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Performance, energy, and nutrient utilization benefits with exogenous enzyme supplementation of wheat-soybean meal-based diets fed to 22-day-old broiler chickens

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ABSTRACT

This study was designed to investigate the performance, bone quality, and nutrient and energy utilization in broiler chickens fed wheat-soybean meal-based diets with a dietary multi-enzyme complex. The positive control (PC) diet met or exceeded energy and nutrient needs, while the negative control (NC) diet was formulated to contain 3.0, 16.7, and 33.3% less of AMEn, Ca, and avP, respectively. Enzyme complex was supplemented to the NC diet at 0, 150, 200, or 250 mg/kg during the pre-starter (d 0-9) and starter (d 9-22) phases. A total of 300 d-old male broiler chicks were assigned to the 5 treatments in a randomized complete block design with 10 replicate cages of 6 chicks per cage. Data were analyzed using the GLM procedures of SAS. Birds fed the NC diet had lower ($P < 0.01$) average daily gain, average daily feed intake, and feed efficiency (FE) compared to those fed the PC diet (d 0-22), while the chickens fed enzyme supplemented diets had similar ($P > 0.05$) performance to those fed the PC diet. Average daily feed intake (d 9-22 and 0-22) and FE (d 9-22) rose linearly ($P < 0.05$) with the level of enzyme supplementation. There were linear and quadratic ($P < 0.001$) relationships for jejunal digesta viscosity, tibia bone breaking strength and tibia ash with enzyme supplementation level. Birds fed the NC diet had lower ($P < 0.05$) apparent ileal digestibility and utilization of DM, N, digestible energy, and AMEn, but higher ($P < 0.05$) Ca digestibility and utilization compared with birds fed the PC diet. Higher levels of enzyme supplementation resulted in a quadratic relationship ($P = 0.005$) with P digestibility and a linear relationship ($P < 0.001$) with P utilization. Ileal digestibility of nonessential amino acids was quadratically related ($P < 0.05$) with enzyme supplementation except for Glu and Tyr ($P < 0.01$). This study showed that growth performance, energy and nutrient utilization, and bone mineralization were maintained with a supplemental multi-enzyme complex in a reduced energy and nutrient wheat-soybean meal-based diet.

Introduction

Sustainable broiler meat production requires the bird's ability to optimally digest and utilize the energy and nutrients contained in their feed. Wheat is the second most widely used feed ingredient after corn as a source of energy in broiler diets. The plant-based feed ingredients which account for more than 90 % of the diet contains phytic acid and non-starch polysaccharides (NSP). These two important antinutritional factors (phytic acid and NSP) make it difficult for poultry to efficiently utilize the energy and the nutrients in the feed ingredients (Naghshbandi and Moghimi, 2020). There are several exogenous enzymes in the market today that are supplemented to poultry feeds to enhance the digestion of energy and nutrient. The advantages of this are two-fold, first they help to reduce feed cost and secondly, they make poultry feeding operations more sustainable by reducing nutrient excretion into the environment.

The three most common exogenous enzymes are the phytases, carbohydrases, and proteases (Naghshbandi and Moghimi, 2020). Most of

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these enzymes are available singly which makes it easier for poultry nutritionists to select enzymes based on their particular needs. However, it is important to note that a corn-soybean meal (SBM) - or wheat-SBM-based diet contains antinutritional factors such as phytic acid and NSP, hence the birds' ability to break down these antinutritional factors may require the combined efforts of several exogenous enzymes rather than a single enzyme.

After energy and protein (amino acid, AA), phosphorus (P) is the third most expensive component of a typical poultry diet. Phytase supplementation can allow P to be used at a lower level by reducing the inclusion rate of inorganic phosphate. One of the advantages of enzyme supplementation to diets is to reduce feed cost. In addition to a reduction in feed cost, the use of phytase has also been shown to make poultry production more environmentally friendly by minimizing the output of P in excreta (Chakraborty et al., 2021).

Carbohydrases on the other hand are a group of exogenous enzymes that enhance how birds digest and utilize energy from fractions of plant-based feed ingredients that otherwise would not have been available to them. Wheat contains a high level of arabinoxylan, which is the major component of NSP, hence without supplemental carbohydrase enzyme, the birds are unable to efficiently utilize the energy embedded in the NSP. Studies have shown that wheat-based diets benefit more than corn-based diets from supplemental exogenous carbohydrase because of the ability of the water soluble portion of the arabinoxylan to convert the digesta into a gel-like structure. This process limits the ability of endogenously produced enzymes to have direct access to the digesta leading to a decrease in digestibility of energy and nutrients (Ravindran and Amerah, 2009; Adeola and Cowieson, 2011). This increase in viscosity also increases the quantity of nutrient such as nitrogen (N) from undigested protein or peptides and undigested polysaccharides that escape into the hindgut and could lead to the proliferation of potentially pathogenic bacteria (Olojede and Adedokun, 2019; Cowieson, 2010). The excreta from highly viscous digesta have also been shown to result in wet litter with the potential for an increase in incidence of footpad dermatitis (Liu et al., 2021).

One of the commercially available carbohydrase enzymes that is able to enhance the breakdown of NSP is xylanase, with its ability to improve energy and nutrient digestibility via a reduction in digesta viscosity (Engberg et al., 2004; Munyaka et al., 2016; Dunaway and Adedokun, 2021). Despite the fact that there are many commercially available exogenous enzymes on the market, efforts are still ongoing by different labs and companies to come up with an improved single product or a product that combines the activities of two or more enzymes into one product. The use of enzyme cocktail is becoming common because of the potential for reduced cost of adding two or more enzyme from the same product as well as the possibility of taking advantage of potential synergistic effect of multiple enzymes. The hypothesis of this study was that increasing level of supplemental enzyme would result in an increase in the response variables being evaluated. Hence, the objective of this study was to examine the growth performance, bone mineralization, energy and nutrient digestibility in broiler chickens fed a wheat-SBM-based diets with varying levels of a dietary exogenous enzyme with multiple enzyme activities. The enzyme that was evaluated in this study was added to diets containing reduced energy, calcium (Ca) and P levels because of the anticipated actions of the enzymes in increasing nutrient and energy digestibility from their respective substrates.

Materials and methods

Animal housing, management, and experimental design

The experimental procedures and management of birds were approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Three hundred day-old male Cobb by-product breeder chicks were obtained from a commercial hatchery. The study was conducted as a randomized complete block design with location in the room as the blocking factor. Chicks were weighed on d 0 and randomly assigned to treatments. Each of the 5 dietary treatments were randomly assigned to cages and replicated 10 times with 6 birds per replicate cage. The birds were housed in cages (0.61 × 0.51 × 0.36 m) in an environmentally controlled room with 22 h of light and 2 h of darkness. The experimental diets consisted of a pre-starter (Table 1) and starter (Table 2) diets. The pre-starter diets were fed from d 0-9 while the starter diets were fed from d 9-22. All birds were given *ad libitum* access to feed and water for the duration of the experiment.

Experimental diets

The enzyme product used in this experiment was Allzyme® Spectrum (Alltech Inc., Nicholasville, KY). Prior to diet mixing, the enzyme was analyzed and confirmed to contain 4.2 million XU/lb of Xylanase (*Trichoderma longibrachiatum*) and 454,000 SPU/lb of Phytase (*Aspergillus niger*). One xylanase unit is the amount of enzyme that releases 1 mmol of xylans per minute under the conditions of the assay. Enzyme supplementation to the experimental diets was based on the analyzed activity levels of the enzyme product. The solid-State fermentation phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay (McKinney et al., 2015).

Each of the feeding phases consisted of 5 diets. A positive control (PC), negative control (NC), NC + 150, NC + 200, and NC + 250 mg of the enzyme per kg of the diet. The positive control (PC) diet for both the pre-starter and starter phases was a wheat-SBM-based reference diet comparable to what was recommended by the breeder company that met or exceeded the energy and nutrient requirements of birds of this

Table 1
Ingredients composition of the experimental pre-starter diets fed to broiler chickens from day 0-9 (on as-fed basis)[1].

Ingredient, g/kg	Positive control (Treatment A)	Negative control (Treatment B)
Wheat (hard red)	712.4	722.20
Soybean meal (47 % CP)	231.4	222.1
Wheat bran	0.0	20.6
Soy oil	14.0	0.0
L-Lysine HCl	5.20	5.20
DL-Methionine	2.60	2.60
L-Threonine	1.40	1.40
Salt (NaCl)	3.40	3.40
Limestone	10.6	11.8
Dicalcium phosphate	16.7	8.4
Vitamin-mineral premix[2]	1.50	1.50
Choline chloride (60 %)	0.8	0.8
Total	1,000.0	1,000.0

[1] The three enzyme containing dietary treatments (C, D, and E) were mixed from the same negative control basal diet. Exogenous enzyme (Allzyme® Spectrum) was added to the basal diet at the rate of 150, 200, and 250 mg/kg to produce diets containing increasing levels of enzyme activity. Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (Aspergillus niger) and 4.2 million XU/lb of Xylanase (Trichoderma longibrachiatum). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme that will release 1 mmol of xylans per minute under the conditions of the assay. The calculated enzyme activity for each enzyme containing diet is 150 SPU and 1,395 XU, 200 SPU and 1,860 XU, and 250 SPU and 2,325 XU for the 150, 200, and 250 mg/kg, respectively.

[2] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: 32 mg of iron from iron sulfate; 8 mg of copper from copper sulfate; 51 mg of manganese from manganese oxide; zinc oxide, 60 mg; iodine (EDDI), 1.48 mg; sodium selenite, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D_3 (cholecalciferol), 2,822 IU; vitamin E ($_{dl}$-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B_{12}, 0.02 mg; choline, 480 mg.

Table 2
Ingredients composition of the experimental starter diets fed to broiler chickens from day 9-22 (on as-fed basis)[1].

Ingredient, g/kg	Positive control (Treatment A)	Negative control (Treatment B)
Wheat (hard red)	759.9 0	779.20
Soybean meal (47 % CP)	174.3	164.4
Wheat bran	0.0	14.0
Soy oil	20.9	4.6
L-Lysine HCl	6.00	6.00
DL-Methionine	2.30	2.30
L-Threonine	1.90	1.90
Salt (NaCl)	3.20	3.20
Limestone	9.8	10.9
Dicalcium phosphate	14.4	6.2
Vitamin-mineral premix[2]	1.50	1.50
Choline chloride (60 %)	0.8	0.8
Titanium dioxide	5.0	5.0
Total	1,000.0	1,000.0

[1] The three enzyme containing dietary treatments (C, D, and E) were mixed from the same negative control basal diet. Exogenous enzyme (Allzyme® Spectrum) was added to the basal diet at the rate of 150, 200, and 250 mg/kg to produce diets containing increasing levels of enzyme activity. Allzyme® Spectrum contains 454,000 SPU/lb of Phytase (*Aspergillus niger*) and 4.2 million XU/lb of Xylanase (*Trichoderma longibrachiatum*). One Solid State Fermentation Phytase unit (SPU) is defined as the amount of enzyme that will release 1 mmol of inorganic P per minute under the conditions of the assay. One xylanase unit is the amount of enzyme will release 1 mmol of xylans per minute under the conditions of the assay. The calculated enzyme activity for each enzyme containing diet is 150 SPU and 1,395 XU, 200 SPU and 1,860 XU, and 250 SPU and 2,325 XU for the 150, 200, and 250 mg/kg, respectively.

[2] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: 32 mg of iron from iron sulfate; 8 mg of copper from copper sulfate,; 51 mg of manganese from manganese oxide; zinc oxide, 60 mg; iodine (EDDI), 1.48 mg; sodium selenite, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D_3 (cholecalciferol), 2,822 IU; vitamin E ($_{dl}$-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B_{12}, 0.02 mg; choline, 480 mg.

age (Cobb-Vantress, 2022). The negative control (NC) diet was similar to the PC diet except that the energy (AMEn), Ca, and avP were reduced by 3.0 (90 kcal), 16.7, and 33.3 %, respectively. These values were based on internal data from previous studies (unpublished). The NC diet and all enzyme supplemented diets were mixed from a single basal diet, divided into different portions to which different amount of exogenous enzymes were added to the apportioned NC diets to produce the respective enzyme supplemented diets. Titanium dioxide (5 g/kg diet) was added to all the starter diets as an indigestible marker for digestibility and utilization calculations. The ingredient composition and the formulated and analyzed energy and nutrient contents of the experimental diets are reported in Tables 1 and 3 (pre-starter) and Table 2 and 4 (starter).

Sample collection

Excreta samples were collected from each cage on d 21 and 22 and dried for 5 days at 55°C in a forced-air oven. The dried samples were ground using a Wiley Mill Laboratory Standard (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA) fitted with a 1 mm screen and then stored in airtight plastic bags at 4 °C before being analyzed for DM, gross energy (GE), Ca, N, P, and titanium (Ti). On d 22, two birds per cage were euthanized by argon asphyxiation prior to tibia and digesta collection. The right tibia from two birds per cage were removed from birds with body weights closest to the average cage body weight. All bone samples were stored at - 20 °C until bone-breaking strength (BBS) and bone ash determination. Additionally, jejunal digesta samples were collected from the same two birds. The digesta was placed in a pre-labeled 15 mL plastic tube. The jejunal samples were frozen at -20°C until measured for viscosity. Ileal digesta from the distal two-thirds of the ileum was collected from all the birds within each cage by flushing with distilled water into clean pre-labeled plastic containers. All ileal digesta samples from each cage were combined in a single container before being stored at -20°C until they were freeze-dried. The dried ileal digesta samples were ground using a coffee grinder before storing in airtight bags in a refrigerator at 4 °C until they were analyzed for GE, DM, AA, Ti, Ca, P, and N.

Table 3
Formulated and analyzed energy and nutrient contents of the experimental pre-starter diets fed to broiler chickens from day 0-9.

Nutrient and energy	Positive control	Negative control[1]
Formulated value		
Crude protein, g/kg	220.3	220.4
MEn, kcal/kg	2,975	2,885
Ca, g/kg	9.0	7.5
P, g/kg	7.5	6.2
Non-phytate P, g/kg	4.5	3.0
SID[2] amino acid, g/kg		
Lys	12.1	12.0
Met	5.2	5.3
Met + Cys	8.2	8.3
Thr	6.7	6.7
Trp	2.3	2.3
Val	8.7	8.6
Analyzed value		
Crude protein, g/kg	212.5	213.5
Gross energy, kcal/kg	3,938	3,888
Ca, g/kg	12.4	10.6
P, g/kg	6.5	5.4
Lys, g/kg	13.6	13.7
Met, g/kg	5.6	5.2
Met + Cys, g/kg	9.1	8.7
Thr, g/kg	8.2	8
Trp, g/kg	2.3	2.3
Val, g/kg	9.6	9.4

[1] The negative control diet and diets containing supplemental exogenous enzymes (C, D, and E) were mixed from a single basal diet.
[2] Standardized ileal digestibility (digestible)

Table 4
Formulated and analyzed energy and nutrient contents of the experimental starter diets fed to broiler chickens from day 9-22.

Nutrient and energy	Positive control	Negative control[1]
Formulated values		
Crude protein, g/kg	199.8	201.1
MEn, kcal/kg	3,056	2,966
Ca, g/kg	8.0	6.5
P, g/kg	6.9	5.6
Non-phytate P, g/kg	4.0	2.5
SID[2] amino acid, g/kg		
Lys	11.4	11.3
Met	4.7	4.7
Met + Cys	7.6	7.6
Thr	6.5	6.4
Trp	2.1	2.1
Val	7.9	7.9
Analyzed values		
Crude protein, g/kg	199.6	201.4
Gross energy, kcal/kg	3,964	3,927
Ca, g/kg	10.6	7.7
P, g/kg	6.0	4.4
Lys, g/kg	12.9	12.7
Met, g/kg	4.7	4.5
Met + Cys, g/kg	8.2	7.8
Thr, g/kg	7.6	8.3
Trp, g/kg	2.1	2.1
Val, g/kg	8.6	8.5

[1] The negative control diet and diets containing supplemental exogenous enzymes (C, D, and E) were mixed from a single basal diet.
[2] Standardized ileal digestibility (digestible)

Sample analysis

Diets (collected immediately after mixing at the feed mill) were analyzed in triplicate while excreta, ileal digesta, and jejunal digesta samples were analyzed in duplicate. Five percent coefficient of variation (CV) was used as the cutoff point with any samples with CV above 5% subjected to repeat analysis. The DM contents of the diets, ileal digesta, and excreta were determine by drying at 105°C for 24 h (method 934.01; AOAC, 2006). Gross energy of the diets, ileal digesta, and excreta was determined using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr Instruments, Moline, IL, USA) with benzoic acid serving as the calibration standard. Nitrogen in the diets, excreta, and ileal digesta was analyzed using the LECO analyzer (model FP2000, LECO, St. Joseph, MI; AOAC, 2006; method 990.03), with EDTA as the internal standard. The Ti content of the diets, ileal digesta, and excreta were analyzed using the Short et al. (1996) method and then determined using a UV-visible spectrophotometer (UV-1800, Shimadzu Scientific, Kyoto, Japan) at a wavelength of 410 nm. The Ca and P of the diets, ileal digesta, and excreta as well as the AA of the diets and ileal digesta were determined at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO). The Ca and P contents were determined using Inductively Coupled Plasma-optical emission spectroscopy (method 990.08; AOAC, 2006). Concentrations of AA were analyzed using method 982.30 E(a,b,c) (AOAC, 2006). The concentration of Ti in the diets, ileal digesta, and excreta samples was determined as described by Myers et al. (2004) at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO).

To determine the viscosity of the jejunal sample, the digesta samples were first centrifuged for 10 min at 5,000 rpm. The supernatant was then removed and centrifuged again for 8 min at 10,000 rpm. That viscosity of the supernatant was then determined using a viscometer (Vibro Viscometer SV-1A, A&D Weighing, Ann Arbor, MI). The tibias were cleaned thoroughly during collection, following thawing all additional tissue and fat were removed. The BBS was measured using an Instron Materials tester (model 4301, Instron Corp., Canton, MA) at a loading rate of 50 mm/min. Following BBS determination, the bones were dried in an oven at 105°C for 24 h (Precision Scientific Co., Chicago, IL). The dried bones were then soaked in a glass jar with petroleum ether for four extraction periods with the fresh complete replacement of the petroleum ether with a clean fresh petroleum ether every 24 h for a total of 96 h. After no color change of the petroleum ether solution was observed following the final extraction, bones were removed and allowed to dry for 8 h under the hood at room temperature. They were then dried in the 105°C oven overnight to ensure there was no moisture left. These dried, de-fatted bones were then weighed by cage (2 tibias/cage) and placed in a pre-weighed porcelain crucible for ashing in a muffle furnace overnight at 600 °C. The porcelain crucible containing the bones were then weighed back to determine bone ash percentage.

Calculations and statistical analysis

All the diets were individually analyzed for GE, Ca, P, N, Ti, and AA, the values of the NC and all the enzyme supplemented diets were averaged because they were mixed from a single basal diet prior to the addition of the enzyme. The average values of the diets mixed from the NC were used for digestibility and utilization calculations while the analyzed values for the PC diet were used for PC calculations.

The apparent ileal nutrient and energy digestibility (AID) and energy and nutrient utilization (TTU) of DM, energy, N, Ca, and P were calculated using the method of Kong and Adeola (2014) with the following equation:

$$\text{AIDorTTU}(\%) = 100 - [100 \times (\text{Ti}/\text{To})\text{x}(\text{No}/\text{Ni})]$$

Where To is the concentration of titanium in the ileal digesta or excreta (%), Ti is the titanium concentration in the feed, No is the concentration of energy or nutrients in ileal digesta or excreta, and Ni is the concentration of energy or nutrients in the feed.

The AME and apparent digestible energy (ADE) were calculated using the equation below:

AME or *ADE* (*kcal/kg* DM) = *Calculated* energy utilization or ileal energy digestibility (%) × *GE of* the *diet* (*kcal/kg*).

The AME corrected for N (AMEn) was obtained using the Hill and Anderson (1958) method.

Data was analyzed using the PROC GLM procedure of SAS 9.4 v 4 (2011, SAS Institute Inc., Cary, NC) appropriate for a randomized complete block design. PROC IML was used to generate the coefficients used for orthogonal polynomial contrasts. Orthogonal polynomial contrasts were utilized to determine polynomial effects of increasing dietary supplemental levels of enzyme. A simple contrast between the PC and NC diets was also performed. Additionally, a contrast between the PC and the enzyme supplemented diets was also performed. All outliers (data that deviated from first or third quartiles by more than 3 times the interquartile range within each dietary treatment) were removed prior to statistical analysis. The level of significance was defined as $P < 0.05$. Digestibility and utilization data is presented on a DM basis.

Results

Nutrient composition of experimental diets

The pre-starter and starter diets were analyzed for N, GE, Ca, and P contents and the starter diets were also analyzed for Ti. The analyzed Ca in the starter diets was 32% higher than the formulated value (10.6 vs 8.0 g/kg) for the PC diet (Table 4) while for the NC diet, it was 18% higher than the formulated value (7.7 vs 6.5 g/kg; Table 4). Similar trends were observed for the pre-starter diets as well (Table 3).

Growth performance and viscosity

The treatment effect on performance are reported in Tables 5 and 6. Birds fed the NC diet had lower ($P < 0.01$) average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE) compared to those fed the PC diet for the entire experimental period (22 days), while the ADG, ADFI, and FE of birds fed diets supplemented with enzymes were not different from that of birds fed the PC diet. Average daily feed intake of birds from d 9-22 and 0-22 and FE from d 9-22 linearly increased ($P < 0.05$) with increasing level of supplemental enzyme. There was a quadratic relationship ($P < 0.05$) of enzyme supplementation and BWG d 9-22 and 0-22 and FE d 9-22 and 0-22.

The treatment effect on jejunal digesta viscosity among treatments are reported in Table 6. Both the linear and quadratic effects were significant ($P < 0.001$) for jejunal digesta viscosity, with enzyme supplementation resulting in lower viscosity. Enzyme supplemented diets had lower ($P < 0.05$) digesta viscosity compared to PC diets.

Energy and nutrient digestibility

Enzyme supplementation resulted in higher ($P < 0.05$) Ca and P digestibility compared to birds on the PC diet (Table 7). There was also a quadratic relationship ($P < 0.01$) with DM, N, P digestibility, and ADE for birds fed the enzyme supplemented diets. Birds fed the NC diet had lower ($P < 0.05$) apparent ileal digestibility of DM, N, and ADE but higher ($P < 0.05$) Ca digestibility compared with those fed the PC diet. There was a positive linear relationship ($P = 0.002$) for Ca digestibility and increasing enzyme supplementation (Table 7).

There was no difference ($P > 0.05$) in apparent ileal digestibility of essential AA when comparing the PC diet to the enzyme supplemented diets except for Thr ($P < 0.001$) where enzyme supplementation resulted in higher ($P < 0.05$) apparent ileal Thr digestibility compared to

Table 5
Effects of increasing level of Allzyme Spectrum® supplementation on the performance of 22-d-old broiler chickens fed wheat-soybean-meal-based diets[1].

Treatment	Diet	Allzyme Spectrum®, mg/kg	IBW, g/b	Feed intake, g/b/d			Body weight gain, g/b/d		
				d 0-9	d 9-22	d 0-22	d 0-9	d 9-22	d 0-22
A	PC	0	40.8	21.5$_z$	75.7	53.3	17.7	48.8	35.9
B	NC	0	40.4	20.0	67.7	47.8	15.2	38.9	29.0
C	NC	150	40.7$_z$	21.5	76.2	53.6	17.9	47.2	35.1
D$_z$	NC	200	39.9	20.4	75.0	52.2	16.4	47.6	34.6
E	NC	250	41.3	21.3	76.2$_z$	53.1	16.9	47.9$_z$	34.8
Pooled standard deviation			0.355	0.584	1.483	1.093	0.704	1.171	0.896
P-value			0.120	0.260	0.001	0.002	0.065	<0.001	<0.001
			Probability						
PC vs. NC			0.409	0.076	<0.001	0.001	0.018	<0.001	<0.001
PC vs. Enzyme supplemented diets			0.591	0.485	0.978	0.773	0.472	0.391	0.328
Linear effect of enzyme supplementation			0.418	0.157	<0.001	<0.001	0.089	<0.001	<0.001
Quadratic effect of enzyme supplementation			0.247	0.568	0.083	0.070	0.111	0.049	0.026

[1] PC = Positive control, NC = Negative control, IBW = Initial body weight. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
[z]n = 10 replicate cages with 6 birds/replicate, subscript z where n = 9

Table 6
Effects of increasing level of Allzyme Spectrum® supplementation on feed efficiency and jejunal digesta viscosity of 22-d-old broiler chickens fed wheat-soybean-meal-based diets[1].

Treatment	Diet	Allzyme Spectrum®, mg/kg	Feed efficiency			Viscosity, mPa
			d 0-9	d 9-22	d 0-22	
A	PC	0	0.820$_z$	0.644	0.673	5.2
B	NC	0	0.758$_z$	0.574	0.606	6.2
C	NC	150	0.832	0.620$_z$	0.656$_z$	3.1
D	NC	200	0.811	0.634	0.660$_z$	2.9
E	NC	250	0.791	0.629	0.656	3.0
Pooled standard deviation			0.02	0.01	0.01	0.38
P-value			0.016	<0.001	<0.001	<0.001
			Probability			
PC vs. NC			0.008	<0.001	<0.001	0.078
PC vs. Enzyme supplemented diets			0.638	0.112	0.086	<0.001
Linear effect of enzyme supplementation			0.041	<0.001	<0.001	<0.001
Quadratic effect of enzyme supplementation			0.006	0.158	0.046	<0.001

[1] PC = Positive control, NC = Negative control. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
[z]n = 10 replicate cages with 6 birds/replicate, except for subscript z where n = 9

the PC diet (87.2 vs. 85.0%; Table 8). There was a quadratic relationship ($P < 0.05$) for ileal digestibility of Arg, His, and Val with increasing enzyme supplementation, as well as a linear relationship ($P = 0.001$) for ileal digestibility of Thr (Table 8). Birds on the PC diets had higher ($P < 0.05$) nonessential AA digestibility compared to birds on the NC diets except for Glu and Tyr where there was no difference (Table 9). Increasing enzyme supplementation resulted in quadratic change ($P < 0.05$) in all the nonessential AA investigated in this study except for Glu and Tyr where the difference was not significant (Table 9).

Energy and nutrient utilization

The effects of exogenous enzyme supplementation on energy and nutrient retention are reported in Table 10. Birds fed the NC diet had lower ($P < 0.05$) utilization of DM, N, and AME but higher ($P < 0.05$) Ca and P utilization compared with those fed the PC diet. Birds fed the enzyme supplemented diets had higher ($P < 0.001$) DM, N, Ca, P, and energy utilization compared to the birds fed the PC diet. Enzyme supplementation level had a quadratic relationship ($P < 0.001$) with DM, N, energy retention, AME, and AMEn. Calcium and P utilization also rose linearly ($P < 0.001$) with enzyme supplementation level.

Bone mineralization

The effect of exogenous enzyme supplementation on BBS and bone ash are reported in Table 11. Birds fed the PC diet had higher BBS ($P = 0.003$) and bone ash ($P = 0.013$) compared to birds fed the NC and enzyme supplemented diets. There was a linear and quadratic increases ($P < 0.001$) in percent bone ash and BBS with exogenous enzyme supplementation.

Discussion

The current study shows that feeding a reduced energy and nutrient

Table 7
Effects of increasing level of Allzyme Spectrum® supplementation on apparent ileal nutrient and energy digestibility of 22-d-old broiler chickens fed wheat-soybean-meal-based diets (DM basis)[1]

Treatment	Diet	Allzyme Spectrum®, mg/kg	DM, %	N, %	Ca, %	P, %	Energy, %	ADE, kcal/kg
A	PC	0	78.3	87.7	61.7	59.3	77.1	3,454
B	NC	0	71.9	86.2	71.2	56.4	69.8	3,139
C	NC	150	78.4	88.4	75.4$_z$	62.3	76.6	3,404
D	NC	200	79.0	88.3	73.2	65.5	77.4	3,434
E	NC	250	78.7	88.5	76.2	66.0	77.2	3,426
Pooled standard deviation			1.35	1.20	9.25	5.39	0.46	60.83
P-value			<0.001	<0.001	0.008	<0.001	<0.001	<0.001
			Probability					
PC vs. NC			<0.001	0.011	0.027	0.232	<0.001	<0.001
PC vs. Enzyme supplemented diets			0.442	0.091	<0.001	0.011	0.991	0.157
Linear effect of enzyme supplementation			0.370	0.162	0.002	0.021	0.927	0.228
Quadratic effect of enzyme supplementation			<0.001	0.003	0.337	0.005	<0.001	<0.001

[1] PC = Positive control, NC = Negative control, ADE = apparent digestible energy. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
[z]n = 10 replicate cages with 6 birds/replicate, except for subscript z where n = 9

Table 8
Effects of increasing level of Allzyme Spectrum® supplementation on apparent ileal digestibility of essential amino acids of 22-d-old broiler chickens fed wheat-soybean-meal-based diets (DM basis)[1]

Treatment	Diet	Allzyme Spectrum®, mg/kg	Arg, %	His, %	Ile, %	Leu, %	Lys, %	Met, %	Phe, %	Thr, %	Trp, %	Val, %
A	PC	0	88.0	87.8	87.8	87.7	90.5	93.6$_z$	89.2	85.0	88.5	85.6
B$_z$	NC	0	86.3	86.1	86.6	86.4	89.8	92.2	87.9	84.7	87.6	83.9
C	NC	150	88.1$_z$	88.1$_z$	89.0	88.8	91.3	93.2	90.1	87.4	89.1	86.1$_z$
D$_z$	NC	200	88.4	88.2	88.3	88.2	90.9	92.9	89.5	86.9	89.0	86.3
E	NC	250	88.6	88.4	89.0	88.7$_z$	91.5	93.3	90.2	87.3$_z$	89.4	86.7
Pooled standard deviation			1.72	1.61	1.84	1.85	1.80	1.10	1.49	1.87	1.74	1.82
P-value			0.032	0.027	0.042	0.049	0.264	0.089	0.013	0.004	0.160	0.022
Probability												
PC vs. NC			0.035	0.026	0.153	0.122	0.391	0.009	0.070	0.943	0.246	0.045
PC vs. Enzyme supplemented diets			0.606	0.490	0.178	0.216	0.272	0.275	0.172	<0.001	0.276	0.307
Linear effect of enzyme supplementation			0.709	0.587	0.363	0.355	0.458	0.200	0.377	0.001	0.405	0.449
Quadratic effect of enzyme supplementation			0.017	0.014	0.165	0.117	0.362	0.080	0.082	0.146	0.158	0.020

[1] PC = Positive control, NC = Negative control. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
[z]n = 10 replicate cages with 6 birds/replicate, except for subscript z where n = 9

Table 9
Effects of increasing level of Allzyme Spectrum® supplementation on apparent ileal digestibility of nonessential amino acids of 22-d-old broiler chickens fed wheat-soybean-meal-based diets (DM basis)[1]

Treatment	Diet	Allzyme Spectrum®, mg/kg	Ala, %	Asp, %	Cys, %	Glu, %	Gly, %	Pro, %	Ser, %	Tyr, %
A	PC	0	83.8	85.0	86.2	93.8	84.2	92.8	86.2	88.5
B$_z$	NC	0	81.7	83.0	82.3	93.2	81.9	91.7	84.1	87.2
C	NC	150	84.2$_z$	86.1	85.9	94.6	84.5$_z$	93.5	87.2	89.8
D$_z$	NC	200	84.5	85.4	85.8	94.3	84.8	93.2	86.7	89.1
E	NC	250	84.8	86.0	85.5	94.6	84.9	93.4	87.3	89.7
Pooled standard deviation			2.20	1.92	1.79	0.90	1.82	0.85	1.99	1.75
P-value			0.032	0.005	<0.001	0.006	0.004	<0.001	0.009	0.014
Probability										
PC vs. NC			0.040	0.022	<0.001	0.126	0.007	0.010	0.030	0.095
PC vs. Enzyme supplemented diets			0.395	0.252	0.452	0.058	0.462	0.054	0.233	0.122
Linear effect of enzyme supplementation			0.501	0.445	0.555	0.140	0.548	0.114	0.390	0.240
Quadratic effect of enzyme supplementation			0.018	0.032	<0.001	0.069	0.003	0.004	0.029	0.088

[1] PC = Positive control, NC = Negative control. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
[z]n = 10 replicate cages with 6 birds/replicate, except for subscript z where n = 9

Table 10
Effects of increasing level of Allzyme Spectrum® supplementation on apparent nutrient and energy utilization of 22-d-old broiler chickens fed wheat-soybean-meal-based diets (DM basis)[1]

Treatment	Diet	Allzyme Spectrum®, mg/kg	DM, %	N, %	Ca, %	P, %	Energy, %	AME, kcal/kg	AMEn, kcal/kg
A	PC	0	72.8$_z$	63.1	65.6	61.8	76.6$_z$	3,352$_z$	3,249$_z$
B	NC	0	68.3	60.8	69.9	68.4	71.8$_z$	3,099$_z$	2,989$_z$
C	NC	150	74.8	69.6	72.7	75.2	77.2	3,329	3,244
D	NC	200	74.9	68.2	72.3	73.6$_z$	77.3$_z$	3,337$_z$	3,249$_z$
E	NC	250	74.8	68.8	74.4	75.2	77.1	3,325	3,237
Pooled standard deviation			0.62	3.79	3.65	1.81	1.55	21.04	22.05
P-value			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Probability									
PC vs. NC			<0.001	0.044	0.012	<0.001	<0.001	<0.001	<0.001
PC vs. Enzyme supplemented diets			<0.001	<0.001	<0.001	<0.001	0.002	0.009	0.495
Linear effect of enzyme supplementation			<0.001	<0.001	<0.001	<0.001	0.001	0.044	0.833
Quadratic effect of enzyme supplementation			<0.001	<0.001	0.598	0.262	<0.001	<0.001	<0.001

[1] PC = Positive control, NC = Negative control, AME = apparent metabolizable energy, AMEn = AME corrected for nitrogen. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22
[z]n = 10 replicate cages with 6 birds/replicate, except for subscript z where n = 9

wheat-soybean meal-based diet supplemented with a multi-enzyme complex to broiler chickens can result in growth performance and nutrient and energy digestibility and utilization not different from that of birds receiving a full nutrient diet. Birds fed the enzyme supplemented diets had an overall better performance than those fed only the reduced energy and nutrient diet (NC). The poor performance by birds on the NC diet could be attributed to the reduction in nutrient and energy levels in the diet (compared to the PC diet) coupled with the difficulty of releasing energy and nutrient from the NSPs and phytate, whose negative effects were offset by the carbohydrase and phytase enzymes, respectively, in the multi-enzyme complex that was supplemented to the NC diets. The performance of birds given the enzyme supplemented diets was not different from birds fed the PC diet.

Wheat is high in arabinoxylans, a form of hemi-cellulose NSPs. Wheat's soluble arabinoxylan content is 17 times that of corn (Choct, 1997); the only other cereal grain with a higher soluble arabinoxylan content than the wheat is rye (Choct, 1997). Xylanase has been shown to offset the anti-nutritive factors of arabinoxylans by hydrolyzing the β-1, 4 glycosidic bonds of xylan into xylose (Adeola and Cowieson, 2011). Alongside phytase, the exogenous enzyme responsible for hydrolyzing

Table 11
Effect of increasing level of Allzyme Spectrum® supplementation on the tibia quality of 22-d-old broiler chickens fed wheat-soybean-meal based diets[1].

Treatment	Diet	Allzyme Spectrum®, mg/kg	Bone breaking strength, kgf	Bone ash, %
A	PC	0	17.7	53.4$_z$
B	NC	0	11.2	50.1
C	NC	150	15.6	51.4$_y$
D	NC	200	15.4	51.2$_z$
E	NC	250	15.4	53.0
Pooled standard deviation			1.98	1.62
P-value			<0.001	<0.001
Probability				
PC vs. NC			<0.001	<0.001
PC vs. Enzyme supplemented diets			0.003	0.013
Linear effect of enzyme supplementation			0.006	0.002
Quadratic effect of enzyme supplementation			<0.001	0.011

[1] PC = Positive control, NC = Negative control. Pre-starter diet fed from d 0-9 and starter diet fed from d 9-22

[z]n = 10 replicate cages with 6 birds/replicate, except for y & z where n = 8&9, respectively

phytate, these enzymes supported digestion and improved birds' ability to utilize energy and nutrients in wheat-based diets.

The ability of xylanase to alleviate challenges associated with high digesta viscosity has been well documented (Engberg et al., 2004; Munyaka et al., 2016). The lower digesta viscosity in the current study (51%) is in line with what Engberg et al. (2004) reported (50%) for digesta viscosity when xylanase was added to wheat-based diets. Munyaka et al. (2016) also reported a decrease of 31% in digesta viscosity of broiler chickens fed wheat-based diets supplemented with xylanase. Likewise, Anwar et al., (2023) reported a greater reduction in digesta viscosity in birds fed wheat-based diets supplemented with both xylanase and phytase compared to birds supplemented with only xylanase (37 vs. 56% reductions). The results from the use of a combination of phytase and xylanase showed that there is a potential additive effects of both enzymes in reducing digesta viscosity. The high difference in digesta viscosity (51%) observed in the current study can be attributed primarily to xylanase's ability to break down arabinoxylans but also to the interactive effects of both enzymes (xylanase and phytase).

The enzyme complex use in this study allowed the birds to maintain performance on a low nutrient diet. The linear and quadratic increases in performance (ADFI, ADG, and FE) of the birds fed enzyme supplemented diets in the current study is consistent with published manuscripts (Gonzalez-Ortiz et al., 2016; Gonzalez-Ortiz et al., 2017; Arczewska-Wlosek et al., 2019; Olukosi et al., 2020). Anwar et al., (2023) reported birds fed diets supplemented with xylanase and phytase had an improved FI, BWG, and feed conversion ratio (FCR) compared to those birds who did not receive enzyme supplemented diet. The growth performance benefits seen in the current study can be attributed to phytases' ability to cleave phytate bound nutrient, making them more available to the birds. The lower digesta viscosity observed with enzyme supplementation allowed for greater interactions between the endogenously secreted digestive enzymes such as the protein and energy digesting enzymes likely leading to greater digestion and absorption of these nutrients, indicated in this study by the higher nutrient digestibility values (Palander et al., 2005; Rutherfurd, et al., 2007).

The AME and nutrient utilization improvement observed in the enzyme supplemented diets in the current study can be associated with the partial or total cleavage of arabinoxylans in the wheat. The lower digesta viscosity with enzyme inclusion allowed for better absorption of nutrients in the mid gut. Digestible and metabolizable energy gains with enzyme supplementation is well documented (Walters et al., 2019; Anwar et al., (2023)). The AMEn benefit (8%) in this study is comparable to the improvement (5%) in AMEn that Selle et al. (2009) reported when feeding xylanase and phytase in combination to broilers being fed low-P wheat-based diets. Similarly, Gallardo et al. (2018) reported similar increases (9.7%) in AME due to supplemental phytase and xylanase. Despite these documented improvements in energy digestibility and utilization, there are speculations that the improvement in energy may be independent of its effect on AA digestibility. It has been suggested that Ca phytate may increase the formation of metallic soaps in the lumen of the GIT, resulting in reduced digestibility (Adeola and Cowieson, 2011; Cowieson et al., 2017).

Nonstarch polysaccharides are known to bind multivalent cations (Gallardo et al., 2018), so the addition of phytase and xylanase would allow for better availability of these cations to the birds, thus improving digestibility and utilization. Phytase has also been shown to improve mineral digestibility and utilization due to its ability to hydrolyze mineral-phytate complexes. This is likely the reason for the linear increases in P and Ca digestibility and utilization in enzyme supplemented birds in this study. The results of the current study agree with Gallardo et al. (2018) who reported similar findings on Ca (5 vs. 8%) and P (10 vs. 13%) retention (control vs. enzyme supplemented diet) when xylanase and phytase were added to wheat-based broiler diets. Selle et al. (2009) also reported an improvement in Ca and P retention with phytase and xylanase supplementation while Walters et al. (2019) reported an improvement in Ca and P digestibility when phytase was added to low-P diets. In the current study, apparent ileal Ca digestibility and Ca and P utilization were higher in the NC diet compared to the PC diet, however the lower apparent ileal P digestibility of the NC diet although difficult to explain, may be attributed to the fact that unlike in the PC diet, where the analyzed level of P (starter diet) was 13% below the formulated level, the corresponding value for the NC diet was 21%. This means the NC diet probably had much less nPP (from dicalcium phosphate) than formulated. This could explain why the NC diet that was not supplemented with exogenous enzymes resulted in lower P digestibility value compared to birds on the PC diet.

Greater DM digestibility is consistent with reports from other researchers (Anwar et al., 2023). The higher N digestibility (2.6%) and retention (11.8%) reported in the current study (average of enzyme supplemented diets vs. the NC diet) can be associated with lower endogenous losses of N due to the action of the carbohydrase and phytase enzymes on NSPs and phytate (Selle et al., 2006; Cowieson et al., 2017). Both phytate and NSPs are known to bind to proteins so the addition of exogenous enzymes can increase dietary protein hydrolysis. Selle et al. (2009) observed an 11% increase in N retention in broiler diets supplemented with phytase and xylanase. However, they reported that N retention improved more (10 vs. 6%) when only phytase was supplemented compared to when only xylanase was supplemented. This is likely due to phytases' ability to cleave phytate-bound proteins. Gallardo et al. (2018) reported similar improvements of 8% in N retention when broilers were fed wheat-based diets with phytase and xylanase supplementation.

The effect of exogenous enzymes on apparent ileal AA digestibility is inconsistent. Selle et al. (2006) observed that improved AA digestibility was associated with reduction of endogenous AA losses. It has been suggested that the negative effect of phytate on AA digestibility may be due to increased losses of endogenous AA from the intestine rather than a direct impact on dietary protein utilization (Cowieson et al., 2017; Gallardo et al., 2018). Woyengo et al. (2008) reported an improvement in apparent AA digestibility in pigs fed wheat-based diets supplemented with phytase and xylanase. Cowieson et al. (2017) reported that Met and Glu showed lower responses to phytase addition compared to other AA. Similar studies have shown improvement in apparent AA digestibility in wheat-based diets with enzyme supplementation (Gallardo et al., 2018; Anwar et al., 2023). It has been suggested that NSPs impair the digestibility of AA by encapsulating the AA in grains, thereby preventing digestion and absorption of those AAs (Anwar et al., 2023). The higher apparent digestibility of some AA in this current study may be attributed to xylanases ability to degrade NSPs thus enhancing protein digestibility.

Bone quality response variables such as BBS and bone ash have been

shown to improve when enzymes are supplemented to reduced nutrient diets (Lalpanmawia et al., 2014; Leyva-Jimenez et al., 2019; Walters et al., 2019). Al-Qahtani et al. (2020) reported a comparable improvement of 23% in BBS in broilers fed wheat-based diets supplemented with xylanase and phytase. The beneficial changes in BBS and percent bone ash are likely due to phytase hydrolyzing the phytate-bound minerals, especially P, and making them more available to the bird leading to an increase in bone mineralization. Xylanase's ability to reduce digesta viscosity thereby allowing for better energy and nutrient digestion and absorption through an increase in digesta surface area and better interactions between the digesta and digestive enzymes may also have contributed to the improvement in BBS and bone ash percentages through an increase in the digestion and absorption of P.

The addition of xylanase and phytase to broiler diets allows birds to optimize nutrient digestion supporting greater nutrient absorption. One of the ways through which broiler chickens could benefit from the combination of the two exogenous enzymes is the potential ability of increased interaction of exogenous enzymes, in this case phytase, with digesta in the small intestine after a reduction in digesta viscosity through the action of xylanase leading to an increase in nutrient release This means formulated crude protein, energy, and P levels in the diet can be adjusted for the expected release of these nutrients by the supplemental exogenous enzymes. This reformulation would, in turn, allow for lower feed costs and less environmental pollution as a result of significant decrease in P and N excretion into the environment (Chakraborty et al., 2021). Furthermore, the effect of exogenous enzymes on nutrient and energy digestibility allows nutritionists to use cheaper alternative feed ingredients and more by-products in broiler diets, subsequently providing more economic benefits to producers and reducing wastes (Williams, 2006).

Conclusion

Allzyme® Spectrum enzyme supplementation to broiler chickens fed wheat-based, low nutrient diets in the current study resulted in growth performance, energy and nutrient digestibility and utilization, and tibia bone mineralization on par with birds fed full nutrient diets. This study shows that the use of an enzyme complex containing phytase and xylanase in wheat-SBM-based diets offer the ability to producers to feed reduced energy and nutrient (Ca and P) diets while still maintaining performance standards equivalent to that of birds fed a standard commercial diet. The use of the combination of exogenous phytase and xylanase shows potential as a useful way for a sustainable, environmentally friendlier, lower cost diet for poultry production.

Disclosures

T. Ao and R. F. Power are employees of Alltech Inc. Their role was limited to the study design and to review of the manuscript. All other authors declare no conflict of interest.

References

Adedokun, S.A., Olojede, O.C., 2019. Optimizing gastrointestinal integrity in poultry: the role of nutrients and feed additives. Front. Vet. Sci. 5, 348. https://doi.org/10.3389/fvets.2018.00348.

Adeola, O., Cowieson, A.J., 2011. Board invited review: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J. Anim. Sci. 89 (10), 3189–3218. https://doi.org/10.2527/jas.2010-3715.

Al-Qahtani, M., Ahiwe, E.U., Abdallh, M.E., Chang'a, E.P., Gausi, H., Bedford, M.R., Iji, P. A., 2020. Endogenous enzyme activities and tibia bone development of broiler chickens fed wheat-based diets supplemented with xylanase, β-glucanase, and phytase. Anim. Biosci. 34, 1049–1060. https://doi.org/10.5713/ajas.19.0885.

Anwar, U., Riaz, M., Farooq Khalid, M., Mustafa, R., Farooq, U., Ashraf, M., Munir, H., Auon, M., Hussain, M., Hussain, M., Ayaz Chisti, M.F., Bilal, M.Q., Rehman, A.U., Rahman, M.A.U., 2023. Impact of exogenous xylanase and phytase, individually or in combination, on performance, digesta viscosity and carcass characteristics in broiler birds fed wheat-based diets. Animals 13 (2). https://doi.org/10.3390/ani13020278.

AOAC, 2006. Official Methods of Analysis of AOAC International, 18th Ed. Association of Official Analytical Chemists, Gaithersburgs, MD.

Arczewska-Wlosek, A., Swiatkiewicz, S., Bederska-Lojewska, D., Orczewska-Dudek, S., Szczurek, W., Boros, D., Fras, A., Tomaszewska, E., Dobrowolski, P., Muszynski, S., Kwiecien, M., Schwarz, T., 2019. The efficiency of xylanase in broiler chickens fed with increasing dietary levels of rye. Animals 9 (2), 46. https://doi.org/10.3390/ani9020046.

Chakraborty, D., Prasad, R., Bhatta, A., Torbert, H.A., 2021. Understanding the environmental impact of phosphorus in acidic soils receiving repeated poultry litter applications. Sci. Total. Environ. 779, 146267. https://doi.org/10.1016/j.scitotenv.2021.146267.

Choct, M., 1997. Feed non-starch polysaccharides: chemical structures and nutritional significance. Feed Mill. Int. 191, 13–26.

Cobb-Vantress, 2022. Cobb500 Broiler Performance and Nutrition Supplement (2022). Cobb-Vantress, Inc, Siloam Springs, AR.

Cowieson, A.J., 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. The J. Poult Sci. 47, 1–7. https://doi.org/10.2141/jpsa.009045.

Cowieson, A., Ruckebusch, J., Sorbara, O., Wilson, J., Guggenbuhl, P., Roos, F., 2017. A systematic view on the effectof phytase on ileal amino acid digestibility in broilers. Anim. Feed Sci. Technol. 225, 182–194. https://doi.org/10.1016/j.anifeedsci.2017.01.008.

Dunaway, A., Adedokun, S.A., 2021. Coccidia vaccine challenge and exogenous enzyme supplementation in broiler chickens 1. Effect on digesta viscosity, diet energy utilization, and apparent metabolizable energy value of wheat. Animals 11 (3), 641. https://doi.org/10.3390/ani11030641, 2021.

Engberg, R.M., Hedemann, M.S., Steenfeldt, S., Jensen, B.B., 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83 (6), 925–938. https://doi.org/10.1093/ps/83.6.925.

Gallardo, C., Dadalt, J.C., Trindade Neto, M.A., 2018. Nitrogen retention, energy, and amino acid digestibility of wheat bran, without or with multicarbohydrase and phytase supplementation, fed to broiler chickens. J. Anim. Sci. 96 (6), 2371–2379. https://doi.org/10.1093/jas/sky062.

Gonzalez-Ortiz, G., Olukosi, O., Bedford, M.R., 2016. Evaluation of the effect of different wheats and xylanase supplementation on performance, nutrient and energy utilisation in broiler chicks. Anim. Nutr. 2 (3), 173–179. https://doi.org/10.1016/j.aninu.2016.06.005.

Gonzalez-Ortiz, G., Sola-Oriol, D., Martinez-Mora, M., Perez, J.F., Bedford, M.R., 2017. Response of broiler chickens fed wheat-based diets to xylanase supplementation. Poult. Sci. 96 (8), 2776–2785. https://doi.org/10.3382/ps/pex092.

Hill, F.W., Anderson, D.L., 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64, 587–603.

Kong, C., Adeola, O., 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27 (7), 917–925. https://doi.org/10.5713/ajas.2014.r.02.

Lalpanmawia, H., Elangovan, A.V., Sridhar, M., Shet, D., Ajith, S., Pal, D.T., 2014. Efficacy of phytase on growth performance, nutrient utilization and bone mineralization in broiler chicken. Anim. Feed Sci. Technol. 192, 81–89. https://doi.org/10.1016/j.anifeedsci.2014.03.004.

Leyva-Jimenez, H., Alsadwi, A.M., Gardner, K., Voltura, E., Bailey, C.A., 2019. Evaluation of high dietary phytase supplementation on performance, bone mineralization, and apparent ileal digestible energy of growing broilers. Poult. Sci. 98, 811–819. https://doi.org/10.3382/ps/pey389.

Liu, X., Xing, K., Ning, R., Carné, S., Wu, X., Nie, W., 2021. Impact of combined αgalactosidase and xylanase enzymes on growth performance, nutrients digestibility, chyme viscosity, and enzymes activity of broilers fed corn-soybean diets. J. Anim. Sci. 99, skab088. https://doi.org/10.1093/jas/skab088.

McKinney, K., Combs, J., Becker, P., Humphries, A., Filer, K., Vriesekoop, F., 2015. Optimization of phytase production from *escherichia coli* by altering solid-state fermentation conditions. Fermentation. 1, 13–23. https://doi.org/10.3390/fermentation1010013.

Munyaka, P.M., Nandha, N.K., Kiarie, E., Nyachoti, C.M., Khafipour, E., 2016. Impact of combined beta-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. Poult. Sci. 95 (3), 528–540. https://doi.org/10.3382/ps/pev333.

Myers, W.D., Ludden, P.A., Nayigihugu, V., Hess, B.W., 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. J. Anim. Sci. 82 (1), 179–183. https://doi.org/10.2527/2004.821179x.

Naghshbandi, M.P., Moghimi, H., 2020. Stabilization of phytase on multi-walled carbon nanotubes via covalent immobilization. Methods Enzymol. 630, 431–451. https://doi.org/10.1016/bs.mie.2019.10.013.

Olukosi, O.A., Gonzalez-Ortiz, G., Whitfield, H., Bedford, M.R., 2020. Comparative aspects of phytase and xylanase effects on performance, mineral digestibility, and ileal phytate degradation in broilers and turkeys. Poult. Sci. 99 (3), 1528–1539. https://doi.org/10.1016/j.psj.2019.11.018.

Palander, S., Nasi, M., Jarvinen, S., 2005. Effect of age of growing turkeys on digesta viscosity and nutrient digestibility of maize, wheat, barley and oats fed as such or with enzyme supplementation. Arch. Anim. Nutr. 59, 191–203.

Ravindran, V., Amerah, A.M., 2009. Wheat: composition and feeding value for poultry. In: Davies, S., Evans, G. (Eds.), Soybean and Wheat Crops. Nova Science Publishers,, Hauppauge, NY, USA., pp. 245–259

Rutherfurd, S.M., Chung, T.K., Moughan, P.J., 2007. The effect of a commercial enzyme preparation on apparent metabolizable energy, the true ileal amino acid digestibility, and endogenous ileal lysine losses in broiler chickens. Poult. Sci. 86, 665–672. https://doi.org/10.1093/ps/86.4.665.

SAS Institute, I., 2011. SAS/STAT User's Guide. SAS Institute Inc, Cary, NC.

Selle, P., Ravindran, V., Partridge, G., 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilization, mineral retention, and growth performance in wheat-based broiler diets. Anim. Feed Sci. Technol. 153, 303–313. https://doi.org/10.1016/j.anifeedsci.2009.06.011.

Selle, P.H., Creswell, D.J., Partridge, G.G., Scott, T.T., 2006. Phytase supplementation of wheat-based broiler diets reduces dependence on meat-and-bone meal. J. Poult. Sci. 43, 330–338.

Short, F.J., Gorton, P., Wiseman, J., Boorman, K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59 (4), 215–221. https://doi.org/10.1016/0377-8401(95)00916-7.

Walters, H.G., Coelho, M., Coufal, C.D., Lee, J.T., 2019. Effects of increasing phytase inclusion levels on broiler performance, nutrient digestibility, and bone mineralization in low-phosphorus diets. J. Appl. Poult. Res. 28, 1210–1225. https://doi.org/10.3382/japr/pfz087.

Williams, W.P. 2006. Economic implications of phytase use in layer nutrition. Engormix. https://en.engormix.com/poultry-industry/articles/economic-implications-phytase-use-t33453.htm. Accessed (5 January 2023).

Woyengo, T.A., Sands, J.S., Guenter, W., Nyachoti, C.M., 2008. Nutrient digestibility and performance responses of growing pigs fed phytase- and xylanase-supplemented wheat-based diets. J. Anim. Sci. 86 (4), 848–857. https://doi.org/10.2527/jas.2007-0018.