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

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 (AMEn), 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, AMEn, 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 AMEn. The XYL included as low as 1 ppm resulted in a significant increase in AMEn 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 calorific content of the diet influenced the increase in AMEn 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 AMEn, and improved bird performance represented by either BW gain or FCR.

Key words: xylanase, enzyme, AMEn, digesta viscosity, broiler

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.
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 (Chcott and Annison, 1992; Chcott 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). 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 (AMEn).

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; Chcott et al., 1996, 1999; Wu et al., 2004; Gonzalez-Ortiz et al., 2016). However, others have demonstrated that this correlation does not always occur (Chcott 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-β-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, AMEn, 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).
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 AMEn.

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 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 to produce 1 nanomol of reducing sugars from 5% beechwood xylan per second at 50°C in 50 mM 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 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.

| Ingredient | Exp. 1 | Exp. 2 | Exp. 3 |
|------------|--------|--------|--------|
| 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 |
| 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 |

1. 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.
2. 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.
3. NaSeO₃ premix provided 0.3 mg Se/kg of complete feed.
4. Diatomaceous earth, inert reference material included in feed for analysis of nitrogen-corrected metabolizable energy (AMEᵦ) (World Minerals, Inc., Santa Barbara, CA).
5. 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.

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

| Ingredient | Experiment 1 | Experiment 2 | Experiment 3 |
|------------|--------------|--------------|--------------|
| Xylanase¹ | ppm | 0, 1, 2, 4, 8, 16 | 0, 20 | 0, 20 | 0, 5, 10, 20, 40 |
| Form | Mash | Crumble⁵ | Mash | Crumble⁵ |
| Energy² | 2,770 | 2,770 | 2,920 | 3,070 | 2,770 |
| Kcal/kg | 0 | 0 | 4.0 | 8.0 | 0 |
| Cornstarch³ | 0 | 0 | 0 | 0 | 0 |

¹Mono-component endo-β-1,4-xylanase engineered to be thermostable (BioResource International Inc. Durham, NC)
²ME poultry.
³Added 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.
⁴Pelleted 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 carbohydrate cocktail (CC) (Rovabio Excel, Adisseo, Antony, France). The enzyme cocktail contained enzyme activities of endo-1,4-β-xylanase, endo-1,3(4)β-glucanase, and endo-1,4-β-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 and higher (6.20 vs. 2.24%) poultry fat content than the soybean meal (23%)-corn DDGS (5.5%)-based crumble mash and crumble form (Table 2).

Each XYL inclusion level was prepared in both pelleted and crumbled form (Table 2). Divided in half, and one half received XYL to produce the 5 experimental levels of XYL (0, 5, 10, 20, 40 ppm). The 0 and 40 XYL feed were blended to create the 5 experimental levels of XYL (0, 5, 10, 20, 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 aliquot 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 pelletized 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 ileocecal 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 μL 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⁻¹ and 45 sec⁻¹.

Apparent Metabolizable Energy

To measure AMEn, 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 (Nifeed) and excreta (Niecreta) 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 AMEn was calculated using the following equations:

\[
N_{\text{Retained}} = N_{\text{feed}} - \left( (N_{\text{excreta}} \times AIA_{\text{feed}}) / AIA_{\text{excreta}} \right)
\]

\[
\text{AME}_{\text{n}} = \frac{GE_{\text{feed}} - \left( (GE_{\text{excreta}} \times AIA_{\text{feed}}) / AIA_{\text{excreta}} \right)}{8.22 \times N_{\text{Retained}}}
\]
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 AMEn; 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 NL MIXED). 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 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 the pancreas.

| Source of variation | P values |
|---------------------|---------|
| Treatment           |         |
| Regression          |         |
| SEM (25)            | 0.005  |

| Treatment | AME  | AvgVisc  |
|-----------|------|----------|
| NC        | 2776 | 13.29    |
| 1 ppm     | 2814 | 9.89     |
| 2 ppm     | 2853 | 7.91     |
| 4 ppm     | 2874 | 8.08     |
| 8 ppm     | 2903 | 5.95     |
| 16 ppm    | 2918 | 6.76     |
| CC        | 2848 | 6.79     |

| Source of variation | P values |
|---------------------|---------|
| Treatment           | 0.06    |
| Regression          | 0.005   |
| SEM (28)            | 0.995   |

1Values are means of 5 replicate pens of ca. 8 birds per pen.
2One 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.
3SEM (25) = Standard error of the mean with 25 degrees of freedom.
4$^{a-c}$Means within a column with no common superscript are significantly different ($P \leq 0.05$).

### 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.

| Treatment | BWG | FI  | FCR |
|-----------|-----|-----|-----|
| NC        | 210 | 308 | 306 |
| 1 ppm     | 232 | 334 | 323 |
| 2 ppm     | 217 | 304 | 310 |
| 4 ppm     | 215 | 309 | 311 |
| 8 ppm     | 226 | 317 | 323 |
| 16 ppm    | 231 | 310 | 326 |
| CC        | 225 | 305 | 321 |

| Source of variation | P values |
|---------------------|---------|
| Treatment           | 0.85    |
| Regression          | 0.86    |
| SEM (25)            | 2.8     |

### 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 (AMEn) and ileal digesta viscosity at 21 d.

| Treatment | AME  | AvgVisc  |
|-----------|------|----------|
| NC        | 2776 | 13.29    |
| 1 ppm     | 2814 | 9.89     |
| 2 ppm     | 2853 | 7.91     |
| 4 ppm     | 2874 | 8.08     |
| 8 ppm     | 2903 | 5.95     |
| 16 ppm    | 2918 | 6.76     |
| CC        | 2848 | 6.79     |

1Values are means of 5 replicate pens of ca. 8 birds per pen.
2One 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.
3SEM (25) = Standard error of the mean with 25 degrees of freedom.
4$^{a-c}$Means within a column with no common superscript are significantly different ($P \leq 0.05$).
duodenum, jejunum, or ileum neither as actual weight with bird weight used as a covariate nor when expressed as a percentage of BW.

Based on regression analysis, dietary AMEn increased with increasing level of XYL inclusion with AMEn leveling off at the highest inclusion (Table 4).

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.1

| Treatment3 | Percentage of body weight | Length2 | Weight2 |
|------------|---------------------------|---------|---------|
|            | (%)                       | (cm)    | (g)     |
| 1 ppm      | 1.01                      | 1.86    | 1.43    | 0.36    | 22.2 | 52.6 | 52.1 | 7.78 | 13.20 | 10.32 | 2.39 |
| 2 ppm      | 1.08                      | 1.82    | 1.37    | 0.38    | 21.0 | 53.5 | 53.3 | 6.95 | 12.79 | 9.85  | 2.50 |
| 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 | 53.2 | 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 |

P values

| Treatment3 | 0.18 | 0.87 | 0.40 | 0.68 | 0.37 | 0.87 | 0.55 | 0.27 | 0.89 | 0.45 | 0.67 |
| SEM (95)4 | 0.040 | 0.065 | 0.051 | 0.014 | 0.048 | 1.28 | 1.28 | 0.28 | 0.450 | 0.346 | 0.089 |

1Values are means of 15 replicate birds per treatment. D, duodenum; J, jejunum; I, ileum; Panc, pancreas.
2Weight and length were analyzed using bird bodyweight as a covariate.
3One 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.
4SEM (95) = Standard error of the mean with 95 degrees of freedom.

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

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.1

| Days of age | BWG | FI | FCR |
|-------------|-----|----|-----|
| 0−7         |     |    |     |
| 7−14        |     |    |     |
| 14−21       |     |    |     |
| 0−14        |     |    |     |
| 14−21       |     |    |     |
| 0−21        |     |    |     |

| Effect Energy | 2770 | 2920 | 3070 | SEM (2) |
|---------------|------|------|------|---------|
| 0 ppm         | 112a | 107a | 101b | 2.4     |
| 20 ppm        | 109  | 106  | 101b | 5.8     |
| SEM (1)       | 1.9  | 1.9  | 1.9  | 0.8     |

| Xylanase2     | 0 ppm | 20 ppm | SEM (28)3 |
|---------------|-------|--------|-----------|
| 0 ppm         | 110   | 106    | 3.3       |
| 20 ppm        | 115   | 106    | 9.7       |
| SEM (28)3     | 1.0   | 0.7    | 0.03      |

| Source of variation | P values |
|---------------------|----------|
| Energy              | 0.008    | 0.13   | 0.70    |
| Xylanase            | 0.004    | 0.98   | 0.56    |
| Energy*Xylanase     | 0.004    | 0.08   | 0.18    |

1Values are means of 6 replicate cages of ca. 6 birds per cage.
2One 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.
3SEM (28) = Standard error of the mean with 28 degrees of freedom.
4Means within a column with no common superscript are significantly different (P ≤ 0.05).
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 (AMEn) at 21 d.1

| Effect       | Energy kcal ME/kg | Days of age |
|--------------|-------------------|-------------|
| Source of variation |                  | 21          |
| Energy       |                   |             |
| 2.770        | 3.191             |             |
| 2.920        | 3.226             |             |
| 3.070        | 3.274             |             |
| SEM (2)      |                   |             |
| 0 ppm        | 3147              |             |
| 20 ppm       | 3320              |             |
| SEM (1)      |                   |             |
| Energy       | Xylanase2         |             |
| 2770         | 0 ppm             | 3159 b      |
| 2770         | 20 ppm            | 3224 a b    |
| 2920         | 0 ppm             | 3109 b      |
| 2920         | 20 ppm            | 3363 a      |
| 3070         | 0 ppm             | 3175 b      |
| 3070         | 20 ppm            | 3374 a      |
| SEM (28)     |                   | 37          |
| Source of variation |                  | P values    |
| Energy       | 0.10              |             |
| Xylanase     | <0.0001           |             |
| Energy×Xylanase |                | 0.05        |

1Values are means of 6 replicate cages.
2One 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.
3SEM (28) = Standard error of the mean with 28 degrees of freedom.
4Means within a column with no common superscript are significantly different (P ≤ 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 d), 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 AMEn 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 AMEn values. Higher AMEn 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 AMEn 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 AMEn (Table 9). Somewhat unexpectedly, the main affect mean for the mash AMEn was greater than for the main affect mean for crumble AMEn. However, there was a form by enzyme interaction. For the interaction means at the
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.

| Feed form | Days of age | BWG (grams/bird) | FI (grams/bird) | FCR (grams FI/grams BWG) |
|-----------|-------------|------------------|-----------------|--------------------------|
|           | 0–7         | 7–14             | 14–21           | 0–14                     | 0–21                     | 0–7           | 0–21                     |
| **Mash**  |             |                  |                 |                          |                          |               |                          |
| 0 ppm     | 98          | 274              | 445             | 375                      | 820                      | 251           | 1,400                     | 2.653         | 1.730         |
| 5 ppm     | 118         | 301              | 462             | 418                      | 884                      | 239           | 1,480                     | 2.134         | 1.677         |
| 10 ppm    | 116         | 283              | 436             | 417                      | 829                      | 240           | 1,421                     | 2.171         | 1.709         |
| 20 ppm    | 119         | 299              | 462             | 417                      | 890                      | 238           | 1,444                     | 2.199         | 1.647         |
| 40 ppm    | 124         | 303              | 462             | 434                      | 890                      | 253           | 1,488                     | 2.162         | 1.696         |
| SEM (4)   | 2.9         | 9.2              | 8.0             | 8.6                      | 17.8                     | 8.7           | 34.0                      | 0.124         | 0.036         |
| **Crumble** |            |                  |                 |                          |                          |               |                          |
| 0 ppm     | 118         | 301              | 462             | 418                      | 884                      | 239           | 1,480                     | 2.134         | 1.677         |
| 5 ppm     | 116         | 283              | 436             | 417                      | 829                      | 240           | 1,421                     | 2.171         | 1.709         |
| 10 ppm    | 119         | 299              | 462             | 417                      | 890                      | 238           | 1,444                     | 2.199         | 1.647         |
| 20 ppm    | 124         | 303              | 462             | 434                      | 890                      | 253           | 1,488                     | 2.162         | 1.696         |
| 40 ppm    | 122         | 308              | 489             | 434                      | 906                      | 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         |

| Source of variation | P values |
|---------------------|----------|
| Feed form           | <0.0001  |
| Xylanase            | 0.40     |
| Feed form*Xylanase  | 0.79     |
| RegressionMASH      | 0.22     |
| RegressionCRUMBLE   | 0.22     |

1 Values are means of 6 replicate cages of ca. 6 birds per cage.
2 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.
3 SEM (43) = Standard error of the mean with 43 degrees of freedom.
4 a-c Means within a column with no common superscript are significantly different (P ≤ 0.05).

nonenzyme supplemented level (0 ppm), the mash and crumble AMEn means are not significantly different. While it is generally accepted that heat processing and pelleting of feed improve bird performance and AMEn, 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. 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

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 results of this experiment further support the findings of Abdollahi et al. (2019) and show that the inclusion of xylanase in both mash and crumbled wheat-based diets have a better effect on growth and feed efficiency compared to those fed the equivalent mash diet. 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.
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 AMEn, 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, AMEn in the mash diet was not affected by enzyme supplementation.

Similarly to the response in AMEn, 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 AMEn. A reduction in digesta viscosity, associated with improvement in AMEn, can result in improved performance (Bedford and Classen, 1992; Almirall et al., 1995; Choc et al., 1996; Choc et al., 1999; Wu et al., 2004; Gonzalez-Ortiz et al., 2016). However, sometimes there is no correlated improvement in performance (Choc 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 AMEn 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 Chesn, 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 (Choc 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 produce increased proliferation rates of enterocytes (Smits and

**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 (AMEn) at 21 d.1

| Feed form | kCal/kg | Effect 21 | SEM (4) |
|-----------|---------|------------|---------|
| Mash 0 ppm | 2.928abc | 0.001 | 0.17 |
| Mash 5 ppm | 2.930abc | 0.002 | 0.04 |
| Mash 10 ppm | 3.019abc | 0.002 | 0.04 |
| Mash 20 ppm | 2.975abc | 0.001 | 0.17 |
| Mash 40 ppm | 2.925abc | 0.001 | 0.17 |
| Crumble 0 ppm | 2.846abc | 0.002 | 0.04 |
| Crumble 5 ppm | 2.829abc | 0.001 | 0.17 |
| Crumble 10 ppm | 2.907abc | 0.002 | 0.04 |
| Crumble 20 ppm | 2.944abc | 0.001 | 0.17 |
| Crumble 40 ppm | 2.969abc | 0.002 | 0.04 |

1Values are means of 6 replicate cages.
2One 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.
3SEM (43) = Standard error of the mean with 43 degrees of freedom.
4Values are means of 6 replicate cages.
5Means within a column with no common superscript are significantly different (P ≤ 0.05).
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; Chocòt 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 carbohydrates (Ikemani 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 AMEn comparable to that provided by a commercial carbohydrate 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

The authors declare no conflicts of interest. Funding sources had no role in any aspect of the preparation of this article.

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