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Comparing a single dose of xylanase to a double dose or cocktail of non-starch polysaccharide-degrading enzymes in broiler chicken diets

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Abstract

This study compared supplementation with a single dose of xylanase to a double dose of xylanase or a non-starch polysaccharide (NSP) degrading enzyme cocktail (NSP-ase cocktail) on productive performance, nutrient utilisation and the gastrointestinal environment in broilers fed commercial diets. Cobb 500 broilers (n=1,080) were fed 12 dietary treatments; four Australian commercial diets (based on wheat-barley, wheat-maize, wheat-sorghum or wheat only) with three different enzyme treatments (single dose of xylanase (16,000 BXU/kg), double dose of xylanase (32,000 BXU/kg) or NSP-ase cocktail (xylanase, β-glucanase, cellulase, pectinase, mannanase, galactanase, arabinofuranosidase). There were 108 pens, nine replicates per dietary treatment, with 10 birds per pen. Performance (total pen body weight, feed intake and feed conversion ratio corrected for mortality) was determined at d 0-35. On d 35, one male and one female were weighed individually and used to determine breast meat, thigh and drumstick weight, dry matter (DM) contents from the gizzard, jejunum and ileum, ileal protein, energy, starch and dry matter digestibility, ileal viscosity and xylo-oligosaccharide (XOS) concentration, caecal microbiota and short chain fatty acid (SCFA) composition. The double dose of xylanase and NSP-ase cocktail had no effect on bird performance, meat yield, ileal viscosity, ileal starch, energy or DM digestibility or digesta DM content. The double xylanase dose and NSP-ase cocktail increased protein digestibility in birds fed the wheat-sorghum based diet (P=0.041) and increased caecal concentration of butyric acid in birds fed the wheat-maize based diet (P=0.040), and propionic, valeric and lactic acid and *Bifidobacteria* and *Enterobacteria* spp. in birds fed the wheat-based diet (P<0.05). The double xylanase dose increased XOS production, particularly in birds fed the wheat-barley based diets (P<0.05). The lack of performance effects observed when feeding the double xylanase dose or NSP-ase cocktail suggested that the current recommended xylanase dose (16,000 BXU/kg) is sufficient.

Keywords: fibre, nutrition, non-starch polysaccharides, digestion, enzymes, xylanase

1. Introduction

It is well established that the presence of dietary non-starch polysaccharides (NSP) in poultry diets influences digesta passage rate, gastrointestinal health, tract development and microbiome composition, therefore influencing nutrient utilisation and productive performance (Kheravii *et al.*, 2018; Mahmood and Guo, 2020; Mateos *et al.*, 2012). Modern, commercial broiler diets contain approximately 10-12% total NSP (Bach Knudsen, 2001; Morgan *et al.*, 2021). The impact of dietary NSP in the gastrointestinal tract is dependent on its quantity and physio-chemical composition and structure (Jha *et al.*, 2019). Water-soluble NSP increases digesta viscosity, which, at high levels, results in lessened nutrient digestibility by reducing accessibility of enzymes to substrates and physically hindering absorption through the gastrointestinal wall (Choct, 2015). However, at moderate levels, soluble NSP can ensure digesta transit

rate is not too fast, allowing more time for nutrients to be absorbed. Moreover, a significant proportion of soluble NSP can be fermented, providing a source of fuel for beneficial microbiota, resulting in production of an additional source of energy in the form of short-chain fatty acids (Craig *et al.*, 2020). Insoluble NSP acts as a nutrient diluent and physical barrier to digestive enzymes, which reduces nutrient utilisation. It increases bulk in the digesta and absorbs water, which helps maintain digesta flow rate and motility (Hetland *et al.*, 2004), and stimulates gizzard and proventriculus function, resulting in increased peptic digestion and grinding capacity (Yokhana *et al.*, 2016). Moreover, insoluble NSP can be converted into soluble NSP (Bautil *et al.*, 2019). This demonstrates that dietary NSP in poultry diets has both advantages and disadvantages, suggesting strategies need to be developed that target attaining the greatest benefits from these NSP. One such strategy is enzyme application.

Commercial application of NSP-degrading enzymes is now common practice in poultry diets. Originally, these enzymes were used as a tool to reduce issues associated with litter quality and energy utilisation from feeding wheat- and barley-based diets. Focus then shifted towards using these enzymes to alleviate the negative effects of NSP, particularly arabinoxylans, on digesta viscosity (Aftab and Bedford, 2018). It was then identified, primarily by Choct *et al.* (2004), that the success of these enzymes could not be attributed to viscosity-reduction alone, as positive effects of their application were seen when feeding non-viscous grains. Consequently, alternative mechanisms are currently being explored, including production of prebiotic oligosaccharides and modulation of the gastrointestinal microbiota. A variety of NSP-degrading enzymes are currently on the market, ranging from mono-component single enzymes to cocktails of multiple enzymes with differing activities. These enzymes should be selected based on the substrate present and mode of action. Xylanase is the most widely used NSP-degrading enzyme in poultry diets, because arabinoxylans are the major NSP fractions present in diets (Choct, 2015). The NSP-degrading enzyme cocktails generally contain xylanase as the main activity, in combination with other enzymes (Aftab, 2012, Massey O'Neill *et al.*, 2014). The hypothesis behind providing multiple enzyme activities, as opposed to just xylanase, is that there will be enhanced disruption of cell walls and solubilisation of NSP, resulting in increased nutrient release and utilisation and producing a more diverse range of prebiotic oligosaccharides. However, there is a deficit of data available, and lack of consistency, to address this hypothesis. This is heightened by the fact that few research trials have a control containing just xylanase. Nonetheless, Cowieson *et al.* (2010) found that xylanase and glucanase alone each improved feed conversion ratio (FCR), but combining the two enzymes resulted in no further improvements. In addition, Stefanello *et al.* (2015) found that feeding combined amylase and xylanase enhanced jejunum and ileum starch digestibility, but feeding amylase alone did not. These examples challenged the assumption that feeding additional enzymes on top of xylanase induced additional benefits. However, Jimoh and Atteh (2018) observed that feeding cellulase and β-glucanase in combination with xylanase resulted in improved apparent metabolisable energy compared to feeding xylanase alone in diets containing brewers dried grains. Furthermore, Mathlouthi *et al.* (2002) conducted analysis of viscosity in a range of grains in the presence of xylanase and β-glucanase alone or in combination, and found that viscosity in wheat, triticale, rye, barley, oat and pea diet digesta samples was significantly lower with the combination of the two enzymes, compared to singly. This suggested that efficacy of an NSP-ase cocktail is dictated by the specific enzyme combination and substrates, and that a deeper understanding of the mechanisms and interrelationships between enzymes within a cocktail is required.

An additional hypothesis in this study was that increasing the dose of xylanase in the diet would increase bird performance and gastrointestinal health, primarily through enhanced production of prebiotic xylo-oligosaccharides (XOS). Liu and Kim (2017) supplemented wheat-based diets with 0, 1,875, 3,750, and 5,625 XU/kg xylanase and observed linear improvements in bird performance, digesta viscosity, ileal nutrient digestibility, gastrointestinal tract morphology and microbiota composition. Van Hoeck *et al.* (2021) found that feeding 90,000 U/kg xylanase resulted in higher caecal volatile fatty acid concentration and digestibility of organic matter, protein, fat, acid detergent fibre and energy, coupled with a lower FCR value, compared to feeding 30,000 U/kg xylanase. Moreover, Nusairat and Wang (2021) observed that feeding 15 XU/g xylanase was advantageous over feeding 10 XU/g in terms of body weight gain (BWG) at d 1-42 and apparent metabolisable energy at d 21 and 42, when supplemented into reduced-energy diets, and Singh *et al.* (2021) observed a linear increase in body weight gain when feeding 0, 8,000 and 16,000 BXU/kg xylanase. Conversely, Olukosi *et al.* (2007) saw no differences in performance between feeding 3,200 and 32,000 U/kg xylanase to broiler chickens fed wheat-rye-soybean meal-based diets. Also, Rabello *et al.* (2021) found that feed conversion was poorer when feeding 32,000 U/kg compared to when feeding 8,000 or 16,000 U/kg in broilers fed a maize-based diet. These findings suggested there may be advantages to feeding higher doses of xylanase, but there was a lack of constancy between findings, and further research is warranted in this field.

In the following study, broilers were fed commercial-type meat chicken diets, representing those fed in different states across Australia, with varying ingredient availability and costs. The diets contained either just wheat as the primary grain source, or wheat combined with barley, sorghum, or

maize. This provided a range of NSP concentrations and compositions as substrates. The dietary treatments were formulated to have similar protein and energy values. The aim of this study was to compare and provide the poultry industry with insight into the effects of supplementing these diets with a single dose of xylanase, based on commercial recommendations, or a double dose of xylanase or cocktail of NSP-degrading enzymes, on productive performance, nutrient utilisation and the gastrointestinal environment. The overarching hypothesis was that feeding a double dose of xylanase or NSP-ase cocktail would be superior to feeding a single dose of xylanase.

2. Materials and methods

Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the Animal Ethics Committee at University of New England, New South Wales, Australia.

Birds and husbandry

Cobb 500, mixed-sex broilers (n=1,080 + 30 spare birds) were obtained from a commercial hatchery at one day old. The chicks were randomised by weight and placed in 0.85 m^2 floor pens, 108 pens containing 10 birds per pen giving 90 birds per treatment, bedded on clean wood shavings. All birds were vaccinated against Marek's disease, infectious bronchitis and Newcastle disease at the hatchery, under the Australian Code Of Practice for distribution of broiler chickens. Temperature settings followed Cobb 500 recommendations of 33-34 °C on arrival, followed by a gradual decrease by approximately 0.5 °C daily, until a temperature of 21-22 °C was reached by d 21. The lighting regimen used was 24 h light on d 1, with darkness increasing by 1 h a day until 6 h of darkness was reached, which was maintained throughout the remainder of the study. Mortality was recorded daily, and any birds culled or dead were weighed.

Dietary treatments

The dietary treatments replicated commercial diet formulations from different states across Australia. The primary grain source was either just wheat, or wheat combined with barley, maize or sorghum (labeled as Wheat-based, Barley-based, Maize-based and Sorghum-based, respectively). All feed ingredients were analysed by Near InfraRed Analysis (Model 6500 scanning reflectance detector near infrared spectrophotometer, Vision 3.5 Service Pack 4 software), and these values were used in the feed formulation. Within each of these four treatments, the diets were supplemented with either a commercial recommended single dose of mono-component xylanase (16,000 BXU/kg Econase XT 5P, AB Vista, Marlborough, UK; single), a double dose of xylanase (32,000 BXU/kg Econase XT 5P, AB Vista; double) or an NSP-ase cocktail (xylanase (16,000 U/kg Econase XT 5P, AB Vista), β-glucanase (20,000 U/kg Econase GT, AB Vista), cellulase (2,000 U/kg Sigma-Aldrich Pty. Ltd, Castle Hill, Australia), pectinase (1,400 U/kg Deltagen, Kilsyth, Australia), mannanase (250 U/kg Deltagen), galactanase (20 U/kg Deltagen) and arabinofuranosidase (10,000 U/kg Deltagen; cocktail). One BXU was defined as the amount of enzyme that produces 1 nmol reducing sugars from birchwood xylan in 1 s at 50 °C and pH 5.3. The enzymes and doses used in the NSP-ase cocktail were selected based on analysis of the NSP composition of the diets, coupled with meta-analysis of NSP-ases tested in meat chicken diets in the last 5 years. The majority of the enzyme activities in the dietary treatments were measured using Megazyme assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland); the kit codes were K-XylX6-2V for xylanase activity, K-CellG5-2V for cellulase activity, E-EXBGOS for beta-glucanase activity, T-MNZ for mannanase activity and S-AGALP for galactanase activity. Pectinase activity was measured using the Sigma Aldrich recommended method for P4716. This resulted in 12 dietary treatments. Protein and energy level were formulated to be the same across all dietary treatments. All diets contained 0.5% titanium dioxide (TiO_2) as a digestibility marker and supplemental phytase at commercial levels (Quantum Blue, AB Vista). Soluble and insoluble NSP and free oligosaccharides were measured in the dietary treatments using the procedure described in Morgan *et al.* (2022). Birds had *ad libitum* access to water and feed throughout the trial period. The diets were fed as starter from d 0-12, grower from d 12-23 and finisher from d 23-35. The diets were cold pelleted and fed as crumble (ø0.1-0.2 mm) from d 0-7 and then pellet (ø3 mm pellet) for the remainder of the trial period. The nutrient composition of the test diets is presented in Table 1.

Response variables

Total pen weight and feed intake (FI) was determined on arrival and d 35 post-hatch and used to calculate feed conversion ratio corrected for mortality (cFCR) from d 0-35. On d 35, the number of males and females per pen was recorded (56% female and 44% male) and one male and one female per pen were weighed individually and euthanised by electrical stunning followed by cervical dislocation. Breast, thigh and drumstick were collected and weighed per bird.

Gizzard, jejunum and ileum digesta samples were collected per bird, and pooled per pen. Dry matter content was determined in these digesta samples where a sub-sample of digesta sample was weighed into duplicate crucibles and oven dried at 105 °C to a constant weight and then reweighed, and dry matter was calculated. Sub-samples of fresh ileum digesta were collected for viscosity analysis, and the remaining ileum digesta sample was frozen at -20 °C,

Table 1. Nutrient composition of Starter (S), Grower (G) and Finisher (F) dietary treatments.

Ingredient (g/kg)[1]	Barley			Maize			Sorghum			Wheat		
	S	G	F	S	G	F	S	G	F	S	G	F
Wheat	386.3	445.9	491.3	304.2	292.2	326.1	202.6	295.1	328.6	556.9	591.0	646.3
Barley	149.3	149.2	149.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maize	0.0	0.0	0.0	248.8	298.5	298.5	0.0	0.0	0.0	0.0	0.0	0.0
Sorghum	0.0	0.0	0.0	0.0	0.0	0.0	298.5	298.5	298.5	0.0	0.0	0.0
Canola seed	79.6	47.6	69.7	69.7	19.9	69.7	64.7	20.5	69.7	69.7	43.1	69.7
Soybean meal	289.7	257.4	202.2	300.4	292.7	222.7	282.1	284.2	214.5	290.4	262.9	215.7
Canola meal	29.9	29.8	20.7	29.9	29.8	29.9	79.6	29.8	29.9	24.9	29.8	5.0
Canola oil	23.3	28.5	29.9	5.2	23.7	17.2	23.7	28.8	22.7	15.8	29.8	26.3
Limestone	13.9	15.0	12.0	13.9	15.5	11.7	17.5	15.3	11.7	13.9	15.2	12.0
Monocalcium phosphate	7.8	7.0	5.8	8.0	7.7	5.8	11.1	7.6	5.8	7.8	7.5	5.8
Na bicarb	3.6	3.7	3.8	3.3	4.2	3.2	3.5	4.3	3.3	3.7	4.7	3.7
DL-methionine	2.9	3.0	3.3	2.8	3.1	3.3	2.8	3.1	3.2	2.8	3.1	3.3
L-lysine HCl	2.8	2.6	2.6	2.8	2.2	2.2	2.9	2.4	2.4	3.1	2.7	2.6
Salt	1.6	1.7	1.4	1.8	2.1	1.9	1.9	2.0	1.7	1.6	1.8	1.5
L-threonine	1.3	1.3	1.0	1.2	1.1	0.8	1.1	1.1	0.9	1.4	1.3	1.0
Copper sulphate	0.4	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0	0.4	0.0	0.0
Choline chloride	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.2	0.0	0.0	0.1	0.0
Phytase[2]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[3]	1.0	0.8	0.8	1.0	0.8	0.8	1.0	0.8	0.8	1.0	0.8	0.8
Mineral premix[4]	1.3	1.0	1.0	1.3	1.0	1.0	1.3	1.0	1.0	1.3	1.0	1.0
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salinomycin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Probiotic[5]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Analysed composition												
Dry matter (g/100 g)	90.53	89.30	88.20	88.73	88.07	87.49	90.46	89.50	88.25	89.70	89.02	88.55
Protein (g/100 g DM)	23.91	23.27	21.36	23.69	22.47	21.14	23.92	22.64	21.50	25.24	23.48	22.72
Energy (MJ/kg DM)	17.53	17.10	17.00	16.80	16.48	16.59	17.21	16.78	16.85	17.08	16.93	17.00
Soluble NSP (g/kg DM)	19.09	22.67	21.06	19.01	17.91	16.59	19.65	17.51	15.71	21.30	20.73	23.39
Insoluble NSP (g/kg DM)	77.67	78.60	65.62	63.26	71.16	66.07	67.93	65.84	60.71	76.38	69.30	70.51
Free oligosaccharides (g/kg DM)	41.72	38.31	40.14	41.58	39.85	38.41	38.99	36.83	36.05	36.13	41.54	35.32
Calculated composition												
AME (MJ/kg)	12.57	12.76	13.18	12.57	12.76	13.18	12.57	12.76	13.18	12.57	12.76	13.18
d Lys (%)	1.28	1.13	1.00	1.28	1.13	1.00	1.28	1.13	1.00	1.28	0.79	1.00
d Met (%)	0.61	0.58	0.57	0.61	0.59	0.58	0.62	0.59	0.58	0.60	1.13	0.58
d Met + Cys (%)	0.95	0.86	0.84	0.95	0.86	0.84	0.95	0.86	0.84	0.95	0.58	0.84
d Tryp (%)	0.26	0.25	0.23	0.24	0.25	0.22	0.26	0.25	0.23	0.26	0.86	0.23
d Arg (%)	1.37	1.21	1.07	1.41	1.21	1.07	1.37	1.21	1.07	1.37	0.25	1.08
d Threo (%)	0.86	0.76	0.67	0.86	0.76	0.67	0.86	0.76	0.67	0.86	1.21	0.67
d Leu (%)	1.46	1.30	1.17	1.54	1.42	1.29	1.67	1.55	1.42	1.46	0.76	1.18
d Iso (%)	0.93	0.78	0.69	0.93	0.80	0.70	0.96	0.81	0.72	0.93	1.29	0.69
d Val (%)	0.97	0.88	0.81	0.97	0.85	0.80	0.98	0.89	0.83	0.97	0.77	0.82

[1] AME = apparent metabolisable energy; NSP = non-starch polysaccharide.
[2] Quantum Blue, AB Vista.
[3] Formulated to supply 5,040 mg retinol, 17.5 mg cholecalciferol, 105 mg tocopheryl acetate, 4 mg menadione, 4 mg thiamine, 11 mg riboflavin, 77 mg niacin, 18 mg pantothenate, 7 mg pyridoxine, 0.35 mg biotin, 3.0 mg folate, 0.02 mg cyanocobalamin per kg of finished feed.
[4] Formulated to supply 23 mg copper, 1.79 mg iodine, 57 mg iron, 171 mg manganese, 0.43 mg selenium and 143 mg zinc per kg finished feed.
[5] Alterion, Adisseo.

freeze-dried to constant weight and then ground through a 0.5 mm screen. Samples of the diets were ground through a 0.5 mm screen. Caeca samples per pen were also collected on d 35, and frozen at -20 °C until further analysis.

The TiO_2 marker was quantified in the diets and ileum digesta by UV-spectroscopy at 410 nm (Cary 50 Bio UV-Visible spectrophotometer equipped with a Cary 50 MPR microplate reader, Varian Inc., Palo Alto, CA, USA), using the method described by Short *et al.* (1996). Nitrogen content of the diets and ileum samples was determined using the combustion method (LECO Corp., St. Joeseph, MI, USA), and the value was multiplied by a factor of 6.25 to determine protein content. Gross energy was determined in the diets and ileum samples using an adiabatic bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA), standardised with benzoic acid. Starch was measured in the diets and ileum samples using the Megazyme total starch assay (Megazyme International Ireland Ltd). Xylanase activity was measured in the diets using the Megazyme Xylanase Assay Kit (XylX6 Method; Megazyme International Ireland Ltd), and confirmed activity was as predicted (±1.2% at 16,000 BXU/kg and ±0.8% at 32,000 BXU/kg).

Ileal digestibility of dry matter, protein, energy and starch was determined using the following equation:

$$\text{Digestibility (\%)} = \frac{1 - (\text{Nutrient}_{\text{ileum digesta}} \times \text{TiO}_{2\text{ diet}})}{\text{TiO}_{2\text{ ileum digesta}} \times \text{Nutrient}_{\text{diet}}} \times 100$$

For viscosity analysis, the ileal digesta samples were collected into 2 ml Eppendorf tubes, which were centrifuged at 10,000×*g* for 10 minutes at room temperature. Viscosity was measured in 0.5 ml of the resulting supernatant, using a Brookfield DV3T Rheometer (Brookfield Ametek, Instrumentation and Specialty Controls Division, Middleboro, MA, USA), with CPA-40Z Spindle, at 35 °C. Viscosity measurements were expressed in centipoise (cPs) unit (1 cPs = 1/100 dyne sec/cm^2 =1 mPa.s) prior to statistical analysis. Measurements were collected at 0.5, 1, 5 and 10 revolutions per min. The XOS were extracted from samples using a multi-step solid phase extraction. Extracted XOS were derivatised using 1-phenyl-3-methyl-5-pyrazolone (PMP). Analysis of the PMP-XOS was carried out on an Agilent Single Quad LCMS equipped with Agilent ZORBAX SB-C18 column (3.0×150 mm, 1.8 micron), and separated using mobile phases -A: 0.1% formic acid in H_2O and B: 0.1% formic acid in acetonitrile.

Short chain fatty acids were measured in the d 35 caeca samples using the method described by Jensen *et al.* (1995), with some modifications. Briefly, approximately 0.8 g of fresh homogenised caecal digesta sample maintained at approximately 5 °C was homogenised with 1 ml of internal standard (0.01 mol/l ethylbutyric acid). The sample was then centrifuged at 3,900×*g* for 20 min at 5 °C, and 1 ml of resulting supernatant was mixed with 0.5 ml concentrated HCl (36%) and 2.5 ml of ether. The sample was centrifuged at 2,000×*g* for 15 min at 5 °C, and 400 µl of resulting supernatant was transferred into a GC vial. Following this, 40 µl of N-tert-butyldimethlsilyl-N-methyltrifuoroacetamide (MTBSTFA) was added to the vial and the sample was heated at 80 °C for 20 min, and then maintained at room temperature for 48 h, prior to analysis on a Varian CP3400 CX gas chromatograph (Varian Analytical Instruments). The short chain fatty acid (SCFA) concentration in the samples was expressed as µmol/g digesta.

Analysis of microbiota composition was determined in duplicate in the d 35 caecal digesta samples. The DNA extraction from the samples was performed using an Isolate II Plant DNA Kit (Bioline, Alexandria, Australia) and QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) with slight modification, as described by Keerqin *et al.* (2017) and Kheravii *et al.* (2017). The purity of the extracted DNA was assessed by a Nano-Drop ND-8000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Only DNA elution that emitted ratios from 1.8 and above at a wavelength of 260/280 nm were used for PCR analysis. Following a 20× dilution with sterilised water, the extracted DNA was analysed for total anaerobic bacteria, *Bacillus*, *Bacteriodes*, *Bifidobacterium*, *Ruminococcus*, *Lactobacillus* and *Enterobacteria* spp. by quantitative real-time PCR analysis, using a Rotorgene 6500 real-time PCR machine, and quantification was determined using Rotorgene 6000 series software 1.7 (Corbett, Sydney, Australia). A threshold cycle averaged from the duplicate samples was used for quantification analysis. The number of target DNA copies was calculated using a standard curve constructed with plasmid DNA cloned with the amplicons. Copy numbers of plasmid DNA were calculated according to its mass, taking into account the size of the plasmid with amplicon insert. The resulting values were expressed as log10 (genomic DNA copy number)/g digesta. The species-specific 16 rRNA primers utilised are described in detail by Kheravii *et al.* (2017).

Data analysis

All data was analysed using IBM SPSS statistics version 25 (IBM, Armonk, NY, USA). Pen represented the replicate unit for statistical analysis. After Kolmogorov-Smirnov testing to confirm normality, ANOVA analysis was used to evaluate the impact of the enzyme treatment within each of the four dietary treatments with differing primary grain sources. The percentage of male birds per pen was applied as a co-variate for analysis of bird performance. Treatment means were separated using Tukey post-hoc test where appropriate. Statistical significance was declared at $P<0.05$.

3. Results

Performance

Table 2 shows that the enzyme treatments had no impact on FI, BWG or cFCR in broilers at d 0-35, or on female BW at d 35 ($P>0.05$). In male birds fed the sorghum- based diet, BW at d 35 was greater when feeding the single dose of xylanase compared to the double xylanase dose or NSP-ase cocktail ($P=0.047$). The enzyme treatments had no impact on male BW in birds fed the barley-, maize- or wheat-based diets ($P>0.05$).

Breast, thigh and drumstick weight

Table 3 shows that the enzyme treatments had no impact on d 35 breast meat weight ($P>0.05$). In birds fed the barley-based diet, thigh weight at d 35 was lower when feeding the NSP-ase cocktail compared to feeding either the single or double dose of xylanase ($P=0.026$). The enzyme treatments had no impact on thigh weight in birds fed the maize-, sorghum- or wheat- based diets ($P>0.05$). In birds fed the maize-based diet, drumstick weight at d 35 was higher in birds fed the single dose of xylanase compared to those fed the NSP-ase cocktail ($P=0.003$). The enzyme treatments had no impact on drumstick weight in birds fed the barley-, sorghum- or wheat- based diets ($P>0.05$).

Table 2. Effect of grain type and xylanase level (16,000 BXU/kg (Single) or 32,000 BXU/kg (Double)) or NSP-ase cocktail (Cocktail) on individual feed intake (FI), body weight gain (BWG) and feed conversion ratio corrected for mortality (cFCR) in broilers at d 0-35[1] and male and female body weight (BW) at d 35.[2]

Grain	Enzyme	FI	BWG (g)	cFCR	Male BW (g)	Female BW (g)
Barley	Single	3,526	2,560	1.38	2,853	2,542
	Double	3,499	2,531	1.38	2,791	2,452
	Cocktail	3,300	2,406	1.37	2,764	2,382
SEM		50.26	37.93	0.01	30.31	48.57
P-value		0.143	0.231	0.727	0.501	0.431
Maize	Single	3,637	2,573	1.41	2,918	2,531
	Double	3,510	2,524	1.39	2,870	2,520
	Cocktail	3,571	2,551	1.40	2,692	2,545
SEM		58.41	41.60	0.01	46.34	40.30
P-value		0.703	0.903	0.344	0.118	0.972
Sorghum	Single	3,482	2,552	1.37	2,983[a]	2,381
	Double	3,543	2,557	1.39	2,752[b]	2,355
	Cocktail	3,351	2,396	1.40	2,738[b]	2,389
SEM		42.88	34.35	0.01	45.74	46.90
P-value		0.192	0.100	0.247	0.047	0.958
Wheat	Single	3,445	2,514	1.37	2,698	2,536
	Double	3,315	2,427	1.37	2,728	2,408
	Cocktail	3,318	2,450	1.35	2,777	2,470
SEM		45.86	31.15	0.01	46.78	38.52
P-value		0.447	0.525	0.552	0.803	0.430

[1] Represents ratio of 56% female and 44% male birds. Percentage males per pen was used applied as a covariate.
[2] Means within the same column, within the same parameter, with no common subscript, differ significantly. Means represent 9 replicates per treatment

Table 3. Effect of grain type and xylanase level (16,000 BXU/kg (Single) or 32,000 BXU/kg (Double)) or NSP-ase cocktail (Cocktail) on weight of breast, drumstick and thigh meat in male and female broilers at d 35.[1]

Grain	Enzyme	Breast (g)	Thigh (g)	Drumstick (g)
Barley	Single	235.33	127.17[a]	113.36
	Double	218.61	125.03[a]	110.61
	Cocktail	222.67	111.17[b]	107.17
SEM		4.08	2.67	1.49
P-value		0.238	0.026	0.259
Maize	Single	239.61	125.19	121.72[a]
	Double	236.22	120.50	115.78[ab]
	Cocktail	227.19	119.06	108.28[b]
SEM		4.34	2.31	1.80
P-value		0.514	0.556	0.003
Sorghum	Single	222.58	118.22	113.03
	Double	216.00	118.14	107.00
	Cocktail	220.97	118.14	108.25
SEM		4.44	2.53	1.91
P-value		0.837	0.998	0.428
Wheat	Single	220.72	126.78	112.97
	Double	218.22	117.56	108.44
	Cocktail	232.89	125.50	110.86
SEM		6.77	2.08	1.55
P-value		0.667	0.158	0.521

[1] Means within the same column, within the same parameter, with no common subscript, differ significantly.

Ileal viscosity and nutrient digestibility

Data in Table 4 shows that the enzyme treatments had no impact on dry matter content of the gizzard, jejunum or ileum, or on ileal viscosity, in broilers at d 35 (P>0.05). These results showed that the enzyme treatments had no impact on ileal protein, energy, starch or dry matter digestibility at d 35 in birds fed the barley-, maize- or wheat-based diets (P>0.05). In birds fed the sorghum-based diet, protein digestibility was lower in birds fed the single xylanase dose compared to those fed the double xylanase dose or NSP-ase cocktail (P=0.041). The enzyme treatments had no impact on ileal energy, starch or dry matter digestibility in birds fed the sorghum-based diet (P>0.05).

Caecal short chain fatty acids

The data in Table 5 shows that the enzyme treatments had no impact on caecal SCFA concentration at d 35 in birds fed the barley- or sorghum-based diets (P>0.05).

Table 4. Effect of grain type and xylanase level (16,000 BXU/kg (Single) or 32,000 BXU/kg (Double)) or NSP-ase cocktail (Cocktail) on ileum digesta viscosity (cP) and ileal digestibility of dry matter, protein, energy and starch (%) in broilers at d 35.[1]

Grain	Enzyme	Dry matter (%)			Ileal viscosity (cP)	Ileal digestibility			
		Gizzard	Jejunum	Ileum		Protein (%)	Energy (%)	Starch (%)	Dry matter (%)
Barley	Single	29.23	20.06	19.45	2.00	69.00	77.45	93.40	81.07
	Double	26.47	20.49	19.02	2.08	76.61	81.70	94.93	81.67
	Cocktail	27.68	19.03	18.97	2.11	71.80	79.49	91.07	80.32
SEM		0.55	0.33	0.34	0.05	0.09	0.04	0.85	1.96
P-value		0.571	0.358	0.901	0.771	0.172	0.301	0.197	0.971
Maize	Single	31.14	18.14	18.61	1.85	71.24	82.10	93.10	80.72
	Double	34.42	19.15	19.09	1.76	75.69	83.39	94.51	81.53
	Cocktail	30.90	18.97	18.90	2.16	77.39	81.99	94.20	81.55
SEM		0.88	0.37	0.22	0.06	0.08	0.13	0.58	1.77
P-value		0.395	0.711	0.836	0.101	0.918	0.394	0.621	0.509
Sorghum	Single	31.14	18.88	20.81	2.20	68.77^{b}	80.20	90.14	80.41
	Double	33.66	18.63	18.84	2.05	74.43^{a}	82.54	92.60	82.30
	Cocktail	34.26	20.27	20.31	2.15	75.94^{a}	82.41	92.86	81.20
SEM		0.56	0.32	0.27	0.07	0.05	0.08	0.64	2.16
P-value		0.613	0.293	0.122	0.774	0.041	0.624	0.176	0.515
Wheat	Single	30.06	20.08	20.01	2.12	73.50	80.87	93.05	81.19
	Double	27.60	19.86	18.00	2.45	77.38	81.54	93.56	82.37
	Cocktail	25.72	18.25	17.60	2.42	77.81	82.77	96.07	82.86
SEM		0.65	0.36	0.48	0.09	0.06	0.06	0.71	2.01
P-value		0.449	0.252	0.346	0.455	0.541	0.368	0.197	0.714

[1] Means within the same column, within the same parameter, with no common subscript, differ significantly. Means represent 9 replicates per treatment, with samples collected from one male and one female per replicate.

Table 5. Effect of grain type and xylanase level (16,000 BXU/kg (Single) or 32,000 BXU/kg (Double)) or NSP-ase cocktail (Cocktail) on short-chain fatty acid concentration (µmol/g) in the caeca at d 35.[1]

Grain	Enzyme	Total	Formic	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Lactic	Succinic
Barley	Single	69.76	0.16	45.67	4.09	0.34	16.00	0.03	0.53	1.31	1.62
	Double	81.43	0.27	54.03	4.12	0.41	17.46	0.12	0.67	0.70	3.66
	Cocktail	76.81	0.26	52.37	3.18	0.48	17.71	0.11	0.50	0.62	1.58
SEM		5.49	0.04	4.08	0.40	0.07	1.28	0.02	0.09	0.25	0.49
P-value		0.701	0.586	0.774	0.553	0.893	0.911	0.301	0.781	0.670	0.408
Maize	Single	49.85	0.03	36.02	2.16	0.14	9.16^{b}	0.00	0.15	0.11	2.09
	Double	76.03	0.11	51.35	2.90	0.23	17.39^{a}	0.05	0.29	0.21	3.50
	Cocktail	84.50	0.09	52.68	1.44	0.14	21.71^{a}	0.00	0.22	0.42	7.80
SEM		7.99	0.03	4.73	0.45	0.05	2.23	0.01	0.07	0.09	1.48
P-value		0.134	0.650	0.165	0.585	0.758	0.040	0.374	0.713	0.445	0.417
Sorghum	Single	37.14	0.09	25.73	1.95	0.41	6.85	0.11	0.27	0.49	1.22
	Double	65.98	0.23	46.78	2.80	0.42	12.11	0.10	0.42	0.46	2.66
	Cocktail	59.48	0.22	42.66	2.11	0.27	12.37	0.05	0.29	0.66	0.85
SEM		6.27	0.05	4.03	0.43	0.08	1.55	0.02	0.07	0.08	0.56
P-value		0.335	0.694	0.222	0.832	0.828	0.485	0.717	0.787	0.838	0.602
Wheat	Single	52.34	0.02	38.39	0.47^{c}	0.05	12.73	0.00	0.09^{c}	0.02^{c}	0.58
	Double	78.85	0.28	53.70	2.16^{b}	0.28	17.75	0.04	0.37^{b}	0.66^{b}	3.61
	Cocktail	83.18	0.44	53.56	3.71^{a}	0.41	19.21	0.08	0.71^{a}	1.33^{a}	3.73
SEM		7.91	0.09	5.14	0.47	0.06	2.14	0.02	0.09	0.11	0.88
P-value		0.444	0.357	0.596	0.039	0.445	0.646	0.343	0.049	0.016	0.478

[1] Means within the same column, within the same parameter, with no common subscript, differ significantly. Means represent 9 replicates per treatment, with samples collected from one male and one female per replicate.

In birds fed the maize-based diet, caecal butyric acid concentration was greater in bird fed the double xylanase dose or NSP-ase cocktail compared to those fed the single xylanase dose (*P*=0.040). In birds fed the wheat-based diet, caecal propionic, valeric and lactic acid concentrations were higher in birds fed the NSP-ase cocktail, and lower in birds fed the single xylanase dose, compared to those fed any other enzyme treatment (*P*=0.039, *P*=0.049 and *P*=0.016, respectively).

Microbiota composition

Results in Table 6 demonstrated that the enzyme treatments had no impact on caecal microbiota composition at d 35 in birds fed the barley-, maize- or sorghum- based diets

(P>0.05). In birds fed the wheat-based diet, *Bifidobacteria* spp. abundance was lower in birds fed the single xylanase dose compared to those fed the double xylanase dose or NSP-ase cocktail (P=0.037). Additionally, *Enterobacteria* spp. abundance was greater in birds fed the NSP-ase cocktail compared to those fed the double xylanase dose in birds fed the wheat-based diet (P=0.010).

Ileal xylo-oligosaccharide concentration

Table 7 shows the levels of XOS, arabinose and xylose produced in the ileum as a consequence of the enzyme supplement treatments. In birds fed the barley-based diet supplementation with a double dose resulted in consistently greater production of XOS (X_2-X_6) compared to feeding the single xylanase dose (P<0.05). Furthermore, ileal X_5, arabinose and xylose production was greater in birds fed

Table 6. Effect of grain type and xylanase level (16,000 BXU/kg (Single) or 32,000 BXU/kg (Double)) or NSP-ase cocktail (Cocktail) on microbiota composition in the caeca at d 35.[1]

Grain	Enzyme	Total	*Bacillus*	*Bacteriodes*	*Bifidobacteria*	*Ruminococcus*	*Lactobacillus*	*Enterobacteria*
Barley	Single	11.36	9.13	6.50	8.84	9.88	11.57	9.33
	Double	11.51	8.97	6.69	8.71	10.00	11.31	9.42
	Cocktail	11.60	8.83	6.69	9.07	10.04	11.42	6.62
SEM		0.05	0.06	0.05	0.06	0.05	0.07	0.08
P-value		0.139	0.168	0.232	0.137	0.363	0.345	0.374
Maize	Single	11.47	8.68	6.69	8.79	9.95	11.10	9.24
	Double	11.37	8.96	6.56	8.93	9.79	11.19	9.01
	Cocktail	11.47	9.01	6.66	8.80	9.96	11.15	9.14
SEM		0.04	0.09	0.05	0.03	0.05	0.06	0.11
P-value		0.555	0.350	0.565	0.602	0.375	0.872	0.750
Sorghum	Single	11.50	9.06	6.75	8.84	10.07	11.27	9.62
	Double	11.43	9.25	6.66	8.82	10.02	11.48	9.18
	Cocktail	11.35	8.66	6.47	8.72	9.94	11.12	9.50
SEM		0.05	0.13	0.03	0.04	0.03	0.08	0.12
P-value		0.438	0.765	0.170	0.363	0.644	0.266	0.363
Wheat	Single	11.50	8.74	6.61	8.56^{b}	9.98	11.17	9.55^{ab}
	Double	11.57	8.90	6.72	8.77^{a}	10.06	11.29	9.29^{b}
	Cocktail	11.50	8.88	6.65	9.04^{a}	10.03	11.04	9.90^{a}
SEM		0.04	0.09	0.07	0.08	0.03	0.07	0.09
P-value		0.662	0.765	0.420	0.037	0.570	0.409	0.010

[1] Means within the same column, within the same parameter, with no common subscript, differ significantly. Means represent 9 replicates per treatment, with samples collected from one male and one female per replicate.

Table 7. Effect of grain type and xylanase level (16,000 BXU/kg (Single) or 32,000 BXU/kg (Double)) or NSP-ase cocktail (Cocktail) on proportion of arabinoxylan converted into xylose (X_1), arabinose (Ara) or xylobiose (X_2), xylotriose (X_3), Xylotetraose (X_4), Xylopentaose (X_5) and Xylohexaose (X_6) (mg/TiO_2 g) in the ileum at d 35.[1]

Grain	Enzyme	Ara	X_1	X_2	X_3	X_4	X_5	X_6	XOS total (X_2-X_6)
Barley	Single	5.52^{b}	8.60^{b}	12.58^{b}	9.51^{b}	3.79^{b}	1.28^{b}	0.60^{b}	27.75^{b}
	Double	6.52^{ab}	14.55^{ab}	40.81^{a}	32.76^{a}	13.35^{a}	4.55^{a}	2.07^{a}	93.53^{a}
	Cocktail	18.39^{a}	23.87^{a}	25.13^{b}	20.15^{b}	8.75^{ab}	3.34^{a}	1.39^{ab}	58.75^{ab}
SEM		2.20	2.34	3.34	2.83	1.24	0.42	0.18	7.85
P-value		0.024	0.021	<0.001	0.001	0.002	0.002	0.001	<0.001
Maize	Single	6.86	8.93	14.69	11.60	4.70^{b}	1.70	0.79	33.48
	Double	13.72	18.58	29.79	22.80	9.16^{a}	3.35	1.33	59.55
	Cocktail	12.46	17.75	26.77	20.76	8.39^{ab}	2.69	0.95	66.42
SEM		3.54	4.09	3.03	2.12	0.76	0.30	0.14	6.07
P-value		0.740	0.612	0.107	0.075	0.035	0.083	0.313	0.067
Sorghum	Single	8.37	9.90	11.58	8.03	3.35	1.30^{ab}	0.34^{ab}	24.60
	Double	3.59	8.91	11.43	8.42	2.89	1.70^{a}	0.50^{a}	24.93
	Cocktail	3.01	5.80	7.94	5.43	1.87	1.00^{b}	0.28^{b}	16.51
SEM		1.72	1.84	0.95	0.54	0.25	0.12	0.04	1.57
P-value		0.420	0.699	0.265	0.060	0.053	0.044	0.042	0.069
Wheat	Single	7.82	14.43	13.45	10.68	4.60	1.35	0.58	30.66
	Double	5.49	13.90	21.95	19.25	7.96	2.75	0.97	52.87
	Cocktail	7.11	13.13	12.95	10.90	4.18	1.47	0.74	30.23
SEM		0.80	1.65	1.93	1.95	0.87	0.32	0.12	5.08
P-value		0.525	0.957	0.110	0.136	0.175	0.152	0.487	0.126

[1] Means within the same column, within the same parameter, with no common subscript, differ significantly. Means represent 9 replicates per treatment, with samples collected from one male and one female per replicate.

the NSP-ase cocktail compared to those fed the single xylanase dose (P<0.05). In birds fed the maize-based diet, manufacture of X_4 in the ileum was greater in birds fed the double compared to single dose of xylanase (P=0.035). Ileal X_5 and X_6 production was lower in birds fed the NSP-ase cocktail compared to those fed the double xylanase dose in birds fed the sorghum-based diet (P=0.044 and P=0.042, respectively). The enzyme treatments had no impact on ileal XOS, arabinose or xylose production in birds fed the wheat-based diet.

4. Discussion

In this study, the double xylanase and NSP-ase cocktail failed to induce any improvement in overall performance in broilers aged d 0-35, compared to feeding a single xylanase dose. In fact, performance was worse in male birds fed the sorghum-based diet as a consequence of applying double inclusion levels of xylanase and the NSP-ase cocktail. A possible explanation for this was that the sorghum-based diet was lacking in fermentable fibre, meaning that the fermentation capacity of the microbiota was low, so there was less production of endogenous enzymes (Ribeiro *et al.*, 2018). In addition, it was predicted that, in older birds, the small amounts of xylan that could be fermented were fully degraded or absorbed in the ileum, so did not make it to the caeca to be utilised by the beneficial microbiota (Bautil *et al.*, 2019; González-Ortiz *et al.*, 2020). In the presence of the double xylanase, more X_5 and X_6 were manufactured, which likely stimulated the xylan-degrading bacteria, but there was insufficient substrate to use, due to the low soluble NSP concentration in the sorghum-based diets (González-Ortiz *et al.*, 2021). It may have been possible that the single xylanase dose induced production of soluble xylan, which provided sufficient fuel for these bacteria, but the double dose and NSPase cocktail produced too much, which had a detrimental impact on microbiota balance and the gastrointestinal environment. The additional oligosaccharides produced, as a consequence of the NSPase cocktail, may have had detrimental effects, possibly because the probiotic bacteria that could utilise them were not present, so they were instead used as a substrate by pathogenic bacteria species. This may have resulted in competition between the host and bacteria for valuable nutrients, such as amino acids. This possibly explains why thigh and drumstick weight in birds fed the barley- and maize-based diets, respectively, was worse when feeding the NSP-ase cocktail compared to the single xylanase dose. The NSP-ase cocktail may have increased solubilisation of the insoluble NSP, which increased digesta viscosity and thus reduced absorption of amino acids, calcium and phosphorus (Muszyński *et al.*, 2020), as well as potentially reducing phytase efficacy (Poernama *et al.*, 2021). The insignificant impact of enzyme treatments on breast meat was in agreement with Dos Santos *et al.* (2017) and Arczewska-Wlosek *et al.* (2019), and may have been largely due to how crude the determination of breast meat weight was, resulting in high variability between samples (Bianchi and Fletcher, 2002). The lack of effect of the NSP-ase cocktail on performance and ileal nutrient digestibility in birds fed the maize-based diet was in contrast to the work of Ko *et al.* (2021), who fed male broilers an NSP-ase cocktail containing mannanase, β-glucanase and xylanase and observed that it significantly improved feed conversion and apparent ileal digestibility of DM, soluble NSP and protein. This was likely because the diets in this study contained almost half the amount of maize and double the amount of wheat, and notably less soybean meal, compared to those used by Ko *et al.* (2021), so the NSP compositions were very different, namely less pectic polysaccharides and more xylan. This observation was, however, in agreement with Cowieson *et al.* (2010), who noted a lack of any positive effects on performance when combining xylanase and glucanase. Furthermore, Buchman *et al.* (2007) saw no notable effects on broiler performance when feeding an NSP-ase cocktail containing beta-glucanase, pentosanase and hemicellulose activities. This suggested the current recommended dose of xylanase was sufficient for alleviating the anti-nutritional effects of dietary NSP on broiler performance and meat production. Further research is warranted comparing these enzyme treatments to diets without supplemental xylanase, to confirm that the observations were in fact a consequence of xylanase presence. Moreover, the capacity of phytase to mask the effects of the NSP-degrading enzymes needs to be examined. This was illustrated by Tiwari *et al.* (2010), who fed an NSP-ase cocktail containing xylanase and amylase with phytase and observed that phytase was predominantly responsible for the positive effects on bird performance.

The lack of any significant effect of the double xylanase dose or NSP-ase cocktail on ileal viscosity and digesta dry matter content was surprising, as it was predicted that the double xylanase dose and NSP-ase would increase degradation of soluble NSP, reducing viscosity and water-holding in the gastrointestinal tract (Liu and Kim, 2017; Mathlouthi *et al.*, 2002). This again suggested that the current commercial dose of xylanase is adequate at alleviating the anti-nutritional effects of the soluble NSP on viscosity. In birds fed the sorghum-based diet, both the double dose of xylanase and NSP-ase cocktail increased ileal protein digestibility. The sorghum used in this trial had an analysed insoluble NSP content of 5.03% and soluble NSP content of 0.43%, which highlighted that, even though the NSP content of sorghum was low, improvements could still be made to accessibility of nutrients as a consequence of NSP hydrolysis. This observation was probably not attributable to prebiotic oligosaccharide production, due to the lack of impact of these enzyme treatments on ileal XOS concentration, caecal microbiota or SCFA composition. This suggested hydrolysis of the insoluble NSP released

entrapped amino acids and may have been responsible for improved protein utilisation.

The increased butyric acid concentration seen in digesta from birds fed the maize-based diet due to feeding the double xylanase dose or NSP-ase cocktail may have been a consequence of the observed heightened X_4 production, which suggested that this XOS fraction specifically was successfully utilised by probiotic bacteria species. This output was in agreement with Singh *et al.* (2021), who observed a linear increase in total caecal SCFA with increasing xylanase level, including a numerical increase in butyrate, in birds fed maize-based diets. Furthermore, Van Hoeck *et al.* (2021) found that butyrate production was increased by feeding 30,000 U/kg xylanase, similar to the double xylanase level fed in this study, but there were no further increases when feeding 45,000 or 90,000 U/kg. The beneficial effect on butyric acid was specifically notable, given the role it plays as an energy source for enterocytes (Guilloteau *et al.*, 2010). The heightened concentrations of caecal propionic, valeric and lactic acid and increased abundance of probiotic *Bifidobacteria* and *Enterobacteria* spp. observed when supplementing the NSP-ase cocktail into the wheat-based diet indicated successful hydrolysis of multiple NSP, and suggested that manufacturing a range of different oligosaccharides increases the diversity of probiotic bacteria species that are stimulated in the caeca (Yadav and Jha, 2019). This agreed with Meng *et al.* (2005), who observed that feeding a cocktail containing xylanase, cellulase, pectinase, glucanase and mannanase, improved feed-to-gain ratio and protein digestibility compared to feeding a combination of xylanase, glucanase and cellulase, or xylanase, glucanase, cellulase and pectinase. However, the positive effects on SCFA production and prevalence of some beneficial bacteria species appeared to not be extensive enough to translate into beneficial impacts on performance or nutrient utilisation in this study.

In conclusion, the results demonstrated that there were some benefits to feeding a double dose of xylanase and NSP-ase cocktail, particularly in birds fed wheat-based diets. However, generally it appeared that the current recommended commercial doses of xylanase are adequate at combatting anti-nutritional effects from dietary NSP on broiler performance and gastrointestinal health.

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Conflict of interest

The authors declare no conflict of interest.

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