Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value

C. A. Flores,[*] M. P. Williams,[*] K. Smith,[*] J. Pieniazek,[*] R. Latham,[*] J. J. Wang,[†] J. Tyus,[†] and J. T. Lee[*,1]

[*]*Poultry Science Department, Texas A&M AgriLife Research, College Station, TX; and* [†]*BioResource International, Inc., Durham, NC*

Primary Audience: Live Production Personnel and Nutritionists

SUMMARY

Two experiments were conducted to evaluate the impact of a thermotolerant xylanase on male broiler performance and dietary ileal digestible energy (IDE). The first experiment consisted of 3 treatment groups with 12 replications per treatment each containing 35 Cobb 500 males for a total of 1,260 broilers placed in floor pens for a 42 d grow-out. The experiment treatments included a corn/soy diet with DDGS control formulated at a low energy level, and the control supplemented with one of 2 concentrations of xylanase (20,000 XU/kg [XYL20] and 40,000 XU/kg [XYL40]). No significant differences in body weight were observed with the inclusion of xylanase when compared to the control diet throughout the experiment. At d 28, the inclusion of XYL20 improved ($P < 0.05$) mortality corrected feed conversion ratio (FCR) compared to the control diet. Feed conversion ratio was also improved ($P < 0.01$) at d 42 for birds fed XYL20 when compared to the control. At d 42, inclusion of XYL20 and XYL40 significantly ($P < 0.05$) increased IDE compared to the control. Experiment 2 consisted of 4 treatment groups with 10 replications per treatment each containing 44 Cobb 500 males for a total of 1,760 broilers placed in floor pens for a 41 d grow-out. The dietary treatments included a positive control (PC) based on a corn/soy diet containing DDGS and phytase, a negative control (NC) diet (PC -150 kcal/kg in AME), NC + xylanase at 10,000 XU/kg (XYL10), and NC + xylanase at 20,000 XU/kg (XYL20). A significant increase ($P < 0.05$) in BW was observed in broilers fed the inclusion of XYL20 in the NC diet increased ($P < 0.05$) on d 14. A significant increase in cumulative body weight gain was observed on d 27 and d 41 with xylanase (XYL20) inclusion compared to the NC. These data demonstrate that xylanase inclusion increased energy utilization through improvements in IDE, which improved broiler performance.

Key words: xylanase, broiler, performance, digestible energy

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DESCRIPTION OF PROBLEM

The primary ingredients used in the majority of U.S. broiler diets include corn and soybean meal (**SBM**) with corn inclusion reaching in excess of two-thirds of the total formulation. Due to gastrointestinal limitations and minimal endogenous enzyme production, broilers lack the digestive capacity to utilize the full nutritive value of the diet. In an effort to maximize nutrient

[1]Corresponding author: jlee@poultry.tamu.edu

utilization, the use of exogenous enzymes has become common in order to improve the nutritive value of feedstuffs predominantly through solubilization of non-starch polysaccharides (**NSP**) [1].

Non-starch polysaccharides are a major dietary fiber component comprised of both cellulosic and non-cellulosic polysaccharides within the cell wall of cereal grains [1]. Although NSP concentration in corn is less than that of SBM, the presence of NSP in corn is much more impactful due to the high inclusion rates within a typical corn/SBM diet [2]. High concentrations of NSP (arabinoxylans, β-glucans, mannans, and galactomannans) within the endosperm cell wall of these grains have the ability to impede nutrient absorption and energy utilization in the digestive tract, reducing overall broiler performance. Cowieson [3] estimated that these anti-nutritive properties are responsible for approximately 400 to 450 kcal/kg of the diet not being digested when birds are fed a standard corn/soy ration with the energy range being partitioned at 18% fat, 45% protein, and 37% starch. An effective method of reducing the anti-nutritive properties of NSP is through proper supplementation of xylanase and other carbohydrases.

Xylanase is a type of hemicellulase that is effective in diets containing viscous (wheat) and non-viscous (corn/SBM) cereals and specifically acts on xylan, the major hemicellulosic polysaccharide present in plant cell walls [4]. The β-1, 4 linkages present along the backbone of xylan gives the molecule structure and aids in the entrapment of nutrients, which directly impedes broiler performance by reducing the proportion of nutrients available for absorption and utilization. Degradation of these linkages breaks down the backbone of xylan into individual xylose units allowing for utilization of normally inaccessible nutrients. The impact of xylanase in wheat-based diets has been widely explored and has shown consistent results; however, xylanase supplementation into corn/SBM based diets has provided results that have not been as definitive. Multiple reports have confirmed that broiler performance, as well as ileal digestible energy (**IDE**), is significantly improved with xylanase supplementation, which may be correlated with an increase in feed efficiency and utilization [5–7].

Distillers' dried grains with solubles (**DDGS**) have become a commonly used alternative feed ingredient in broiler diets. Typical DDGS may have up to 12.55% oil content with low-oil DDGS (**LO-DDGS**), a by-product of oil extraction from DDGS, containing as little as 2.1% fat after the extraction process [8,9]. In a study conducted by Loar et al. [10], it was concluded that DDGS may be included up to 15% without adverse effects on performance as long as appropriate nutrient profiles specific to the DDGS were used and diets were formulated on a digestible amino acid basis [10,11]. Incorporation of DDGS into the diet also increases the overall concentration of NSP with reports indicating that DDGS may contain up to 12% insoluble arabinoxylans [12]. However, through proper xylanase supplementation, adverse nutritional effects associated with high NSP concentration may be alleviated leading to improved nutrient utilization and broiler performance. Thus, the objective of the 2 experiments was to evaluate the effect of a new form of *Pichia pastoris*-based xylanase[1] in corn-soybean meal-based diets with DDGS on broiler performance and IDE value. The working hypothesis used during both studies was that the inclusion of xylanase would increase IDE leading to an improvement of growth performance.

MATERIALS AND METHODS

Experimental Design

Experiment 1. On d of hatch, 1,260 Cobb 500 male broiler chicks were placed in floor pens to determine the effect of xylanase inclusion into a low energy corn/soy diet with DDGS to assess the impact on broiler growth performance and IDE. The experiment was conducted in a completely randomized block design and consisted of 3 experimental treatments with 12 replicates per treatment, each containing 35 chicks for a 42 d assay period.

Chicks were reared on used litter in 6 × 6 ft. (1.8 m^2) floor pens, provided age appropriate heat and ventilation, and given access to feed and water ad libitum. The lighting program

[1]Xylamax™ - BioResource International, Inc., Durham, NC,

included continuous light through 3 d of age at 25 lux, 23 h of light from d 4 to 7 at 25 lux, 20 h of light from d 8 to 14 at 15 lux, 16 h of light from d 15 to 28 at 10 lux, 18 h of light from d 29 to 38 at 7 lux, and 23 h of light for the remaining 3 d at 7 lux. On d 14, 28, and 42, at each dietary change, birds and remaining feed were weighed for determination of average body weight and feed consumption for the calculation of feed conversion ratio (**FCR**). Ileal contents were collected and pooled per replicate pen for determination of IDE.

Experiment 2. On d of hatch, 1,760 Cobb 500 male chicks were placed in floor pens to determine the effect of xylanase inclusion into a low energy corn/soy diet with DDGS to evaluate the impact on broiler growth performance and IDE. The experiment was conducted in a completely randomized block design and consisted of 4 experimental treatments with 10 replicates per treatment, each containing 44 chicks for a 41 d assay period.

Chicks were reared on used litter in 6 × 6 ft. (1.8 m^2) floor pens, provided age appropriate heat and ventilation, and given access to feed and water ad libitum. The lighting program was similar to that of the first experiment. On d 14, 27, and 41, at each dietary change, birds and remaining feed were weighed for determination of average body weight and feed consumption for the calculation of FCR. Ileal contents were collected and pooled per replicate pen for determination of IDE. Animal care for both experiments was provided in accordance with a protocol approved by the Texas A&M University Institutional Animal Care and Use Committee (*IACUC*).

Experimental Diets

Experiment 1. The 3 dietary treatments consisted of a control, and the remaining 2 treatments consisted of an endo-β-1, 4-xylanase with increasing levels at 20,000 XU/kg (XYL20) and 40,000 XU/kg (XYL40), respectively. The xylanase was derived from a *Pichia pastoris* strain with an optimum enzyme activity temperature range of 50 to 60° C. One unit of xylanase activity was defined as the amount of enzyme needed for the release of one nmol of reducing sugars (with xylose standard) per s from 0.5% xylan (Sigma X4252, from Beechwood) at 50° C in 50 mM trisodium citrate buffer at pH 6.0. The control diet was formulated to a lower energy diet compared to the industry diets with an estimated reduction of 150 kcal/kg AME. All diets were corn-soybean meal based with varying energy levels. Concentration of DDGS increased from 5% during the starter phase, 7.5% during the grower, and 10% during the finisher phase (Table 1). Titanium dioxide was included at 0.4% of the diet and utilized as an indigestible marker for the determination of IDE. Three dietary phases were fed through the duration of the trial including a starter (d zero to 14, crumble), grower (d 15 to 28, pellet), and finisher (d 29 to 42, pellet). One large diet was manufactured and then subsequently divided among treatments with the pelleting temperature ranging between 83 and 85° C and a conditioning time of approximately 25 seconds. Temperature of the mash feed was continuously monitored with a probe strategically placed between the condition and the pellet dye.

Experiment 2. The **four** treatments consisted of a positive control (**PC**), a negative control (**NC**) formulated to an energy density 150 kcal/kg lower than PC, and the NC supplemented with either 10,000 XU/kg (XYL10) or 20,000 XU/kg (XYL20) per kg of finished feed (Table 2). Diets were corn-soybean meal based and contained DDGS. Titanium dioxide (0.4%) was added to all diets as an indigestible marker for determination of IDE. Dietary inclusion of DDGS increased in each successive phase from 5% in the starter phase (d zero to 14) to 7.5% in the grower phase (d 15 to 27), and then to 10% in the finisher phase (d 28 to 41). In each phase a single, larger batch of NC was divided and combined with the appropriate amounts of xylanase. Starter phase diets were fed in crumble form, while grower and finisher phase diets were pelleted. Pelleting temperatures ranged between 83 and 85° C with a 25 s conditioning time.

Similar to the first experiment, temperature of the mash feed was continuously monitored with a probe strategically placed between the condition and the pellet dye. Feed samples for both experiments were collected during feed manufacturing for nutrient analysis, which was conducted in triplicate. Crude protein was determined by

Table 1. Experiment 1 ingredient profile, calculated and analyzed nutrient concentration of the control basal diet for the starter (d 0 to 14), grower (d 15 to 28), and finisher (d 28 to 42) dietary phases.

Ingredient profile	Starter (%)	Grower (%)	Finisher (%)
Corn	56.82	60.64	60.74
Soybean meal (48%)	32.80	26.89	24.74
DL – Methionine	0.22	0.19	0.14
L - Lysine HCL	0.19	0.19	0.06
L – Threonine	0.07	0.06	N/A
Animal/Vegetable blend fat	0.70	0.50	0.50
Limestone	1.52	1.53	1.53
Mono-calcium phosphate	1.49	1.30	1.13
Sodium chloride	0.44	0.38	0.40
Sodium bicarbonate	N/A	0.06	0.03
Trace minerals[1]	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25
Coban 90[3]	0.05	0.05	0.05
LO Distillers dried grains w/ solubles[4]	5.00	7.50	10.00
Titanium dioxide	0.40	0.40	0.40
Calculated nutrient concentration			
Protein	22.42	20.52	19.94
Crude fat	3.59	3.68	3.87
Calcium	0.92	0.88	0.85
Available phosphorous	0.45	0.41	0.38
Metabolizable energy (kcal/kg)	2930	2970	2984
dig Methionine	0.53	0.48	0.41
dig TSAA	0.82	0.75	0.67
dig Lysine	1.17	1.03	0.88
dig Tryptophan	0.23	0.22	0.20
dig Threonine	0.78	0.70	0.62
dig Arginine	1.30	1.11	1.06
Sodium	0.20	0.20	0.20
Analyzed nutrient concentration			
Moisture	11.87	12.10	12.66
Dry matter	88.13	87.90	87.34
Crude protein	20.4	22.5	18.5
Crude fat	2.86	3.66	3.46
Fiber	3.6	4.2	3.5
Ash	5.38	4.93	4.75

[1]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3]Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria mivati*, and *Eimeria maxima*.

[4]Analyzed to contain 12.1% moisture, 28.4% protein, 4.95% crude fat, 10.0% ADF, and 4.20% ash.

combustion using an AOAC method (AOAC method 990.03) (AOAC), total phosphorous was determined by wet ash inductively coupled with plasma spectroscopy (AOAC method 985.01M), ADF was determined using an Ankom digestion unit (AOAC method 973.18) (Ankom Technology), and an ether extraction method was used to determine crude fat (AOAC method 920.39).

Ileal Digestible Energy

IDE was determined on multiple d of age during both experiments using titanium dioxide as an indigestible marker. Ileal contents were taken from 5 birds per replicate on d 14, 4 birds per replicate on d 28 during the first experiment and on d 27 during the second experiment, and

Table 2. Experiment 2 ingredient profile, calculated and analyzed nutrient concentration of the negative control (NC) and positive control (PC) basal diet for the starter (d 0 to 14), grower (d 15 to 27), and finisher (d 27 to 41) dietary phases.

	Negative control (%)			Positive control (%)		
Ingredient profile	Starter	Grower	Finisher	Starter	Grower	Finisher
Corn	57.80	62.80	66.32	54.40	59.01	62.53
Soybean meal (48%)	32.59	25.19	18.86	32.91	25.87	19.54
DL - Methionine	0.22	0.19	0.15	0.22	0.19	0.15
L - Lysine HCL	0.19	0.25	0.26	0.19	0.23	0.24
L – Threonine	0.07	0.08	0.08	0.07	0.08	0.08
Animal/vegetable blend fat	0.50	0.65	0.99	3.56	3.77	4.11
Limestone	1.54	1.40	1.40	1.54	1.39	1.39
Mono-calcium phosphate	0.90	0.72	0.68	0.91	0.73	0.69
Sodium chloride	0.44	0.33	0.19	0.44	0.34	0.20
Sodium bicarbonate	N/A	0.14	0.32	N/A	0.13	0.31
Trace minerals[1]	0.05	0.05	0.05	0.05	0.05	0.05
Vitamins[2]	0.25	0.25	0.25	0.25	0.25	0.25
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05
LO Distillers dried grains w/ solubles [4]	5.00	7.50	10.00	5.00	7.50	10.00
Phytase[5]	0.01	0.01	0.01	0.01	0.01	0.01
Titanium oxide	0.40	0.40	0.40	0.40	0.40	0.40
	Calculated nutrient concentration					
Protein	22.53	20.13	18.07	22.40	20.13	18.07
Crude fat	3.26	3.64	4.18	6.18	6.61	7.14
Calcium	0.92	0.82	0.80	0.92	0.82	0.80
AV phosphorous	0.45	0.41	0.40	0.45	0.41	0.40
Metabolizable energy (kcal/kg)	2910	2970	3020	3060	3120	3170
dig Methionine	0.53	0.48	0.42	0.53	0.48	0.42
dig TSAA	0.82	0.75	0.66	0.82	0.75	0.66
dig Lysine	1.17	1.03	0.89	1.17	1.03	0.89
dig Tryptophan	0.22	0.21	0.17	0.23	0.21	0.17
dig Threonine	0.78	0.70	0.62	0.78	0.70	0.62
dig Arginine	1.30	1.07	0.90	1.30	1.08	0.90
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
	Analyzed nutrient concentration					
Moisture	12.56	10.55	10.47	12.43	11.25	10.83
Dry matter	87.44	89.45	89.53	87.57	88.75	89.17
Crude protein	21.60	19.70	18.20	23.20	19.90	17.50
Crude fat	4.26	6.19	4.61	5.57	3.59	5.53
Fiber	2.60	3.30	4.10	2.60	2.90	2.30
Ash	5.47	4.60	4.79	5.20	5.06	4.49

[1]Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2]Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[3]Active drug ingredient monensin sodium, 90 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria mivati*, and *Eimeria maxima*.

[4]Analyzed to contain 12.1% moisture, 28.4% protein, 4.95% crude fat, 10.0% ADF, and 4.20% ash.

[5]OptiPhos® PF- Huvepharma. Peachtree City, GA.

3 birds per replicate at the conclusion of each experiment for determination of IDE. Samples were then dried at 100°C for 24 h and gross energy of ileal contents was determined using a Parr 6400 bomb calorimeter.[2] Ileal contents were removed 4 centimeters caudal to Meckel's

[2]Parr Instrument Company, Moline, IL

diverticulum and 4 centimeters rostral to the ileo-cecal junction and pooled per replicate pen. Ileal and feed samples were dried at 100°C for 24 hours. Samples were ground for gross energy and titanium concentration determination.

Titanium concentration was determined using a modified protocol outlined by Short et al. [13]. Half a gram of each dried sample was weighed and placed in an ashing oven at 450°C for 12 hours. Following ashing, each sample was titrated with 10 mL of 7.4 M sulfuric acid and boiled at 200°C for 3 h until dissolved. Samples were then titrated with 10 mL of 30% hydrogen peroxide. A total sample volume of 100 mL was achieved using distilled water. Samples were analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis (Model 10S UV-Vis) Spectrophotometer[3] at 410 nm. Gross energy of feed and ileal samples was determined using a Parr 6400 bomb calorimeter. For both experiments, ileal contents were sampled and collected using the same procedure.

Statistical Analysis

All data was analyzed via one-way ANOVA using the General Linear Model (SPSS software) with means deemed significantly different at $P < 0.05$. Further means determined to be different were analyzed via Fishers' LSD. The parameters evaluated during both experiments included body weight (**BW**), body weight gain (**BWG**), feed consumption (**FC**), mortality-corrected FCR, and IDE.

RESULTS AND DISCUSSION

Experiment 1

As shown in Table 3, no significant differences in feed consumption were observed during the starter or grower phases. During the finisher phase, broilers fed xylanase at both inclusion levels consumed at a significantly ($P < 0.05$) lower rate than the control. No significant differences were observed cumulatively through d 28 or d 42. The inclusion of xylanase did not significantly impact body weight or body weight gain throughout the experiment compared to the control diet.

No significant differences were observed in mortality corrected FCR throughout the starter or finisher phase. During the grower phase, the inclusion of xylanase did not impact FCR compared to the control diet; however, the supplementation of XYL20 significantly ($P < 0.05$) reduced FCR when compared to the treatment containing XYL40. Cumulatively through d 28, the inclusion of XYL20 significantly reduced FCR compared to the control diet. Through d 42, the inclusion of XYL20 reduced FCR compared to the control and the inclusion of XYL40 yielded similar results to the inclusion of XYL20, however, was not statistically different from the control diet. No significant differences were observed in weight corrected FCR through d 42.

No significant differences in IDE were observed during the starter phase through d 14. On d 28, the inclusion of xylanase increased ($P < 0.05$) IDE by 88 kcal/kg compared to the control diet, whereas the inclusion of XYL40 did not. At the conclusion of the trial on d 42, the inclusion of XYL20 and XYL40 yielded significantly ($P < 0.05$) higher IDE values than the control, 174 and 180 kcal/, respectively.

Experiment 2

During the starter phase, the PC fed broilers yielded a significantly higher feed consumption (**FC**) compared to the NC. The inclusion of xylanase at both inclusion rates did not influence FC when compared to the NC; however, XYL20 increased FC to levels that were comparable to the PC diet. Although no differences were observed in FC between the PC and NC diets during the grower phase, the inclusion of XYL20 significantly increased FC compared to the control diets with XYL10 yielding intermediate results. Cumulatively through d 27, the NC significantly reduced FC compared to the PC diet while the inclusion of XYL20 increased ($P < 0.05$) cumulative FC compared to the NC, yielding similar results to the PC. The inclusion of XYL10 yielded similar results to the PC diet, however, was not statistically different from the NC. During the finisher phase, no significant difference in FC was observed between the PC and NC diet. However, the inclusion of XYL10 increased

[3]Thermo Fisher Scientific, Waltham, MA

Table 3. Experiment 1, effect of xylanase at multiple levels compared to a control diet on male broiler body weight, feed consumption, body weight gain, mortality corrected feed conversion ratio, and ileal digestible energy.

	Treatment[1]			
Item	Control	XYL20	XYL40	PSEM
Body weight (kg/bird)				
14 d	0.339	0.333	0.334	0.003
28 d	1.370	1.419	1.370	0.013
42 d	2.458	2.530	2.496	0.028
Feed consumption (g/bd/d)				
Starter (0 to 14 d)	30.5	29.9	30.4	0.2
Grower (15 to 28 d)	110.0	112.8	112.7	1.0
Finisher (29 to 42 d)	228.5[a]	212.0[b]	213.4[b]	3.5
Cumulative feed consumption (g/bd/d)				
0 to 28 d	61.8	62.2	63.0	0.5
0 to 42 d	80.1	80.5	81.1	0.6
Body weight gain (kg)				
0 to 14 d	0.300	0.293	0.294	0.003
15 to 28 d	1.033	1.081	1.036	0.013
29 to 42 d	1.086	1.106	1.12	0.027
Cumulative BWG (kg)				
0 to 28 d	1.333	1.379	1.330	0.013
0 to 42 d	2.418	2.486	2.451	0.029
Mortality corrected feed Conversion ratio (FCR) (feed:gain)				
Starter (0 to 14 d)	1.355	1.345	1.350	0.009
Grower (15 to 28 d)	1.490[a,b]	1.456[b]	1.496[a]	0.008
Finisher (29 to 42 d)	2.196	2.114	2.068	0.032
Cumulative mortality Corrected FCR (feed:gain)				
0 to 28 d	1.454[a]	1.424[b]	1.459[a]	0.006
0 to 42 d	1.733[a]	1.689[b]	1.702[a,b]	0.009
0 to 42 d[2]	1.749	1.678	1.704	0.018
Ileal digestible energy (IDE)				
14 d	3123	3074	3132	22
28 d	3040[b]	3128[a]	2989[b]	20
42 d	2930[b]	3104[a]	3110[a]	27

[a,b]Means with different superscripts within a row differ significantly at ($P < 0.05$).

[1]Control, with 150 kcal/kg reduction in energy; XYL20, control plus 20,000 units of xylanase; XYL40, control plus 40,000 units of xylanase.

[2]Weight-corrected FCR, 2.5kg correction factor with 27g of BW equal to 1 point in FCR.

($P < 0.05$) FC compared to the NC. Cumulatively through d 41, inclusion of XYL20 significantly ($P < 0.05$) increased consumption compared to the NC while XYL10 was intermediate.

The reduction of energy in the NC diet significantly ($P < 0.05$) reduced BW and BWG compared to the PC on d 14 and d 27 (Table 4). On d 14, the inclusion of xylanase XYL20 increased BW and BWG to levels that were similar to the PC diet. The inclusion of xylanase XYL10 did not impact BW and BWG compared to the NC diet. On d 27, the inclusion of xylanase at both inclusion rates significantly ($P < 0.05$) increased BW and BWG compared to the NC while yielding similar results to the PC. The reduction of energy in the NC diet significantly ($P < 0.05$) reduced cumulative BWG through d 27 compared to the PC diet. The inclusion of xylanase at both inclusion levels significantly ($P < 0.05$) increased cumulative BWG through d 27 when compared to the NC diet to levels that were similar to the PC. No significant differences in BW and BWG were observed among any of the treatments at the conclusion of the trial on d 41. Cumulatively through d 41, no significant difference was observed between

Table 4. Experiment 2, effect of xylanase at multiple levels compared to a standard U.S. corn/SBM-based and low energy diet on male broiler body weight, feed consumption, body weight gain, mortality corrected feed conversion ratio, and ileal digestible energy.

	Treatment[1]				
Item	PC	NC	XYL10	XYL20	PSEM
Body weight (kg/bird)					
14 d	0.395[a]	0.378[b]	0.372[b]	0.392[a]	0.003
27 d	1.466[a]	1.417[b]	1.455[a]	1.475[a]	0.007
41 d	2.896	2.838	2.897	2.912	0.014
Feed consumption(g/bd/d)					
Starter (0 to 14 d)	36.8[a]	35.2[b]	35.0[b]	35.8[a,b]	0.3
Grower (15 to 27 d)	119.9[b]	120.2[b]	122.5[a,b]	124.6[a]	0.8
Finisher (28 to 41 d)	191.5[a,b]	190.0[b]	195.4[a]	194.5[a,b]	1.0
Cumulative feed Consumption (g/bd/d)					
0 to 27 d	69.8[a]	68.2[b]	69.2[a,b]	70.3[a]	0.355
0 to 41 d	97.3[a,b]	95.3[b]	96.9[a,b]	97.8[a]	0.468
Body weight gain (kg)					
0 to 14 d	0.355[a]	0.338[b]	0.332[b]	0.351[a]	0.003
15 to 27 d	1.071[a]	1.039[b]	1.083[a]	1.083[a]	0.006
27 to 41 d	1.430	1.421	1.441	1.437	0.01
Cumulative BWG (kg)					
0 to 27 d	1.426[a]	1.376[b]	1.415[a]	1.434[a]	0.008
0 to 41 d	2.856[a,b]	2.797[b]	2.856[a,b]	2.871[a]	0.014
Mortality corrected feed Conversion Ratio (FCR) (feed:gain)					
Starter (0 to 14 d)	1.450[a,b]	1.454[a,b]	1.472[a]	1.420[b]	0.009
Grower (15 to 27 d)	1.443[b]	1.489[a]	1.465[a,b]	1.480[a]	0.006
Finisher (28 to 41 d)	2.037	2.038	2.043	2.057	0.013
Cumulative mortality Corrected FCR(feed:gain)					
0 to 27 d	1.445[b]	1.480[a]	1.467[a,b]	1.463[a,b]	0.004
0 to 41 d	1.740	1.763	1.756	1.759	0.006
0 to 41 d[2]	1.671[b]	1.720[a]	1.677[a,b]	1.679[a,b]	0.008
Ileal digestible energy (IDE)					
14 d	3378[a]	3134[b]	3255[a,b]	3272[a]	28
27 d	3365[a]	3199[b]	3285[a,b]	3216[b]	23
41 d	3367[a]	3111[b]	3195[a,b]	3277[a,b]	33

[a,b]Means with different superscripts within a row differ significantly at ($P < 0.05$).

[1]Positive Control; Negative Control, with 150 kcal/kg reduction in energy; XYL10, NC plus 10,000 units of xylanase; XYL20, NC plus 20,000 units of xylanase.

[2]Weight-corrected FCR, 3kg correction factor with 27g of BW equal to 1 point in FCR.

the PC and NC diets; however, the treatment containing XYL20 yielded a significantly ($P < 0.05$) higher BWG than the NC.

During the starter phase, no significant differences in FCR were observed between the PC and NC diets. The inclusion of xylanase at both inclusion rates did not impact FCR compared to the control diets; however, the inclusion of XYL20 significantly reduced starter FCR compared to the inclusion of XYL10. The NC significantly increased FCR compared to the PC during the grower phase. XYL10 reduced FCR to levels that were similar to the PC, however, not statistically different from the NC diet. Cumulatively through d 27, a significant increase in FCR was observed with the NC compared to the PC diet. The inclusion of xylanase at both inclusion rates reduced FCR to levels comparable to the PC diet, however, not significantly different from the NC. During the finisher phase, no significant differences were observed in FCR between the PC and NC diets or with the inclusion of xylanase at both inclusion rates. At the conclusion of the trail on d 41, no differences were observed in

cumulative FCR among any of the dietary treatments. A weight correction factor (3 kg) was applied to cumulative FCR through d 41 in order to normalize differences in BW with 27 g of BW equal to one point of FCR. A significant ($P < 0.05$) reduction in weight corrected FCR was observed between the PC (1.671) and NC (1.720), which corresponds with the 150 kcal/kg reduction in energy in the NC compared to the PC. The inclusion of both XYL10 and XYL20 (1.677 and 1.679, respectively) produced similar results to the PC and resulted in approximately a 4-point reduction in FCR.

A significant reduction in IDE was observed in the NC compared to the PC at d 14, 27, and 41, due to the 150 kcal/kg reduction in energy in the NC diet. At d 14, the inclusion of xylanase at both inclusion rates increased IDE to levels that were comparable to the PC diet; however, the inclusion of XYL20 significantly increased IDE compared to the NC. On d 27, the inclusion of XYL10 increased IDE to levels that were comparable to the PC diet, however, did not differ from the NC. At the conclusion of the trial on d 41, the inclusion of xylanase at both inclusion rates increased IDE to levels similar to the PC diet, however, were not significantly different from the NC.

The potential performance improvements with xylanase supplementation and its impact on broilers fed various diet types (corn/soy, wheat, etc.) have been extensively researched. The incorporation of wheat into diets has been noted to possess several advantages over corn in that wheat contains a higher crude protein content than corn (13 vs. 8.6%, respectively) and contains proportionally more lysine [14]. It does, however, have disadvantages, including less metabolic energy and a higher concentration of NSP than corn, which inhibit nutrient utilization and absorption. The major components of NSP found in wheat are arabinoxylans composed of a backbone chain of β-(1,4)-linked xylose units with sidechains of arabinose attached [15]. Crouch et al. [15] noted that the negative impact of arabinoxylans on performance is attributed to the water-soluble NSP fraction causing an entanglement of long polymers resulting in a high water-holding capacity and increased intestinal viscosity [16]. Previous research has shown that supplementation of xylanase-based feed additives, which degrade various NSPs in the cell wall causing a reduction in gut viscosity, is an effective way to mitigate the anti-nutritive properties of NSPs in wheat. This reduction in viscosity has been shown to increase BW and improve feed efficiency as well as nutrient utilization leading to reduced FCR values and increased IDE [11,17–19]. The consistent improvements in broiler performance with xylanase supplementation in wheat-based diets are potentially a result of the high concentration of NSP in wheat, allowing for adequate substrate. Consistent results with xylanase supplementation in corn-based diets, however, have not been observed.

The concept of introducing xylanase-based enzymes into corn-based diets began in the late 1990s following the success achieved in wheat-based diets [5]. The mode of action by which xylanase acts on the arabinoxylans within the endosperm cell wall of corn is the same as it is in wheat. However, as mentioned previously, there have been notable and inconsistent results observed between xylanase supplementation in wheat- and corn-based diets, primarily due to the higher NSP concentration in wheat as compared to corn [20]. Results in corn-based diets have been shown to vary in effectiveness, ranging from having no impact [2,7,21,22] to significantly improving broiler performance [5,18,23].

When comparing the results from both experiments, significant improvements were observed in BW during the second experiment while improvements in FCR as well as IDE were observed during both studies. The inconsistencies in BW to the response of xylanase correlate with previous research in which xylanase inclusion showed no impact on BW [2,5,23]; however, there have been reports that indicate the effectiveness of xylanase resulting in increased broiler BW [18,24]. During the first experiment, no significant differences in BW were observed throughout the duration of the trial at either inclusion level (XYL20 or XYL40) but there were numerical increases with XYL20 supplementation. The increases in BW with XYL20 supplementation were again observed in the second trial; however, results were significant ($P < 0.05$) at d 14 and 27 as well as cumulatively through d 27 and d 41. It is important to note that the low dose of xylanase in Experiment 1 (XYL20)

was the high dose in Experiment 2, suggesting that there may have been a dose response to the level of xylanase supplemented. The impact of xylanase supplementation on feed consumption (g/bird/day) was more prevalent during experiment 2 compared to experiment 1. During the first experiment, no significant differences in feed consumption were observed throughout the duration of the trial with the exception of the finisher phase in which both inclusion levels of xylanase (XYL20 and XYL40) significantly ($P < 0.05$) increased feed consumption compared to the control. During the second experiment, significant differences were observed during all phases of growth as well as cumulatively through d 27 and 41. The PC consumed significantly ($P < 0.05$) more feed than the NC during the starter phase as well as cumulatively through d 27; however, this trend was lost throughout the remainder of the trial. This was an interesting phenomenon considering that it has been accepted that birds eat to meet their energy requirements; however, based on these data, this does not seem to be the case. Based on that logic, the NC that was formulated to have a 150 kcal reduction in energy compared to the PC would have been consumed more than the PC but that was not the case, suggesting that the birds may have been eating to meet another requirement other than energy. The incorporation of xylanase significantly affected feed consumption during all phases of the second experiment. Supplementation of XYL10 significantly ($P < 0.05$) increased feed consumption compared to the NC during the finisher phase while supplementation of XYL20 significantly ($P < 0.05$) increased feed consumption during the grower phase as well as cumulatively through d 27 and 41. Similar to the results seen with feed consumption, the impact of xylanase supplementation was more pronounced during the second experiment compared to the first experiment. The inclusion of XYL20 during the first experiment significantly ($P < 0.05$) reduced FCR during the grower phase and cumulatively through d 27. Cumulatively through d 42, a significant reduction in FCR was observed between XYL20 and the control, which resulted in a 4-point reduction in FCR (1.73 vs. 1.69). During the grower phase and cumulatively through d 27 of the second experiment, the PC yielded a significantly lower FCR than the NC, which was expected due to the 150 kcal/kg reduction in energy between the PC and NC. Cumulatively through d 41, supplementation of xylanase at both levels reduced weight corrected FCR values similar to the PC resulting in a 4-point reduction in FCR, which was similar to the results seen in the first experiment. Supplementation with XYL20 during the first experiment significantly ($P < 0.05$) increased IDE on d 28 and 42 compared to the control (88 kcal/kg, 176 kcal/kg, respectively). Xylanase inclusion XYL40 also significantly increased IDE compared to the control on d 42 (180 kcal/kg). During the second experiment, the PC consistently had significantly ($P < 0.05$) higher IDE values than the NC throughout the duration of the trial, which correlates with the 150 kcal/kg reduction in energy between the 2 treatments. Supplementation of xylanase increased IDE values and consistently produced results that were similar to that of the PC throughout the trial.

As mentioned previously, the effectiveness of xylanase in wheat-based diets as well as other viscous cereals is well established and has been confirmed to be a result of gut viscosity reduction; however, the mechanisms by which xylanase acts within corn-based diets has been inconclusive. It has been suggested through previous research that one effective mechanism of xylanase in corn-based diets could be a result of an increase in feed efficiency through the release of encapsulated nutrients, coupled with increases in BW and minimal effects on feed intake [6]. The significant improvements in IDE during both experiments with xylanase supplementation confirms the improvements in feed efficiency, and based on previous research, can help confirm the improvements in FCR that were observed during both studies [5,25]. These data also show a potential dose response to the level of xylanase supplemented and the resulting effect on broiler growth performance. Results from both studies indicate that inclusion of XYL20, which was the low dose in experiment 1 and the high dose in experiment 2, produced consistent improvements in performance and nutrient utilization during both experiments, while the supplementation of XYL40 and XYL10 produced inconsistent results.

Based on these findings, consistent performance improvements in broilers fed corn-based

diets including DDGS supplemented with xylanase may be achieved by utilizing the proper dosage of the enzyme in order to achieve maximum energy release. Further research is needed in order to confirm the dose responses on the performance parameters and IDE values with regard to this new type of xylanase; however, at this time, we were able to accept our original hypotheses in that during both experiments, a new form of xylanase with DDGS significantly improved broiler performance and IDE.

CONCLUSIONS AND APPLICATIONS

1. Over the course of 2 experiments, inclusion of a new xylanase product significantly improved feed efficiency in broilers fed a corn-based diet with DDGS.
2. When xylanase was supplemented at 10,000, 20,000, and 40,000 units, a clear dose response with regard to broiler performance was observed.
3. Supplementation of xylanase at 20,000 units produced consistent improvements in BW and IDE, and over the course of the 2 trials, showed improvements in FCR, which led to improvements in feed efficiency and overall broiler performance.

REFERENCES AND NOTES

1. Slominski, B. A. 2011. Recent advances in research on enzymes for poultry diets. Poult. Sci. 90:2013–2023.

2. Yegani, M., and D. R. Korver. 2013. Effects of corn source and exogenous enzymes on growth performance and nutrient digestibility in broiler chickens. Poult. Sci. 92:1208–1220.

3. Cowieson, A. J. 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. The Journal of Poultry Science 47:1–7.

4. Kolenová, K., M. Vršanská, and P. Biely. 2006. Mode of action of endo-β-1,4-xylanases of families 10 and 11 on acidic xylooligosaccharides. J. Biotechnol. 121:338–345.

5. Cowieson, A. J., M. R. Bedford, and V. Ravindran. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. Br Poult Sci 51:246–257.

6. Masey O'Neill, H. V., N. Liu, J. P. Wang, A. Diallo, and S. Hill. 2012. Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of China. Asian-Australasian Journal of Animal Sciences 25:515–523.

7. Romero, L. F., J. S. Sands, S. E. Indrakumar, P. W. Plumstead, S. Dalsgaard, and V. Ravindran. 2014. Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn- and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease. Poult. Sci. 93:2501–2513.

8. Liu, K. 2010. Selected factors affecting crude oil analysis of distillers dried grains with solubles (DDGS) as compared with milled corn. Cereal Chemistry Journal 87:243–249.

9. Ganesan, V., K. Rosentrater, and K. Muthukumarappan. 2009. Physical and flow properties of regular and reduced fat distillers dried grains with solubles (DDGS). Food Bioprocess Technol 2:156–166.

10. Loar, R. E., J. S. Moritz, J. R. Donaldson, and A. Corzo. 2010. Effects of feeding distillers dried grains with solubles to broilers from 0 to 28 days posthatch on broiler performance, feed manufacturing efficiency, and selected intestinal characteristics. Poult. Sci. 89: 2242–2250.

11. Kiarie, E., L. F. Romero, and V. Ravindran. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult. Sci. 93: 1186–1196.

12. Knudsen, Bach, and K. E. 2011. Triennial Growth Symposium: Effects of polymeric carbohydrates on growth and development in pigs. J. Anim. Sci. 89:1965–1980.

13. Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

14. Leeson, S., and J. D. Summers. 1991. Commercial Poultry Nutrition University Books.

15. Crouch, A. N., J. L. Grimes, P. R. Ferket, L. N. Thomas, and A. E. Sefton. 1997. Enzyme supplementation to enhance wheat utilization in starter diets for broilers and turkeys. The Journal of Applied Poultry Research 6:147–154.

16. Ward, N. E. 1996. Intestinal viscosity, broiler performance. Poultry Digest 4:12–16.

17. Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925–938.

18. Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolisable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. Br. Poult. Sci. 45:76–84.

19. Kalmendal, R., and R. Tauson. 2012. Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poult. Sci. 91:1387–1393.

20. Choct, M. 2006. Enzymes for the feed industry: Past, present and future. World's Poultry Science Journal 62: 5–16.

21. Kocher, A., M. Choct, G. Ross, J. Broz, and T. K. Chung. 2003. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. The Journal of Applied Poultry Research 12:275–283.

22. Gehring, C. K., M. R. Bedford, and W. A. Dozier. 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poult. Sci. 92:979–991.

23. O'Neill, H. V. M., G. Mathis, B. S. Lumpkins, and M. R. Bedford. 2012. The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. Poult. Sci. 91:1356–1360.

24. Cowieson, A. J., and V. Ravindran. 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci. 49:37–44.

25. Zanella, I., N. K. Sakomura, F. G. Silversides, A. Fiqueirdo, and M. Pack. 1999. Effect of enzyme supplementation of broiler diets based on corn and soybeans. Poult. Sci. 78:561–568.