Xylanase supplementation of pelleted wheat-based diets increases growth efficiency and apparent metabolizable energy and decreases viscosity of intestinal contents in broilers

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ABSTRACT This study was designed to test graded supplementation of a thermostable xylanase in pelleted, wheat-based diets fed to broiler chickens over a 28-d period. A total of 600 Ross 708 male broilers were allotted to 1 of 5 dietary treatments: positive control (**PC**), negative control (**NC**; 125 kcal of AME/kg diet reduction relative to PC), and NC supplemented with 10, 15, or 30 g/ton of xylanase. Wheat-soybean meal-based diets were pelleted and fed in 2 feeding phases (14-d each). Study outcomes included growth performance, AME, and ileal digesta viscosity with 20 battery cages of 6 birds per treatment. Data were analyzed by 1-way ANOVA along with estimation of Pearson correlation coefficients. Whereas no difference between NC and PC was observed for BW gain, NC birds exhibited increased ($P < 0.05$) feed intake during each feeding phase and overall, which caused improvements ($P < 0.05$) in feed conversion ratio (**FCR**) for PC vs. NC birds. The analyzed AME of PC birds was 112 kcal/kg of diet greater ($P < 0.05$) than for NC birds, though no differences in digesta viscosity were observed. Xylanase supplementation of the NC diet at 15 or 30 g/ton elicited overall improvements ($P < 0.05$) in BW gain beyond the PC, while the 30 g/ton level equalized feed intake with the PC. Regardless of level, xylanase supplementation improved ($P < 0.05$) the FCR relative to the NC, thereby equalizing the response with the PC. Similarly, supplementation with any xylanase level increased ($P < 0.05$) AME over the NC, making all treatments synonymous with the PC. Digesta viscosity of all xylanase-supplemented treatments was decreased relative to both the NC and PC treatments. Overall, this study provided clear evidence that addition of a thermostable xylanase to pelleted wheat-based diets elicited improvements in growth performance of broilers concomitant with a reduction in digesta viscosity and elevation of analyzed dietary AME content.

Key words: apparent metabolizable energy, broiler, exogenous enzyme, viscosity, xylanase

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INTRODUCTION

Given the importance of optimizing nutrient utilization as part of environmental and economic sustainability in poultry production (Leinonen and Kyriazakis, 2016), there exists a need for continual development of nutritional technologies to support the digestive capacity of modern broiler chickens. Because the supply of feed represents a majority of the total production cost of broilers, there are clear benefits to mitigating antinutritional factors that reduce the nutritional value of common ingredients. This is particularly important in broilers, where the bird's digestive capacity and microbiota must mature quickly to maximize growth rate and efficiency within a relatively short production timeline. Cereal grains, such as corn, wheat, barley, and sorghum, are commonly used to supply energy in the form of digestible starch, but such ingredients also deliver nondigestible carbohydrates (i.e., dietary fiber), which cannot be hydrolyzed by the bird's digestive enzymes.

With regard to wheat, the major form of dietary fiber that exerts an antinutritional effect is the soluble nonstarch polysaccharide (**NSP**) class of arabinoxylans (Courtin and Delcour, 2002). These soluble arabinoxylans tend to increase the viscosity of luminal contents passing through the alimentary tract, which can limit hydrolytic enzymes from releasing dietary nutrients for absorption and thereby reduce the AME content of diets predominated by high-NSP wheat (Choct and Annison, 1992;

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Scott et al., 1998). Owing to the relatively fast intestinal transit time of broilers, the soluble arabinoxylan-induced reductions in nutrient digestibility, digesta passage rate, and dietary AME content may also be associated with sticky excreta, increased litter moisture content, and increased incidence of footpad lesions (Choct et al., 1999). To correct these issues, the use of exogenous NSP-degrading enzymes has long been commonplace in the poultry industry (Masey O'Neill et al., 2014).

The efficacy of exogenous NSP-degrading enzymes, including those that hydrolyze 1,4-β-D-xylosidic linkages (i.e., xylanases), to reduce viscosity of intestinal contents through depolymerization of soluble NSP is well documented (Bedford and Schulze, 1998; Adeola and Cowieson, 2011; Slominski, 2011; Kiarie et al., 2014). Supplementation of wheat-based diets with exogenous xylanase activity increases the dietary AME content by enhancing nutrient extraction, possibly through microbiota-related mechanisms (Mendis et al., 2016). However, inconsistent results have been reported on whether xylanase supplementation benefits the rate and efficacy of growth by altering feed intake in broilers. Moreover, variability in the nutritional profile of wheat (Scott et al., 1998; González-Ortiz et al., 2016) and differences in the design of studies investigating xylanase efficacy suggest there remains room for improvement in this nutritional technology.

Widespread use of xylanase products has driven innovation in terms of optimization for pH and thermal stability to prevent a diminution of activity under practical conditions, including those involved with pelleting of broiler diets. The digestibility of wheat-derived starch appears to be particularly low and variable between individual birds when fed in pelleted form, and may relate to an inability for broilers to properly regulate feed intake when consuming low-AME, wheat-based, pelleted diets (Hughes, 2008; Svihus et al., 2010; Svihus, 2011). Therefore, our objective was to determine the dose-response relationship of an exogenous xylanase on growth performance, AME content, and viscosity of intestinal luminal contents when included in pelleted, wheat-based diets fed to broiler chickens over a 28-d period. We hypothesized that the positive and negative control diets, differing in their AME content, would elicit differences in growth performance but not intestinal viscosity, and that supplementation with xylanase would overcome the designed deficit of 125 kcal of AME/kg of diet by reducing intestinal viscosity and improving the efficiency of growth in broilers.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to initiation of the experiment. Additionally, the dedicated research space, including the brooder batteries described below, met or exceeded environmental standards for conducting agricultural research as described in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2020).

Bird Husbandry and Experimental Design

Day-old Ross 708 male broiler chicks were obtained from a commercial hatchery (Hoover's Hatchery, Rudd, IA) and transported to the University of Illinois Edward R. Madigan Laboratory (Urbana, IL). Chicks were placed in thermostatically-controlled cages (model SB5T; Alternative Design Manufacturing, Siloam Springs, AR) with raised wire flooring in an isolated, environmentally controlled room with continuous lighting. Ambient room conditions, including temperature, humidity, ventilation, and lighting were controlled by centralized systems and met environmental standards for biomedical research. Upon arrival, chicks were fasted overnight in battery cages with access only to water to permit study commencement at 2 d post-hatch.

At 2-d post-hatch (i.e., study d 0), a total of 600 male chicks were weighed, selected, wing-banded, and assigned to 1 of 5 dietary treatment groups with a total of 6 birds allotted to each of 20 replicate cages (99 cm × 34 cm of floor space or 545 cm^2 floor space per bird) per treatment. Each cage was outfitted with 1 trough feeder and 2 nipple drinkers; equipment and birds were checked twice-daily throughout the study. Before bird arrival, the battery cage temperature was set at 34.5°C, which was gradually decreased to 27.0°C by study conclusion based on visual observation of bird comfort. Feed and water were provided ad libitum throughout the 28-d study.

At study initiation, average group weights and weight distributions were similar across treatments, and treatments were arranged in a randomized complete block experimental design. Birds were randomly allotted to cages with each battery of 20 cages serving as a block (i.e., 4 replicated cages per treatment assigned randomly within battery). Allotment occurred based on initial BW of individual birds in such a way that all birds were within a maximum of 15 g of the overall cage mean for that particular block. Battery cage was considered the experimental unit for all study outcomes with 20 replicate cages of 6 birds per dietary treatment (n = 20 replicate cages per each of 5 dietary treatments; N = 100 cages total). All personnel involved in daily study activities remained blinded to treatment identity throughout study duration. As described below, all diets were manufactured off-site and identified with a blinded (i.e., non-descriptive) color-coding system that was also applied to cage identification cards to ensure assigned dietary treatment were correctly provided to the birds.

Test Article and Dietary Treatments

Xygest HT (Kemin Industries, Inc., Des Moines, IA) was the test article used in this experiment. Xygest HT is an intrinsically thermostable, monocomponent xylanase produced by *Thermopolyspora flexuosa* expressed

in *Pichia pastoris* and is a beta 1-4, endo-xylanase belonging to the GH11 family. It is a commercially available xylanase developed to be intrinsically heat-stable based on standard pelleting conditions for broiler feed (Van Hoeck et al., 2021). One unit of xylanase activity was defined as the amount of enzyme that released 1 μg of xylose-equivalents per minute from a 0.50% birchwood xylan solution at pH 5.3 and 50°C (Nelson, 1944; Somogyi, 1952).The guaranteed activity for the commercial enzyme used herein was 3×10^6 units of xylanase activity per g of product. Enzyme activity of the product lot used in this study was confirmed by analysis prior to incorporation of the test article in experimental diets.

Positive (**PC**) and negative (**NC**) control diets were formulated for both the starter and finisher feeding phases, with the NC designed to contain a lower AME content. All wheat-soybean meal-based diets (Table 1) were formulated to meet or exceed nutrient requirements of broilers (National Research Council, 1994) for starter (study d 0−14; 3,025 kcal AME/kg of diet) and finisher (study d 14−28; 3,100 kcal AME/kg of diet) feeding phases. The source of wheat used in this study was classified as a soft, red winter cultivar, and it was ground to an average particle size of 537 ± 3.68 μM (mean ± SD). A reduction of 125 kcal AME/kg of diet was achieved in the NC diet within each feeding phase by replacing a portion of the wheat and soybean oil with powdered cellulose (Solka-floc; J. Rettenmaier USA LP, Schoolcraft, MI). Additionally, titanium dioxide was added to finisher formulations to permit calculation of AME values as described below. Within each feeding phase, the NC diet was amended by adding the xylanase test article on top of the formulation at graded concentrations (10, 15, or 30 g/ton, as designed to deliver 30,000, 45,000, or 90,000 units of xylanase activity per kg of diet, respectively) to produce the remaining experimental diets.

Table 1. Ingredient and calculated nutrient composition of control diets[1].

	Starter (d 0−14)		Finisher (d 14−28)	
Ingredient, %	PC	NC	PC	NC
Wheat	56.29	55.40	63.56	62.68
Soybean meal	33.50	33.50	25.80	25.80
Powdered cellulose[2]	-	2.00	-	2.00
Soybean oil	5.43	4.32	5.82	4.70
Sodium chloride	0.40	0.40	0.40	0.40
Limestone	1.15	1.15	1.00	1.00
Dicalcium phosphate	1.90	1.90	1.80	1.80
Vitamin premix[3]	0.20	0.20	0.20	0.20
Mineral premix[4]	0.15	0.15	0.15	0.15
Choline chloride[5]	0.20	0.20	0.20	0.20
L-Lys HCl	0.33	0.33	0.30	0.30
DL-Met	0.31	0.31	0.25	0.25
L-Thr	0.14	0.14	0.12	0.12
Titanium dioxide	-	-	0.40	0.40
Calculated composition[6]				
AME_n, kcal/kg	3,025	2,900	3,100	2,975
CP, %	23.1	23.0	20.2	20.1
SID Lys, %	1.26	1.26	1.07	1.07
SID Met, %	0.59	0.59	0.50	0.50
SID TSAA, %	0.91	0.91	0.80	0.80
SID Thr, %	0.80	0.80	0.69	0.69
Ca, %	1.00	1.00	0.90	0.90
Non-phytate P, %	0.48	0.48	0.45	0.45
Analyzed composition[6]				
Dry matter, %	91.0	91.6	91.5	90.8
Crude fiber, %	3.02	4.50	2.96	4.47
Crude fat, %	9.09	7.92	8.92	8.41
Ash, %	6.38	6.77	6.79	6.62
CP, %	25.4	25.9	22.8	23.0
Lys, %	1.58	1.59	1.40	1.39
Met, %	0.63	0.61	0.50	0.48
TSAA, %	1.05	1.03	0.88	0.85
Thr, %	1.00	1.00	0.91	0.89

[1]Abbreviations: NC, negative control; PC, positive control; SID, standardized ileal digestible.

[2]Solka-floc®; J. Rettenmaier USA LP, Schoolcraft, MI.

[3]Vitamins provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; menadione sodium bisulfite complex, 2.33 mg; niacin, 22 mg; D-calcium pantothenate, 10 mg; riboflavin, 4.41 mg; vitamin B_{12}, 0.01 mg.

[4]Minerals provided per kilogram of diet: Mn, 75 mg from MnO; Fe, 75 mg from $FeSO_4 \bullet H_2O$; Zn, 75 mg from ZnO; Cu, 5 mg from $CuSO_4 \bullet 5H_2O$; I, 0.75 mg from ethylenediamine dihydroiodide; Se, 0.1 mg from Na_2SeO_3.

[5]Contained 60% choline.

[6]Components expressed on an as-fed basis. Additional analytical outcomes can be found in Supplementary Tables 1 and 2.

All experimental diets for the starter and finisher feeding phases were manufactured in pelleted form from a single batch of basal within each feeding phase (Kansas State University O.H. Kruse Feed Technology Innovation Center & Feed Safety Research Center; Manhattan, KS). A portion of each starter pelleted diet was crumbled, with all birds receiving their assigned starter dietary treatment in crumble form from study d 0 to 7 and pelleted form from study d 7 to 14; finisher treatments were only provided in pelleted form. Pellets were produced from mash diets via steam conditioning (model 150 twin staff pre-conditioner; Wenger Corp., Sabetha, KS) and subsequently pelleted using a 1-ton, 30-horsepower pellet mill (model 1012-2 HD Master; California Pellet Mill, Crawfordsville, IN) equipped with a 3.97 mm diameter × 31.75 mm pellet die. The pellet mill feeder was set at a constant rate to achieve approximately 9.07 kg per min, and conditioning targets of 83.9 to 86.7°C for 30 s were achieved by adjusting (i.e., increasing) steam addition using 172 to 207 kPa steam pressure. All pellets were cooled in a counter-flow cooler for 20 min prior to bulk packaging.

Homogenous and representative samples of each mash and pelleted dietary starter and finisher treatments (8 replicate aliquots) were collected and analyzed prior to study initiation. A slightly modified version of a commercially available test kit (Xylazyme AX Tablets from Megazyme International, Ireland) was used to confirm xylanase activity specifications prior to birds being allotted to experimental treatments (Table 2). Moreover, the total content of arabinoxylan was quantified in each experimental treatment using high-performance liquid chromatography using an extraction method and equipment identical to that used previously (Liu and Rochfort, 2014). Diets were also analyzed for proximate composition [(Association of Official Analytical Chemists (**AOAC**) method reference number and year provided in parentheses: dry matter (934.01, 2006), crude protein (based on total N; 990.03, 2006), ash (942.05, 2006), crude fiber (978.10, 2006), and crude fat (by ether

Table 2. Formulated and analyzed xylanase activity (enzyme units/kg) and total arabinoxylan content (g/kg of dry matter) of experimental diets in mash and pelleted forms[1].

	Dietary treatment[2]				
Item	PC	NC	NC + 10 g/ton	NC + 15 g/ton	NC + 30 g/ton
Starter, mash					
Formulated xylanase activity	0	0	30,000	45,000	90,000
Analyzed xylanase activity	4,394 ± 5	2,180 ± 14	49,801 ± 8	62,474 ± 8	101,401 ± 36
Starter, pelleted					
Formulated xylanase activity	0	0	30,000	45,000	90,000
Analyzed xylanase activity	3,197 ± 1	3,649 ± 33	32,347 ± 17	40,598 ± 7	95,861 ± 16
Total arabinoxylan content	63.3	68.9	66.4	65.0	68.7
Finisher, mash					
Formulated xylanase activity	0	0	30,000	45,000	90,000
Analyzed xylanase activity	8,836 ± 36	1,721 ± 3	38,615 ± 61	48,863 ± 13	92,485 ± 137
Finisher, pelleted					
Formulated xylanase activity	0	0	30,000	45,000	90,000
Analyzed xylanase activity	10,791 ± 21	2,540 ± 14	39,920 ± 2	39,329 ± 29	95,853 ± 54
Total arabinoxylan content	70.0	69.1	69.4	69.0	70.2

Abbreviations: NC, negative control; PC, positive control.
[1]Homogenous diet samples (n = 8 aliquots for each form per dietary treatment) and results are expressed as means ± SEM.
[2]The exogenous xylanase product had a guaranteed analysis of ≥ 3×10^6 units of xylanase activity/g and was included at graded levels in the experimental diets. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μg of xylose-equivalents per minute from a 0.50% birchwood xylan solution at pH 5.3 and 50°C.

extract; 920.39 A, 2006); Eurofins Scientific Inc., Des Moines, IA] and total amino acids [982.30 E(a,b,c), 2006; University of Missouri Experiment Station Chemical Laboratory, Columbia, MO] as shown in Tables 1 and 2 for the starter and finisher feeding phases, respectively. Additionally, the complete analyzed nutrient profile of individual starter and finisher diets can be found in Supplementary Tables 1 and 2, respectively.

Experimental Outcomes

- *Growth performance*: Individual bird and cage feeder weights were recorded on study d 0, 14, and 28 to permit calculation of BW gain, feed intake, and feed conversion ratio (**FCR**; g feed intake per g BW gain) as metrics of growth performance. Mortality and culls were monitored daily and used to adjust feed intake and FCR data. A representative sample of excreta was collected from pans beneath each cage on study d 26 and 27 and were frozen at −20°C pending analysis. On study d 28, all birds were humanely euthanized following carbon dioxide asphyxiation to permit collection of a homogenous sample of luminal contents from the distal portion of the ileum (i.e., last one-third of the section extending from Meckel's diverticulum to the ileocecal juncture) from all birds remaining at study conclusion. Approximately 3 g of composited ileal digesta from each cage of birds was frozen at −20°C pending viscosity analysis as described below.
- *Apparent metabolizable energy*: Frozen excreta samples were lyophilized and, along with finisher dietary treatment samples, ground via mortar and pestle to a powdered consistency and analyzed for the following: dry matter (AOAC 934.01, 2006), gross energy (using adiabatic bomb calorimetry; model 1216, Parr Instruments, Moline, IL), and titanium dioxide (Short et al., 1996). Excreta samples collected separately on study d 26 and 27 were analyzed in duplicate, used to calculate AME plus AME_n values (assuming a correction factor of 8.22 kcal/g of N) (Hill and Anderson, 1958), and then averaged across successive collection days within cage.
- *Viscosity of luminal contents from the distal ileum*: Ileal digesta viscosity analysis was conducted in duplicate for each sample composited from all birds remaining at study conclusion. As such, frozen digesta samples were thawed, and a representative and homogenous 3 g aliquot of ileal digesta was placed in a sterile tube and centrifuged at 12,000 × *g* at 4°C for 5 min. Supernatant fluid was immediately collected and stored on ice until viscosity measurements could be quantified using a LVDV-I digital cone plate viscometer fitted with a CP-40 Spindle (AMETEK Brookfield, Middleboro, MA). All samples and the viscometer cup were maintained at 40°C during viscosity measurements, and samples underwent analysis for a total of 3 min with the viscometer spindle set at 20, 25, or 30 rpm for sequential 60-s intervals. To ensure accurate viscosity comparisons, torque was monitored and only values generated when torque ranged between 10 and 99% were recorded. Each sample (in duplicate) was averaged across the 3 conditions (i.e., combination of spindle centrifugal speed and recording time) to generate a single viscosity measurement of ileal digesta composited per cage of birds.

Statistical Analysis

A cage of birds served as the experimental unit for all outcomes with 20 replicate cages per each of 5 dietary treatments (n = 20) as arranged in a randomized complete block design. Outliers were identified as having an absolute Studentized residual value of 3 or greater and were removed prior to conducting the ANOVA. Growth

performance data were corrected for mortality, and all outcomes were subjected to an ANOVA using the MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC). A 1-way ANOVA was used to determine whether the model was significant, and in that case, specific contrasts were applied as follows: 1) PC vs. NC diets, and 2) orthogonal polynomial contrasts (linear and quadratic effects) for the NC and enzyme-supplemented diets. Statistical significance was accepted at $P \leq 0.05$ and trends were defined as $0.05 < P < 0.10$. The IML procedure of SAS was used to generate polynomial contrast coefficients due to the unequal spacing of enzyme activities between supplemented treatments.

For viscosity measurements alone, the data were $\log_{10}$ transformed to stabilize the variance structure prior to conducting statistical comparisons. For outcomes where there were one or more missing values, the highest SEM was reported as the pooled SEM for that outcome. Finally, Pearson correlations were evaluated for the following outcomes using the CORR procedure of SAS (version 9.4; SAS Institute): FCR (overall, d 0−28), AME, and viscosity. The dataset used for correlation analysis did not include birds assigned to the PC diet, and thus, focused only on the NC diet without and with graded xylanase supplementation. No differences in correlation coefficients or their statistical significance were observed for AME vs. AME_n or raw vs. transformed viscosity values when related with FCR (data not shown).

RESULTS

Birds remained healthy through the study and average mortality levels were not associated with particular treatments, but were within acceptable limits for the broiler industry for each treatment group (% of total birds allotted per treatment at study initiation): PC, 0.8%; NC, 1.7%; NC + 10 g/ton, 3.3%; NC + 15 g/ton, 4.2%; and NC + 30 g/ton, 5.0%. Although birds did not have access to diets until 2 d post-hatch due to transport, the observed growth performance throughout the study was consistent with breeder standards.

Growth Performance

No overall treatment effects were observed for BW or BW gain responses during the starter or finisher phases, but both outcomes were influenced ($P = 0.044$) by diet when considering the overall experimental period (Table 3). In this context, no differences between PC and NC diets were observed for final BW and overall BW gain of birds, but both tended to increase linearly ($P = 0.06$) with graded supplementation of xylanase

Table 3. Growth performance of broiler chicks fed wheat-soybean meal-based diets containing graded levels of exogenous xylanase activity[1].

	Dietary treatments[2]						*P*-value			
									Enzyme supplementation[3]	
Item	PC	NC	NC + 10 g/ton	NC + 15 g/ton	NC + 30 g/ton	Pooled SEM	Overall model	PC vs. NC	Linear	Quadratic
BW, g/chick										
d 0	38	38	38	38	38	0.01	0.85	0.67	0.50	1.00
d 14	524	519	522	538	522	5.0	0.06	0.50	0.58	0.026
d 28	1,503	1,508	1,536	1,564	1,553	16.7	0.044	0.84	0.06	0.14
BW gain, g/chick										
Starter phase (d 0−14)	485	481	484	497	484	4.9	0.18	0.48	0.59	0.06
Finisher phase (d 14−28)	980	989	1,014	1,026	1,027	14.3	0.07	0.63	0.07	0.29
Overall (d 0−28)	1,465	1,470	1,498	1,526	1,514	16.7	0.044	0.83	0.06	0.14
Feed intake[4], g/chick										
Starter phase (d 0−14)	530	542	531	551	533	5.0	0.018	0.09	0.37	0.40
Finisher phase (d 14−28)	1,422	1,485	1,502	1,515	1,471	17.0	0.002	0.010	0.49	0.07
Overall (d 0−28)	1,951	2,027	2,027	2,062	1,987	19.5	0.001	0.007	0.15	0.06
FCR[4], g/g										
Starter phase (d 0−14)	1.093	1.129	1.099	1.103	1.110	0.0070	0.004	< 0.001	0.13	0.006
Finisher phase (d 14−28)	1.454	1.502	1.484	1.479	1.456	0.0101	0.003	< 0.001	0.001	0.93
Overall (d 0−28)	1.334	1.380	1.355	1.352	1.331	0.0085	< 0.001	< 0.001	< 0.001	0.48

Abbreviations: C, cubic polynomial contrast; FCR, feed conversion ratio (g feed intake per g of BW gain); L, linear polynomial contrast; NC, negative control; PC, positive control, Q, quadratic polynomial contrast.

[1]Values are least-square means derived at 2−30 d post-hatch (study d 0−28) from 20 replicate cages per treatment each with 6 chicks at study initiation.

[2]The exogenous xylanase product had a guaranteed analysis of $\geq 3 \times 10^6$ units of xylanase activity/g and was included at graded levels in the experimental diets. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μg of xylose-equivalents per minute from a 0.50% birchwood xylan solution at pH 5.3 and 50°C.

[3]Includes the negative control diet without and with graded enzyme inclusion levels (4 treatments total). Orthogonal polynomial contrasts represent linear and quadratic effects based on unequal spacing of supplemental enzyme activity levels.

[4]Values were corrected for mortality.

activity with the highest numerical responses occurring in diets containing 15 or 30 g/ton of product. While an overall treatment effect ($P < 0.02$) was observed for feed intake during the starter, finisher, and overall feeding phases, most of this variability was explained by differences ($P \leq 0.09$) between the PC and NC treatments with birds fed the NC diet consuming more feed than those fed the PC diet. With regard to polynomial contrasts of enzyme-supplemented diets, quadratic effects tended to occur in the finisher ($P = 0.07$) and overall ($P = 0.06$) feeding phases.

These effects on BW gain and feed intake resulted in consistent increases ($P < 0.001$) in FCR (i.e., poorer efficiency) for NC compared with PC during the starter, finisher, and overall feeding phases. Additionally, supplementation of the NC diet with graded xylanase activity in the starter phase elicited improved (quadratic effect, $P = 0.006$) feed efficiency (i.e., lower FCR), resulting in FCR responses that were numerically similar between the PC and NC + 30 g/ton treatments. Moreover, clear improvements (i.e., lower FCR) (linear effect, $P < 0.001$) were also observed in the finisher and overall phases with graded addition of dietary xylanase activity to the NC diet.

Dietary AME and Viscosity of Intestinal Contents

Clear reductions ($P < 0.001$) in analyzed AME and AME_n content for the NC diet were observed relative to the PC diet (Figure 1). Supplementation of the NC diet with exogenous xylanase activity elicited a linear increase ($P < 0.01$) in AME and AME_n, which caused all xylanase-supplemented diets to have values similar to PC. Viscosity measurements of luminal contents from the distal ileum showed no differences ($P = 0.83$) when comparing the NC and PC diets. However, each xylanase supplementation of the NC diet elicited clear decreases (linear and quadratic effects, $P < 0.01$) in ileal digesta viscosity, which meant that all xylanase-supplemented treatments were numerically similar to each other and distinct from both the NC and PC diets.

The Pearson correlation coefficients between FCR (d 0−28) and AME and viscosity measurements on samples collected at study conclusion (d 28) are presented in Table 4. A negative relationship was detected between FCR and analyzed dietary AME ($r = -0.332$; $P = 0.003$), while a positive correlation was detected between FCR and viscosity of distal ileum contents ($r = 0.411$; $P < 0.001$). Moreover, a negative correlation was observed between analyzed dietary AME and ileal digesta viscosity ($r = -0.376$; $P < 0.001$).

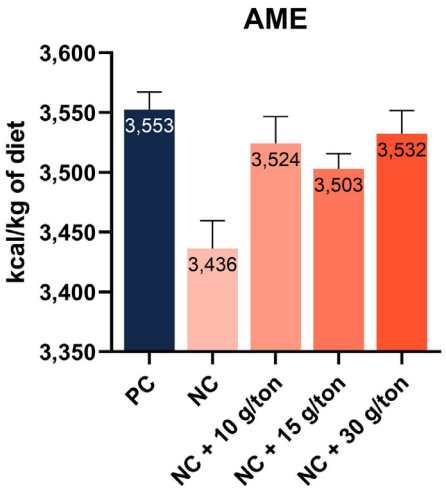
DISCUSSION

The efficacy of exogenous xylanase products when included in wheat-based diets for poultry is well-documented (Bedford and Schulze, 1998; Cowieson et al., 2006; Kiarie et al., 2014; Amerah, 2015; Raza et al., 2019). We sought to identify whether a dose-response relationship existed when including a thermostable xylanase in pelleted wheat-based diets designed with a 125 kcal of AME/kg reduction in the negative control (NC) relative to the positive control (PC) without changes in the total arabinoxylan content within and between phases. This planned reduction in AME was achieved by replacing wheat and soybean oil with 2% powdered cellulose in the NC diet within each feeding phase. Our intent was to reduce AME without a concomitant change in the viscosity of intestinal contents between the NC and PC diets, in which case the restoration of analyzed AME and benefits to growth performance due to xylanase supplementation of the NC diet would prove efficacy of this nutritional intervention.

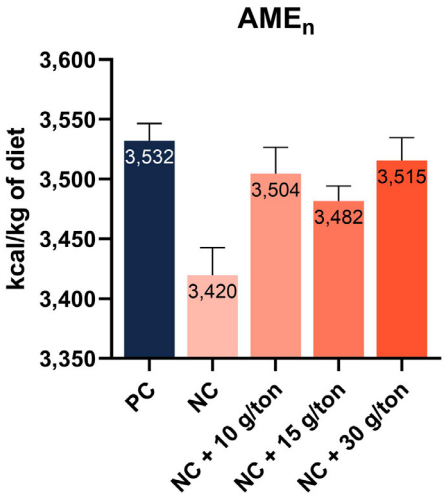
Analysis of xylanase activity in both the mash (i.e., before pelleting) and pelleted experimental diets confirmed that the formulation objectives were met prior to study initiation. The unsupplemented NC and PC starter and grower diets were analyzed to contain some xylanase activity, which was expected given inherent enzyme activity commonly found in wheat sources (Courtin and Delcour, 2002; Gebruers et al., 2010). Additionally, all xylanase-supplemented diets were analyzed to possess enzyme activities that were near or above the formulated activity levels both prior to and after pelleting. Moreover, the analyzed total arabinoxylan content of the experimental pelleted diets did not differ within or between feeding phases, which further instills confidence that adequate substrate was available to be acted upon by the exogenous xylanase.

The planned decrease of 125 kcal/kg in dietary AME between the PC and NC diets did not elicit changes in BW gain during any study phase, however, birds receiving the NC diet did exhibit increased feed intake, compared with the PC diet. The difference in feed intake response between NC and PC led to lower FCR for the PC diet during each of the starter, finisher, and overall feeding phases. The observation that birds respond to lower AME diets by increasing feed intake is not novel, though there is evidence that modern broiler genetic lines may have less ability to respond to dietary energy content (Havenstein et al., 2003; Pym, 2005; Classen, 2017). Also important is evidence suggesting that pelleted diets based predominantly on ground wheat may be associated with high individual bird variability in starch digestibility, potentially due to inadequate stimulation of gizzard development and function, thereby altering satiety signals and negatively influencing digesta passage rates in broilers (Hughes, 2008; Svihus et al., 2010; Svihus, 2011).

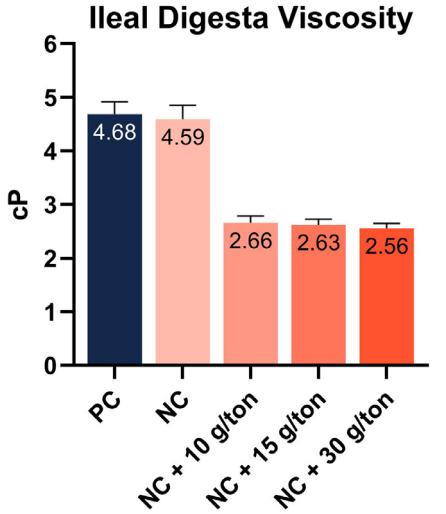
While no differences in overall BW gain existed between the NC and PC diets, supplementation of the NC diet with xylanase at 15 or 30 g/ton improved BW gain by an average of 3.8% compared with the PC. Moreover, while only quadratic trends were observed for FI, addition of 30 g/ton xylanase to the NC diet elicited numerically similar overall feed intake compared with that of the PC diet. Collectively, our data suggest that xylanase-supplemented diets improved FCR compared



Effect	P-value
Overall Model	< 0.001
PC vs. NC	< 0.001
ES - Linear	0.002
ES - Quadratic	0.07



Effect	P-value
Overall Model	< 0.001
PC vs. NC	< 0.001
ES - Linear	0.001
ES - Quadratic	0.10



Effect	P-value
Overall Model	< 0.001
PC vs. NC	0.79
ES - Linear	< 0.001
ES - Quadratic	< 0.001

Figure 1. Dietary AME and AME$_n$ content and viscosity of distal ileum luminal contents from broiler chicks fed wheat-soybean meal-based diets containing graded levels of exogenous xylanase activity. The exogenous xylanase product had a guaranteed analysis of $\geq 3 \times 10^6$ units of xylanase activity/g and was included at graded levels in the experimental diets. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μg of xylose-equivalents per minute from a 0.50% birchwood xylan solution at pH 5.3 and 50°C. Displayed values are least-square means plus individual SEM derived from 20 replicate cages per treatment each with 6 chicks at study initiation. The viscosity data were subjected to a $\log_{10}$ transformation to stabilize the variance structure prior to ANOVA, but raw means and standard error values are displayed along with the *P*-value and means separation based on the transformed dataset. Orthogonal polynomial contrasts represent linear and quadratic effects based on unequal spacing of supplemental enzyme activity levels when added to the NC diet, representing 4 treatments total. Abbreviations: cP, centipoise; ES, effect of enzyme supplementation of the negative control diet; NC, negative control; PC, positive control.

Table 4. Pearson correlation coefficients in broiler chicks fed reduced AME, wheat-soybean meal-based diets containing graded levels of exogenous xylanase activity[1].

Item	FCR	AME	Viscosity
FCR, overall (d 0−28)	1	−0.332*	0.411*
AME, d 28	−0.332*	1	−0.376*
Viscosity, d 28	0.411*	−0.376*	1

Abbreviation: FCR, feed conversion ratio (g feed intake per g of BW gain).

[1]Correlation coefficients were derived from all replicate cages of birds receiving the negative control diet that was either unsupplemented or supplemented with graded levels of exogenous xylanase activity during the entire 28-d feeding period (N = 77-79 data-points).

*Pearson correlation coefficient was significant ($P < 0.003$).

with the NC diet (linear or quadratic effects), which translated into improvements in FCR that were similar to the PC diet. As hypothesized, the improvement in FCR due to xylanase supplementation was concomitant with an increase in analyzed dietary AME and AME_n, such that xylanase supplementation caused linear increases, resulting in AME and AME_n values that were greater than the NC and indistinguishable from the PC. As such, the PC diet elicited increases of 117 and 112 kcal/kg of diet of AME and AME_n, respectively, when compared with the NC diet. Comparatively, the xylanase-supplemented diets increased AME and AME_n an average of 84 and 80 kcal/kg of diet, respectively, over the NC diet. We recognize that the reduction in viscosity of intestinal contents would have additional benefits beyond that of increasing AME in wheat-based diets (i.e., likely improved nutrient digestibility), and acknowledge that the correlation between AME and viscosity explained less than 40% of the total variability, so further studies are warranted to explain these important relationships.

In rationalizing how the relatively small changes in feed intake elicited clear improvements in FCR due to xylanase supplementation, it is unclear how to proportionally distribute this to dietary vs. bird effects. Certainly, the cultivar and growing conditions of the soft, red winter wheat used in our study would have affected the dietary content of soluble NSP, along with inherent xylanase and anti-xylanase activities (Courtin and Delcour, 2002; Gebruers et al., 2010). Additionally, grinding of the wheat to an average particle size of 537 ± 3.68 μM (mean ± SD) and incorporation into pelleted diets would also influence how this source of nutrients was utilized by the bird. It has widely been reported that variability exists in starch digestion of wheat by broiler chickens, likely owing to a relationship between wheat particle size, soluble NSP content, and individual variability in gizzard development and function between birds (Svihus and Hetland, 2001). The relatively low level of gizzard stimulation expected in broilers consuming ground wheat-based diets can partially be overcome by pelleting (Scott et al., 2003), as was done in our study, so it is encouraging to note that the overall rate and efficiency of growth in birds receiving xylanase-supplemented diets was able to meet performance objectives for this genetic line of birds.

As per our formulation objectives, the PC and NC diets differed in analyzed AME and AME_n content without causing changes to digesta viscosity in the distal ileum of broilers upon conclusion of the 28-d feeding study. Following the extensive evidence for the efficacy of xylanase supplementation in wheat-based diets (Bedford and Schulze, 1998; Adeola and Cowieson, 2011; Slominski, 2011; Kiarie et al., 2014), all levels of xylanase addition to the NC diet elicited a clear reduction in ileal digesta viscosity in our study. Whether this particular benefit was the result of increased digesta passage rate, improved macronutrient digestion, generation of arabinoxylan derivatives that induce prebiotic effects or other beneficial shifts in microbiota profiles remains to be seen (Choct et al., 1999; Engberg et al., 2004; Wu et al., 2004; Carré et al., 2007; Adeola and Cowieson, 2011; Munyaka et al., 2016).

As has been historically reported in feeding studies of this sort, we observed clear associations among outcomes including FCR, analyzed dietary AME content, and digesta viscosity in the distal ileum of broilers receiving the NC diet without or with xylanase supplementation. The high replication used in our study design (n = 20 cages of 6 birds per treatment) instills confidence that the highly significant relationships between these outcomes are real, though we acknowledge that said correlations explain no more than 17% of the total variation observed when relating FCR and digesta viscosity. Similar correlation coefficients were observed by others investigating xylanase supplementation of wheat-based diets (González-Ortiz et al., 2016), despite the contention that measurement of dietary AME content may be skewed when evaluating diets containing viscosity-inducing properties, like wheat (Bedford, 1996; Choct et al., 1996; Bedford and Schulze, 1998). Given that broilers in our study were fed wheat-based diets for a 28-d period, there was adequate time for the birds to acclimate to the high-NSP diets (Kiarie et al., 2017) and allow xylanase supplementation to elicit benefits in productive performance.

In conclusion, supplementation of pelleted diets containing ground wheat as the sole cereal grain source with exogenous xylanase improved dietary AME and AME_n content, reduced viscosity of luminal contents in the distal ileum, and improved FCR in broiler chickens.

DISCLOSURES

Vanessa Iseri and Jon Rubach are employees of Kemin Industries, Inc. (Des Moines, IA), which is affiliated with the commercial product evaluated in this manuscript. No other others have a conflict of interest to declare.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102220.

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