

# The effect of a heat-stable xylanase on digesta viscosity, apparent metabolizable energy and growth performance of broiler chicks fed a wheat-based diet

I. B. Barasch, PhD and J. L. Grimes, PhD<sup>1</sup>

*Prestage Department of Poultry Science, NC State University, Raleigh, NC 27695-7608*

**ABSTRACT** Feed costs represent a significant portion of the cost of poultry production. This study, in 3 experiments, was conducted to evaluate the effectiveness of a heat-stable xylanase (XYL) as a dietary supplement and its effect on digesta viscosity, nitrogen-corrected apparent metabolizable energy (AME<sub>n</sub>), and live performance in broiler chicks. Experiment 1: the objective was to determine the effects of the amount and type of enzyme supplementation on digesta viscosity, AME<sub>n</sub>, and bird performance using 7 diets. The dietary treatments were: no supplementation (C), 5 levels of XYL (1 to 16 ppm), or supplementation with a carbohydrase cocktail (CC). Experiment 2: the objective was to determine the interaction of the dietary XYL and the energy content of the feed. There were 2 levels of XYL (0 and 20 ppm) and 3 dietary energy levels (2,770, 2,920, and 3,070 kcal/kg ME). Experiment 3: the objective was to determine the interaction of the dietary XYL and feed form. The treatments were: 5 levels of XYL (0 to 40 ppm) and 2 feed forms (mash and crumble). Broiler chicks were reared in battery cages to 21 d. Statistical analysis of

the data was completed using Proc GLM of SAS (9.2) (SAS Institute, Cary, NC).

In experiment 1, increasing XYL (0 to 16 ppm) resulted in a decrease in digesta viscosity and an increase in AME<sub>n</sub>. The XYL included as low as 1 ppm resulted in a significant increase in AME<sub>n</sub> which reached 5% with 16 ppm XYL. In contrast, increase in BWG (4%) above values with the basal diet was greatest with 1 ppm XYL. In experiment 2, the caloric content of the diet influenced the increase in AME<sub>n</sub> with inclusion of XYL, 8% and 6% increases with 2,920 kcal/kg and 3,070 kcal/kg diets, respectively. Without addition of XYL, BWG was significantly lower when fed the diet with the highest energy content. In experiment 3, feed form x XYL influenced the effect of XYL on BWG. The BWG was greater when birds were fed the crumble diet with XYL vs when they were fed the mash feed with XYL. The xylanase proved effective for broilers to 21 d when fed the diets used herein with changes in digesta viscosity, increased dietary AME<sub>n</sub>, and improved bird performance represented by either BW gain or FCR.

**Key words:** xylanase, enzyme, AME<sub>n</sub>, digesta viscosity, broiler

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## INTRODUCTION

Feed costs represent a significant portion of the cost of animal production. Donohue and Cunningham (2009) reported that feed costs could be up to 80% of production costs. Feed costs can be influenced by demands from other markets such as an increased proportion of corn going to ethanol production rather than animal feed as well a global increase in the demand for feed grains and fuel (Donohue and Cunningham, 2009; Masey O'Neill et al., 2012). In response to rising costs of feed ingredients, poultry, and swine producers in the

United States might increase the inclusion of alternative, lower-cost ingredients to the traditional corn-soybean meal based diets such as wheat or dried distillers grains with soluble (DDGS) (Leeson et al., 2000; Mathews and McConnell, 2009; Adeola and Cowieson, 2011; Yanez et al., 2011). The DDGS, a coproduct of ethanol production, are an alternative source of both protein and energy for nonruminant diets (Lumpkins et al., 2004; Wang et al., 2007). These alternative ingredients may have reduced nutrient digestibility values compared to corn; however, they have some benefits besides their lower cost. For example, while the energy values associated with wheat may be lower compared to corn, wheat has higher crude protein content and higher lysine concentrations than corn (Crouch et al., 1997; Cowieson, 2005; Wang et al., 2005). DDGS can serve as an alternative source of protein as well as energy.

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<sup>1</sup>Corresponding author: [jgrimes@ncsu.edu](mailto:jgrimes@ncsu.edu)

Exogenous enzymes are one type of feed additive for improving the digestibility and feeding value of traditional and alternative low-cost feed ingredients. They have been used commercially in swine and poultry rations since the 1980s when carbohydrase enzymes entered the market. Their use has been expanded to maximize utilization of less costly raw feed materials as the prices of corn, soy, fat, and mineral phosphates increase (Bedford and Partridge, 2010). Lower-cost ingredients as an alternative to corn, e.g., wheat, barley, and DDGS can contain high concentrations of soluble nonstarch polysaccharides (NSP) (Annison, 1993; Peron and Partridge, 2010) that impair nutrient digestibility and negatively affect bird performance (Choct and Annison, 1992; Choct et al., 1996).

The main reason for inclusion of a NSP-degrading enzyme (NSPase) is to degrade the complex carbohydrates in NSP and reduce the associated antinutritive effects. Consumption of diets containing high concentrations of soluble NSP can increase digesta viscosity thought to be the main cause of reduction in nutrient digestion. The inclusion of exogenous carbohydrases can breakdown NSP and may therefore reduce digesta viscosity and, consequently, aid digestion (Campbell and Bedford, 1992; Zhang et al., 2014), and result in increased apparent metabolizable energy (AME) of a diet (Nian et al., 2011). Carbohydrase enzymes were originally introduced in diets containing “viscous grains” such as wheat and barley to reduce to antinutritive effects associated with them (Choct and Annison, 1992; Bedford and Classen, 1992). The addition of carbohydrases can improve the AME value of a feed ingredient through improvements in fat and starch digestibility. The enzyme xylanase can breakdown arabinoxylans, the main NSP in wheat; therefore, so xylanase activity is the favored carbohydrase as an enzymatic supplement for wheat-based diets.

Thermostability can be a concern when supplementing animal feed with some exogenous enzymes because feed can be exposed to temperatures high enough to denature and inactivate some enzymes during feed processing such as conditioning, pelleting, extrusion, or expansion (Svihus et al., 2005; Horton et al. 2006). Selecting enzymes from thermophilic organisms which survive higher temperatures (Chesson, 1993; Horton et al., 2006) and genetic engineering have allowed for great advancement in production of more thermal stable exoenzymes (Turner et al., 2007).

Evaluating the effectiveness of enzymes as a dietary supplement requires in vivo testing, because there are unknown and uncontrollable factors present in vivo such as inhibitors, variations in pH, endogenous enzymes, available substrate, and changing rate of movement of digesta. In addition, it is important to investigate mechanisms responsible for the influence, e.g., effect on digesta viscosity and nitrogen-corrected apparent metabolizable energy (AME<sub>n</sub>).

While NSPase supplementation has been demonstrated to reduce digesta viscosity, this does not always correlate with differences in growth performance. Some

authors have reported significant reductions in digesta viscosity with NSPase supplementation, correlating with weight gain and improved feed conversion efficiency (Bedford and Classen, 1992; Almirall et al., 1995; Choct et al., 1996, 1999; Wu et al., 2004; Gonzalez-Ortiz et al., 2016). However, others have demonstrated that this correlation does not always occur (Choct and Annison, 1992; Crouch et al., 1997; Leeson et al., 2000; Woyengo et al., 2008).

In poultry diets, NSPases are typically supplemented throughout the whole production cycle. However, most of the earlier research focused on the starter period when the viscous grains pose the most challenge to the immature gut of the birds. As a bird ages and the digestive tract develops, the bird is better able to tolerate NSP (Chesson, 1993; Bedford, 1995; Peterson et al., 1999). Increased digestive capacity in older birds (greater than 2 wk of age) due to gut maturity may also result in a reduced response to NSPase supplementation (Campbell and Bedford, 1992). However, there has been more investigation into supplementing poultry diets in the later stages of production (Cowieson and Masey O’Neill, 2013). Birds consume the greatest quantity of feed toward the end of production which could result in more potential savings if energy digestion and feed efficiency can be improved by exogenous enzyme inclusion during this time.

The objective of this study was to test, in vivo, the efficacy of a mono-component endo- $\beta$ -1,4-xylanase (XYL) that was developed to be thermostable so it could withstand the conditioning and pelleting processes during feed manufacturing. Three experiments were conducted to investigate the effect of XYL on digesta viscosity, AME<sub>n</sub>, and broiler chick performance (0–21 d) including the response to increasing levels of XYL, energy content of the diet, and feed form (crumble vs. mash).

## MATERIALS AND METHODS

Three experiments were conducted with male broiler chicks (Ross 708, Aviagen, Huntsville, AL) reared to 21 d to investigate the effect of enzyme supplementation to wheat-soybean-corn DDGS-based diets with a xylanase (XYL). The XYL used was a mono-component endo- $\beta$ -1,4-xylanase bioengineered (BioResource International, Inc., Durham, NC) to be thermostable so as to withstand elevated temperatures during the conditioning and pelleting processes during feed manufacturing. In each experiment, treatment diets were created from common basal diets. The XYL was added to the diets in a dry form during feed manufacturing prior to the pelleting process. Crumble feed was produced by pelleting mash feed at 85°C and then crumbling the pelleted feed.

In experiment 1 the objective was to determine the effects of the amount and type of enzyme supplementation on digesta viscosity, AME<sub>n</sub>, and bird performance using 7 diets. The treatments were: no enzyme supplementation (C), increasing levels of XYL (1, 2, 4, 8, or

16 mg xylanase/kg finished feed), or addition of a commercially available carbohydrase cocktail (CC). In experiment 2 the objective was to determine the effect of the energy content of a crumble feed on the impact of supplementation using a 2 × 3 factorial design with 2 levels of XYL (0 and 20 ppm) and 3 dietary energy levels (2770, 2920, and 3070 kcal/kg ME). In experiment 3 the objective was to determine the effect of feed form and XYL supplementation to diet using a 2 × 5 factorial design with 2 feed forms (mash vs. crumble) and 5 levels of XYL inclusion (0, 5, 10, 20, 40 ppm).

## Bird Husbandry

All bird handling procedures were approved by the NC State University Institutional Animal Care and Use Committee. Chicks were randomly distributed among Alternative Design battery cages (Alternative Design Manufacturing & Supply, Inc, Siloam Springs, AR). Feed and water were provided ad libitum. Each cage was equipped with 2 adjustable-height nipple drinkers and one feed trough. Birds were provided with 23 h of light and 1 h of dark per d. Temperatures were provided at 32°C for the first 48 h after birds were placed. Temperature was then decreased 0.5°C per d for an additional 5 d, after which it was decreased an additional 2.5°C per wk until 21°C was reached. In experiment 1, chicks were randomly assigned to cages, 5 replicate cages/treatment (8 birds/cage). In experiments 2 and 3, birds were randomly assigned to cages, 6 replicates/treatment (6 birds/cage).

## Dietary Treatments

The basal diets, formulated based on breeder recommendations, were mixed at the North Carolina State University Feed Mill Education Unit. They were all wheat-soybean meal-corn DDGS-based. An inert reference material, diatomaceous earth (Celite, World Minerals, Inc., Santa Barbara, CA) was included in all treatments for analysis of AME<sub>n</sub>.

In experiment 1, the basal diet was a wheat (60%)-soybean meal (20%)-corn DDGS (10%)-based mash diet (Table 1) that was divided into 7 aliquots (Table 2). The basal diet served as the control (C, 0 ppm XYL). Five aliquots were supplemented with increasing levels

**Table 1.** Composition and nutrient content of the wheat-soybean meal-corn DDGS-based basal diets (C) fed from placement to 21 d of age in experiments 1, 2, and 3.

Ingredients	% of total diet		
	Exp. 1	Exp. 2	Exp. 3
Wheat	59.40	49.30	57.10
Soybean meal	20.20	23.40	23.40
Corn DDGS	10.00	5.50	10.00
Poultry fat	2.29	6.20	2.00
Limestone (Calcium carbonate)	1.875	1.70	1.50
Monocalcium dicalcium phosphate (21% P)	1.70	1.50	1.60
L-lysine	0.71	0.50	0.50
D,L-methionine	0.50	0.40	0.40
L-threonine	0.275	0.40	0.40
Trace mineral premix <sup>1</sup>	0.15	0.15	0.15
Vitamin premix <sup>2</sup>	0.15	0.15	0.15
Choline chloride 60%	0.20	0.30	0.30
Sodium selenite premix <sup>3</sup>	0.10	0.10	0.10
Sodium chloride	0.25	0.20	0.20
Sodium bicarbonate	0.20	0.20	0.20
Celite <sup>4</sup>	2.00	2.00	2.00
Sand <sup>5</sup>	0	8.00	0
Nutrient content			
ME poultry, kcal/kg	2,900	2,770	2,770
Crude protein, %	21.00	20.02	22.15
Crude protein, % (analyzed)	20.80	21.0	22.45
Crude fat, %	4.26	7.67	4.11
Crude fat, % (analyzed)	4.40	7.59	3.78
Available phosphorus, %	0.50	0.43	0.47
Calcium, %	1.11	0.94	0.95
Sodium, %	0.19	0.20	0.20
Total lysine, %	1.43	1.27	1.33
Total Met + Cys, %	1.08	0.90	0.97
Threonine, %	0.94	0.98	1.02

<sup>1</sup>The mineral premix provided the following per kg of diet: manganese, 90 mg; zinc, 90 mg; iron, 60 mg; copper, 7.5 mg; iodine, 1.9 mg; cobalt, 0.75 mg.

<sup>2</sup>The mineral premix provided the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.

<sup>3</sup>NaSeO<sub>3</sub> premix provided 0.3 mg Se/kg of complete feed.

<sup>4</sup>Diatomaceous earth, inert reference material included in feed for analysis of nitrogen-corrected metabolizable energy (AME<sub>n</sub>) (World Minerals, Inc., Santa Barbara, CA).

<sup>5</sup>To create the medium (2920 kcal/kg) and high (3070 kcal/kg) energy diets in experiment 2, sand was replaced with cornstarch; 1/2 of the sand in the medium energy diet and all of the sand in the high energy diet.

of XYL (1, 2, 4, 8, or 16 ppm). One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM

**Table 2.** Design of experiments 1, 2, and 3.

Ingredient	Experiment 1 <sup>4</sup>		Experiment 2		Experiment 3	
Xylanase <sup>1</sup> ppm	0, 1, 2, 4, 8, 16	0, 20	0, 20	0, 20	0, 5, 10, 20, 40	
Form	Mash	Crumble <sup>5</sup>			Mash	Crumble <sup>5</sup>
Energy <sup>2</sup>	2,770	2,770	2,920	3,070	2,770	
Kcal/kg						
Cornstarch <sup>3</sup>	0	0	4.0	8.0	0	0

<sup>1</sup>Mono-component endo-β-1,4-xylanase engineered to be thermostable (BioResource International Inc. Durham, NC)

<sup>2</sup>ME poultry.

<sup>3</sup>Added to increase energy content. Sand content was adjusted by treatment to compensate for changes in starch content in order to maintain total weight of ingredients constant.

<sup>4</sup>Included 7th treatment: 0 XYL + commercial carbohydrase cocktail containing endo-1,4-β-xylanase, endo-1,3(4)-β-glucanase, and endo-1,4-β-glucanase (Rovabio Excel, Adisseo, Antony, France).

<sup>5</sup>Pelleted at 85°C.

trisodium citrate buffer at pH 6.0. The XYL was included in a raw, dry form and did not contain any carrier or filler. To compare the effectiveness of XYL with a commercial exoenzyme, the seventh treatment was supplementation with a liquid carbohydrase cocktail (CC) (Rovabio Excel, Adisseo, Antony, France). The enzyme cocktail contained enzyme activities of endo-1,4- $\beta$ -xylanase, endo-1,3(4)- $\beta$ -glucanase, and endo-1,4- $\beta$ -glucanase in liquid form. The CC was added according to the manufacturers' recommendations.

The basal diet in experiment 2 was a wheat (50%)-soybean meal (23%)-corn DDGS (5.5%)-based crumble diet that had a lower wheat and corn DDGS content and higher (6.20 vs. 2.24%) poultry fat content than the basal diet in experiment 1 (Table 1). The 2  $\times$  3 design of treatments (Table 2) were produced by dividing the basal feed in half; one half received 20 mg xylanase/kg finished feed and the remaining half did not receive enzyme supplementation (C). Each of the aliquots was further divided into thirds, and the energy content was adjusted using sand and/or corn starch to achieve a low, medium, and high ME diet (Kcal/kg). Cornstarch replaced sand to produce the dietary energy levels (2770, 2920, and 3070 kcal/kg ME). Fat was not used to increase energy so that fat would not confound results by affecting palatability or feed efficiency independently of the xylanase. Sand was used in the base diet to avoid changes in energy dilution in the diet. All diets were pelleted at 85°C to produce the pellets and were then crumbled.

The basal diet in experiment 3 was very similar to the one in experiment 1 (Table 1). To produce the 5 levels of XYL inclusion (0, 5, 10, 20, 40 ppm), the basal diet was divided in half, and one half received XYL to produce 40 ppm XYL. The 0 and 40 XYL feed were blended to create the 5 experimental levels of XYL (0, 5, 10, 20, 40 ppm). Each XYL inclusion level was prepared in both mash and crumble form (Table 2).

## Feed Analysis

Feed samples were analyzed by BioResource International, Inc. (Durham, NC) to determine and confirm the xylanase activity both pre- and post pelleting (data not shown). Proximate analysis of feed was conducted by the North Carolina Department of Agriculture and Consumer Services (Raleigh, NC).

## Live Performance

For all 3 experiments, individual bird and feeder weights were recorded at 7, 14, and 21 d to obtain bird body weights (BW) and measure feed disappearance which is reported as feed intake (FI). Body weight gain (BWG), FI, and feed conversion ratio (FCR) were calculated by each pen of birds. All birds were checked twice daily for mortality and morbidity, which was less than 3%. The FCR was calculated as FI divided by

BWG, for each cage of birds, plus the weight of culls and mortalities.

## Digesta Viscosity and Intestinal Samples

In experiment 1, 15 birds/treatment (3/cage) were euthanized at 3 wk by cervical dislocation for intestinal and pancreas sampling. The small intestine was removed from each bird and was cut into segments: duodenum (duodenal loop), jejunum (duodenal loop to Meckel's diverticulum), and ileum (Meckel's diverticulum to ileal-cecal junction). Ileal contents were collected for viscosity evaluation and then all 3 segments of the small intestine were then flushed with 0.9% saline solution to remove any remaining contents and a longitudinal cut was made along the length of the segments so they could lay flat for more accurate measurements. The pancreas and intestinal segment length and weight were recorded. Digesta contents of fresh ilea were manually expressed and stored on ice. Ileal contents from each individual bird were mixed, subsampled, and centrifuged at 5.9 RCF for 5 min to separate the supernatant from the solid digesta contents. The supernatant was collected and placed in a clean 2 mL tube. Viscosity, in centipoise (cP), was measured using 500  $\mu$ L aliquots of the supernatant using a LVDV-II+ Brookfield digital viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a CP-40 cone spindle at shear rates of 22.5 sec<sup>-1</sup> and 45 sec<sup>-1</sup>.

## Apparent Metabolizable Energy

To measure AME<sub>n</sub>, an inert reference material, diatomaceous earth (Celite, World Minerals, Inc., Santa Barbara, CA), was included in feed. At the end of each experiment, fresh excreta samples were collected from a pan beneath each cage taking care to avoid excreta contaminated with feed particles or feathers. Samples were pooled by cage, homogenized, and stored at -20°C until analysis. The fecal samples were dried at 55°C in a forced air oven (Blue M, Thermal Product Solutions) and ground using a small electric grinder. Gross energy (GE, kcal/kg) was measured on feed and excreta samples using an adiabatic bomb calorimeter (IKA calorimeter C 5000, IKA Works, Inc., Wilmington, NC). Nitrogen of both feed (N<sub>feed</sub>) and excreta (N<sub>excreta</sub>) were measured by combustion analysis (LECO Corporation, St. Joseph, MI). To determine acid-insoluble ash content (AiA), feed and excreta were analyzed for Celite recovery using a procedure reported by Vogtmann et al. (1975). The AME<sub>n</sub> was calculated using the following equations:

$$N_{\text{Retained}} = N_{\text{feed}} - ((N_{\text{excreta}} * AiA_{\text{feed}}) / AiA_{\text{excreta}})$$

$$AME_n = GE_{\text{feed}} - ((GE_{\text{excreta}} * AiA_{\text{feed}}) / AiA_{\text{excreta}}) - (8.22 * N_{\text{retained}})$$

**Table 3.** Experiment 1: Effect of dietary supplementation of a novel exogenous xylanase in increasing concentrations to broiler chickens in wheat-based diets on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) from placement until 21 d.<sup>1</sup>

Treatment <sup>2</sup>	Days of age														
	BWG					FI					FCR				
	0-7	7-14	14-21	0-14	0-21	0-7	7-14	14-21	0-14	0-21	0-7	7-14	14-21	0-14	0-21
	grams/bird					grams/bird					grams FI:grams BWG				
NC	94	210 <sup>c</sup>	308	306	613 <sup>b</sup>	134	368	495 <sup>b</sup>	506	999 <sup>bc</sup>	1.429	1.751	1.610	1.641	1.626
1 ppm	94	232 <sup>a</sup>	334	323	663 <sup>a</sup>	132	371	535 <sup>a</sup>	511	1074 <sup>a</sup>	1.445	1.643	1.578	1.557	1.560
2 ppm	93	217 <sup>bc</sup>	304	310	615 <sup>b</sup>	128	358	485 <sup>b</sup>	485	971 <sup>c</sup>	1.377	1.654	1.597	1.572	1.584
4 ppm	94	215 <sup>bc</sup>	309	311	620 <sup>b</sup>	134	369	489 <sup>b</sup>	510	999 <sup>bc</sup>	1.429	1.718	1.577	1.616	1.600
8 ppm	97	226 <sup>ab</sup>	317	323	641 <sup>ab</sup>	139	379	503 <sup>b</sup>	518	1022 <sup>b</sup>	1.432	1.676	1.584	1.605	1.593
16 ppm	94	231 <sup>a</sup>	310	326	636 <sup>ab</sup>	129	376	493 <sup>b</sup>	508	1003 <sup>bc</sup>	1.362	1.630	1.598	1.547	1.571
CC	98	225 <sup>ab</sup>	305	321	627 <sup>b</sup>	133	378	497 <sup>b</sup>	512	1009 <sup>bc</sup>	1.372	1.683	1.624	1.592	1.607
Source of variation	P values														
Treatment	0.85	0.03	0.13	0.38	0.04	0.55	0.54	0.008	0.53	0.002	0.68	0.31	0.83	0.42	0.57
Regression	0.86	0.14	0.46	0.20	0.95	0.80	0.27	0.26	0.63	0.45	0.49	0.33	0.95	0.50	0.70
SEM (25) <sup>3</sup>	2.8	4.8	7.7	7.6	10.8	4.1	7.8	8.6	11.2	14.0	0.042	0.038	0.026	0.033	0.025

<sup>1</sup>Values are means of 5 replicate pens of *ca.* 8 birds per pen.

<sup>2</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>3</sup>SEM (25) = Standard error of the mean with 25 degrees of freedom.

<sup>a-c</sup>Means within a column with no common superscript are significantly difference ( $P \leq 0.05$ ).

## Statistical Analysis

Statistical analyses of the data were completed using Proc GLM of SAS (9.2) (SAS Institute, Cary, NC). Linear regression (Proc REG) was also used to analyze live performance, digesta viscosity, and AME<sub>n</sub>; the CC treatment (commercial carbohydrase cocktail) was not included in linear regression analysis. Digesta viscosity was also analyzed using nonlinear regression, or segmented regression (Proc NLMIXED). Effects were considered significant at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Experiment 1

**Live Performance** Chick weight at placement was 39.8 ± 0.34 g and there were no coincidental differences due to treatment. Diet had a significant effect on feed intake during 14 to 21 and 0 to 21 d in that greater consumption was observed for birds fed the diet supplemented with 1 ppm XYL. There were no differences in BWG among treatments during the first (0–7 d) or third (14–21 d) week of the experiment. However, during the second week (7–14 d), birds fed 1, 8, or 16 ppm XYL or the CC diet had significantly greater BWG (10%) than those fed the basal diet or intermediate levels of XYL. During 0 to 21 d, only the 1 ppm XYL diet resulted in a significant increase in BWG (8%). In contrast to FI, dietary treatment did not affect FCR (Table 3).

**Digesta Viscosity and Intestinal Samples** The addition of XYL significantly decreased the digesta viscosity even at the lowest addition of 1 ppm (Table 4). The decrease was nonlinear with inclusion of XYL until digesta viscosity stabilized at roughly 50% of the

**Table 4.** Experiment 1: Effect of dietary supplementation of a novel exogenous xylanase in increasing concentrations to broiler chickens in wheat-based diets on nitrogen-corrected apparent metabolizable energy (AME<sub>n</sub>) and ileal digesta viscosity at 21 d.

Treatment <sup>1</sup>	AME <sub>n</sub> <sup>2</sup> kcal/kg	AvgVisc <sup>3</sup> cP
NC	2776 <sup>c</sup>	13.20 <sup>a</sup>
1 ppm	2814 <sup>bc</sup>	9.89 <sup>b</sup>
2 ppm	2853 <sup>abc</sup>	7.91 <sup>bc</sup>
4 ppm	2874 <sup>ab</sup>	8.08 <sup>bc</sup>
8 ppm	2903 <sup>ab</sup>	5.95 <sup>c</sup>
16 ppm	2918 <sup>a</sup>	6.76 <sup>c</sup>
CC	2848 <sup>abc</sup>	6.79 <sup>c</sup>
Source of variation	P values	
Treatment	0.06	<0.0001
Regression	0.005	0.0002
SEM	32 <sup>4</sup>	0.995 <sup>5</sup>

<sup>1</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>2</sup>Values are means of 5 replicate pens.

<sup>3</sup>Values are means of 15 replicate birds per treatment.

<sup>4</sup>SEM (28) = Standard error of the mean with 28 degrees of freedom.

<sup>5</sup>SEM (95) = Standard error of the mean with 95 degrees of freedom.

<sup>a-c</sup>Means within a column with no common superscript are significantly difference ( $P \leq 0.05$ ).

viscosity of the basal diet with XYL inclusion of 8 or 16 ppm. The addition of the carbohydrase cocktail resulted in an equivalent decrease in digesta viscosity. No treatment effect was observed on pancreas weight as a percentage of body weight (Table 5). Actual weight of the pancreas was also analyzed, using bird body weight as a covariate; however, no difference was observed. No treatment effect was observed on weight or length of

**Table 5.** Experiment 1: Effect of dietary supplementation of a novel exogenous xylanase in increasing concentrations to broiler chickens in wheat-based diets on intestinal weight and length and pancreas weight at 21 d.<sup>1</sup>

Treatment <sup>3</sup>	Percentage of body weight				Length <sup>2</sup>			Weight <sup>2</sup>				
	(%)				(cm)			(g)				
	D	J	I	Panc	D	J	I	D	J	I	Panc	
NC	1.15	1.95	1.52	0.36	22.2	52.6	52.1	7.78	13.20	10.32	2.39	
1 ppm	1.01	1.86	1.43	0.36	21.0	53.5	53.3	6.95	12.79	9.85	2.50	
2 ppm	1.08	1.82	1.37	0.38	22.2	51.6	50.0	7.30	12.35	9.30	2.55	
4 ppm	1.03	1.87	1.38	0.37	21.2	53.4	53.7	6.98	12.78	9.44	2.54	
8 ppm	1.09	1.88	1.44	0.38	21.3	52.3	52.6	7.37	12.77	9.80	2.56	
16 ppm	1.03	1.84	1.47	0.36	21.0	51.1	52.2	6.91	12.43	9.95	2.39	
CC	1.03	1.88	1.41	0.37	21.5	52.5	51.7	7.01	12.77	9.62	2.50	
<i>P</i> values												
Treatment	0.18	0.87	0.40	0.68	0.37	0.87	0.55	0.27	0.89	0.45	0.67	
SEM (95) <sup>4</sup>	0.040	0.065	0.051	0.014	0.048	1.28	1.28	0.28	0.450	0.346	0.089	

<sup>1</sup>Values are means of 15 replicate birds per treatment. D, duodenum; J, jejunum; I, ileum; Panc, pancreas.

<sup>2</sup>Weight and length were analyzed using bird bodyweight as a covariate.

<sup>3</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>4</sup>SEM (95) = Standard error of the mean with 95 degrees of freedom.

**Table 6.** Experiment 2: Effect of dietary supplementation of a novel exogenous xylanase to broiler chickens in wheat-based diets with 3 dietary energy levels on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) from placement until 21 d.<sup>1</sup>

Effect	Days of age														
	BWG					FI					FCR				
	0–7	7–14	14–21	0–14	0–21	0–7	7–14	14–21	0–14	0–21	0–7	7–14	14–21	0–14	0–21
Energy	grams/bird														
2770	112 <sup>a</sup>	293 <sup>a</sup>	293 <sup>a</sup>	398 <sup>a</sup>	689 <sup>a</sup>	195 <sup>a</sup>	474 <sup>a</sup>	463 <sup>a</sup>	668 <sup>a</sup>	1123 <sup>a</sup>	1.704	1.628	1.624 <sup>a</sup>	1.662	1.629 <sup>b</sup>
2920	107 <sup>ab</sup>	296 <sup>a</sup>	299 <sup>a</sup>	403 <sup>a</sup>	704 <sup>a</sup>	184 <sup>ab</sup>	470 <sup>a</sup>	452 <sup>a</sup>	657 <sup>a</sup>	1115 <sup>a</sup>	1.725	1.570	1.530 <sup>a</sup>	1.613	1.575 <sup>b</sup>
3070	101 <sup>b</sup>	262 <sup>b</sup>	281 <sup>b</sup>	361 <sup>b</sup>	643 <sup>b</sup>	177 <sup>b</sup>	433 <sup>b</sup>	423 <sup>b</sup>	611 <sup>b</sup>	1033 <sup>b</sup>	1.770	1.618	1.507 <sup>b</sup>	1.657	1.591 <sup>ab</sup>
SEM (2)	2.4	7.3	3.4	8.8	9.4	3.6	9.3	7.4	15.1	11.8	0.035	0.028	0.018	0.027	0.014
Xylanase <sup>2</sup>	grams/bird														
0 ppm	105	284	285 <sup>b</sup>	384	670	190 <sup>a</sup>	458	443	648	1,092	1.795 <sup>a</sup>	1.636	1.579 <sup>a</sup>	1.688 <sup>a</sup>	1.630 <sup>a</sup>
20 ppm	109	284	297 <sup>a</sup>	390	687	181 <sup>b</sup>	460	449	642	1,096	1.671 <sup>b</sup>	1.574	1.531 <sup>b</sup>	1.600 <sup>b</sup>	1.567 <sup>b</sup>
SEM (1)	1.9	5.8	2.7	7.2	7.7	2.9	7.5	6.0	12.2	9.5	0.029	0.023	0.014	0.022	0.011
Energy Xylanase <sup>2</sup>	grams FI:grams BWG														
2770 0 ppm	110	300	282	399	681	206	492 <sup>a</sup>	448	698 <sup>a</sup>	1145	1.783	1.725 <sup>a</sup>	1.645	1.767 <sup>a</sup>	1.687
2770 20 ppm	115	287	303	397	697	185	456 <sup>ab</sup>	477	638 <sup>bc</sup>	1120	1.624	1.531 <sup>b</sup>	1.604	1.556 <sup>b</sup>	1.572
2920 0 ppm	106	291	298	397	698	184	462 <sup>ab</sup>	458	647 <sup>abc</sup>	1106	1.742	1.576 <sup>ab</sup>	1.545	1.621 <sup>ab</sup>	1.584
2920 20 ppm	107	300	300	409	710	183	478 <sup>a</sup>	447	667 <sup>ab</sup>	1124	1.708	1.564 <sup>ab</sup>	1.520	1.605 <sup>ab</sup>	1.567
3070 0 ppm	98	260	274	357	631	181	419 <sup>b</sup>	424	601 <sup>c</sup>	1025	1.86	1.608 <sup>ab</sup>	1.545	1.676 <sup>ab</sup>	1.619
3070 20 ppm	104	264	287	364	655	174	446 <sup>ab</sup>	423	621 <sup>bc</sup>	1043	1.68	1.628 <sup>ab</sup>	1.469	1.639 <sup>ab</sup>	1.563
SEM (28) <sup>3</sup>	3.3	9.7	4.6	12.4	13.2	5.1	12.5	10.4	21.2	16.0	0.050	0.040	0.024	0.039	0.020
Source of variation	<i>P</i> values														
Energy	0.008	0.004	0.003	0.004	0.0004	0.005	0.008	0.002	0.0002	<0.0001	0.43	0.33	0.0002	0.40	0.03
Xylanase	0.13	0.98	0.004	0.58	0.13	0.03	0.84	0.49	0.55	<0.76	0.01	0.07	0.03	0.01	0.001
Energy*Xylanase	0.70	0.56	0.18	0.86	0.92	0.12	0.05	0.15	0.004	0.33	0.29	0.03	0.56	0.03	0.06

<sup>1</sup>Values are means of 6 replicate cages of ca. 6 birds per cage.

<sup>2</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>3</sup>SEM (28) = Standard error of the mean with 28 degrees of freedom.

<sup>a-c</sup>Means within a column with no common superscript are significantly difference ( $P \leq 0.05$ ).

duodenum, jejunum, or ileum neither as actual weight with bird weight used as a covariate nor when expressed as a percentage of BW.

**AME<sub>n</sub>** Based on regression analysis, dietary AME<sub>n</sub> increased with increasing level of XYL inclusion with AME<sub>n</sub> leveling off at the highest inclusion (Table 4).

The 16 ppm inclusion level resulted in a 140 kcal/kg increase in AME<sub>n</sub> above the basal diet. While the CC resulted in dietary AME<sub>n</sub> (2848 kcal/kg) similar to the added XYL increase, this AME<sub>n</sub> was not included in the regression analysis and was not statistically different from the control diet.

**Table 7.** Experiment 2: Effect of dietary supplementation of a novel exogenous xylanase to broiler chickens in wheat-based diets with 3 dietary energy levels on nitrogen-corrected apparent metabolizable energy (AME<sub>n</sub>) at 21 d.<sup>1</sup>

Effect	Days of age	
	21	
	kcal ME/kg	
Energy		
2,770		3,191
2,920		3,226
3,070		3,274
SEM (2)		27
Xylanase <sup>2</sup>		
0 ppm		3147 <sup>b</sup>
20 ppm		3320 <sup>a</sup>
SEM (1)		22
Energy	Xylanase <sup>2</sup>	
2770	0 ppm	3159 <sup>b</sup>
2770	20 ppm	3224 <sup>ab</sup>
2920	0 ppm	3109 <sup>b</sup>
2920	20 ppm	3363 <sup>a</sup>
3070	0 ppm	3175 <sup>b</sup>
3070	20 ppm	3374 <sup>a</sup>
SEM (28) <sup>3</sup>		37
Source of variation		P values
Energy		0.10
Xylanase		<0.0001
Energy*Xylanase		0.05

<sup>1</sup>Values are means of 6 replicate cages.

<sup>2</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>3</sup>SEM (28) = Standard error of the mean with 28 degrees of freedom.

<sup>a,b</sup>Means within a column with no common superscript are significantly difference ( $P \leq 0.05$ ).

### Experiment 2 – Effect of Calorie Content of Diet

In experiment 2, chick weight at placement was 43.7 ± 0.27 g and there were no coincidental differences due to treatment. In this experiment there were 3 dietary energy levels. At 7, 14, and 21 d, BWG was significantly lower for birds fed the high energy diets compared to those fed either the low or mid energy diets. The BWG (0–21 d) for birds fed the high energy diet were 7% and 9% lower than those fed the low and mid energy diets (Table 6). In contrast, the addition of XYL did not affect BWG (0–21), and affected BWG only during 14 to 21 d when BWG of birds fed the supplemented diet was 4% greater than birds fed the unsupplemented diets.

Birds on the high ME diets consumed less feed compared to those fed the medium and low ME diets (Table 6). The reduced FI of the birds consuming high ME diets was associated with lower BWG. Overall, the birds fed the low ME had poorer FCR (Table 6); birds fed low ME diets had to consume a greater amount of feed in order to gain the same amount of BW as birds fed the medium ME treatment. The birds fed the medium ME diets had the best FCR, possibly indicating this energy level was closest to optimal for the bird. Throughout the study, FCR was improved with XYL supplementation; therefore, there was potentially

improved nutrient digestibility with xylanase. This was supported by the measured improvement in AME<sub>n</sub> with xylanase supplementation (Table 7). Xylanase is often included in poultry diets to increase AME. For example, in some cases, the energy level of the diet is reduced and a xylanase is included in a diet and given a matrix value for energy, or AME, uplift. Further improvement in FCR was observed when XYL was supplemented in low ME diets compared to unsupplemented low ME diets. This improvement with the XYL might not be observed at higher dietary energy levels if the bird is already able to metabolize adequate energy from the diet. In these cases, the potential improvements gained from enzyme supplementation may be smaller and thus less noticeable (Cowieson, 2010; Adeola and Cowieson, 2011). However, an unexpected response was the improved FCR in the low energy diets with XYL supplementation which did not correlate with the results of AME<sub>n</sub> values. Higher AME<sub>n</sub> was observed with XYL supplementation, but at the medium and high level, not at the low ME, where improvements in FCR were observed. Improvements in FCR and AME<sub>n</sub> observed with XYL supplementation indicate the XYL activity was still present after the pelleting process.

### Experiment 3 – Effect of Feed form and XYL Inclusion Level

In experiment 3, diets were formulated based on the low ME energy level used in experiment 2, where the improvement with xylanase supplementation was observed most clearly. In experiment 3, xylanase concentrations were included above and below the inclusion level in experiment 2. The purpose of this was to directly compare diets in mash vs. crumble form across a greater range of XYL supplementation and to demonstrate enzyme efficacy subsequent to the pelleting process. The chick weight at placement was 44.3 ± 0.40 g and there were no coincidental differences due to treatment. It was evident that birds fed diets in crumble form had greater BWG and FI (Table 8) over those consuming mash diets of the same composition. This result was not unexpected since it has long been understood that offering feed in pelleted or crumble vs. mash form allows the bird to expend less energy and spend less time feeding to consume the same amount of nutrients (Jensen et al., 1962). There is also less segregation in pelleted and crumbled diets resulting in more uniform consumption and performance of birds. In the crumble diets, XYL supplementation resulted in a linear improvement in BWG and FCR (Table 8) demonstrating that the xylanase was still efficacious and increasing levels resulted in increased improvements. As in experiment 2, in experiment 3, improvements in performance due to XYL were supported by correlated improvements in AME<sub>n</sub> (Table 9). Somewhat unexpectedly, the main affect mean for the mash AME<sub>n</sub> was greater than for the main affect mean for crumble AME<sub>n</sub>. However, there was a form by enzyme interaction. For the interaction means at the

**Table 8.** Experiment 3: Effect of dietary supplementation of a novel exogenous xylanase in increasing concentrations to broiler chickens in both mash and crumbled wheat-based diets on body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) from placement until 21 d.<sup>1</sup>

Effect Feed form	Days of age									
	BWG					FI		FCR		0-21 grams FI:grams BWG
	0-7	7-14	14-21 grams/bird	0-14	0-21	0-7 grams/bird	0-21	0-7		
Mash	98	274 <sup>b</sup>	445 <sup>b</sup>	375 <sup>b</sup>	820 <sup>b</sup>	251	1,400 <sup>b</sup>	2.653 <sup>b</sup>	1.730 <sup>b</sup>	
Crumble	141	323 <sup>a</sup>	480 <sup>a</sup>	473 <sup>a</sup>	939 <sup>a</sup>	235	1,524 <sup>a</sup>	1.670 <sup>a</sup>	1.609 <sup>a</sup>	
SEM (1)	1.8	5.7	5.1	5.5	11.1	5.4	21.9	0.077	0.022	
Xylanase <sup>2</sup>										
0 ppm	118	301	462 <sup>ab</sup>	418	884 <sup>ab</sup>	239	1,480	2.134	1.677	
5 ppm	116	283	436 <sup>b</sup>	417	829 <sup>b</sup>	240	1,421	2.171	1.709	
10 ppm	119	299	462 <sup>ab</sup>	417	890 <sup>ab</sup>	238	1,444	2.199	1.647	
20 ppm	124	303	462 <sup>ab</sup>	434	890 <sup>ab</sup>	253	1,488	2.162	1.696	
40 ppm	122	308	489 <sup>a</sup>	434	906 <sup>a</sup>	248	1,478	2.143	1.619	
SEM (4)	2.9	9.2	8.0	8.6	17.8	8.7	34.0	0.124	0.036	
Feed form Xylanase <sup>2</sup>										
Mash 0 ppm	95	274	456 <sup>bc</sup>	369	829	236	1,417	2.550	1.714	
Mash 5 ppm	97	269	427 <sup>c</sup>	368	778	252	1,362	2.643	1.767	
Mash 10 ppm	96	265	452 <sup>bc</sup>	361	832	243	1,403	2.765	1.732	
Mash 20 ppm	100	284	440 <sup>bc</sup>	394	828	267	1,415	2.685	1.746	
Mash 40 ppm	98	280	450 <sup>bc</sup>	384	836	239	1,403	2.622	1.693	
Crumble 0 ppm	141	327	468 <sup>bc</sup>	466	938	241	1,543	1.717	1.640	
Crumble 5 ppm	135	296	446 <sup>bc</sup>	466	881	228	1,481	1.699	1.652	
Crumble 10 ppm	141	333	473 <sup>abc</sup>	474	949	232	1,485	1.633	1.562	
Crumble 20 ppm	147	322	483 <sup>ab</sup>	474	952	240	1,560	1.639	1.646	
Crumble 40 ppm	143	336	528 <sup>a</sup>	483	976	239	1,553	1.664	1.546	
SEM (43) <sup>3</sup>	4.1	12.9	11.2	12.0	25.0	11.9	48.0	0.173	0.050	
Source of variation	<i>P</i> values									
Feed form	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.06	0.0002	<0.0001	0.0004	
Xylanase	0.40	0.37	0.002	0.37	0.04	0.65	0.61	1.00	0.36	
Feed form*Xylanase	0.79	0.53	0.05	0.76	0.96	0.65	0.96	0.93	0.88	
Regression <sub>MASH</sub>	0.22	0.13	0.41	0.18	0.14	0.48	0.44	0.89	0.57	
Regression <sub>CRUMBLE</sub>	0.22	0.58	0.04	0.39	0.0002	0.97	0.84	0.35	0.02	

<sup>1</sup>Values are means of 6 replicate cages of *ca.* 6 birds per cage.

<sup>2</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>3</sup>SEM (43) = Standard error of the mean with 43 degrees of freedom.

<sup>a-c</sup>Means within a column with no common superscript are significantly difference ( $P \leq 0.05$ ).

nonenzyme supplemented level (0 ppm), the mash and crumble AME<sub>n</sub> means are not significantly different. While it is generally accepted that heat processing and pelleting of feed improve bird performance and AME<sub>n</sub>, the final results can be variable and possibly inconsistent (Mateos et al., 2019). Much of the advantage of pelleted diets are in the mechanics of feed intake and also diet nutrient density. As pellet quality decreases, the resulting advantages of having pelleted the feed also decrease. In addition, the feed conditioning process can have negative effects on AME<sub>n</sub> especially in wheat-based diets (Abdollahi et al., 2019).

There was an interaction effect of feed form and XYL on BWG (Table 8). As might be expected, birds reared on crumbled feed had improved performance compared to those reared on mash feed. However, during 14 to 21 d, birds fed 40 ppm XYL inclusion in the crumble diet had greater BWG (17%) than those fed the equivalent mash diet. For the 0 to 21 d interval, by regression analysis, BWG increased when XYL was included in the crumble diet but not when included in the mash diet. Therefore, the XYL had a greater impact on bird performance when included in crumble feed vs. mash feed. Hosseini and Afshar (2017) fed broilers wheat based

diets with and without xylanase to 42 d. At 21 d there were no effects on gain due to xylanase regardless of feed form. Birds fed the processed feed had a better feed:gain when the enzyme was added whereas those fed mash did not have improved feed efficiency when the dietary enzyme was included. Therefore, the enzyme seemed to have a slightly better effect on performance when included in processed feed.

The use of carbohydrases provides an opportunity to improve feed utilization by monogastric animals as well as allow for more flexibility in the inclusion of alternative or lower quality feed ingredients in formulated rations. As ethanol production has expanded, dried distillers grains (DDGS) have become a more common alternative ingredient in U. S. poultry diets (Lumpkins et al., 2004). Incorporating exogenous enzymes into diets might not improve the digestibility of a good quality feed ingredient; however, it can improve nutrient digestibility from lower quality ingredients (Bedford, 2000). Exogenous enzymes are not usually included in corn-soybean based diets because these diets are considered highly digestible (Odetallah et al., 2003; Cowieson, 2005). However, because the expense of feed accounts for such a great proportion of production, there is increased interest to



**Table 9.** Experiment 3: Effect of dietary supplementation of a novel exogenous xylanase in increasing concentrations to broiler chickens in both mash and crumbled wheat-based diets on nitrogen-corrected apparent metabolizable energy (AME<sub>n</sub>) at 21 d.<sup>1</sup>

Effect		Days of age
		21
Feed form		kcal/kg
Mash		2,955 <sup>a</sup>
Crumble		2,899 <sup>b</sup>
SEM (1)		11
Xylanase <sup>2</sup>		
0 ppm		2,887 <sup>bc</sup>
5 ppm		2,880 <sup>c</sup>
10 ppm		2,963 <sup>a</sup>
20 ppm		2,959 <sup>ab</sup>
40 ppm		2,947 <sup>abc</sup>
SEM(4)		18
Feed form	Xylanase <sup>2</sup>	
Mash	0 ppm	2,928 <sup>abc</sup>
Mash	5 ppm	2,930 <sup>abc</sup>
Mash	10 ppm	3,019 <sup>a</sup>
Mash	20 ppm	2,975 <sup>a</sup>
Mash	40 ppm	2,925 <sup>abc</sup>
Crumble	0 ppm	2,846 <sup>bc</sup>
Crumble	5 ppm	2,829 <sup>c</sup>
Crumble	10 ppm	2,907 <sup>abc</sup>
Crumble	20 ppm	2,944 <sup>abc</sup>
Crumble	40 ppm	2,969 <sup>ab</sup>
SEM (43) <sup>3</sup>		25
Source of variation		<i>P</i> values
Feed form		0.001
Xylanase		0.002
Feed form*Xylanase		0.04
Regression <sub>MASH</sub>		0.17
Regression <sub>CRUMBLE</sub>		0.004

<sup>1</sup>Values are means of 6 replicate cages.

<sup>2</sup>One ppm xylanase inclusion provided 667 XU/kg finished feed. One xylanase unit of activity (XU) is the amount of xylanase needed for the release of 1 nanomol of reducing sugars from 0.5% beechwood xylan per second at 50°C in 50 mM trisodium citrate buffer at pH 6.0.

<sup>3</sup>SEM (43) = Standard error of the mean with 43 degrees of freedom.

<sup>a-c</sup>Means within a column with no common superscript are significantly difference ( $P \leq 0.05$ ).

improve nutrient utilization from all dietary ingredients. Cowieson (2010) reported, that for a standard corn-soy diet with average digestibility, there is a loss of about 440 kcal/kg of energy from undigested starch, protein, and fat at the ileal level. This may represent undigested energy that is potentially available for improved digestibility and absorption by use of exogenous enzymes. There may not be a highly measurable improvement compared to those seen in wheat and barley based diets; however, even small improvements could have economically significant value when put on the scale of a large, integrated poultry company.

Based on measured responses in bird performance, digesta viscosity, and AME<sub>n</sub>, it was evident that the xylanase and the carbohydrase were active in both mash and pelleted/crumbled diets used herein. The addition of the xylanase improved energy digestibility of the diets in each experiment. While some level of performance was improved in all 3 trials, the improvements were not manifested the same way in all 3 trials. Total cumulative BW gain was improved in 2 trials. In the other trial, BW gain was improved for one period of growth. While there

was no effect on FCR in the first experiment, FCR was improved in both experiments 2 and 3. It is not unusual for bird response to enzyme supplementation in wheat or barley diets to be variable (Leeson et al., 1996, 2000). Leeson et al. (1996) supplemented both turkey and broiler diets with commercial enzyme. Improvement in body weight gain was variable without any effect on feed:gain. The authors concluded that the effect of the enzyme was greater during the starter periods. Leeson et al. (2000) supplemented broiler diets with several commercial enzymes in both mash and pelleted/crumbled diets. Positive responses were observed but not for every parameter and not always throughout the production period. In addition, AME<sub>n</sub> in the mash diet was not affected by enzyme supplementation.

Similarly to the response in AME<sub>n</sub>, in experiment 1, there was a linear reduction in digesta viscosity as xylanase inclusion level increased in concentration. Reduction in digesta viscosity can be associated with improved nutrient digestion (Bedford, 2000; Zhang et al., 2014), which could explain the uplift in AME<sub>n</sub>. A reduction in digesta viscosity, associated with improvement in AME<sub>n</sub>, can result in improved performance (Bedford and Classen, 1992; Almirall et al., 1995; Choct et al., 1996; Choct et al., 1999; Wu et al., 2004; Gonzalez-Ortiz et al., 2016). However, sometimes there is no correlated improvement in performance (Choct and Annison, 1992; Crouch et al., 1997; Leeson et al., 2000; Woyengo et al., 2008). There can be observed improvements in performance and reduced digesta viscosity without observed dietary AME<sub>n</sub> effects (Leeson et al., 2000).

An increase in digesta viscosity reduces the ability of the gut contents to mix, an action that is critical for micelle formation and the absorption of fat and fat-soluble nutrients (Edney et al., 1989; Wallace and Chesson, 1995; Santos et al., 2004). Increased gut viscosity can also slow digesta gut passage rate as well as limit the accessibility of the digestive enzymes to their substrates (Campbell and Bedford, 1992). A reduction in viscosity could allow endogenous enzymes better access to nutrients in the lumen, as well as improved contact for mucosal surface enzymes. The increased bulk of the digesta due to increased viscosity reduces the diffusion rate of the nutrients to the mucosal surface and limits the interaction between enzyme and substrate (Hesselman and Aman, 1986; Ikegami et al., 1990). The reduction in gut passage rate due to the viscous digesta has also been suggested to increase mucus secretion produced by goblet cells (Choct et al., 1996; Classen, 1996; Smits and Annison, 1996; Bedford and Cowieson, 2012). This may further inhibit the rate nutrient uptake due to the reduced ability of nutrients, especially fat or fat-soluble, to cross the water to reach the mucosal surface (Johnson and Gee, 1981; Classen, 1996; Smits and Annison, 1996). Increased viscosity due to NSP can also have an effect on the integrity of the intestinal morphology itself. This can result in decreased villous height and surface areas (Teirlynch et al., 2009) as well as increased proliferation rates of enterocytes (Smits and

Annison, 1996). Increased proliferation rates of enterocytes can decrease activity of specific epithelial surface enzymes. Not only does this negatively affect the uptake of nutrients, it also increases the maintenance cost of the animal (Zhang et al., 2005; Parsaie et al., 2007). The addition of NSPases also allows NSP to be broken down in a more anterior portion of the small intestine (Hesselman and Aman, 1986; Classen, 1996). This may move the site of digestion of starch and protein to a more anterior portion of the small intestine, which allows more opportunity to absorb the nutrients and leaves a smaller fraction of undigested nutrients energy available to the microflora (Hesselman and Aman, 1986; van der Klis et al., 1993; Bedford, 2000). This might support an increase in the population of undesirable, or pathogenic, microflora (Campbell and Bedford, 1992; Choct et al., 1996; Gehring et al., 2013; Liu and Kim, 2017).

It has been reported that both the intestinal tract and digestive organs such as the pancreas can increase in size to adapt to diets high in indigestible polysaccharides, an effect that can be reversed when those diets are supplemented with carbohydrases (Ikegami et al., 1990; Almirall et al., 1995; Gao et al., 2008). However, in the current study, no differences were observed in the size or weight of the intestine or pancreas. Therefore, the diets were likely digestible enough that the pancreas and digestive tract did not undergo hypertrophy. Although xylanase inclusion beneficially impacted viscosity and energy metabolism, there are indications that the diet overall was digestible enough to provide birds with adequate nutrients for adequate growth.

In conclusion, given the entirety of the data across 3 bird trials, the supplementation of a xylanase in diets containing alternative ingredients resulted in reduced digesta viscosity and an uplift in AME<sub>n</sub> comparable to that provided by a commercial carbohydrase resulting in improved broiler chick growth and feed efficiency (FCR) to 21 d. The bird response to this xylanase was more consistent and measureable for birds fed pelleted/crumbled diets than birds fed mash diets.

## DISCLOSURES

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## REFERENCES

- Abdollahi, M. Reza, Faegheh Zaefarian, and Velmurugu Ravindran. 2019. Maximising the benefits of pelleting diets for modern broilers. *Anim. Prod. Sci.* 59:2023–2028.
- Adeola, O., and A. J. Cowieson. 2011. BOARD-INVITED REVIEW: opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J. Anim. Sci.* 89:189–3218.
- Almirall, M., M. Francesch, A. M. Perez-Vendrell, J. Brufau, and E. Esteve-Garcia. 1995. The differences in intestinal viscosity produced by barley and beta-glucanase alter digesta enzyme activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *Am Inst. Nutr.* 125:947–955.
- Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. *Aust. J. Agric. Res.* 44:405–422.
- Bedford, M. R., and A. J. Cowieson. 2012. Exogenous enzymes and their effects on intestinal microbiology. *Anim. Feed Sci. Technol.* 173:76–85.
- Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition—their current value and future benefits. *Anim. Feed Sci. Technol.* 86:1–13.
- Bedford, M. R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. *Anim. Feed Sci. Technol.* 53:145–155.
- Bedford, M. R., and H. L. Classen. 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. *J. Nutr.* 122:560–569.
- Bedford, M. R., and G. G. Partridge. 2010. Feed enzymes, the future: bright hope or regulatory minefield? Pages 304–311 in *Enzymes in Farm Animal Nutrition*. M. R. Bedford and G. G. Partridge, eds. 2nd ed. CABI, Cambridge, MA.
- Campbell, G. L., and M. R. Bedford. 1992. Enzyme applications for monogastric feeds: a review. *Can. J. Anim. Sci.* 72:449–466.
- Chesson, A. 1993. Feed enzymes. *Anim. Feed Sci. Technol.* 45:65–79.
- Choct, M., and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. *Brit. Poult. Sci.* 33:821–834.
- Choct, M., R. J. Hughes, and M. R. Bedford. 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. *Brit. Poult. Sci.* 40:419–422.
- Choct, M., R. J. Hughes, J. Wang, M. R. Bedford, A. J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *Brit. Poult. Sci.* 37:609–621.
- Classen, H. L. 1996. Cereal grain starch and exogenous enzymes in poultry diets. *Anim. Feed Sci. Technol.* 62:21–27.
- Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. *Anim. Feed Sci. Technol.* 119:293–305.
- Cowieson, A. J. 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. *J. Poult. Sci.* 47:1–7.
- Cowieson, A. J., and H. V. Masey O'Neill. 2013. Effects of exogenous xylanase on performance, nutrient digestibility and caecal thermal profiles of broilers given wheat-based diets. *Brit. Poult. Sci.* 54:346–354.
- 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 or broilers and turkeys. *J. Appl. Poult. Res.* 6:147–154.
- Donohue, M., and D. L. Cunningham. 2009. Effects of grain and oil-seed prices on the costs of US poultry production. *J. Appl. Poult. Res.* 18:325–337.
- Edney, M. J., G. L. Campbell, and H. L. Classen. 1989. The effect of  $\beta$ -glucanase supplementation on nutrient digestibility and growth in broilers given diets containing barley, oat groats or wheat. *Anim. Feed Sci. Technol.* 25:193–200.
- Gao, F., Y. Jiang, G. H. Zhou, and Z. K. Han. 2008. The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diets. *Anim. Feed Sci. Technol.* 142:173–184.
- Gehring, C. K., M. R. Bedford, and W. A. Dozier III. 2013. Interactive effects of phytase and xylanase supplementation with extractable salt-soluble protein content of corn in diets with adequate calcium and nonphytate phosphorus fed to broilers. *Poult. Sci.* 92:1858–1869.
- Gonzalez-Ortiz, G., O. Olukosi, and M. R. Bedford. 2016. Evaluation of the effect of different wheats and xylanase supplementation on performance, nutrient and energy utilisation in broiler chicks. *Anim. Nutr.* 2:173–179.
- Hesselman, K., and P. Aman. 1986. The effect of beta-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low- or high-viscosity. *Anim. Feed Sci. Technol.* 15:83–93.

- Horton, H. R., L. A. Moran, K. G. Scrimgeour, M. D. Perry, and J. D. Rawn. 2006. *Principles of Biochemistry* (4th ed). Neil Patterson Publishers, Prentice Hall Inc., Hoboken, NJ.
- Hosseini, S. M., and M. Afshar. 2017. Effects of feed form and xylanase supplementation on performance and ileal nutrients digestibility of heat-stressed broilers fed wheat–soybean diet. *J. Appl. Animal Res.* 45:550–556.
- Ikegami, S., F. Tsuchihashi, H. Harada, N. Tsuchihashi, E. Nishide, and S. Innami. 1990. Effect of viscous indigestible polysaccharides on pancreatic-biliary secretion and digestive organs in rat. *J. Nutr.* 120:353–360.
- Jensen, L. S., L. H. Merrill, C. V. Reddy, and J. McGinnis. 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poult. Sci.* 41:1414–1419.
- Leeson, S., L. J. Caston, and D. Yungblut. 1996. Adding Roxazyme to wheat diets of chicken and turkey broilers. *J. Appl. Poult. Res.* 5:167–172.
- Johnson, I. T., and J. M. Gee. 1981. Effect of gel-forming gums on the intestinal unstirred layer and sugar transport in vitro. *Gut* 22:398–403.
- Leeson, S., L. Caston, M. M. Kiaei, and R. Jones. 2000. Commercial enzymes and their influence on broilers fed wheat or barley. *J. Appl. Poult. Res.* 9:242–251.
- Liu, W. C., and I. H. Kim. 2017. Effects of dietary xylanase supplementation on performance and functional digestive parameters in broilers fed wheat-based diets. *Poult. Sci.* 96:566–573.
- Lumpkins, B. S., A. B. Batal, and N. M. Dale. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. *Poult. Sci.* 83:1891–1896.
- Masey O'Neil, H. V., 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.
- Mateos, G. G., L. Camara, G. Fondevila, and R. P. Lazaro. 2019. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. *J. Appl. Poult. Res.* 28:506–525.
- Mathews, K. H. Jr., and M. J. McConnell. 2009. Ethanol Co-Product use in U.S. Cattle Feeding: Lessons Learned and Considerations. *A report from the Economic Research Service*. Economic Research Service, USDA, Washington, DC A report from the Economic Research Service.
- Nian, F., Y. M. Guo, Y. J. Ru, F. D. Li, and A. Peron. 2011. Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. *Asian-Aust. J. Anim. Sci.* 24:400–406.
- Odetallah, N. H., J. J. Wang, J. D. Garlich, and J. C. H. Shih. 2003. Keratinase in starter diets improves growth of broiler chicks. *Poult. Sci.* 82:664–670.
- Parsaie, S., F. Shariatmadari, M. J. Zamiri, and K. Khajeh. 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers compared with a maize-based diet. *Brit. Poult. Sci.* 48:594–600.
- Peron, A., and G. G. Partridge. 2010. Other enzyme applications relevant to the animal feed industry. Pages 231–248 in *Enzymes in Farm Animal Nutrition*, Volume 9. M. R. Bedford and G. G. Partridge, eds. 2nd ed. CABI, Cambridge, MA.
- Peterson, S. T., J. Wiseman, and M. R. Bedford. 1999. Effects of age and diet on the viscosity of intestinal contents in broiler chicks. *Brit. Poult. Sci.* 40:364–370.
- Ross 708 Broiler Nutrition Supplement, 2009.
- Santos, A. A. Jr., P. R. Ferket, J. L. Grimes, and F. W. Edens. 2004. Dietary supplementation of endoxylanases and phospholipase for turkeys fed wheat-based rations. *Inter. J. Poult. Sci.* 3:20–32.
- Smits, C. H. M., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition—towards a physiologically valid approach to their determination. *World Poult. Sci. J.* 52:203–221.
- Svihus, B., A. K. Uhlen, and O. M. Harstad. 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review. *Anim. Feed Sci. Technol.* 122:303–320.
- Teirlynch, E., L. Bjerrum, V. Eeckhaut, G. Huygebaert, F. Pasmans, F. Haesebrouck, J. Dewulf, R. Ducatelle, and F. V. Immerseel. 2009. The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chicks. *Br. J. Nutr.* 102:1453–1461.
- Turner, P., G. Mamo, and E. Nordberg Karlsson. 2007. Potential and utilization of thermophiles and thermostable enzymes in biorefining. *Microb. Cell Fact.* 6:9.
- Van der Klis, J. D., M. W. A. Verstegen, and A. Van Voorst. 1993. Effect of a soluble polysaccharide (carboxy methyl cellulose) on the absorption of minerals from the gastrointestinal tract of broilers. *Brit. Poult. Sci.* 34:985–997.
- Vogtmann, H., H. P. Pfirter, and A. L. Prabucki. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. *Brit. Poult. Sci.* 16:531–534.
- Wallace, R. J., and A. Chesson. 1995. *Biotechnology in animal feeds and animal feeding*. p 301.
- Wang, Z. R., S. Y. Qiao, W. Q. Lu, and D. F. Li. 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poult. Sci.* 84:875–881.
- Wang, Z., S. Cerrate, C. Coto, F. Yan, and P. W. Waldroup. 2007. Utilization of distillers dried grains with solubles (DDGS) in broiler diets using a standardized nutrient matrix. *Int. J. Poult. Sci.* 6:470–477.
- Woyengo, T. A., W. Guenter, J. S. Sands, C. M. Nyachoti, and M. A. Mirza. 2008. Nutrient utilisation and performance responses of broilers fed a wheat-based diet supplemented with phytase and xylanase alone or in combination. *Anim. Feed Sci. Technol.* 146:113–123.
- 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 and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *Brit. Poult. Sci.* 45:76–84.
- Yanez, J. L., B. Beltranena, M. Cervantes, and R. T. Zijlstra. 2011. Effect of phytase and xylanase supplementation or particle size on nutrient digestibility of diets containing distillers dried grains with solubles cofermented from wheat and corn in ileal-cannulated grower pigs. *J. Anim. Sci.* 89:113–123.
- Zhang, A. W., B. D. Lee, S. K. Lee, K. W. Lee, G. H. An, K. B. Song, and C. H. Lee. 2005. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. *Poult. Sci.* 84:1015–1021.
- Zhang, L., J. Xu, L. Lei, Y. Jiang, F. Gao, and G. H. Zhou. 2014. Effects of xylanase supplementation on growth performance, nutrient digestibility and non-starch polysaccharide degradation in different sections of the gastrointestinal tract of broilers Fed Wheat-based Diets. *Asian-Aust. J. Anim. Sci.* 855–861 2014 Jun; 27.