	*NS*	7.86
C.V.[#]	*7.22*	*7.17*	*8.02*	*7.03*	*NS*	
FCR (feed/gain)						
10 to 25 days	1.338[a]	1.355[b]	1.387[c]	1.392[c]	0.009	0.006
25 to 34 days	1.638	1.637	1.635	1.605	*NS*	0.007
10 to 34 days	1.487	1.492	1.509	1.496	*NS*	0.005
Carcass yield % of live weight @ d 35, *n* = 240						
Breast	20.3	20.4	20.5	20.7	*NS*	
Breast C.V. %	*7.81*	*8.64*	*8.07*	*7.61*	*NS*	
Thigh	11.1	11.1	11.2	10.9	*NS*	
Thigh C.V. %	*6.05*	*7.30*	*6.95*	*7.35*	*NS*	
Fat pad	1.09[b]	1.16[b]	1.21[a]	1.12[b]	0.03	
Fat C.V %	*20.06*	*20.50*	*20.63*	*21.53*	*NS*	

** Trt 1 – GME / FME; Trt 2 – 60% GME 40% GNE / 60% FME 40% FNE;*
Trt 3 – 40% GME 60% GNE / 40% FME 60% FNE; Trt 4 – GNE / FNE
NS not significant (P > 0.05); Figures having different superscripts were significantly different (P < 0.05)
#Coefficient of variation (standard deviation/mean) of the flock.

The cost of the AME diet was higher than NE diet, but the live weight gain by the birds on both these diets were similar. Since NE diet was cheaper than AME diet, equal live weight gain made the NE diet significantly ($P < 0.01$) cost-effective. Production cost for NE diet was \$0.59/kg live weight when compared to birds offered AME diet costing \$0.62/kg live weight. Table 7 shows a summary of the costs per kg live weight for the various diets.

Table 7 Feed cost analysis when formulating using AME vs NE.

Item	Trt 1*	Trt 2*	Trt 3*	Trt 4*	P-value
Grower diet \$/kg	428.66	421.36	417.72	410.42	
Finisher diet \$/kg	404.18	395.4	391.01	382.23	
Grower diet \$/10.46 MJ NE	428.32	424.35	422.34	418.28	
Finisher diet \$/10.46 MJ NE	396.25	390.90	388.18	382.69	
Cost per kg live weight (\$/kg)	0.62[a]	0.61[a]	0.61[a]	0.59[b]	0.004

Trt 1 – GME / FME; *Trt 2 – 60% GME 40% GNE / 60% FME 40% FNE;*

Trt 3 – 40% GME 60% GNE / 40% FME 60% FNE; *Trt 4 – GNE / FNE*

DISCUSSION

The results of this study agreed with the findings of De Groot (1974).

When formulating least-cost diets for AME versus NE, the ingredient hierarchy changes, and the database selects to use different raw materials. On this occasion, NE diets used maize and AME diets used canola seed while maize was missing in the AME diet and vice versa. When formulating using NE the way the formulation software values ingredients differs when compared to the least-cost formulation for AME. This result is consistent with findings reported by De Groote (1974) and Noblet (2007). The ingredient hierarchy and hence its usage is dependent on the ingredient market price and nutritional profile. Hence, the dynamics of the current ingredient market may change if the industry chooses to change from AME system to use the NE system for feed formulation.

Even with the use of different ingredients, the performance of birds on both NE and AME diets were comparable at 35 days. Since the NE diets were cheaper than

the AME diets, there was a cost-benefit per kilogram of meat for NE diet. NE diet may not always be cheaper as it depends on the ingredient costs to determine which diet is cheaper. However, within the range of AME and NE tested, both energy systems balanced to other nutrients, can be used to formulate diets for optimum performance.

The current reality in the stock feed industry is that the nutritionist formulates to use ingredients available to them. The diet consists of grains, vegetable proteins, animal proteins, fat sources and minerals. Having the option of using more than 2 or 3 grains is rare. For example, wheat, barley and sorghum are the grains commonly found in Australia. Maize may be available in some locations but is rare and may not be available in adequate quantities.

The ingredient availability and cost are of greater importance in diet formulation than the difference of formulating using NE versus AME. With more options available for ingredients, the difference between NE and AME diets is greater.

Since NE is closer to 'true' energy, there is speculation that flock variation reduces when formulating using NE, as seen in pigs (Cadogan et al., 2005). However, in this study, there was no difference in the coefficient of variation for live weight, breast meat yield, thigh yield and percentage fat pad for birds offered NE versus AMEn diets. Similar observations were made by Cerrate et al. (2013) when both NE and AME energy systems showed similar variability in their study.

Broilers fed both diets formulated for NE and AMEn gave similar performance. Similar to the findings of Carré et al. (2002) and Noblet, et al. (2009, 2010) formulating diets with significantly different measured NE levels seems difficult. At constant AME, the change in NE due to fat and crude protein is restricted.

The AMEn to amino acids ratio in both diets were the same, and birds had comparable performance in feed intake, weight gain and FCR at 35 days of age. However, responses in the younger broilers were different. The FCR was lower for AME diets ($P < 0.01$) which was at a higher nutrient density only until d 25. Wiseman et al. (1998) also noted the difference in the response of the younger versus older broilers. During their study on DE, older broilers and pigs of all ages had similar results, but these were significantly different from younger broilers.

The various ratios of the diet showed that until d 25 the birds responded to diet density which correlated with FCR. Growth, however, was similar at all ages, consistent with observations reported by Carré and Juin (2015). They predicted FCR using linear regression and determined that growth had a higher correlation with water-insoluble cell wall content rather than AME in the diet. Further to this, comparing R^2 values of the regression for FCR and growth, lead Carré and Juin (2015) to speculate that the variability among individuals was higher for growth than for FCR. Comparative performance for all diets suggests that a high nutrient density in earlier broiler diets may be of greater impact to FCR than using high-density diets later.

During d 10 to 24, the feed intake for treatment 1 was the lowest (P value not significant) while the birds offered this diet also had the lowest FCR ($P < 0.05$). At 25 to 34 days, birds offered treatment 4 had the lowest feed intake ($P < 0.05$) and FCR (P value not significant). An increase in feed intake did not eventuate in FCR improvement in birds. Further investigation is required to establish what is causing the birds' response to the feed intake.

There was no difference observed in breast yield, nor thigh yield for broilers offered diets formulated for NE or AME. Relative fat pad, however, showed a higher yield for treatment 3 (2GME: 3GNE). A significant correlation can explain this atypical result with the weight of birds selected ($P < 0.001$) and no significant correlation with the treatment. Hence the higher fat pad in treatment three may be due to bigger birds selected for carcass work in this treatment.

CONCLUSION

The results observed in this experiment were identical to those of De Groot (1974), showing birds fed NE diet had a higher financial return with a lower cost per kg live weight.

If industry changes to net energy for feed formulation, the procurement strategy for ingredients may change as formulating with NE prioritises ingredients differently to AME. Limiting the number of ingredients reduce the variations seen when formulating using AME versus NE.

Diets formulated using NE and AME systems both resulted in similar flock variation and performance.

High nutrient density may be more beneficial and have a more significant impact on performance in earlier diets rather than later stages in broiler production.

Chapter 3: CHAMBER STUDY – THE EFFECT OF FEED NUTRIENT DENSITY ON NET ENERGY

ABSTRACT

To study the effect of diet density on respiratory quotient (RQ), heat production (HP) and net energy (NE), high-density AME and a low-density NEwere fed to broilers. The hypothesis was that birds fed high-density AME diet would have higher RQ, HP and NE when compared to those offered a low-density NE diet. Two diets, a high-density AME diet and a low-density NE, were formulated. Two new diets of different AME levels were created by mixing these diets in varying ratios. The common standard starter diet was fed to Ross 308 birds from d 0 to 10. On day 10 the grower diets were offered to broilers. Four chambers, each containing two birds, were allocated to each diet. The data collected was used to determine the RQ, HP and NE of feed. The layout was a completely randomised design with data analysed by ANOVA.

The results showed that birds offered diets formulated for NE had higher RQ ($P < 0.05$) and lower HP ($P < 0.05$) when compared to birds offered the diet formulated on an AME basis. Even with the difference in HP, the measured NE of diets was not different.

Although there was a lack of measured difference in NE, the study showed that energy intake caused a variation in RQ and HP. While there were differences seen between NE and AME diets, these were not adequate to conclude the benefit of one system over the other. Both NE and AME can be used to formulate broiler diets.

INTRODUCTION

During the NE study, De Groote (1974) did not measure the energies and used the calculated values for NE. De Groote's focus on the commercial advantage of formulating using the two different energy systems sounds convincing. However, since the NE levels reported has not been replicated by any studies, the accuracy of data without supporting measured energy values is debatable.

In this current study, NE of diets produced earlier in chapter 2 is determined using a closed-circuit indirect calorimetric (CIC) chamber.

A closed-circuit chamber is a sealed chamber that does not allow any gas exchange with the surroundings. The broilers housed in the chamber consume oxygen and release carbon dioxide. An oxygen cylinder is placed in line to replenish the oxygen used via a calibrated flow meter, while the carbon dioxide produced is removed by a potassium hydroxide scrubber. A pump is also fitted to the chamber to ensure a consistent circulation of gases.

The weight loss of the oxygen cylinder measures oxygen consumption. The release of carbon dioxide is determined using the barium sulphate precipitation method (published by Annison and White, 1961), to analyse carbonate trapped in the scrubber.

Brouwers equation used to determine HP.

$$HP = 3.866\ O_2 + 1.2\ CO_2$$

$$HI = HP - FHP$$

Fasting heat production is HP during the resting stage. Since the biochemical pathway of fasting birds is different, it is not an accurate picture of a resting HP, if fasted birds are used (Sturkie, 1986). Hence to overcome this, for this study, the asymptotic HP at resting, reported as 450 $kJ/BW^{0.70}$ per day, by Noblet et al. (2015) was used.

NE (production) = ME retained – total heat production + fasting heat production

$$NEp = ME\ intake - HI$$

Further details on the chamber are in publications by Swick et al. (2013), Barekatain et al. (2014) and Wu et al. (2019).

Using broilers placed in a CIC chamber, RQ, HP and HI of the diets formulated for AME and NE were measured.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England, approved this experiment (Approval No: AEC 14-004) conducted in the chamber room of the animal house at the University of New England, Armidale.

Two grower diets formulated to meet Ross 308 nutrient specifications (Aviagen, 2007) were pelleted. The first diet formulated using AMEn, control diet, while the second diet formulated for NE, test diet. The control AMEn diet (treatment 1), GME, was formulated for 13.15 MJ/kg AMEn. The test NE grower diet (treatment 4), GNE, was formulated for 10.26 MJ/kg NE. Two new diets were created by mixing different ratios of the two original diets; treatment 2; 60% GME and 40% GNE and treatment 3; 40% GME and 60% GNE. Table 8 shows the grower diet composition.

Using the equation published by Wu et al. (2019) NE was calculated.

The ratio of amino acids to AMEn and NE to AMEn were the same for both diets.

A common standard starter diet was fed to day old Ross 308 male chickens until d10. Table 8 shows the starter diet composition. On d 10, pens, each with ten chicks, were assigned to each of the four grower diets. The arrangement of treatments was in a completely randomised design.

On d 21, sixteen birds, eight from each treatment, were selected for CIC chamber. The details of the CIC chamber are below.

There were four chambers assigned to each of the four diets and two birds placed in each chamber set up in a climate control room. The birds in the chamber continued feeding on the grower diets.

Closed-circuit indirect calorimetric chamber

Sixteen chambers, each measuring 100 cm long x 70 cm wide x 65 cm high, were set up for NE determination at UNE. The details on chamber operation are in publications by Swick et al. (2013) and Barekatain et al. (2014).

A diagrammatic representation of the chamber setting is below.

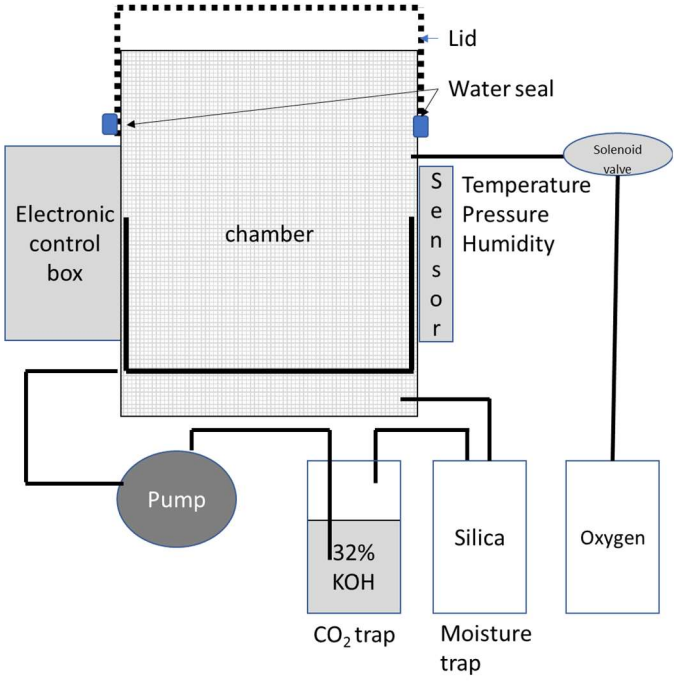


Figure 4 A diagram of CIC chamber.

At d 21 birds were left to acclimatise in the calorimetric chambers for three days with the pumps turned on. Birds in the chambers had access to food and water at all times. On d 24 the gas flow was turned on, and the lids to the chambers were closed

The loss in the weight of oxygen cylinder during the run was taken as weight of oxygen and converted to the volume of O_2 consumed (L) by dividing this with oxygen density of 1.331 g/L. The CO_2 output was determined by trapping CO_2 in a potassium hydroxide scrubber. The gases in the chamber were pumped through the potassium hydroxide scrubber to trap CO_2. Barium chloride precipitation method, as described by Annison and White (1961), was used to calculate the volume of CO_2 output.

The RQ of the birds was calculated as the ratio of CO_2 volume exhaled to O_2 consumed. Brouwers equation (1965), using the volume of these gases, was used to determine total HP.

Feed consumption (FI) was measured, and all excreta were collected and weighed. Excreta was homogenized and subsampled for oven moisture testing (85ºC for 48 hours). Homogenized excreta was freeze-dried for crude protein and gross energy (GE) analysis. Gross energy in the feed was measured to determine the

gross energy intake of the bird (GEI). Metabolisable energy was determined using the equation below:

$$ME\ (kcal/kg) = (GEI - GEE) / FI$$

Where GEI is the gross energy intake (kcal/kg DM)

GEE is the gross energy output of excreta (kcal/kg DM)

FI is the feed intake (kg DM).

The NE kcal/kg of diet calculated as:

$$NE = (RE+FHP) / FI$$

Where NE net energy, kcal/kg DM feed

RE retained energy, kcal/d

FHP fasting heat production (450 kJ/$BW^{0.70}$ per day, Noblet et al., 2015)

FI feed intake, kg/d DM

Table 8 Diet composition and the nutritional profile.

Ingredients (%)	Diets		
	Starter	Grower GME	Grower GNE
Wheat	61.9	66.8	59.1
Maize			13.4
Soybean meal	22.5	16.1	16.5
Canola seed	4.8	6.0	
Meat and bone meal	8.0	7.6	7.8
Canola oil	1.0	1.8	1.6
Limestone	0.47	0.35	0.34
Xylanase	0.005	0.005	0.005
Salt	0.108	0.048	0.037
Sodium bicarbonate	0.104	0.200	0.200
L-lysine HCl	0.381	0.344	0.347
D, L-methionine	0.357	0.357	0.304
L-threonine	0.210	0.180	0.181
Sacox® 120****	0.050	0.050	0.050
Premix***	0.130	0.130	0.130
AMEn **MJ/kg**	12.66	13.15	12.99
Calculated analysis			
NE MJ/kg*	8.98	10.55	10.40
Crude protein %	23.9	22.0	22.2
Dig** lysine %	1.27	1.1	1.06
Dig methionine & cysteine %	0.94	0.84	0.81
Dig arginine %	1.34	1.14	1.10
Dig threonine %	0.83	0.73	0.70
Dig isoleucine %	0.85	0.75	0.72
Dig valine %	0.99	0.87	0.84
Dig tryptophan %	0.24	0.21	0.20
Calcium %	1.05	0.84	0.83
Available phosphorous %	0.50	0.42	0.41
Sodium %	0.16	0.16	0.15
Chloride %	0.23	0.18	0.19
Crude protein analysed %	25.3	23.7	23.1
Crude fat analysed %	4.5	8.7	8.8

*Calculated using the equation of Wu et al. 2019. ** Dig digestible

*** Vitamins and mineral mix supplied the following amounts per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg.

****Sacox® 120 - 120 g/kg sodium salinomycin

RESULTS

Shown in table 9 are the calorimetric results from the chambers.

The GE and the nitrogen content of the AME and NE diets were significantly different ($P < 0.01$). The birds offered diet formulated for NE had a higher RQ and lower HP when compared to birds offered AME diet. The amount of nitrogen excreted ($P < 0.05$) and the amount retained ($p<0.001$) were significantly different for both diets.

Increase in ME intake had a positive correlation in increase in oxygen consumption ($r = 0.99$, $P < 0.01$). There was a positive correlation between nitrogen retain and HP ($r = 0.95$, $P < 0.05$). Higher oxygen consumption in broilers caused higher RQ ($r = 0.94$, $P < 0.05$) and higher HP ($r = 0.98$, $P < 0.05$).

Table 9 Indirect calorimetric measurements from d 25 to 28.

Item	Trt 1*	Trt 2*	Trt 3*	Trt 4*	P value	SEM
Feed intake (g/bird/day)	182	174	181	171	NS	9.24
GE feed as is (kJ/kg feed)	17.74[a]	17.41[b]	17.29[b]	16.99[c]	< 0.01	0.27
N as is % feed	3.79[a]	3.70[b]	3.72[b]	3.69[b]	< 0.01	0.04
Oven dried excreta N %	5.20[a]	5.15[a]	5.04[a]	5.44[b]	0.049	0.22
Retained N (g/bird/day)	4.03[a]	3.77[b]	4.04[a]	3.68[b]	0.001	1.24
AMEn correction factor kJ#	830[a]	778[b]	835[a]	760[b]	0.001	42.8
MEn intake (kJ/b/d)	2188[a]	2054[b]	2120[a]	2006[b]	0.049	111.8
O_2 L (bird/day)	61[a]	58[b]	59[ab]	57[b]	0.032	2.5
CO_2 L (bird/day)	59	58	59	58	NS	1.7
RQ[##]	0.966[a]	0.995[b]	1.00[b]	1.02[b]	0.025	0.03
Heat Production (kJ/b/d)[###]	307[a]	293[b]	301[a]	288[b]	0.04	11.0
REn kJ (bird/day) [####]	901	826	860	800	NS	80.8
NE feed as is MJ/kg calculated	10.55	10.49	10.46	10.40	NS	48.3
NE feed as is MJ/kg measured	9.59	9.48	9.43	9.49	NS	8.3
ME as is feed MJ/kg calculated	13.15	13.09	13.05	12.99	NS	17.4
ME as is feed MJ/kg measured	12.03	11.81	11.72	11.74	NS	17.1

**Trt 1 – GME / FME; *Trt 2 – 60% GME 40% GNE / 60% FME 40% FNE;*
**Trt 3 – 40% GME 60% GNE / 40% FME 60% FNE; *Trt 4 – GNE / FNE*
 # For correction to zero N retention used 8.22 kcal/g of N retained (Hill and Anderson, 1958).

RQ Respiratory Quotient = Volume CO2 / volume O2
modified Brouwer equation (Brouwer, 1965; McLean, 1972)
Retained energy nitrogen corrected = (nitrogen corrected ME intake - heat production)

DISCUSSION

Broilers offered both diets formulated for NE and AME had similar performances reported in the earlier experiment. Similar to the findings of Carré et al. (2002) and Noblet, et al. (2009, 2010), formulating diets with significantly different measured NE levels seems difficult. While significant differences observed in nitrogen excretion, nitrogen retention, RQ and HI indicted a possible variation in the biochemical pathway of the birds, there was no significant difference in determined NE.

While carbon dioxide output was constant, oxygen consumption changed with the diet. The RQ was lower while the HI was higher for the high-density AME diets, which were at a high protein and energy levels. Noblet et al. (2003) studied the effect of protein on HP in pigs and poultry. Total HP in poultry was not affected by protein level in diet nor the age of the birds. The observations made by Noblet et al. are consistent with the observations made in the current study. However, while there is no correlation between the nitrogen content of the feed and HP, a positive correlation was seen between nitrogen retained by the birds and HP. Results of a study reported by Koh and Macleod (1999) showed that HP was affected by the level of feeding with higher numbers seen in ad libitum feeding versus restricted feeding. Ohtani and Leeson (2000) reported higher HP in birds on intermittent lighting with higher AME intake. The relationship between feed intake and HP is consistent with the findings of the current study. Increased ME intake showed an increase in oxygen consumption and higher heat production. The findings of a study by Latshaw and Moritz (2009) showed that AME partitioning into PE, HI and maintenance energy was dependent on the AME daily intake. In agreement with Koh and Macleod (1999); Ohtani and Leeson (2000) and Latshaw and Moritz (2009), in the current study, AME diet with high AME intake had higher HP ($P < 0.05$). Heat production seems to be affected by the number of nutrients flowing through the gut achieved either by higher feed intake or increasing nutrient density of the diet and also by the activity level of the bird.

Studies show that dietary macronutrients may affect the biochemical pathway within the bird (Shapiro and Wertheimer, 1948; Swennen et al., 2004). Swennen

et al. 2004 showed that broilers responded to low protein diet by increasing feed intake and removing the excess energy by increasing de novo lipogenesis and fat deposition. In the current study, NE diet with lower protein and energy had a higher RQ of 1.02, while the higher protein and energy, AME diet, had RQ of 0.966 ($P < 0.05$). High RQ in low protein diets formulated by Carré et al. (2002) and Noblet et al. (2003) was consistent with the observation made during this study. Since NE and AME diets differed in their nutrient content, the difference in RQ may be due to a difference in biochemical pathway within the birds, in response to the macronutrient content of the diets.

A higher retained nitrogen ($P < 0.001$), RQ ($P < 0.05$) and heat production ($P < 0.05$) was observed in birds offered treatment 3. It is difficult to conclude if the higher weight was causing the above differences or if the bird's biochemical pathway was different leading to the above-observed differences and hence causing higher weight gain. The highest FCR for this treatment is coherent with observed fat deposited in the bird, which would be energy expensive for the birds. The FCR result, however, was not significantly different while the fat pad results were.

CONCLUSION

While there were differences seen between NE and AME diets, these were not adequate to conclude the benefit of one system over the other. Both NE and AME can be used to formulate broiler diets.

Birds with high fat pads had high FCR. These birds also had high RQ and heat production, showing that fat deposition is energy expensive for the birds. The difference in oxygen consumption, RQ and heat increment indicate that the biochemical pathways of these birds are likely to be different.

While the NE range used in this study was not wide to measure the difference, the study successfully showed that the diets energy intake had caused a variation in the RQ and HI.

Chapter 4: INGREDIENT EFFECT WHEN FORMULATING NET ENERGY DIET

ABSTRACT

The raw material composition of diets may change when formulating diets using NE versus AME system. This study determined the effect of raw material variation in the diet on the feed intake, live weight and FCR of the birds.

During the study, using NE and AME systems and a variety of raw materials, diets were formulated. There were eight grower and eight finisher diets formulated using different raw materials. Each of the eight diets was offered to broilers in four replicate pens with 34 birds per replicate. There was a total of 32 pens. The layout was completely randomized design.

The results indicated that broilers offered accurately formulated diets, that meets the nutritional requirement, performed to the Aviagen target irrespective of the raw materials used to achieve the target nutrients. The nutritionist should be able to confidentially formulate least-cost diets using a wide range of raw materials. Some raw materials have anti-nutritional factors which the nutritionist need to be aware of when formulating.

NE is a better predictor of FCR compared to AME during the early age of birds (d 10 – 21, $P < 0.01$). Also, NE was found to a more accurate predictor of FCR compared to AME at lower diet AME levels of 12.55 MJ/kg ($P < 0.001$) irrespective of the age of birds.

INTRODUCTION

Fraps, 1946, had been one of the first to assign energy (as PE) to ingredients and formulate diets to the productive energy value. Fraps PE was one of the first proposed net energy systems. The productive energy of feed is the energy that goes into increasing body energy stores.

$$E = (PE)\ (F - g * W)$$

Where E: mean gain in body energy cal

PE: productive energy cal/g

F: feed intake g

W: live weight g

g: food required to maintain one gram of live weight g

Working on the assumption that g, food required to maintain one gram of live weight is a constant, this equation was further revised by simultaneously solving equations for ad libitum feeding and a lower feeding as shown below:

$$PE = (E_1W_2 - E_2W_1) / (F_1W_2 - F_2W_1)$$

Where $_1$ is ad libitum feeding

$_2$ is lower feeding (Parkes, 1982)

However, the use of Fraps production energy faded, and the industry switched to using AME for poultry diet formulation. The reason for the change from net energy to ME was the lack of reproducibility of the net energy system (Davidson et al. 1957) and lack of data.

The lack of reproducibility may be explained by some essential differences that the Fraps production energy system did not consider as discussed below.

Studies have shown that (Boekholt et al., 1994) feed energy partitioning into maintenance and production vary with feed intake, which challenges the basis of simultaneous equations solved for productive energy by Fraps. Further to this, Cheng et al. (1997) showed that energy utilisation was affected by environmental temperature and dietary protein levels. Fat addition affects the heat increment, the

difference between ME and NE, as shown in studies conducted by Adams et al. (1962), Cowan and Mitchie (1978), Dale and Fuller (1980) and Cheng et al. (1997). Similar studies have also shown that dietary protein affects energy utilisation (Kamran et al. 2008).

Also, these studies show that feed intake, environmental temperature and the nutrient composition of the diet could explain the lack of reproducibility of Fraps production energy.

Summarised in table 10 are some of the studies conducted to determine net energy using the nutrient composition.

Table 10 Equations using raw material composition to calculate NE.

Authors	Equations to estimate net energy
De Groote (1974)	NE = 13.4 dig CP + 35.3 dig EE + 13.0 dig NFE
Hoffmann and Schiemann (1980)	NE = 10.8 dig CP + 33.5 dig EE + 13.4 (dig NFE + dig CF)
Emmans (1994)	NE = 1.17 AME – 4.2 CP – 2.44

Dig, digestible; CP, crude protein (kg/kg); EE, ether extracted fat (kg/kg); NFE, nitrogen-free extract (kg/kg); CF, crude fibre (kg/kg); AME (MJ/kg).

A similar equation has been derived by Wu et al. 2019.

Net energy equations assume that NE is a factor of the diet's nutrient composition. If the assumption were correct, a change in raw materials used for feed formulation would not have an impact on NE. However, the current practice in the poultry industry identifies raw material as having differences in nutritive value. Hence the performance of the birds is predicted based on the raw materials used, e.g. sorghum, when used in the diet, is calculated as 10% less than maize in nutritive value and assumed to cause lower performance in broilers (Beyer, 2010). There are studies which support the findings that raw materials do cause a variation in performance (Tukei, 1998; Mavromichalis, 2016).

However, recent studies show that raw materials used when processed appropriately are of high quality with high digestibility. Without anti-nutritive factors, diets produced using different raw materials performed equally well, i.e. when formulated accurately for nutritional composition. Raw materials do not have an impact on the performance of the broilers (Beyer, 2010; Bolden, 2015 and Barekatain et al., 2017).

This study was to evaluate the effect on the performance of broilers when formulating commercial feeds using different raw materials at two different AME levels of, 12.55 MJ/kg and 13.39 MJ/kg, and varying NE levels, for the period of d 10 to 35. The target was also to see how much NE varies when formulated using different raw materials and to investigate if AME or NE could predict live weight and FCR with greater accuracy. All diets in the study met the amino acids requirement for Ross 308 nutrient specifications (Aviagen, 2007).

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England approved this experiment conducted at Inghams Enterprises Pty Ltd, Nutrition Research Centre, Leppington, NSW.

Series of broiler grower and broiler finisher diets were formulated to either 13.39 MJ/kg or 12.55 MJ/kg of AME levels, as shown in tables 11 and 12. Diets were created at varying NE levels using various ingredients. All diets met the minimum nutrient requirement as published for Ross 308 broilers (Aviagen, 2007). The NE content of the diets was determined using the equation published by Wu et al. (2019) and preliminary equation by Swick et al. (2013). The equation used by Swick et al. (2013) is below.

NE = 0.836 + 0.731 AMEn MJ/kg - 0.0369 crude protein % + 0.042 crude fat %

(Swick personal communication)

A common standard starter diet was fed to Ross females from d 0 to d 10. On d 10, four pens, each with 34 broilers, were randomly allocated to each dietary treatment. There were eight dietary treatments. Tables 11 and 12 show the composition of these diets. Broiler feeding was ad libitum and recorded daily. On d 10, 21 and 35 the birds were weighed.

The birds received 23 hours of light from 0 to 7 days, reduced to 16 hours from eight days onwards.

RESULTS

Tables 13 and 14 show the nutritional composition of eight grower and finisher diets formulated using different raw materials. Table 15 shows the performance results of the birds at various ages.

There was no treatment difference observed for live weight of the birds at any age ($P > 0.05$). Birds on all the eight treatments had higher weight gains than the Ross standard 2009 for female birds. The Ross 2009 target live weight at 35 days was 1.977 kg, while the live weight of the birds for the study varied between 2.261 kg to 2.386 kg.

The raw material composition of the various diets was different. There were two levels of AME, 13.39 MJ/kg and 12.55 MJ/kg the feed intake was not affected by the AME content of the feed.

Birds offered wheat-based diets had lower FCR while birds offered diets with high levels of pea inclusion and lower nutrient density had higher FCR ($P < 0.001$).

Feed intake comparisons between diets for 10 to 21 d or 21 to 35 d periods showed no differences ($P > 0.05$) except for difference seen between diet B, and F. Results showed feed intake to be higher for diet F (0.937 kg) as compared to diet B (0.878 kg) from 10 to 21 d ($P < 0.01$).

There was poor correlation between NE and FCR for birds offered high AME diets of 13.39 MJ/kg, but a high correlation of NE to FCR (R^2 range from 0.86 to 1) when birds offered low AME diet of 12.55 MJ/kg (graph 3).

Table 11 Raw material composition of diets during d 10 to 21.

Ingredients (%)	A	B	C	D	E	F	G	H
Sorghum	33.5	42.3	30.0		45.5	40.3	30.3	10.0
Wheat	14.0	10.0	28.9	52.5	10.0	15.0	30.0	49.0
Peas	20.0	8.0			20.0	12.0		
Canola meal	5.2	4.2			2.8	5.1	7.4	
Canola seed	5.0	5.0	5.0	1.3	5.0	5.0	5.0	4.4
Soybean meal	8.0	17.8	23.8	31.6	7.3	12.3	16.5	25.7
Meat and bone meal	7.0	7.2	7.6	7.6	4.6	7.0	7.0	7.5
Blood meal	1.5				1.5	1.3	1.5	1.5
Canola oil	4.0	3.9	3.4	6.0		0.3	0.8	0.9
Salt	0.05	0.03	0.06	0.17	0.01	0.04	0.07	0.15
Limestone	0.28	0.20	0.14	0.10	0.75	0.25	0.20	0.15
Mono dicalcium phosphate					0.55			
L-lysine	0.19	0.24	0.22		0.31	0.20	0.18	
D, L-methionine	0.32	0.30	0.27	0.27	0.36	0.29	0.23	0.20
L-threonine	0.11	0.10	0.07		0.16	0.09	0.04	
L-isoleucine	0.12	0.03			0.14	0.06	0.04	
L-tryptophan	0.01				0.03			
L-arginine					0.12			
Sodium bicarbonate	0.34	0.37	0.32	0.16	0.45	0.35	0.31	0.18
Choline chloride	0.05	0.06	0.03		0.06	0.07	0.00	0.03
Grower premix**	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

* Vitamins and minerals supplied per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg.

Table 12 Raw material composition of diets during d 22 to 35.

Ingredients (%)	A	B	C	D	E	F	G	H
Sorghum	21.9	31.3	5.0		48.4	36.0	17.2	
Wheat	32.0	31.1	57.4	56.7	3.2	25.0	50.3	63.5
Peas	16.0	6.0			20.0	6.9		
Canola meal	6.2				8.0	8.0	3.1	
Canola seed	5.0	5.0	5.0		5.0	5.0	5.0	0.03
Soybean meal	5.8	15.7	21.0	29.6	8.2	9.7	15.3	26.1
Meat and bone meal	6.0	6.5	6.6	6.6	2.8	5.9	6.3	6.6
Blood meal	1.5					1.5	1.5	0.5
Canola oil	4.0	2.8	3.7	6.1	1.3	0.45		2.2
Salt	0.08	0.04	0.12	0.18	0.04	0.07	0.10	0.17
Limestone	0.28	0.19	0.15	0.11	0.83	0.25	0.20	0.13
Mono dicalcium phosphate					0.65			
L-lysine	0.17	0.26	0.16		0.26	0.17	0.15	
D, L-methionine	0.24	0.25	0.19	0.25	0.27	0.20	0.18	0.17
L-threonine	0.08	0.09	0.03	0.03	0.12	0.04	0.02	0.09
L-isoleucine	0.09	0.03			0.04	0.04	0.02	
L-tryptophan					0.01			
L-arginine					0.01			
Sodium bicarbonate	0.33	0.38	0.28	0.18	0.47	0.34	0.29	0.19
Choline chloride	0.04	0.07	0.02	0.01	0.04	0.01	0.02	0.02
Finisher premix*	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

* Vitamins and minerals supplied per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B12, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg.

Table 13 Nutritional composition of diets during d 10 to 21.

AMEn MJ/kg	13.39				12.55			
Diet	A	B	C	D	E	F	G	H
NE MJ/kg*	10.89	10.67	10.46	10.25	10.25	10.04	9.83	9.62
NE MJ/kg**	*10.73*	*10.72*	*10.69*	*10.67*	*9.95*	*9.93*	*9.92*	*9.88*
NE**:AME	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79
Dry matter %	89.27	88.85	89.05	89.39	89.00	89.06	89.19	89.50
Crude protein %	21.5	22.0	22.4	24.9	20.0	22.1	22.9	24.6
Crude fat %	8.86	8.77	8.01	8.82	4.77	5.32	5.51	5.15
Available phosphorus %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Calcium %	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Chloride %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sodium %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Dig*** arginine %	1.20	1.22	1.24	1.48	1.20	1.20	1.20	1.39
Dig cysteine %	0.27	0.27	0.29	0.33	0.25	0.28	0.31	0.34
Dig isoleucine %	0.77	0.76	0.77	0.87	0.76	0.76	0.76	0.81
Dig lysine %	1.12	1.12	1.12	1.12	1.12	1.12	1.12	1.12
Dig methionine & cysteine %	0.85	0.85	0.85	0.91	0.85	0.85	0.85	0.85
Dig methionine %	0.58	0.57	0.56	0.57	0.59	0.57	0.53	0.51
Dig threonine %	0.75	0.75	0.75	0.77	0.75	0.75	0.75	0.78
Dig valine %	0.89	0.86	0.88	0.97	0.85	0.92	0.96	1.03

* calculated using equation by Swick et al. 2013. ** calculated using equation by Wu et al. 2019. *** Dig standard ileal digestible

Table 14 Nutritional composition of diets during d 21 to 35.

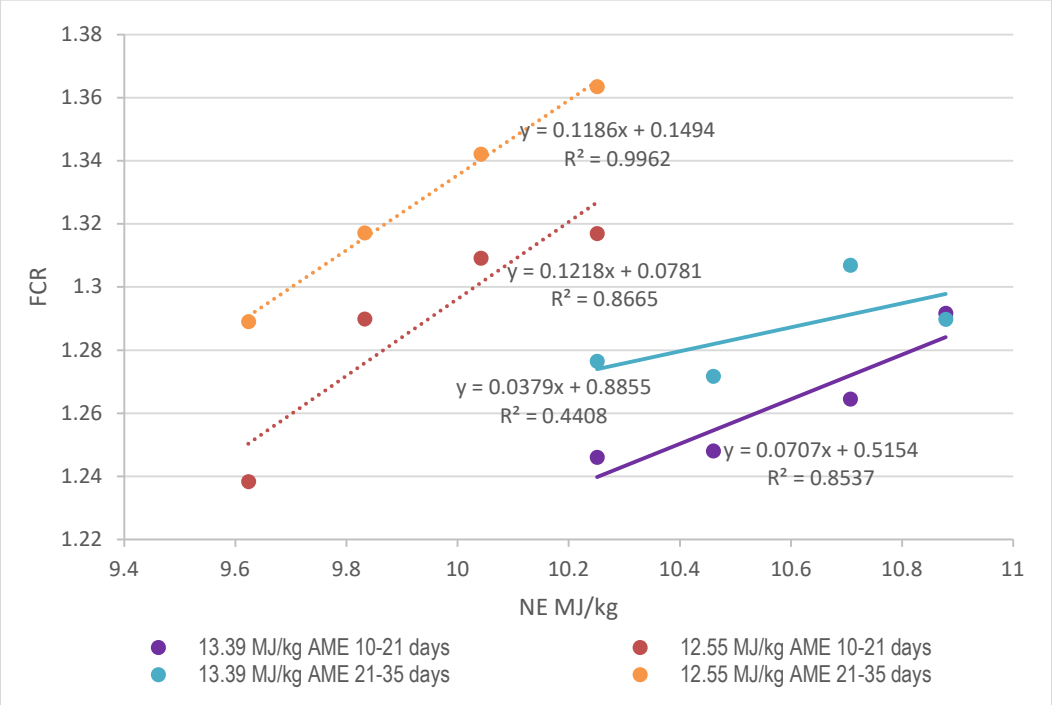
AMEn MJ/kg	13.39				12.55			
Diet	A	B	C	D	E	F	G	H
NE MJ/kg*	10.88	10.67	10.46	10.29	10.29	10.04	9.83	9.62
NE MJ/kg**	10.74	10.72	10.70	10.67	10.00	9.95	9.92	9.89
NE**:AME	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.79
Dry matter %	88.97	89.16	89.61	89.35	88.76	89.17	89.52	89.54
Crude protein %	20.5	19.9	21.3	23.6	19.2	21.0	21.3	23.3
Crude fat %	8.89	7.70	8.01	8.29	6.12	5.45	4.64	4.54
Available phosphorus %	0.4	0.4	0.4	0.39	0.4	0.4	0.4	0.4
Calcium %	0.8	0.8	0.8	0.79	0.8	0.8	0.8	0.8
Chloride %	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium %	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Dig*** arginine %	1.09	1.09	1.2	1.4	1.09	1.09	1.13	1.34
Dig cysteine %	0.27	0.27	0.31	0.33	0.26	0.29	0.31	0.33
Dig isoleucine %	0.7	0.69	0.72	0.83	0.69	0.69	0.69	0.79
Dig lysine %	1.02	1.02	1.02	1.05	1.02	1.02	1.02	1.02
Dig methionine & cysteine %	0.78	0.78	0.78	0.87	0.78	0.78	0.78	0.79
Dig methionine %	0.5	0.5	0.46	0.54	0.51	0.48	0.46	0.46
Dig threonine %	0.68	0.68	0.68	0.76	0.68	0.68	0.68	0.81
Dig valine %	0.87	0.79	0.84	0.93	0.78	0.89	0.9	0.93

* calculated using equation by Swick et al. 2013. ** calculated using equation by Wu et al. 2019. *** Dig standard ileal digestible

Table 15 Weight gain, feed intake and FCR for birds on various diets during the different ages.

Day		13.39 MJ/kg diets				12.55 MJ/kg diets				p-value	SEM	Ross 2009
		A	B	C	D	E	F	G	H			
10 to 21	Weight gain	0.707	0.694	0.717	0.711	0.698	0.715	0.717	0.715	0.155	0.003	0.601
	Feed intake	0.913^{ab}	0.878^{a}	0.894^{ab}	0.887^{ab}	0.919^{ab}	0.937^{b}	0.925^{ab}	0.886^{ab}	0.043	0.006	0.848
	FCR	1.292^{ab}	1.265^{abc}	1.248^{ab}	1.246^{ab}	1.317^{d}	1.309^{cd}	1.290^{bcd}	1.238^{a}	0.002	0.007	
		AME interaction[1]										
	Weight gain	0.707				0.711				0.430	0.003	
	Feed intake	0.893				0.916				0.043	0.006	
	FCR	1.263				1.289				0.050	0.007	
Day 21	Av. Weight	0.971	0.960	0.986	0.975	0.961	0.989	0.989	0.976	0.140	0.003	0.886
	FCR	1.200^{abc}	1.177^{ab}	1.171^{ab}	1.166^{ab}	1.216^{c}	1.208^{c}	1.197^{abc}	1.160^{a}	0.008	0.005	1.293
22 to 35	Weight gain	1.411	1.373	1.381	1.399	1.300	1.364	1.397	1.366	0.140	0.010	1.022
	Feed intake	1.908	1.919	1.854	1.894	1.914	1.964	1.959	1.886	0.637	0.015	1.882
	FCR	1.290^{a}	1.307^{ab}	1.272^{a}	1.276^{a}	1.363^{c}	1.342^{bc}	1.317^{ab}	1.289^{a}	<0.001	0.008	
		AME interaction[1]										
	Weight gain	1.391				1.357				0.010	0.083	
	Feed intake	1.893				1.931				0.205	0.145	
	FCR	1.286				1.328				0.001	0.007	
Day 35	Av. Weight	2.382	2.333	2.366	2.373	2.261	2.353	2.386	2.342	0.074	0.011	1.977
10 to 35	FCR	1.331^{ab}	1.353^{abc}	1.311^{a}	1.317^{a}	1.417^{d}	1.395^{cd}	1.364^{bc}	1.332^{ab}	<0.001	0.007	

Figures having different superscripts were significantly different (P < 0.05) *[1]Mean of all diets at 13.39 MJ/kg and 12.55 MJ/kg AME.*



Graph 3 NE VS FCR at 12.55 MJ/kg and 13.39 MJ/kg AME during various age periods.

DISCUSSION

When formulating diets with different levels of NE and AME, different raw materials had to be selected. Using different raw materials to formulate diet introduces variability inherent of the raw materials to the diet. Even with different raw materials used to formulate diets at two different AME levels, birds fed the various diets had higher weight gain than the Ross standard 2009.

Numerous studies have shown successful inclusion of various raw materials in the broiler diets. In 2015, Bolden presented similar findings, reporting comparable performance in broilers offered maize and wheat diets. While there are historical differences between maize and wheat, with maize diets giving better performances than wheat, Bolden explained these disparities were not due to the grains. The inconsistencies in broilers performances were due to inappropriate processing, raw material quality, inadequate availability of information, especially digestibility and lack of enzymes. Bolden reported that with all of these addressed both maize and wheat diets would give a comparative performance.

Beyer (2010) had reported similar findings on broilers offered sorghum diets. The study found that sorghum is more environmentally friendly, and with proper processing, there would be no performance losses as it replaced maize.

Barekatain et al. (2017) had shown that when formulated to balance nutrients appropriately, canola meal and oil could replace canola seed. A study by Roth-Maier (1999) had shown that canola meal could replace soy meal in the broiler diets. Field pea inclusions of up to 25% were reported as optimum in broiler diets by Perez-Maldonado et al. (1999).

The results from this study are coherent with the above researches. There may have been differences in broiler performances observed due to raw materials in the past. Once the cause for these variations addressed, the performance of broilers offered diets consisting of different raw materials should be parity. In situations where the processing and quality of raw material is not an issue, the nutritionist may formulate to the least cost diet using the raw materials nutritional composition without concerns about the negative impact on performance.

A well-formulated diet contains the balance of nutrients irrespective of the raw materials used. However, as reported by Mavromichalis (2016), anti-nutritional factors could affect the performance, which is not picked during formulation but is evident in the field. The anti-nutritional factors of raw materials need addressing before treating them as equal.

Birds offered diets with high pea inclusions in the low AME diets had a higher FCR. Perez-Maldonado et al. (1999) had reported an optimum level at 25%, and the broiler performance was better than Ross standard (Aviagen, 2009). However, the FCR, when compared to a wheat-based diet, was poorer for high pea inclusion at lower AME.

While the formulated diets were significantly different in raw material inclusion, the NE levels in the diets did not differ much when calculated using equation published by Wu et al. (2019).

The performance of the birds at 13.39 MJ/kg AME was different to the birds at 12.55 MJ/kg with birds offered higher AME having lower FCR at 21 ($P < 0.05$) and 35 ($P < 0.001$) days of age. At 12.55 MJ/kg there was a significant correlation of FCR with the NE of the diet. At 12.55 MJ/kg, NE range 9.88 MJ/kg to 9.95 MJ/kg using equation by Wu et al. (2019) had FCR range of 1.160 to 1.216, respectively at 21 days. Broilers at all ages had lower FCR at lower NE levels in the diet. This correlation was, however, only seen during 10 - 21 days for the 13.39 MJ/kg AME diets.

Studies have reported that AME does not always act as an accurate predictor of performance (Fraps and Carlyle, 1939; Warpechowski, 2004). AME was also seen as a poor predictor of performance here when diets formulated at the same AME level had different FCRs. NE, however, is a better predictor of FCR with $R^2 > 0.86$ for diets at 12.55 MJ/kg for all ages and 10-21 days for diets at 13.39 MJ/kg AME. As speculated (Noblet, 2013) this study shows that NE can be used to predict FCR more accurately than AME.

The NE, when recalculated using equation by Wu et al. (2019), had smaller incremental changes in the value when AME is kept constant. However, even with the small differences, the performance of birds offered these diets were significantly different.

These smaller differences in NE of the diets were not significant in the CIC chambers due to the high coefficient of the variance reported during earlier chamber studies; however, these diets had shown variation in FCR and weight gain in the field.

CONCLUSION

A balanced NE or AME diet may contain a variety of available raw materials. When using different raw materials, a well-formulated diet to meet the nutritional requirement of the broilers should not affect the performance of the broilers. With the anti-nutritional factors accounted for, the nutritionist should be able to formulate least-cost diets using a range of raw materials.

NE was a more accurate predictor of FCR than AME, especially at lower AME levels of 12.55 MJ/kg of diet and early age of the diets formulated at 13.39 MJ/kg.

Chapter 5: NET ENERGY AND AMINO ACID RATIO

ABSTRACT

This study was to determine differences in performance of broilers as a result of varying dietary NE and amino acid levels. The hypothesis was that higher NE and amino acid levels in the diet would cause an increase in weight gain.

Four diets formulated at the same AME level, with two different NE and amino acid levels. The broilers were fed the diets with feed intake, and live weights were measured to determine FCR and weight gain. Ross 308 male day-old chickens were placed on a common standard starter diet and fed ad libitum. On d 10, 24 weighed chicks, were placed into 36 individual pens. Nine pens were randomly assigned to each diet and offered the allocated grower diet.

During the early age, higher amino acid levels caused lower feed intake ($P < 0.007$) and lower FCR ($P < 0.001$) while greater weight gain ($P < 0.0$) and lower FCR ($P < 0.01$) was observed in broilers offered low NE diets ($P < 0.01$). At a later age of d, 41 feed intake difference was due to NE ($P < .007$) and not amino acid levels in the diet.

The results indicated that the broiler response to dietary nutrients might vary with age. The broilers fed diets with a low dietary ratio of NE to amino acid had reduced the FCR and increased the live weight gain.

INTRODUCTION

The earlier studies showed that formulating for NE or AME system lead to comparable growth in broilers. While the nutrient density had affected FCR and feed intake at various ages, it did not affect the live weight of the birds.

Growth rate and efficiency of feed utilisation in broilers is a direct function of nutrient density (Waldroup et al., 1976). However, our early study reported in chapter 1 had failed to see this interaction between nutrient density and performance after 25 days of broiler age. In the study conducted by Waldroup, AME ranged from 12.43 to 15.65 MJ/kg. It may be possible that the range used in our study was not broad enough to pick the response to diet nutrient density however the range used by Waldroup et al.

(1976) is not a reality in commercial broiler diets in Australia. However, birds have changed since 1976, and this could be contributing to the differences in the observation.

During the first study, the ratio of AME to amino acids was the same for all diets. For this study, the AME was kept constant for all diets, while the NE and amino acid levels were varied. The study conducted to identify if NE or amino acid levels could cause an improvement in weight gain in birds offered diets at a constant AMEn level of 13.39 MJ/kg.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England approved this experiment conducted at the Rob Cumming Poultry Innovation Centre at Kirby, University of New England, Armidale, NSW.

Four grower and finisher diets, as shown in table 16, were formulated at different NE and amino acid levels. Diet 1 had a low amino acid density (as recommended for Ross 308) and low NE of 10.21 MJ/kg in grower diets and 10.61 MJ/kg in finisher diets. Diet 2 had a high amino acid density (10% higher than Ross 308 recommended nutrient specification) and low NE of 10.21 MJ/kg in grower diets and 10.61 MJ/kg in finisher diets. Diet 3 had low a low amino acid density and high NE of 10.28 MJ/kg in grower diets and 10.67 MJ/kg in finisher diets. Treatment 4 had a high amino acid density and high NE of 10.28 MJ/kg in grower diets and 10.67 MJ/kg in finisher diets.

All grower diets were formulated to 12.97 MJ/kg AMEn, and finisher diets to 13.39 MJ/kg AMEn. NE was determined using NE equation published by Wu et al. (2019).

Ross 308 male day-old chickens were placed on a common standard starter diet and fed ad libitum. On d 10, nine pens each with 24 chicks, were randomly assigned to each diet and offered the allocated grower diet.

On d 24 the birds were weighed, and feed changed to corresponding finisher diets. On d 35 and 41 the birds and the feed were weighed. On d 42, four birds were euthanised per pen to measure breast meat, thigh yield and fat pad.

Statistical Analysis

PROC GLM was used to determine significance and interactions of main effects (SAS, 2013) with data analysed for one-way ANOVA and 2 × 2 factorial arrangement of treatments.

RESULTS

Shown in table 17 is the nutritional composition of the diets. Tables 18 and 19 show the broiler performance for d 24, 35 and 41.

Birds offered the low nutrient density feed with low NE, and low AA had the highest feed intake of 1322 g while birds offered high NE, and high AA diet had lowest feed intake of 1291 g ($P < 0.05$) at 24 days.

Birds offered the low NE, and high AA diets had the highest weight gain of 1073 g ($P < 0.01$) and lowest FCR of 1.212 ($P < 0.001$) till d 24. These birds also had had the lowest relative fat pad, but this result was not statistically significant. Birds offered the low NE, and high amino acid density diet continued to be the heaviest for the duration of the trial; however, these results were statistically not significant ($P > 0.05$) after 24 days.

Birds offered low NE, and high AA had significantly lower FCR while birds offered high NE, and low AA diet had the highest FCR for the duration of the study ($P < 0.001$ at d 35 and $P < 0.05$ at d 41).

There was no significant difference for breast yield, thigh yield and fat pad for any of the treatments, as shown in table 20.

Table 16 Raw material composition of diets.

Treatment	Starter	Grower				Finisher			
		1	2	3	4	1	2	3	4
Sorghum	29.7	-	-	39.6	39.6	28.6	27.0	39.6	39.6
Maize	11.2	52.6	57.5	1.6	-	36.0	37.8	5.7	8.1
Wheat	9.9	7.6	-	14.3	13.7	2.1	0.9	14.6	11.7
Soybean meal	29.5	24.6	27.4	24.7	25.9	18.8	19.6	19.4	19.8
Canola seed	5.9	5.9	5.9	3.3	4.1	5.9	5.9	5.9	5.9
Canola meal	5.9	-	0.6	5.9	5.9	-	-	5.9	5.9
Meat and bone meal	1.4	6.8	5.8	2.0	2.0	5.2	5.3	2.0	2.0
Canola oil	1.5	-	0.2	3.7	3.7	0.8	0.9	3.1	3.1
Limestone	1.0	0.2	0.2	0.8	0.8	0.2	0.2	0.6	0.6
Dicalcium phosphate	1.5	-		1.6	1.7	-	-	0.9	1.0
Avizyme 1502	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2
Sodium bicarbonate	0.2	0.1	0.1	0.4	0.4	0.2	0.2	0.1	0.1
Premix*	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
L-lysine HCL	0.3	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2
D, L-methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2
L-threonine	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sacox® 120**	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Albac 150***	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033
Water	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

* Vitamins and minerals supplied per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg. ** 120 g/kg sodium salinomycin. ***150g/kg Zinc Bacitracin

Table 17 Nutrient composition of diets.

Treatment	Starter	Grower				Finisher			
		1	2	3	4	1	2	3	4
AMEn (MJ/kg)	12.55	12.97	12.97	12.97	12.97	13.39	13.39	13.39	13.39
Net energy (MJ/kg)**	9.89	10.21	10.21	10.28	10.28	10.61	10.61	10.67	10.67
NE:ME	0.788	0.787	0.787	0.793	0.793	0.793	0.793	0.797	0.796
Crude protein %	24.5	22.9	23.5	21.9	22.5	20.1	22.1	20.9	21.2
Crude fat %	4.45	4.84	4.93	6.67	6.96	5.42	6.38	7.56	7.61
Crude fibre %	2.98	2.37	2.39	2.75	2.83	2.4	2.39	2.99	2.99
Dig* lysine %	1.28	1.15	1.19	1.15	1.19	1.03	1.05	1.03	1.05
Dig methionine %	0.656	0.596	0.62	0.59	0.62	0.56	0.55	0.52	0.53
Dig methionine + cysteine %	0.95	0.87	0.9	0.87	0.9	0.8	0.82	0.8	0.82
Dig tryptophan %	0.23	0.225	0.24	0.24	0.24	0.18	0.22	0.22	0.22
Dig threonine %	0.86	0.77	0.79	0.77	0.8	0.69	0.71	0.69	0.7
Dig arginine %	1.37	1.33	1.39	1.25	1.3	1.1	1.29	1.16	1.18
Dig isoleucine %	0.86	0.8	0.85	0.78	0.81	0.71	0.76	0.71	0.73
Dig valine %	1.02	0.97	1.01	0.95	0.98	0.84	0.94	0.90	0.91
Calcium %	0.96	1.00	0.91	1.00	1.03	0.79	0.92	0.83	0.81
Sodium %	0.16	0.16	0.17	0.23	0.24	0.16	0.16	0.16	0.16
Linoleic acid %	1.61	1.83	1.90	2.22	2.27	2.02	2.18	2.36	2.38
Cost $/t	-	478.85	490.75	478.67	484.46	457.99	463.51	458.06	461.93
Crude protein % (analysed)	23.7	22.3	21.4	22.0	22.9	19.4	20.5	20.7	21.7
Crude fat % (analysed)	4.5	4.8	6.7	7.0	4.9	5.4	7.6	7.6	6.4

*Dig = digestible ** calculated using Wu et al. 2019.

Table 18 Broiler performance d 24, 35 and 41

Treatment	Day 24				Day 35				Day 41			
	Feed intake g	Weight gain g	FCR	Livability %	Feed intake g	Weight gain g	FCR	Livability %	Feed intake g	Weight gain g	FCR	Livability %
Low NE Low AA	1322[a]	1057[ab]	1.250[ab]	100.0	3261	2287	1.426[a]	97.8	4447	2951	1.506[a]	96.9
Low NE high AA	1301[ab]	1073[a]	1.212[c]	99.6	3231	2328	1.388[b]	99.1	4448	3001	1.482[b]	98.7
High NE low AA	1310[ab]	1033[c]	1.268[a]	99.1	3263	2278	1.432[a]	97.8	4522	2970	1.523[a]	96.9
High NE high AA	1291[b]	1048[bc]	1.232[bc]	99.6	3242	2292	1.415[a]	98.2	4477	2971	1.507[a]	98.2
CV %	2.50	2.57	2.45	1.28	1.95	2.27	1.91	2.27	1.91	1.88	1.76	2.99
P value	*0.050*	*0.010*	*0.001*	*0.550*	*0.680*	*0.200*	*0.001*	*0.560*	*0.210*	*0.350*	*0.010*	*0.470*
Main effects												
					Net Energy[1]							
Low NE	1311	1065[a]	1.231[b]	99.8	3246	2308	1.407[b]	98.4	4448[a]	2978	1.494[b]	97.8
High NE	1301	1040[b]	1.250[a]	99.3	3253	2286	1.423[a]	98.0	4499[b]	2970	1.515[a]	97.6
P value (NE)	*0.32*	*0.01*	*0.01*	*0.331*	*0.76*	*0.2*	*0.03*	*0.55*	*0.007*	*0.69*	*0.01*	*0.82*
					Amino acids[2]							
Low AA	1316[a]	1045	1.259[a]	99.6	3262	2283	1.429[a]	97.8	4485	2962	1.514[a]	96.9
High AA	1296[b]	1061	1.222[b]	99.6	3236	2310	1.401[b]	98.7	4463	2986	1.495[b]	98.4
P value (AA)	*0.007*	*0.058*	*0.001*	*1.000*	*0.24*	*0.11*	*0.001*	*0.25*	*0.43*	*0.2*	*0.02*	*0.122*
P value (NE x AA)	*0.95*	*0.944*	*0.91*	*0.31*	*0.93*	*0.44*	*0.158*	*0.56*	*0.41*	*0.22*	*0.59*	*0.822*

[abc] Means sharing the same superscripts are not significantly different from each other at P < 0.05. [1]Low NE = mean of diets low NE low AA (trt1) and low NE high AA (trt2). High NE = mean of high NE low AA (trt3) and high NE high AA (trt4) [2] Low AA = mean of low NE low AA (trt1) and high NE low AA (trt3). High AA = mean of low NE high AA (trt2) and high NE high AA (trt4)

Table 19 Broiler performance d 24 to 41 days

Treatment	Day 24 to 41		
	Feed intake	Weight gain	FCR
Main Effects			
	Net energy[1]		
Low NE	3143[b]	1911	1.646
High NE	3206[a]	1930	1.662
P value (NE)	*0.013*	*0.304*	*0.182*
	Amino acids[2]		
Low AA	3177	1915	1.659
High AA	3172	1926	1.648
P value (AA)	*0.857*	*0.577*	*0.372*
P value (NE x AA)	*0.38*	*0.194*	*0.401*

[abc] Means sharing the same superscripts are not significantly different from each other at P < 0.05.
[1]Low NE = mean of diets low NE low AA (trt1) and low NE high AA (trt2). High NE = mean of high NE low AA (trt3) and high NE high AA (trt4)
[2] Low AA = mean of low NE low AA (trt1) and high NE low AA (trt3). High AA = mean of low NE high AA (trt2) and high NE high AA (trt4)

Table 20 Carcass yield d 42

Treatment	Breast %	Thigh %	Fat pad %
Low NE Low AA	13.06	6.81	1.55
Low NE high AA	10.24	5.47	1.12
High NE low AA	10.23	5.35	1.15
High NE high AA	10.12	5.32	1.18
CV %	36.91	34.31	39.15
P value	*0.34*	*0.32*	*0.21*
Main Effects			
	NE[1]		
Low NE	11.65	6.07	1.33
High NE	10.17	5.41	1.16
P value (NE)	*0.27*	*0.32*	*0.29*
	Amino acids[2]		
Low AA	11.64	6.14	1.34
High AA	10.18	5.33	1.15
P value (AA)	*0.28*	*0.22*	*0.22*
P value (NE × AA)	*0.32*	*0.30*	*0.17*

[1]Low NE = mean of diets low NE low AA (trt1) and low NE high AA (trt2). High NE = mean of high NE low AA (trt3) and high NE high AA (trt4)
[2] Low AA = mean of low NE low AA (trt1) and high NE low AA (trt3). High AA = mean of low NE high AA (trt2) and high NE high AA (trt4)

DISCUSSION

Increased amino acids in diets produced an improvement in FCR in the birds at all ages. This observation is consistent with the results reported by Kidd et al. (2004) and Pesti (2009).

Diet 1, low net energy, low amino acid and diet 4, high net energy high amino acid both with the same NE to amino acid ratio had no significant difference in weight gain nor FCR throughout the life of the birds. This result indicates that the balance of nutrients that is more crucial to performance than the level of amino acid or NE in the diet. When the balance was maintained the live weight gain and the FCR in the birds were the same.

When the net energy in the diet increased, but the same amino acid level was maintained (comparing treatment 1 versus treatment 3), the birds FCR increased for the birds offered high NE diets. Classen (2013) studied the ratios of energy to amino acid looking at the energy range of 11.30 to 12.97 MJ/kg and reduced amino acid inclusion and discovered that broilers consuming a diet with essential amino acids set at 70% of requirement had lower FCR at low energy levels. The current study concurs with the observations made by Classen (2013). Birds had lower FCR when fed diets higher amino acids to NE ratio.

Since all diets were at the same AME level, the NE to amino acid balance seemed more crucial to performance. Diets 1 and 3 at the same AME balanced to amino acid had different NE levels. Diet 3 with higher fat inclusion had high NE, which explains the observation made by Pesti and Smith (1984), that fat had benefits beyond those attributed to ME. However, diet 3 with higher NE did not perform as well indicating amino acid levels became limiting in this diet with high NE.

On d 24 diet 1, low net energy low amino acid had a high feed intake. Diet 2, low net energy high amino acid, diet 4, high net energy high energy, and diet 3, high net energy low amino acid, all had comparable feed intake. During 24 to 41 days the feed intake was higher in diets 3 and 4, both of which were high energy diets. After a detailed look, the methionine level of these diets was significantly correlated ($P < 0.01$) with feed intake with a negative Pearson correlation of -0.453. A study

conducted by Hickling et al. (1990) showed that the methionine requirements for broilers at 3 to 6 weeks of age was higher than the recommendation published by NRC. The age of broilers in Hickling et al. study is similar to the age in the current study. This study did not report the feed intake; however, when calculated using the data reported, feed intake for the group with low methionine seemed higher. One could only speculate if the birds were consuming more feed during this phase to meet the methionine requirement. Increase in feed intake when birds fed low amino acid diets suggests that energy may not be the sole factor determining the feed intake. Energy and diet amino acid levels both seemed are contributing factors towards feed intake.

Fisher and Wilson (1974) reported that only 28% of the variation in feed intake was due to energy. Leeson et al. (1996) and Lemme et al. (2005) reported that feed intake linked to dietary energy. However, Plumstead et al. (2007); Latshaw (2008), Delezie et al. (2010) and Li et al. (2011) failed to see a correlation between feed intake and energy content of the diet. During d 24 to 41 of this study, the birds offered low NE diet also had the lowest feed intake ($P < 0.05$). There was also a negative correlation between methionine levels and feed intake. It seems broilers could be responding to the most limiting nutrient.

Birds fed the various diets had similar results for breast yield, thigh yield and percentage fat pad ($P > 0.05$).

Diets with low NE low amino acid and high NE high amino acid, both at the same NE to amino acid ratio were not significantly different in performance. Diets formulated to high net energy while keeping the same amino acid levels, resulted in performance loss. When cost favours selection of high NE ingredients, the amino acid level in the diet needs increase to ensure consistency in broiler performance.

CONCLUSION

The broiler feed intake may be a response to a limiting nutrient which could be protein, amino acid or energy.

The amino acid content of the diet drives the weight gain in broilers during an early age. Low NE levels lead to a higher amino acid to NE availability which eventuates in lower FCR and higher live weight. Another way to improve performance would be to increase the amino acid content of the diet.

The ratio of NE to the amino acids significantly influences bird performance. For grower diets formulated at 12.97 MJ/kg and finisher at 13.39 MJ/kg, a high amino acid to NE ratio improves FCR and weight gain in broilers.

Chapter 6: CHAMBER STUDY - EFFECT OF ANTIBIOTIC ON HEAT PRODUCTION, HEAT INCREMENT AND NET ENERGY

ABSTRACT

Zinc bacitracin is a commonly used antibiotic for growth promotion in the Australian poultry industry. Studies have shown that zinc bacitracin improves the AME of feed; however, there is no data to show its effect on NE. The hypothesis was, Zn bacitracin inclusion would reduce HP and hence increase NE of the diet.

Wheat and soybean meal-based diets were fed to 50 Ross 308 male broilers from d 0 with and without Zn bacitracin. On d 24, eight chambers, each containing two broilers, were assigned to each diet to determine the HI, HP and NE. The layout was a completely randomised design.

The results showed zinc bacitracin reduced HP from 758 kJ/kg to 713 kJ/kg ($P < 0.05$) but had no effect on HI nor RQ of birds fed the diet ($P > 0.05$) or NE of the diet ($P > 0.05$). These results suggest that while metabolism changed, the effect was not significant enough to be detected in terms of AME, HI or NE of feed. Further investigation into the impact of zinc bacitracin in the diet and the gut microflora, may be required.

INTRODUCTION

While banned in many overseas countries, the use of in-feed antibiotics is still a common practice for conventional broiler production in Australia. Zinc bacitracin produced by *Bacillus licheniformis* is a commonly used antibiotic for poultry production (Sarmah et al., 2006). It consists of a mixture of high molecular weight polypeptides. These polypeptides bind with the lipid carrier, isoprenyl pyrophosphate. The binding of the lipid carrier hinders the transfer of N-acetyl-muramyl-N-acetylglucosamyl-amino acid across the cell membrane. N-acetyl-muramyl-N-acetylglucosamyl-amino acid is the building blocks for cell wall (Phillips, 1999). This mechanism of bactericide activity is unique to bacitracin, compared to other commercial antibiotics.

Studies have shown an improvement in FCR in bacitracin fed chickens (Ao and Choct, 2013; Crisol-Martínez et al., 2017). While the benefits of using in-feed antibiotics are widely accepted, the mechanism is not as well understood. Some studies have reported that the improvement in growth when using antibiotics was due to reduction of total gut microbiota. Reducing the energy cost of the internal system (Gaskins et al., 2002; Collier et al., 2003), hence more energy available for growth, while others showed that there was a change in the gut microbial structure to the bacterial community more conducive to host growth (Dibner and Richards, 2005).

Crisol-Martínez et al. (2017) found that bacitracin reduced the *Lactobacillus* and *Eubacterium* counts, increases in the genus *Faecalibacterium* counts and hence increased the microbiota diversity. The increase in the diversity observed was unique as previous studies had only reported an effect on microbiota composition (Engberg et al., 2000; Pedroso et al., 2006; Gong et al., 2008 and Geier et al., 2009;). Crisol-Martínez et al. (2017) concluded that the low counts of *Lactobacillus* could have promoted the growth of other less-dominant taxa hence increasing the diversity in treated chickens. Torok et al. (2011) also reported a decrease in *Lactobacillus* with antibiotic treatment.

Lactobacillus produces the enzyme which hydrolyses the bile salt (Begley et al., 2006; Ridlon et al., 2006). A reduction *Lactobacillus* count reduces the amount of this enzyme which causes an increase in conjugated bile salts, improving digestion and ultimately growth performance (Lin et al., 2013). Huyghebaert and De Groote (1997) reported that ME corrected for nitrogen (MEn) for zinc bacitracin to be 2,080 and 1,184 Mcal/kg, for broiler chicks and laying hens, respectively.

The suppliers recommend the ME correction for zinc bacitracin for least cost formulation. However, there are no reports on how zinc bacitracin affects the net energy of the diet. This study is to measure the net energy, HP and HI difference due to the addition of zinc bacitracin in the diet.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England approved this experiment conducted in the net chamber room of the animal house at the University of New England, Armidale.

Fifty Ross 308 birds were fed a common standard starter diet from d 0 to d 10. Table 21 shows the composition of the starter diet. On d 10 they were divided into two pens. A control diet, as shown in table 21, was formulated to meet Ross 308 nutrient specification (Aviagen 2007). Fifty mg/kg zinc-bacitracin (Albac 150®, Pfizer Inc., NY, USA) was added to the control diet before pelletising to create the test diet

On d 21, 16 birds per treatment of average weights were placed in the CIC chamber to measure heat production, heat increment, AME and NE.

There were eight chambers assigned to each of the two diets and two birds placed in each chamber. Birds were fed grower diets and placed in chambers in a climate control room. The method for NE determination using CIC chamber is in chapter 3, 'Closed-circuit indirect calorimetric chamber'.

At d 21 birds were left to acclimatise in the calorimetric chambers for three days with the pumps turned on. Broilers always had access to food and water in the chamber. On d 24, the chambers were closed. The total excreta were collected and feed consumption recorded daily. The oxygen cylinder was weighed at the beginning of the trial and end of each run, to determine oxygen consumption. A subsample of solvent from potassium hydroxide scrubber, collected at the end of the run, was further analysed as outline in chapter 3.

The excreta collected was weighed and homogenized. The homogenized sample was freeze-dried, to determine crude protein and GE, and tested for moisture (85ºC for 48 hours).

Statistical analysis

ANOVA analysis performed using IBM SPSS® software was used to determine the statistical significance of data collected.

Table 21 Raw material composition of diets.

Ingredient	Starter %	Grower %
Wheat	60.4	68.1
Soybean meal	29.3	23.1
Meat meal	5.1	2.10
Canola oil	3.0	2.70
Salt	0.04	0.21
Sodium bicarbonate	0.52	0.19
Dicalcium phosphate		1.58
Limestone	0.69	1.13
L-lysine HCl	0.29	0.30
D, L-methionine	0.30	0.22
L-threonine	0.12	0.10
Choline chloride	0.03	0.06
Premix *	0.16	0.16
Sacox® 120**	0.05	0.05

* Vitamins and minerals supplied per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg.

** 120 g/kg sodium salinomycin.

RESULTS

Table 22 summarises the results for broiler response to zinc bacitracin in the diet.

Table 22 Response to zinc bacitracin fed to broilers.

	Control[1]	With zinc bacitracin[1]	P value	SEM
Average bird weight kg	1.511	1.569	NS	0.037
Average daily gain g/d	78.9	81.3	NS	6.203
FCR g/g	1.92	1.87	NS	0.131
ME intake Av. kJ/kg bird weight ^0.7	1098	1077	NS	42.5
HP kJ/kg BW0.7	758	713	0.042	11.39
HI feed kJ/kg	414	361	NS	19.56
RQ	0.95	0.96	NS	0.014
NE MJ/kg feed DM	8.08	8.29	NS	0.207
NE:ME	71.5	75.3	NS	0.011

[1] The values are the means of 8 replicates from each treatment.

There was no significant difference in the NE of feed nor the RQ with the addition of zinc bacitracin. Heat production is, however, significantly different ($P < 0.05$) with the addition of zinc bacitracin reducing the HI.

DISCUSSION

Adding zinc bacitracin to feed did not change the respiratory quotient (RQ) of broilers. Koh and MacLeod (1999) saw that RQ was a function of feed intake and reported constant RQ when feeding birds ad libitum, which is consistent with the observations made here. Even when the RQ did not change the heat production (HP) was lower with the addition of zinc bacitracin. Koh and MacLeod had also reported that HP directly correlated with feed intake, hence with a reduced feed intake HP would reduce. While the zinc bacitracin diet had reduced feed intake, this result was not statistically significant. Supplementation with zinc bacitracin may also reduce fasting heat production (Manner and Wang, 1991). The reduced fasting heat production may be due to a reduction of total gut microbiota, not measured during this study, reducing the energy cost of the internal system (Gaskins et al., 2002; Collier et al., 2003). It also is possible that with the dominant microbiota removed, other microbes are taking their place as reported by Crisol-Martínez et al. (2017). In their study, facultative anaerobe, Lactobacillus and Eubacterium replaced anaerobic

Faecalibacterium and heat production reduced. The addition of zinc bacitracin decreases GI tract inflammation by reducing pathogenic bacteria and endotoxin production (Gaskins et al., 2002; Collier et al., 2003) which would, in turn, reduce HI and the NE:ME ratio.

Even with the significant decrease in the HP ($P < 0.01$), the HI and NE were unaffected. The fasting rate of metabolism subtracted from HP gives the HI, hence NE of diet. Rosenberg and Zilber-Rosenberg (2016) reported that in the human gut, microbial metabolism produces approximately 70% of the total heat production of an average person at rest. Further to this, Manner and Wang (1991) reported that zinc bacitracin reduced the fasting heat production by 4.1% and 7.6% in hens at 20^{o}C and 34^{o}C, respectively. When determining fasting heat production (FHP) Noblet et al. (2015) did not have antibiotics in the diet.

Reviewing the number of replicates used in the study and the effect size due to the observed variation seen in the NE value the type 2 error or a false-negative, there is a 91.2%. The number of replicates used was not enough, and hence due to the low power of the study, it was not possible to deduce small differences in NE between diets.

Further research is needed to determine if infeed antibiotics, affect the gut microbiota, hence the fasting heat production of broilers. Addition of zinc bacitracin did not change the NE of diet in this study. However, the results of this study need reviewing if proven that zinc bacitracin affects fasting heat production, hence HI and NE.

CONCLUSION

Birds offered diets with zinc bacitracin had reduced HP. The addition of zinc bacitracin did not change the HI and NE of the diet.

The lower heat produced by zinc bacitracin indicates that there may be a change in the gut structure or microflora in the broilers, which needs further investigation.

Chapter 7: CHAMBER STUDY - EFFECT OF PHYTASE ON HEAT PRODUCTION, HEAT INCREMENT AND NET ENERGY

ABSTRACT

In the Australian poultry industry, where wheat is the prominent grain and sorghum is seasonal, it is common practice to add phytase in combination with xylanase in the feed. This current study was to investigate the effect of phytase (1000 FTU/kg) added together with xylanase (1220 units/kg) in wheat-based diets on performance and energy partitioning in broilers. The hypothesises was that the addition of phytase to a wheat-based diet with xylanase would increase the NE of the diet.

The base diet was a wheat-based diet supplemented with xylanase. Phytase was added to half of this feed to create a test diet. Birds were offered a common standard starter diet from d 0. On d 10 25 of the birds were fed diets with and without phytase. On d 24 birds were placed into 16 CIC chambers (2 birds per chamber). Eight chambers were assigned to each diet to determine the HI, HP and NE of the diets. The layout was a completely randomised design.

The results showed that there was no difference in HI, HP and NE of diets with the addition of phytase to an existing wheat-based diet with xylanase. However, phytase addition to the diet reduced the nitrogen content in the excreta of the broilers.

INTRODUCTION

Concerns regarding the bioavailability of phytate phosphorus from plant sources, to monogastric, has been documented (Johnson and Tate, 1969; Nelson et al., 1971; Harland and Morris, 1995). Phytate binds with proteins in the diet to form binary protein-phytate compounds hindering digestion (Cowieson et al., 2017). Phytase results in the cleavage of phytate-protein bonds reducing nitrogen excretion (Cowieson and Ravindran, 2007). Similar observations were made by Afsharmanesh et al. (2008) and Gallardo (2018), who reported higher nitrogen retention with the use of phytase.

Several studies have reported the lack of endogenous phytase produced by chickens as the cause for the reduced bioavailability of phosphorus (Maenz and Classen, 1998; Applegate et al., 2003) and this has become a common view in the industry. However, studies are indicating that poultry possesses very effective phytase and phosphatase activity in the gut (Oshima et al., 1964; Birge and Avioli, 1981; Maenz and Classen, 1998). Without the lack of enzymes, Cowieson et al. (2011) proposed that low substrate solubility in the small intestine and not the lack of compatible endogenous enzymes was causing the low bioavailability of phytate. The action of phytase can, therefore, be bimodal; improving the breakdown of food and absorption of nutrients. If the absorption of nutrients is improved, the net energy is higher.

The effect of phytase on energy has shown conflicting results. Some studies showed that phytase affected the dietary AME (Ravindran et al., 1999; Shirley and Edwards, 2003), while others (Tejedor et al., 2001; Pirgozliev et al., 2011) did not. Further to this, Pirgozliev et al. (2011) demonstrated that phytase affected the dietary NE of production in caged chickens.

Using a comparative slaughter technique, Pirgozliev and Bedford (2013) reported that an increase of 100 FTU of phytase increased dietary net energy by 15.4 J (3.4 cal/kg). Olukosi et al. (2008) also showed that phytase supplementation, alone or in combination with other enzymes, improved the net energy of production. Olukosi et al. (2008) used the comparative slaughter technique.

Wu et al. (2015) reported that only 2 out of 3 phytases tested at the inclusion level of 1,000 FTU/kg feed showed an improvement in the dietary NE. Wu et al., (2015) used closed-circuit calorimeter chamber technique to determine NE. The heat production (HP) and heat increment (HI) of the feed was unaffected by phytase. In this current study, NE change when phytase (1000 FTU/kg) is added together with xylanase (1220 units/kg) to the wheat-based diet, is determined. HI, HP and NE are measured using a CIC chamber.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England approved this experiment (Approval No: AEC 17-123) conducted in the net chamber room of the animal house at the University of New England, Armidale.

Fifty Ross 308 were placed on a common standard starter diet on floor pens until d 10. Table 23 shows the starter diet composition. On d 11 they were divided into two pens. A control grower diet, as shown in table 23, formulated to meet Ross 308 nutrient specification, contained 100 g/t of Axtra XB 201TPT (1220 xylanase units/kg). Combined 100 g/t of Axtra XB 201TPT (1220 xylanase units/kg) and 100 g of Axtra PHY 10,000 TPT (containing10,000 FTU/kg active 6-phytase) was added to the test diet. Both enzymes were products of Dupont, NSW, Australia.

Eight chambers, each containing two broilers, were randomly assigned to each diet. On d 21, 16 birds per treatment of average weights were placed in the CIC chamber to measure HI, HP and NE of the diets.

The method to determine NE using CIC chamber is in chapter 3, 'Closed-circuit indirect calorimetric chamber'.

At d 21 birds were left to acclimatise in the calorimetric chambers for three days with the pumps turned on. Broilers always had access to food and water in the chamber. On d 24, the chambers were closed. The total excreta were collected and feed consumption recorded daily. The oxygen cylinder was weighed at the beginning of the trial and end of each run, to determine oxygen consumption. A subsample of solvent from potassium hydroxide scrubber, collected at the end of the run, was further analysed as outline in chapter 3.

The excreta collected was weighed and homogenized. The homogenized sample was freeze-dried, to determine crude protein and GE, and tested for moisture (85ºC for 48 hours).

Statistical analysis

ANOVA analysis performed using IBM SPSS® software was used to determine the statistical significance of data collected.

Table 23 Raw material composition of the diets.

Ingredient	Starter %	Grower %
Wheat	60.4	68.1
Soybean meal	29.3	23.1
Meat and bone meal	5.1	2.1
Canola oil	3.0	2.7
Salt	0.04	0.21
Sodium bicarbonate	0.52	0.19
Dicalcium phosphate		1.58
Limestone	0.68	1.12
L-lysine HCl	0.29	0.30
D, L-methionine	0.30	0.22
L-threonine	0.12	0.10
Choline chloride	0.03	0.06
Premix *	0.16	0.16
Sacox® 120**	0.05	0.05
Axtra XB 201 TPT***	0.01	0.01

* Vitamins and minerals supplied per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg.

**120 g/kg sodium salinomycin; ***1220 xylanase units/kg

RESULTS

Table 24 shows the results reported for the CIC chamber. There was no significant difference in NE, heat production and RQ with the addition of phytase to the diet. The nitrogen content in the excreta dropped significantly ($P < 0.05$) with the addition of phytase.

Table 24 Responses to phytase fed to broilers from d 23 to 25 of age[1].

	Control no phytase[1]	With phytase[1]	SEM	P-value
Average bird weight kg	1607	1584	0.025	0.671
Average daily gain g/d	96.2	89.6	5.657	0.579
FCR g/g	1.639	1.577	0.059	0.614
ME intake Av. kJ/kg bird weight ^0.7	1221	1122	42.8	0.330
N in excreta %	17.7	12.4	0.807	0.039
N excreted / N intake %	40.1	38.4	<0.001	0.012
HP kJ/kg $BW^{0.7}$	753	731	7.96	0.169
HI kJ/kg feed	425	390	14.65	0.256
RQ	1.02	1.01	0.006	0.279
NE MJ/kg feed DM	9.14	9.59	0.273	0.428
NE:ME	75.0	74.6	0.005	0.701

[1] The values are the means of 8 replicates from each treatment.

DISCUSSION

The presence of phytase, in combination with xylanase, did not change the HP nor the HI of the diet. Further to this, the increase in NE observed by Olukosi et al. (2008); Pirgozliev and Bedford (2013) and Wu et al. (2015) failed to replicate in this study. While the NE of the diet with phytase was higher than the diet without phytase, the result was not significant ($P > 0.05$). This result might become significant with more replicates or with reduced coefficient of variation between the chambers.

Wu et al. (2015) had reported that not all phytase were similar and some did not show an improvement in NE while there was an improvement in NE of diet by 548 kJ/kg of diet for one out of 3 phytases at an addition rate of 1,000 FTU/kg active phytase.

The phytase used in this study did not show an improvement in NE. There was a significant reduction in nitrogen ($P < 0.05$) in the excreta for the diets with phytase, consistent with the observations made by Wu et al. (2015). The lack of differences in the HP and HI suggest the biochemical pathway may be unchanged.

Earlier publications reported that enzyme addition improved amino acid digestion of a wheat-based diet. Later it was corrected that the effects with amino acid were likely due to a reduction in endogenous amino acid losses and not due to improvement in digestibility (Selle et al. 2006). Similary Cowieson et al. (2011) reported that phytase improves the solubility of the nutrients. The low nitrogen in the excreta seen in this study may be due to higher absorption of nutrients in the gut and hence reduced endogenous amino acid losses.

During the current study, it was surprising to see that while broilers offered diets with phytase had higher retained nitrogen, they failed to show an improvement in protein gain. The short duration of the CIC chamber study may have contributed towards this. An extended period of feeding may be needed to investigate if phytase addition to the diet could benefit the birds by improving nitrogen retention.

CONCLUSION

Heat increment, HP and RQ were not affected by the addition of phytase indicating that the biochemical pathway may be unchanged. However, phytase addition to the diet reduced the nitrogen content of the excreta.

The reduction in nitrogen content of the excreta indicates that as proposed by the earlier studies, phytase may be changing the solubility of nutrients (hence nutrient absorption) and warrants further investigation into the effect phytase has on the absorption of nutrients.

Chapter 8: CHAMBER STUDY - EFFECT OF PELLET QUALITY ON HEAT PRODUCTION AND NET ENERGY

ABSTRACT

This study was to test if physical characteristics of feed, i.e. particle size, would affect energy partitioning and hence the NE of diets. The hypothesis was that pelleted feed would have higher NE than diets with finer particles due to differences in energy required for prehension of feed.

During the study, a starter and a grower diet were formulated and pelleted. Half of each diet was then passed through a crumble roller to create test diets with finer feed particles. Each of the starter diets was fed to 25 broilers in a separate pen from d 0. The birds were fed the respective grower diets (pellets or crumbles) from d 10. On d 24 birds were placed into 16 CIC chambers (2 birds per chamber). Eight replicate chambers were assigned to each diet to determine the HI, HP and NE of the diets. The layout was a completely randomised design.

The results showed that a reduction in feed particle size of the diet increased the HP from 715 kJ/kg to 799 kJ/kg ($P < 0.001$) and decreased the NE of diet from 11.21 MJ/kg to 9.67 MJ/kg. The HI of both diets were the same. Birds offered pelleted diets had higher live weight ($P < 0.001$) when compared to birds fed a diet with finer feed particles. Variation in feed particle size affected the NE of diet.

INTRODUCTION

Numerous studies have shown that pelleted feeds improve boiler performance by increasing feed intake (Calet, 1965; Choi et al., 1986; Nir et al., 1994a, 1994b, 1995; Lilly et al., 2001 and Engberg et al., 2002). This improved performance may be due to a decrease in energy used to consume pellets as opposed to mash feed. Abdollahi et al. (2011) showed that the variations in the pelleting processes affected broiler performance. The study produced pellets made at various steam-conditioning temperatures of the mash, conditioning temperatures and addition of binders and water to compare the broiler performance.

Abdollahi et al. (2011) showed that changes in pelleting parameters contributed to the performance variations seen in broilers. To study the performance difference caused by particle size only, Lilly et al. (2011) ground the pelleted diets to create a mash and compared the performance of broilers offered mash to birds feeding whole pellets. They observed that broilers consuming pellets had higher carcass weight, higher breast yield and lower FCR than boilers fed mash.

Lilly et al. (2011) concluded that the feed particle size was contributing to the performance variations seen. Feed particle size variation has an impact on the organ development of the broilers (Choi et al., 1986; Nir et al., 1994b; Svihus et al., 1997; Engberg et al., 2002 and Svihus, 2010).

Studies have also shown that pelleting affects the microbiome in the gut of the broilers (Engberg et al., 2002) which is consistent with the observations made in pigs (Jørgensen et al., 1999).

McKinney and Teeter (2004) were more focused on behavioural observations to explain the performance differences observed between mash and pellets. During their study, the effective caloric value was determined using bird body weight and feed conversion. The effective caloric value is the caloric density required to achieve the same live weight and FCR. Even though the mash and pelleted diets were isocaloric, McKinney and Teeter saw 4.60 MJ/kg change in the effective caloric value of diet for the two diets. For a 12.76 MJ/kg ration, the effective caloric value was 10.25 when the birds spent 20% resting time compared to 14.85 when the bird spent 85% of the time resting.

These findings were consistent with Jensen et al. (1962) study who had come to the same conclusion after observing the change in behaviour while feeding on pellets versus mash. Birds on mash were active when compared to birds on pellets, hence leading to lesser weight gains and higher FCR for birds fed mash. However, a study conducted by McIntosh et al. (1962) to determine the factors affecting the ME content of poultry feeds ruled out grinding nor pelleting of cereal grains as possible factors contributing to ME differences, therefore, if there is an energy change due to particle size variation its likely NE and not ME.

The current study was to examine feed texture only. The objective was to determine the NE change caused by grinding pelleted diets. Both ground and pellets diets were identical in the formulation, processing and isocaloric for AME.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England approved this experiment conducted in the net chamber room of the animal house at the University of New England, Armidale.

A starter and grower diet, as shown in table 25, formulated to meet Ross 308 nutrient specification, were pelleted. These were control starter and control grower diets. Half of these diets were processed through a crumble roller to reduce the size of the pellets. These feed with finer particles were the test starter and test grower diets.

Twenty-five Ross 308 birds were placed on each of the starter diets on floor pens until d 10 and switched to the respective grower diets.

On d 21, 16 birds per treatment of average weight were placed in the CIC chamber to measure HI, HP and NE.

The particle size profile of the feeds was analysed using Retsch AS200 Digit CA for 5 minutes at 3.00 mm amplitude.

The method to use CIC chamber to determine NE is in chapter 3, 'Closed-circuit indirect calorimetric chamber'.

At d 21 birds were left to acclimatise in the calorimetric chambers for three days with the pumps turned on. Broilers always had access to food and water in the chamber. On d 24, the chambers were closed. The total excreta were collected and feed consumption recorded daily. The oxygen cylinder was weighed at the beginning of the trial and end of each run, to determine consumption. A subsample of solvent from potassium hydroxide scrubber, collected at the end of the run, was further analysed as outline in chapter 3.

The excreta collected was weighed and homogenized. The homogenized sample was freeze-dried, to determine crude protein and GE, and tested for moisture (85ºC for 48 hours).

Statistical analysis

ANOVA analysis performed using IBM SPSS® software was used to determine the statistical significance of data collected.

Table 25 Raw material composition of diets.

Ingredient	Starter %	Grower %
Wheat	60.36	67.41
Soybean meal	29.3	22.1
Meat meal	5.1	4.5
Canola oil	3.0	4.1
Limestone	0.69	0.48
Salt	0.04	0.15
Sodium bicarbonate	0.52	0.30
Choline chloride	0.03	0.04
L-lysine HCl	0.29	0.30
D, L-methionine	0.30	0.25
L-threonine	0.12	0.12
Avizyme	0.04	0.04
Sacox® 120	0.05	0.05
Broiler vitamins minerals premix *	0.16	0.16

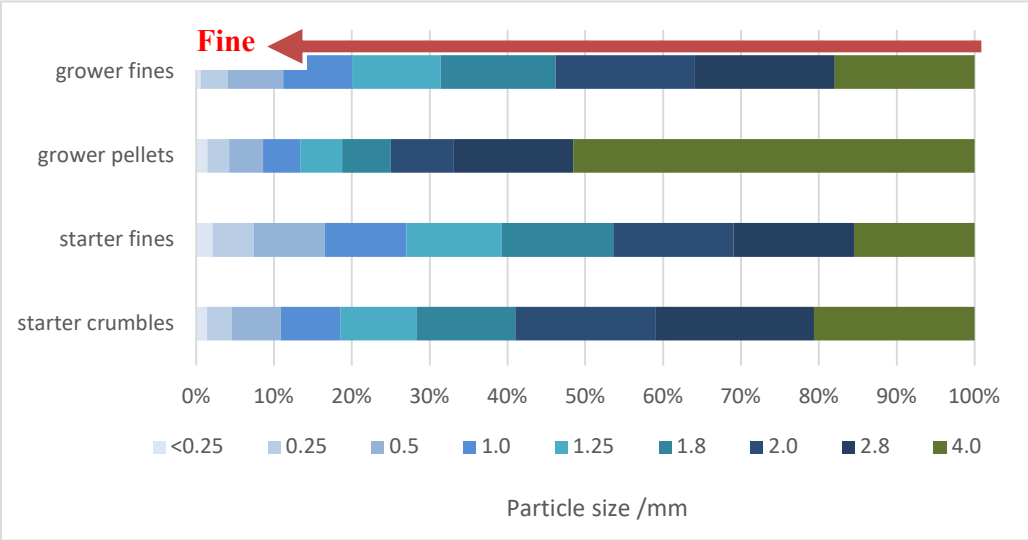
* Vitamins and minerals supplied per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5,000 IU; vitamin E, 75 IU; vitamin K, 3 mg; vitamin B_{12}, 16 mcg; riboflavin, 8 mg; pantothenic acid, 13 mg; nicotinic acid, 55 mg; folic acid, 2 mg; biotin, 0.2 mg, Mn, 120 mg; Zn, 100 mg; Fe, 40 mg; Cu, 16 mg; Se, 0.3 mg; I, 1.25 mg.

RESULTS

Table 26 Response of broilers fed pellet versus fine diets.

	Control pellets	Test fines	SEM	P value
Average bird weight d25 kg	1864	1472	0.056	< 0.001
FCR d 25 g/g	1.32	1.31	0.038	0.939
Data collected from d 23 to 25 of age[1].				
Average daily gain d 25 g/d	121.1	112.3	3.120	0.168
ME intake Av. kJ/kg bird weight ^0.7	1216	1235	23.1	0.682
N in excreta %	12.9	13.5	0.377	0.912
HP kJ/kg $BW^{0.7}$	715	799	18.44	0.001
HI kJ/kg feed	410	456	19.1	0.126
RQ	0.968	0.984	0.017	0.128
NE MJ/kg feed DM	11.21	9.67	0.315	0.003
NE:ME	79.8	73.8	0.015	0.004

[1] The values are the means of 8 replicates from each treatment.



Graph 4 The particle size profile of feeds.

The particle size had a significant effect on live weight with birds offered fines having a lower weight; however, there was no difference in FCR between the treatments. While the AME intake for both diets was not significantly different, the NE pelleted feed was higher than mash ($P < 0.05$). There were no significant differences in the nitrogen in the excreta for birds fed both diets. The RQ of birds fed both diets were also similar. However, birds offered pelleted diets had lower heat production than those offered fines ($P < 0.01$).

DISCUSSION

Consistent with all studies reported earlier, birds offered the pelleted diet had significantly higher weight gains than birds offered a fines diet (1.826 kg and 1.491 kg respectively). The NE of pelleted diet, 11.2 kJ/g, was also significantly higher ($P < 0.05$) than the NE of the mash diet, 10.21 kJ/g. The increase in HP contributed towards the lower NE seen in broilers offered fines.

Birds offered pelleted diet had a significantly lower ($P < 0.001$) HP of 734 kJ/kg $BW^{0.70}$ when compared to birds on mash at 789 kJ/kg $BW^{0.70}$. It is difficult to say if the lower HP in the pelleted offered diet was due to difference in the organ development or a change in microbiome population of the gut (Engberg et al., 2002). The high HP in a mash diet may also be due to increased feeding activity as proposed by McKinney and Teeter 2004 and Jensen et al., 1962. Even with the changes in HP, the RQ was constant. Constant RQ, even with the increase in activity due to feeding mash, could mean the birds were still in their comfort zone.

The significant change observed in NE but not in AME due to particle size indicates that while the feeding behaviour and the energy spent in feeding was affecting the NE, it did not influence AME. A similar observation reported by Hill and Anderson (1958) and Bourdillon et al. (1990) who reported that ME was independent of food intake in the range from 100 to 30% fed ad libitum. They had also noted a relationship between the plane of food intake and production energy, but the relationship was not consistent.

While the improvement in organ development and less time spent feeding is working in favour of pelleted feed, potential improvement in the digestion and absorption due

to greater surface area of fines does not seem to benefit the birds offered finer feed particles. The effect of feed particle size on digestibility and absorption needs further investigation.

A review presented by Noblet (2010) stated that NE could be beneficial and a better predictor for poultry as it is for pigs. However, the benefit was not as clear in poultry, and some areas needed further investigation. During his studies, Noblet did not report on the physical characteristics of the feed. When considering the net energy theory which proposes that HI, net energy for maintenance and net energy for production were all constants of feed ingredients or feed (Latshaw and Moritz, 2009) the assumptions made by Noblet during research seems sound. Further to this, Noblet et al. (2015) predicted the FHP for broilers. This value was used as the literature value for the study conducted by Wu et al. (2019), to generate prediction equations for the energy content of the diet, used during this research. However, since FHP is affected by the energy spent during eating activity, it may not be consistent across diets with varying particle size.

Li et al. (1991) when measuring heat production associated with food intake reported that while 16% ME intake was associated with HP of feeding, 0.8% of ME intake was energy cost of the eating activity. In agreement with Li et al., this study also shows that the particle size causes a change in HP and NE.

While ME is independent of the quality of diet, it seems that the diet quality is playing a role in the NE of the diet.

CONCLUSION

Feed particle size reduction reduced the NE of the diet, which supports previously published research.

There is more than just proximate analysis that affects the NE of the diet. Further investigation to establish if these are to be factored into the NE prediction equation or stated as conditions to fulfil before using the published literature is needed.

Chapter 9: EFFECT OF NET ENERGY ON PERFORMANCE AND CARCASS COMPOSITION OF BROILERS

ABSTRACT

This study was to evaluate diets formulated with the same key ingredients but with differences in energy to amino acid ratios. The hypothesis was that the NE to amino acids ratio but would be a better predictor of bird performance than AME to amino acid ratio. Diets formulated to 3 different AME, NE and protein levels were blended. Diet A had high AMEn of 13.85 MJ/kg; diet E had low AMEn of 13.29 MJ/kg while diet B, C and D were at the same AMEn level of 13.60 MJ/kg. Diets A, B and E had similar protein levels between 19.0% to 19.6%. Diet C had a low protein of 18.4%, and diet D had a high protein of 21.9%. The NE content of diets A and B, and diets C and D were the same (11.05 MJ/kg and 10.79 MJ/kg respectively). The broilers were fed the diets with feed intake, live weights measured. Fifteen Ross 308 day-old male chicks were fed a common standard starter diet in each of the 45 pens and fed *ad libitum*. On d 10, nine pens, each with fifteen birds, were randomly assigned to each of the five grower diets. On d 24 the diet was changed to the finisher.

The results showed that high protein diets had a higher weight gain only when there were adequate NE and AME in the diet. The optimum protein levels for grower diet was 21.5% and NE of 10.75 MJ/kg. It is the ratio of NE to protein (amino acid) that dictates live weight and FCR, and not AME.

Regression analysis indicated that feed intake might be more challenging to predict than the FCR and weight gain of the birds. Feed intake not only varied with the nutrient content of the diet and the age of the birds.

The results also strongly suggested that NE is a better predictor of FCR than AME.

INTRODUCTION

Earlier in the study, both NE and AME systems were successfully used to formulate broiler diets. However, when formulating diets using NE versus the AME system, the raw materials used are different. This variation in raw material introduces an

additional variable in the study. The study showed that, while diets formulated using a range of raw materials achieved the nutritional and performance targets in broilers, there was a significant difference in performance due to raw materials. One of the objectives of this study was to eliminate the raw material variation and formulate diets using the same key ingredients.

This study further investigated the role of NE and amino acid on the performance of the bird without the raw material variability in diets. Since diets formulated to a constant AME resulted in differences in performance, it was apparent that AME was not the driving force for performance.

During the current study, five diets formulated at three different AME, NE and protein levels, contained the same major raw materials. The objective was to determine NE and protein levels to achieve the optimum performance in broilers.

MATERIALS AND METHODS

The Animal Ethics Committee of the University of New England approved this experiment conducted at the Ring Road Shed, University of New England, Armidale.

The birds were fed a common standard starter diet from d 0 to d 10. Tables 27 and 28 show diets formulated at varying NE and AME levels.

Diets were formulated at three AME levels; diet A formulated at a high AME level (13.61 MJ/kg), diets B, C and D at a mid AME level (13.46 MJ/kg), and diet E at a low AME level (11.72 MJ/kg). Diets A and B were at the same protein and NE levels. Diet B and D were at the same protein level of 21.5%, while the protein level in diet C was at18.8%. Diet B was at 10.91 MJ/kg NE level while diet C and D were both had NE of 10.75 MJ/kg. Diet E had the same protein level as diet A, B and D, but different AME and NE levels. The marker used was titanium dioxide at 0.5%.

All diets were fed as mash and formulated to deliver the amino acid requirements outlined for Ross 308 (Aviagen, 2009). The litter used was hardwood shavings.

Fifteen Ross 308 day-old male chicks were fed a common standard starter diet in each of the 45 pens and fed *ad libitum*. On d 10, 9 pens, each with fifteen birds, were

randomly assigned to each of the five grower diets. On d 24 the diet was changed to the finisher. On d 14, 24 and 35 birds and feeders were weighed.

On d 28, three average weight birds were selected and euthanised from every pen. The ileal content was collected by, squeezing out the contents from Meckel's diverticulum to approximately 1 cm proximal to the ileocecal junction. These were then frozen and kept below -20^{o}C until processed. The ileal content was freeze-dried and finely ground to 3 mm screen and analysed for GE and protein. The procedure to determine digestibility is below. The liver was removed and weighed.
On d 35 the birds and the feeders were weighed. Four birds per pen were selected and euthanised to measure breast meat (single breast skin off), thigh (thigh fillet skin off) (Australian Chicken Meat Federation, 2018) and the fat pad.

Weight gain and feed intake were calculated and corrected for the euthanised birds. The digesta and feed samples were analysed for DM by oven drying at 105^{o}C for 24 hours (AOAC 930.15, 1990) and nitrogen content was determined using Leco (Model FP-2000 N analyser, Leco Corp., St Joseph, MI), a combustion analyser using EDTA as a calibration standard. Nitrogen content was multiplied by factor 6.25 to convert to crude protein.

Titanium dioxide concentrations were determined in triplicate for the diets and digesta samples by a colorimetric method (Short et al., 1996).

Digestibility calculations

Ileal nitrogen flow (INF) (mg/kg dry matter intake) was determined by:

$$INF = N \text{ in digesta (mg/kg)} \times TiO_2 \text{ in diet (mg/kg)} / TiO_2 \text{ in digesta (mg/kg)} \quad (1)$$

The apparent ileal digestibility of N (AIDN) calculated using the following equations:

$$AID = [(\text{diet N intake} - \text{total INF}) \times 100] / \text{diet N intake} \quad (2)$$

Ileal energy flow (IEF) (mg/kg dry matter intake) was determined by:

$$IEF = E \text{ in digesta (mg/kg)} \times TiO_2 \text{ in diet (mg/kg)} / TiO_2 \text{ in digesta (mg/kg)} \quad (3)$$

The apparent ileal digestibility of E (AIDE) calculated using the following equations:

$$AIDE = [(\text{diet E intake} - \text{total IEF}) \times 100] / \text{diet E intake} \quad (4)$$

Statistical analysis

Polynomial regressions for FCR, weight gain and FI, and to determine if these correlated with AME, NE and protein content of the diets, was analysed using IBM SPSS® software. Table 34 shows the coefficient estimates and summary statistics.

Table 27 Raw material composition of grower diets.

Ingredient (%)	A	B	C	D	E
Wheat	56.26	37.31	32.38	57.8	37.3
Soybean meal	23.92	22.27	19.38	23.06	22.27
Millrun		12.00	10.44		12.00
Canola oil	7.33	11.00		6.62	3.00
Sunflower oil			0.87		
Canola meal	5.00	6.00	5.22	5.00	6.00
Meat and bone meal	5.00	5.00	4.35	5.00	5.00
Oat hulls	-	3.81	3.31		3.81
Limestone	0.48	0.60	0.52	0.48	0.60
Sodium bicarbonate	0.40	0.39	0.34	0.41	0.39
L-lysine	0.25	0.25	0.31	0.26	0.25
D, L-methionine	0.28	0.29	0.34	0.28	0.29
Premix*	0.18	0.18	0.18	0.18	0.18
Salt	0.19	0.19	0.16	0.19	0.19
L-threonine	0.11	0.11	0.17	0.11	0.11
Choline chloride				0.01	
Sacox® 120**	0.05	0.05	0.05	0.05	0.05
Zinc bacitracin***	0.033	0.033	0.033	0.033	0.033
Axtra XB 201 TPT****	0.01	0.01	0.01	0.01	0.01
Axtra PHY 10,000 TPT *****	0.01	0.01	0.01	0.01	0.01
Corn starch	-		21.18		8.01
L-tryptophan			0.02		
L-arginine			0.14		
L-isoleucine			0.09		
Titanium oxide	0.5	0.5	0.5	0.5	0.5

*Trace vitamin and mineral concentrate supplied per kg of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

**120 g/kg sodium salinomycin; ***150g/kg Zinc Bacitracin; ****1220 xylanase units/kg; *****10,000 FTU/kg active 6-phytase

Table 28 Raw material composition of finisher diets.

Ingredient (%)	A	B	C	D	E
Wheat	40.88	43.41	37.81	50.51	60.29
Soybean meal	17.05	18.10	15.83	24.50	16.20
Millrun	8.94	9.49	8.30	9.21	4.00
Canola oil		10.97		3.33	6.33
Sunflower oil	0.94		0.87		
Canola meal	4.71	5.00	4.37	6.00	5.00
Meat and bone meal	4.33	4.60	4.02	4.60	4.60
Oat hulls	5.27	5.60	4.89		1.03
Limestone	0.52	0.55	0.48	0.52	0.53
Sodium bicarbonate	0.35	0.38	0.33	0.07	0.29
L-lysine	0.72	0.68	0.77	0.04	0.48
D, L-methionine	0.30	0.27	0.32	0.18	0.25
Premix*	0.17	0.17	0.17	0.17	0.17
Salt	0.05	0.05	0.05	0.27	0.11
L-threonine	0.14	0.11	0.17		0.11
Choline chloride	0.02	0.02	0.01		0.01
Sacox® 120**	0.05	0.05	0.05	0.05	0.05
Zinc bacitracin***	0.033	0.033	0.033	0.033	0.033
Axtra XB 201 TPT ****	0.01	0.01	0.01	0.01	0.01
Axtra PHY 10,000 TPT *****	0.01	0.01	0.01	0.01	0.01
Corn starch	14.90		20.68		
L-tryptophan	0.01		0.02		
L-arginine	0.06		0.12		
L-isoleucine	0.04		0.07		
Titanium oxide	0.5	0.5	0.5	0.5	0.5

*Trace mineral concentrate supplied per kg of diet: retinol, 12000 IU; cholecalciferol, 5000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamin, 16 µg; biotin, 200 µg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

**120 g/kg sodium salinomycin; ***150g/kg Zinc Bacitracin; ****1220 xylanase units/kg; *****10,000 FTU/kg active 6-phytase

RESULTS

Table 29 and 30 show the nutritional composition of these diets. Table 31 summarises the performance results.

At d 24 diet C, at similar energy levels as diet A, B and D, also had similar feed intake. Diet E, however, with the lowest AME and NE, had significantly higher feed intake of 104 g/d ($P < 0.001$). Broilers offered diet E also had the low weight gain, 659 g ($P < 0.001$) and high FCR of 1.59 ($P < 0.001$). Birds fed diet C, at the lower protein level of 20.1%, had lower weight gains and higher FCR. Grower A and B were at the same protein and NE levels but different AME levels with diet A being at higher AME of 13.61 MJ/kg while B at 13.46 MJ/kg. The results for weight gain, feed intake and FCR for broilers offered these diets were comparable with no statistical differences.

Diets B, C and D had all been at the same AME value, but the birds had performed differently for weight gain ($P < 0.001$) and FCR ($P < 0001$). Grower B and D had different dietary NE levels. The broilers were consuming more of diet D at lower NE and gaining more weight ($P < 0.001$) and had lower FCR (not significant) compared to birds on diet B.

For d 24 to 35, birds offered diets A, C and E with lower protein (18.4% to 19.2%) levels had a higher feed intake when compared to birds offered diet D with a higher dietary protein of 21.9% ($P < 0.001$). Even with the high feed intake, the weight gain of birds offered diet A was significantly low ($P < 0.001$). Diet A had a high NE level of 10.66 MJ/kg. These birds also had a high FCR of 2.12 ($P < 0.001$). While Diet E had the lowest NE of 10.66 MJ/kg, broilers offered this diet had a lower FCR of 1.48 and higher weight gain at 1171 g ($P < 0.001$).

Finisher A and B; and C and E; had the same NE but different AME and protein levels. Finisher A, which was at a lower protein, had a higher intake ($P < 0.001$) which was similar to the finisher C which was also lower protein with the higher intake (not significant). Even with the higher intake for finisher A and C, the broilers had lower weight gain (finisher A, $P < 0.001$; finisher C, not significant).

Finisher B, C and D had the same AME level, but there was a variation seen in the feed intake ($P < 0.001$) of broilers offered these diets. Broilers offered diet C had a higher feed intake of 146 g while intake of broilers offered D was 128 g ($P < 0.001$). The diets C and D were at similar NE and AME, but diet D was at a higher protein level (diet D at 21.9% and C at 18.4%). There was no significant difference in the weight gain and FCR of these broilers. The feed intake of birds on diet D was significantly higher than broilers offered diet B (128g and 115g, respectively). Both diets were at the same AME. The NE of diet B was 11.05 MJ/kg while diet D was 10.78 MJ/kg. There was a significant correlation between NE and feed intake with an increase in feed intake at lower dietary NE levels.

The broilers offered low-density diet E had significantly lower nitrogen ($P < 0.05$) and energy ($P < 0.001$) digestibility. The birds offered diet E also had significantly larger ($P < 0.001$) liver size. There was a correlation ($r = -0.89$, $P < 0.05$) between the liver size and the thigh muscle yield in the broilers. Birds with smaller livers had higher thigh yield. Table 32 summarises these results.

Broilers offered diet D had the highest fat pad ($P < 0.001$) and lower breast meat ($P < 0.05$). This diet had high protein and NE levels. Table 33 shows the result summary.

Diets A and B had similar NE and protein levels but different AME level. Broilers offered diet A had lower weight gain and reduced breast yield while broilers offered diet B had significantly ($P < 0.05$) higher weight gain and breast yield. High AME in diet A did not eventuate in a higher weight gain and neither carcass yield.

Table 29 Nutritional profile of the grower diets.

Ingredient	Grower A	Grower B	Grower C	Grower D	Grower E
AMEn MJ/kg	13.61	13.46	13.46	13.46	11.72
Crude protein %	21.7	21.5	20.1	21.5	21.5
Crude fat %	8.9	12.8	2.8	5.2	4.8
Linoleic acid %	1.98	2.80	1.20	1.84	1.20
Dig* lysine %	1.14	1.12	1.20	1.12	1.12
Dig methionine %	0.57	0.57	0.57	0.56	0.57
Dig methionine & cysteine %	0.87	0.86	0.86	0.86	0.86
Dig tryptophan %	0.22	0.22	0.22	0.22	0.22
Dig arginine %	1.26	1.26	1.26	1.24	1.26
Dig threonine %	0.75	0.75	0.75	0.75	0.75
Dig isoleucine %	0.83	0.79	0.79	0.81	0.79
Dig valine %	1.00	0.99	0.86	0.99	0.99
NE MJ/kg **	10.91	10.91	10.75	10.75	9.25
NE : protein	120	121	137	120	103
AME : protein	150	150	171	150	130
NE : AME	0.801	0.810	0.799	0.800	0.891

**Dig = digestible ** calculated using equation by Wu et al. (2019).*

Table 30 Nutritional profile of the finisher diets.

Ingredient	Finisher A	Finisher B	Finisher C	Finisher D	Finisher E
AMEn MJ/kg	13.85	13.60	13.60	13.60	13.29
Crude protein %	19.0	19.6	18.4	21.9	19.2
Crude fat %	2.7	12.7	4.0	5.9	8.0
Linoleic acid %	1.40	2.78	1.39	2.21	1.84
Dig* lysine %	1.20	1.33	1.20	1.00	1.13
Dig methionine %	0.52	0.52	0.52	0.48	0.50
Dig methionine & cysteine %	0.78	0.78	0.78	0.78	0.77
Dig tryptophan %	0.19	0.19	0.19	0.23	0.18
Dig arginine %	1.09	1.09	1.09	1.30	1.05
Dig threonine %	0.67	0.67	0.67	0.67	0.66
Dig isoleucine %	0.69	0.69	0.69	0.85	0.68
Dig valine %	0.75	0.75	0.75	0.75	0.76
NE MJ/kg**	11.05	11.05	10.79	10.78	10.66
NE : protein	139	135	138	119	133
AME : protein	174	166	177	148	165
NE : AME	0.798	0.812	0.784	0.803	0.802

*Dig = digestible ** calculated using equation by Wu et al. (2019).

Table 31 Broiler performance for birds on various diets.

Period	Diet	A	B	C	D	E	P-value	SEM
14 to 24	weight gain g	751^{bc}	739^{b}	624^{a}	783^{c}	659^{a}	<0.001	10.62
	Feed intake g/day	93^{a}	93^{a}	94^{a}	96^{a}	104^{b}	<0.001	0.96
	FCR	1.26^{a}	1.28^{a}	1.51^{b}	1.25^{a}	1.59^{c}	<0.001	0.96
24 to 35	weight gain g	884^{a}	1052^{b}	1062^{b}	1077^{bc}	1171^{c}	<0.001	21.23
	Feed intake g/day	141^{c}	115^{a}	146^{c}	128^{b}	141^{c}	<0.001	2.15
	FCR	2.12^{d}	1.58^{b}	1.66^{c}	1.68^{c}	1.48^{a}	<0.001	0.04
14 to 35	weight gain g	1635^{a}	1802^{b}	1686^{a}	1861^{b}	1830^{b}	0.012	429
	Feed intake g/day	234^{bc}	208^{a}	240^{cd}	224^{b}	244^{d}	<0.001	2.44
	FCR	1.60^{c}	1.41^{a}	1.61^{c}	1.42^{a}	1.54^{b}	<0.001	0.02

Table 32 Ileal digestibility on d 28.

Diet	A	B	C	D	E	P-value	SEM
Average weight	1188^{bc}	1171^{b}	1032^{a}	1231^{c}	1078^{a}	<0.001	13.05
Apparent ileal digestibility of N %	81.32^{b}	77.28^{ab}	79.76^{b}	78.48^{ab}	75.53^{a}	0.034	0.63
Apparent ileal digestibility of E %	73.61^{b}	64.36^{ab}	65.47^{b}	68.39^{ab}	55.85^{a}	<0.001	1.18
liver % live weight	2.31^{ab}	2.46^{bc}	2.36^{b}	2.10^{a}	2.65^{c}	<0.001	0.04

Table 33 Carcass yield on d 35.

	A	B	C	D	E	P-value	SEM
Average weight g	2071^{a}	2234^{b}	2094^{a}	2309^{b}	2248^{b}	<0.001	21.42
fat pad % live weight	0.58^{a}	0.56^{a}	0.49^{a}	0.82^{b}	0.61^{a}	<0.001	0.03
breast % live weight	9.62^{a}	10.49^{b}	10.79^{b}	9.89^{a}	10.20^{b}	0.016	0.12
thigh % live weight	4.67	4.59	4.52	4.71	4.41	NS	0.04

Table 34 ANOVA coefficient statistical analysis for performance and protein, NE and AME of the diet.

	14 to 24 days			25 to 35 days		
	weight Gain	Feed intake	FCR	weight Gain	Feed intake	FCR
AME MJ/kg	221.745	-0.962	-0.259	-493.468		1.229
NE MJ/kg	-187.466	-5.029	0.087	-10.818	-33.740	-0.192
Protein %	75.288		-0.120		-3.138	
Intercept	-1822.321	161.498	6.394	7871.351	562.160	-12.911
R^2	0.691	0.376	0.782	0.400	0.316	0.585
R^2 adjusted	0.669	0.330	0.766	0.372	0.283	0.566
P value	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
			Interaction regression			
AME x Protein	3.170	-0.169	-0.008		-0.345	
Intercept	-166.827	142.972	3.714		225.875	
R^2	0.583	0.204	0.781		0.164	
R^2 adjusted	0.573	0.185	0.776		0.144	
P value	*<0.001*	*<0.001*	*<0.001*		*<0.001*	
NE x Protein	3.480	-0.190	-0.009		-0.470	
Intercept	-58.761	138.112	3.444		234.140	
R^2	0.568	0.207	0.774		0.232	
R^2 adjusted	0.558	0.189	0.768		0.214	
P value	*<0.001*	*<0.001*	*<0.001*		*<0.001*	

DISCUSSION

Neither dietary AME, NE, nor protein could singularly explain the broiler performance variations observed in this study. The broiler performance seems to be dictated by interaction with all these nutrients. These nutrients do not perform independently of each other. This interaction explains the observations reported by Cerraete et al. (2013) that AME as poor performance predictor.

The regressions in table 34 show that feed intake would be more challenging to predict than FCR or weight gain. Not only did it have low R^2 ($R^2 = 0.4$) for all regressions, the response during the different life stages (14 to 24 and 24 to 35 days) were also different. FCR and weight gain had a greater correlation to dietary protein, AME and NE.

Broilers consumed more of the low NE diet. As the broilers feed intake increases, there is an increase in the nutrient uptake, and hence birds had a higher weight gain. In cases when the NE is similar, as seen in finisher C and D, broilers consumed more of the low protein diet C ($P < 0.001$). The results suggest that the broilers were consuming more of the diet if either dietary NE or protein levels were low. FI was not in response to dietary AME levels. Similar observations were made by De Groote (1974) who reported that FI was not affected by an increase in diet ME but saw a linear decrease with diet NE.

The protein level in the diet impacts the weight gain showing a significant correlation during 14 to 24 days. Similar findings of lower weight gain, when broilers offered low protein diets, were observed by Swennen et al. (2004). Swennen et al. (2004) did not see a change in absolute feed intake, but when they converted the feed intake to per metabolic body weight$^{0.75}$ where there were significant differences. Bartov and Bornstein (1976); Jones and Smith (1986); Buyse et al. (1992); Bregendahl et al. (2002) and Collin et al. (2003) all reported similar slower growth when broilers offered low protein diets. Similarly, Jackson et al. (1982) reported improved weight gain and FCR with increased dietary protein or energy. When adequate protein delivered via the diet, birds grow to the target weights. Broilers response to low protein in diets is complicated. Slightly reduced protein levels cause a slight increase

in feed intake (hyperphagia as reported by Rosebrough and Steele, 1985 and Carew and Alster, 1997). A more severe protein deficiency in diet could lead to feed rejection (hypophagia as reported by Buyse et al., 1992 and Rosebrough et al., 1996). Deficiency in essential amino acids leads to the same effect.

Earlier studies show that excessive dietary protein produced leaner birds and had poor FCR (Buyse et al., 1992). However, this observation was not coherent with the current study. The selection in the genotype of the modern bird, with significantly higher protein requirement, may explain this difference observed. Adjusting diets to a higher protein level could be used to improve on the weight gain of the present Ross broilers. A similar effect in the diet is achieved by, reducing the NE level, while keeping the protein constant. Lower NE increase the feed intake, which increases the protein intake per se. A well-balanced protein to NE level in the diet may improve the FCR and increase the lean bodyweight of the broilers.

Diet E with the lower nutrient density also reported significantly poor digestibility of nitrogen and energy. The birds offered these diets had significantly larger livers when compared to birds on all other diets. Diet D with high protein content had a significantly smaller liver size.

The liver carries out many vital functions. These include fatty acid synthesis, gluconeogenesis, glycolysis, DNA synthesis, protein synthesis, and Na^+/K^+ transport. Further to this, the liver metabolic rate could account for 10 to 12% of the total bird energy expenditure (Spratt et al., 1990). While de novo synthesis of amino acids occurs in the liver, lipogeneses occur in the adipose tissues. Diet D had a high protein content (21.5%) and lower NE content (10.75 MJ/kg). With high protein available in the diet, there would be no need for de novo synthesis of amino acid in the birds. De novo synthesis of amino acids is a function of the liver, and the birds on this diet had a significantly smaller liver (2.10%).

Broilers offered diet E, however, had significantly lower nitrogen digestibility (75.53%) and larger livers when compared birds offered diets A and C ($P < 0.001$). Birds offered diet C had low nitrogen digestibility and enlarged livers. Due to poor digestibility, de novo synthesis of amino acids may be enhancing liver development.

Studies conducted on organ development, affected by diet, show contradicting results. Noy and Sklan (2002) reported that dietary protein, fat or cellulose influenced the proportional weights of internal organs. Enlarged gizzard development was reported in birds consuming litter (Ali et al., 2009) and whole-grain inclusion in the diet (Hetland et al., 2005). To study the effect of stress on broilers Malheiros et al. (2003) added various levels of corticosterone to the diet. High corticosterone increased the weight of liver, abdominal fat pad, proventriculus and gizzard. However, there was a reduction in the growth of spleen and bursa. The changes in the organ sizes indicated that broilers were adapting to an external stimulus. Further studies have shown that broilers start to adapt to diet at a very young age. Swennen et al. (2010) saw the liver enlargement in birds as young as five days old when offered carbohydrate or fat in the pre-starter diet. The benefit of enlargement of the liver needs further investigation.

In addition to organ development, numerous studies have shown that the diet affects the body composition of chickens (MacLeod 1990, 1992; Buyse et al., 1992; Nieto et al., 1997; Collin et al., 2003 and Swennen et al., 2004). It has been seen that fat retention by boilers increases when offered diets with high ME and high protein. Both energy and protein are essential. Leeson and Summers (1997, 2000) highlighted that the ratio of energy to the protein being high is what caused the excess energy to store as fat. Similarly, in the current study, there an increased fat pad for birds offered high NE and protein diets; however, there was no correlation to AME.

Broilers which had a high amount of fat deposited also had significantly high weight gains and significantly poorer FCR for 25 to 35 days. The higher AME compared to protein requirement is reported for fat deposition in broilers (Petersen, 1970; De Groote, 1974; Boekholt et al., 1994). There is a different biochemical path leading to fat deposition in the broilers. With an understanding of the biochemical pathway, it may be possible to formulate diets to steer the biochemical pathway in the preferred path to target higher yield.

There was a variation in the digestibility levels of the diet. Ingredients like millrun and oat hulls could affect the digestibility by increasing the fibre content of the diet.

High fibre lowered the ME value for chicks seen in a study conducted by Rajaguru and Ravindran (1985). Other studies have shown that even a small proportion of fibre being present, e.g., 1 3 - glucans and arabinoxylans (pentosans), can influence the nutritional value of the diet. It has shown to cause depression of performance by impairment of nutrient digestion. Reduce litter quality, due to sticky droppings causing wet litter, particularly in young broiler birds has also been reported (Classen and Bedford, 1991; Choct and Annison, 1992; Choct et al., 1996a). The fibre content of the diets seems different and hence should have been included in the study. The study also did not investigate the digestibility of corn starch which replaced highly digestible canola oil.

CONCLUSION

The feed intake of broilers is dependent on NE and protein content of the diet. Broilers on lower NE diet had higher feed intake.

The NE to protein ratio affected the carcass composition of the diet. Diets high in protein and NE produced increase fat pad thickness and a smaller liver. The lower bioavailability of proteins and energy caused enlargement in the liver.

Protein balanced with NE led to weight gain and lower FCR. The findings showed that the ratio of NE to essential amino acids and not the ratio of AME to essential amino acids that influences live weight and FCR.

Overall, NE is a better predictor of FCR than AME.

Chapter 10: GENERAL DISCUSSION

Both AME and NE are critical to broiler performance and can be used to formulate broiler diets to meet the production targets.

The age of birds and nutrient density of diet determined the effect NE and protein has on the performance of birds. The response to higher nutrient density was evident in the first three weeks of age. In agreement with this study, Wiseman et al. (1998) and Noblet et al. (2010) have also reported the inconsistency in the response of the younger versus older broilers.

During the early growth period, there was a negative correlation between diets nutrient density and FCR, i.e. high dietary nutrient density resulted in lower FCR. Similar observations were made by Carré and Juin (2015), who predicted FCR using linear regression based on AME and dietary protein. Further to this, comparing R^2 values of the regression for FCR and growth, lead Carré and Juin (2015) to speculate that the flock variability was higher for growth than for FCR. Similar observations with significant FCR trends when compared to weights were observed during the current study. Further research is required to test the optimum nutrient density for the grower, and finisher diets as the values published by Aviagen 2007 may not result in the most cost-effective nutrition levels. The effect of NE at various ages and the differences between males and females require further research.

The current study showed that when AME and protein were constant, the birds consumed more of the low NE diet. Excess protein in the diet may provide energy to broilers. If together with protein, the NE also happens to be high, lipogenesis may take place increasing the fat deposition in broilers. The NE to protein ratio of the diet determines the rate of biochemical processes in broilers. These biochemical processes include; glycolysis, glycogenesis, lipogenesis, and de novo synthesis.

An adequate amount of protein should be delivered in the diet to increase weight gain. Another way to increase the protein intake would be to lower the NE of the diet, which increases the feed intake and hence the protein intake per se.

Excess NE in diet can lead to fat deposition. While the fat deposition improves weight gain, the meat yield in these broilers is low. Low dietary protein leads to de novo synthesis of amino acid. De novo synthesis is energy expensive and causes high FCR. The ideal ratio of NE to protein in the diet is imperative for high meat yield and low FCR in broilers.

Broiler performance improved at higher amino acid levels to levels recommended in Aviagen 2009. The latest Aviagen guide published in 2019 has significant increases in amino acid levels of the grower and finisher diets. These higher amino acid levels may improve the response to diets formulated on net energy.

Table 32 contains a summary of nutrient recommendation for dietary AME, NE and protein levels derived from the current study for Ross 308 broilers. All other nutrients are to be as per the Aviagen 2009 Ross Nutrition specification.

Table 35 Recommended NE for Ross specification for broilers grown to 2.0 – 2.5 kg.

Nutrient	AME MJ/kg	Protein %	Recommended NE MJ/kg
Grower	13.18	21-23	10.54
Finisher	13.39	19-23	10.75

In agreement with the observations made by Classen (2013), this study shows that a balance between amino acid (or protein) and energy is crucial to birds' performance. Protein requirement per unit NE is higher than the protein requirement per unit AME. Protein levels should increase with increasing NE of the diet. Higher NE and protein above the bird requirement threshold likely increases the fat deposit in the birds. Further research is needed to test the NE of the synthetic amino acids.

Feed intake in birds seems to be dictated by the most limiting nutrient. Protein, NE, followed by AME, seem to be the priority order. Increased FI, however, may not eventuate in higher live weight and lower FCR of the broilers if the diets are not nutritionally balanced. Feed intake would be the most difficult to predict using

regression equations. At the same time, FCR showed higher correlations and would be easier to predict using regression equations.

Dietary nutrition may influence the organ development of the bird. Birds offered lower nutrient density, or poorly digestible feed had significantly larger liver when compared to birds on a high protein diet. These changes indicate that broilers may alter their biochemical pathway to the best-utilised nutrient composition of the diets to gain the best outcome. These outcomes may not be the desired production outcome. Hence, with an understanding of the biochemical pathway, it may be possible to formulate diets to steer the biochemical pathway in the preferred path to target performance.

Raw materials available in the region limits the number of ingredients used in the production of broiler diets. When ingredient choices are not available formulating using NE or AME likely results in the same formulation. If there are many ingredient options, formulating using NE system versus the AME system results in the different raw material composition of the diet. While the nutritive value of the diets may not seem different, the raw material composition of the diets when formulating using NE is significantly different to formulating for AME as the two energy systems value raw materials differently. The table below shows the shadow price change when formulating using AME versus NE for various raw materials relative to the value of wheat when wheat was a cost of $0/t.

Table 36 Predicted raw material shadow price relative to wheat at a value of $0/t when diets were formulated for AME vs NE.

Ingredient	Formulated for	Target AME MJ/kg						
		11.30	11.72	12.13	12.34	12.55	12.97	13.39 – 14.64
Soybean meal	AME	166.6	166.6	166.6	247.6	149.1	149.1	149.1
	NE	155.6	155.6	155.6	388.7	388.7	388.7	388.7
Maize	AME	147.9	119.6	119.6	127.7	110.5	110.5	110.5
	NE	111.5	111.5	111.5	137.6	137.6	137.6	137.6
Meat & bone meal	AME	389.4	389.4	389.4	389.3	389.3	389.3	389.3
	NE	392.2	392.2	392.2	393.5	393.5	393.5	393.5
Poultry Tallow	AME	(202.1)	(202.1)	(202.1)	(201.7)	443.4	443.4	443.4
	NE	(206.3)	(206.3)	(206.3)	425.5	425.5	425.5	425.5

Using different raw materials available locally to formulate broiler diets should not cause a performance drop when the quality of the raw material and anti-nutritive factors associated with raw material is addressed.

The addition of zinc bacitracin to feed lowered the heat production in birds. There was a significant decrease in the HP seen in this study, while HI and hence NE was unaffected. Further research is warranted to determine if factors like antibiotics, which affect the gut microbiota, affects FHP. It might also be interesting to measure what portion of HP is due to gut microflora, and hence the effect zinc bacitracin has on HP when it changes the gut microflora.

Phytase present in combination with xylanase, in a wheat-based diet, did not change HP, HI nor the NE of the diet. However, there was a significant reduction in nitrogen ($P < 0.05$) in the excreta for the diets with phytase. Phytase in the diet reduced the nitrogen output and hence is better for the environment. However, in a wheat-based diet with xylanase, phytase addition may not provide the energy benefit. When allocating energy values to enzymes, the nutritionist may need to be careful with the assigning energy value for phytase when multiple enzymes are in the diet.

Consistent with studies reported earlier (Li et al., 1991), birds offered pelleted diets had significantly higher weight gains than birds offered diets with finer particles. The measured NE of the pelleted diet, 11.2 kJ/g being higher than the NE of the mash diet, 10.21 kJ/g with HP of 734 kJ/kg and 789 kJ/kg respectively.

The higher NE, but not AME, due to an increase in feed particle size indicates that while the feeding behaviour and the energy spent in feeding affected the NE, it did not influence AME. A similar observation was reported by Hill and Anderson (1958) and Bourdillon et al. (1990), who reported that ME was independent of the plane of food. They had noted a relationship between the plane of food intake and production energy, but the relationship was not consistent.

NE is affected by external factors (Latshaw and Moritz, 2009; Noblet, 2010). FHP predicted by Noblet et al. (2015) was used for this study and the study conducted by Wu et al. (2019) to generate prediction equations to determine NE. To do that all external factors across all these studies need to be constant. Further research into the external factors affecting FHP and hence NE is warranted.

A single breed, Ross 308 broilers, was used for this study. The data generated here may or may not apply to Cobb birds and needs further research. Further to this, NE value for Ross birds may also change with new, improved genetics. Continuous research may be needed to keep the NE system current.

While ME is independent of the quality of diet, it seems that the diet quality and changes in the gut microbiome may be playing a role in the NE of the diet. These factors could explain the variation previously reported for NE systems (Davidson et al., 1957; Hill and Anderson, 1958; Pirzgoliev and Rose, 1999; Carré, 2002 and Latshaw and Moritz, 2009).

AME and NE both can be used to formulate broiler diets. Depending on the availability of raw materials, there may be a benefit of using one energy system over the other. Changing to formulate for NE from AME changes the current procurement strategy for raw material for the Australian poultry industry. While the formulated diets were significantly different in raw material inclusion, the NE levels in the diets

did not differ much. This lack of difference in NE is coherent with observations made by De Groote (1974) and Noblet (2007).

Broilers fed a well-balanced diet perform well irrespective of the raw materials used or energy system used to formulate the diet. Formulating diets with significantly different measured NE levels seems challenging. While there were significant changes in nitrogen excretion, nitrogen retention, RQ and HI that indicted a possible variation in the biochemical pathway of the birds, there were no significant differences seen in determined NE. The ingredient availability and pellet quality have a greater financial impact than the difference in formulating using NE versus AME.

Chapter 11: CONCLUSION

The present study shows that NE is a superior predictor of feed conversion and carcass yield compared to AME.

The supremacy of the NE system is more remarkable in lower energy diets of AME at 12.55 MJ/kg and during the early age for the broilers.

Diets with varying formulated NE levels failed to show the difference when measured using the CIC chamber. The CIC chamber may not be sensitive enough to pick the small NE changes calculated using the equation published by Wu et al. (2019); however, these diets had shown a variation in FCR and weight gain in the field.

If industry changes to net energy for feed formulation, the procurement strategy for raw materials may change as both systems value ingredients differently. When using different raw materials, a well-formulated diet to meet the nutritional requirement of the broilers should not affect the performance of the broilers. The nutritionist should be able to formulate to the least cost diet confidentially provided the quality is maintained, and the anti-nutritional factors are taken into consideration. Limiting the number of ingredients reduces the differences seen when formulating using AME versus NE.

More than just proximate analysis affects the NE of the diet, and this needs further investigation. The other factors affecting NE need to be somehow incorporated in the NE prediction equation for ingredients or stated as the conditions to fulfil before using published literature.

Birds offered diets with zinc bacitracin had reduced HP, but this did not affect the HI nor NE of the diet. Phytase addition to the diet reduced the nitrogen content of the excreta. NE, HI and HP of the diets were not affected. Feed particle size reduction increased the HP and reduced the NE of the diet.

High nutrient density may be more beneficial and have a greater impact on performance at an earlier age rather than later stages in broiler production.

The broiler feed intake is likely a response to a limiting nutrient which could be protein, an amino acid or energy.

The amino acids content of the diet drives the weight gain in broilers during an early age. Low NE levels lead to a higher amino acid to NE availability hence eventuates in lower FCR and higher weight gain. Another way to improve performance would be to increase the amino acid content of the diet.

NE and protein ratio affected the carcass composition of the diet. High protein and NE in the diet caused high fat pad and smaller size of the liver. Low proteins and energy caused enlargement of the liver. Protein balanced with NE leads to high weight gain and lower FCR. It is the ratio of NE to protein (amino acid) that dictates the performance, live weight and FCR, and not AME. Hence, NE is a better performance predictor than AME.

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