*Indian Journal of Animal Sciences* **88** (1): 105–110, January 2018/Article

# Effects of xylanase on yellow-feather broiler diets

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Received: 5 July 2017; Accepted: 13 July 2017

ABSTRACT

To evaluate the effect of xylanase on broiler diets, we carried out two trials. In experiment 1, broilers were randomly assigned to six wheat-maize-soybean meal diet groups with different xylanase concentrations (0–500 mg/kg). In experiment 2, broilers were randomly assigned to several experimental groups consisting of two metabolic methods (total tract excreta collection and ileal digesta collection) and two xylanase concentrations (0 and 244.23 mg/kg). Based on the results, xylanase supplementation significantly increased the digestibility of dry matter, gross energy and apparent metabolizable energy. These corresponding values were maximized at 300 mg/kg xylanase. The coefficients of variation (CVs) for DM, GE and AME in the ileal digesta collection method were about 10%, whereas those in the total tract collection method were only about 1.7%. Regression analysis showed that a segmented model satisfactorily described the dose-response relationship. Compared to the ileal digesta collection method, the total tract excreta collection method was more efficient for evaluating the effects of xylanase. These results provide valuable information on the optimal nutrition of broilers.

**Key words**: Broiler nutrition, Digestibility, Regression model, Xylanase

Xylanase is an enzyme that is widely used in broiler diets because it improves growth performance (Gonzalez-Ortiz *et al*. 2017, Liu and Kim 2017) and nutrient digestibility (Stefanello *et al.* 2016, Amerah *et al.* 2017, Gonzalez-Ortiz *et al*. 2017). For maximizing nutrient utilization and energy conversion of wheat, distiller's dried grains with solubles (DDGS) (Ayasan and Karakozak 2009) and other cereals, it is important to establish an optimal concentration of xylanase in broiler diet. Even though the relationships between xylanase and nutrient digestibility or apparent metabolizable energy (AME) have been established, there are no models that reliably describe these relationships. Therefore, it is important to determine whether the response of broilers to supplemental xylanase can be predicted with a simple model equation.

Total tract excreta collection, force-feeding after fasting and ileal digesta collection methods are currently used to measure metabolizable energy and nutrient digestibility of feed ingredients or compound feed (Nortey *et al.* 2008). However, it is not clear which metabolic method is the most efficient for evaluating the function of exogenous enzyme xylanase.

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Since xylanase do not show obvious effects on performance when the diet provides adequate energy to broilers (Barbosa *et al.* 2012, Singh *et al.* 2012), we used a low-energy diet as the control in this present study. The objectives of this study were to predict the optimal concentration of xylanase in a wheat-maize-soybean meal by measuring total tract digestibility and AME from excreta and establish the most efficient way to evaluate the function of xylanase by comparing the total tract excreta collection method to the ileal digesta collection method.

## MATERIALS AND METHODS

*Diets:* The diet used (Table 1) met the requirements for Chinese yellow-feather male broilers (Ministry of Agriculture of China, 2004). Beta-1,4-xylanase (activity: 30,000 U·g$^{-1}$) from *Aspergillus niger* was acquired from Youteer Biotechnology Company (Hunan, China). In the control diets, xylanase was replaced with an equal amount of sand as an inert filler. The diets were devoid of drug additives.

*Animal studies:* Animal experiments were conducted at Wen's Foodstuffs Group Co. Ltd. (Guangdong, China) according to the animal care and handling procedures of the College of Animal Science of South China Agricultural University (Guangzhou, China). This study was reviewed and approved by the local animal care committee. Two animal experiments were conducted.

In experiment 1, we studied the effect of xylanase on the digestibility of a wheat-maize-soybean meal diet. One

ninety two, 18-week-old Chinese yellow broilers (a local broiler breed) were sorted according to their initial weight (about 2.3-2.5 kg), to ensure that all groups had similar average weight (2.43±0.12 kg). We placed the broilers in individual cages (0.50 m × 0.42 m × 0.55 m) in an environmentally controlled room (25°C, 12–h photoperiod) and randomly divided them into six groups. Each group was fed with different xylanase concentrations (0, 100, 200, 300, 400 and 500 mg/kg). There were eight replicates in each group, with four broilers in each replicate. Prior to the feeding trial, the broilers were fed the same commercial diet (Table 1) and had *ad libitum* access to water via a suspended nipple drinker line. All broilers remained healthy throughout the study.

In experiment 2, we compared the total tract excreta collection method to the ileal digesta collection method. Eighteen-week-old Chinese yellow broilers (256) were sorted and placed in individual cages as in experiment 1, and randomly divided into four groups with 16 replicates in each group and four broilers in each replicate. Total tract excreta collection was performed with half of the broilers, and ileal digesta collection was performed with the other half. Each group received two treatments of two different xylanase concentrations (0 and 244.23 mg/kg).

*Experimental protocol:* A modified metabolizable energy bioassay was used in the total tract digestibility experiment. The bioassay included a 96-h adaptation period (*ad libitum* amount), a 17–h fasting period and a 72–h excreta collection period, which consisted of a 55-h *ad libitum* intake period and a 17-h fasting period. Feed intake was measured and excreta was collected for analysis every 12 h for 3 d. Additionally, the bioassay was used in the ileal digesta digestibility experiment (Adedokun *et al.* 2008, Kim *et al.* 2011). A 55-h *ad libitum* intake period was carried out (the broilers in this group did not undergo the 17-h fasting). After 4 h, broilers were sacrificed by intracardial administration of sodium pentobarbital (30 mg/kg of BW) and jugular exsanguination. The ileal section between the terminal ileum and Meckel's diverticulum was removed, and the contents of the ileum were flushed with distilled water. Chromic oxide (0.3%) was added to the diets to replace the sand, and nutrient concentrations were calculated by the difference between the concentrations of chromic oxide in the diet and digesta.

Table 1. Ingredients and nutrient composition (as-fed basis) of the wheat-maize-soybean meal control diet.

| Ingredient | Content (%) | Nutrient | Content |
|---|---|---|---|
| Wheat | 40.01 | Dry matter (%) | 87.86 |
| Maize | 39.60 | Gross energy (cal·kg$^{-1}$) | 3,852 |
| Soybean meal | 15.50 | ME‡ (cal·kg$^{-1}$) | 2,950 |
| Soybean oil | 0.81 | Crude protein (%) | 16.01 |
| Dicalcium phosphate | 1.31 | Calcium‡ (%) | 0.80 |
| Limestone | 1.01 | Total phosphorus[b] (%) | 0.59 |
| Premix[a] | 0.60 | | |
| L-Lys HCl | 0.45 | | |
| Sodium bicarbonate | 0.20 | | |
| Salt | 0.16 | | |
| DL-Met | 0.18 | | |
| L-Thr | 0.08 | | |
| Choline chloride | 0.10 | | |
| Total | 100.00 | | |

[a]Supplied per kg of diet: vitamin A (retinyl acetate), 2,700 IU; vitamin D3 (cholecalciferol), 400 IU; vitamin E (dl-a-tocopheryl acetate), 10 IU; vitamin K3, 0.5 mg; thiamine, 2.0 mg; riboflavin, 5.0 mg; pantothenic acid, 10.0 mg; niacin, 30 mg; pyridoxine, 3.0 mg; folic acid, 0.5 mg; biotin, 120 µg; vitamin B12, 10 µg; antioxidant, 120 mg; Mn, 80 mg; Zn, 80 mg; Cu, 8 mg; Fe, 80 mg; I, 0.7 mg; and Se, 0.3 mg. [b]Calculated according to apparent metabolizable energy values of feedstuffs for broiler (Ministry of Agriculture of China, 2004).

*Sample collection and chemical assays:* Excreta or digesta was pooled and stored at –20°C. After thawing, excreta or digesta was oven-dried at 65°C for 48 h and allowed to equilibrate at room temperature for 24 h. Prior to analysis, the samples were ground using a mill (Wenling, Zhejiang, China) coupled to a 0.5-mm mesh screen. The dry matter (DM) of the diets and excreta or digesta were determined by oven drying at 105°C for 5 h. The energy contents of the diets and excreta or digesta were determined by bomb calorimetry using a Parr 6400 automatic adiabatic calorimeter (Parr Instrument Co., Moline, Chicago, American. IL), with benzoic acid as a standard. The amounts of chromic oxide in the diets and digesta were analyzed spectrophotometrically at 440 nm after ashing overnight at 450°C. Each chemical component was determined in duplicate.

*Statistical analysis:* Experiments 1 and 2 had a completely randomized design and a 2 × 2 factorial completely randomized design, respectively. Data were analyzed using SAS software (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was performed to determine the significance of the primary effects. The statistical significance of differences between the individual treatments was assessed using the least significant difference test at $P<0.05$. Mean values were used for regression analysis using linear, quadratic, cubic and segmented procedures. The general models of segmented regression were $Y = a + b \times X + c \times X^2$ ($X < X_0$) and $Y = a + b \times X_0 + c \times X_0^2$ ($X > X_0$). For values of $X < X_0$, the equation relating Y and X was quadratic (a parabola), and for values of $X > X_0$, the equation was a horizontal line. Xylanase supplementation was estimated as the breakpoint. Models were fitted to xylanase data using the general linear model and non-linear procedures of SAS. Mean square error (MSE) and coefficient of determination ($R^2$) were used to assess the goodness of fit of the different models.

## RESULTS AND DISCUSSION

Compared to the control group, the xylanase-supplemented groups had higher total tract digestibility, DM and gross energy (GE) digestibility, and AME ($P<0.05$; Table 2). Regression analysis showed that DM, GE and AME followed a quadratic model ($P<0.05$) with increasing

xylanase concentration. The differences between the treatments (100–500 mg/kg) were not significant (Table 2), and the lack-of-fit tests in the linear models were statistically significant (P< 0.05; Table 3), thereby revealing the unsuitability of the linear models. Compared to the quadratic model, the segmented model had higher $R^2$ values and lower MSE.

Xylanase supplementation from 100 to 500 mg/kg significantly increased DM by 3.85%–6.65%, GE by 3.41%–6.40% and AME by 3.41%–6.39% (P<0.05). These results were consistent with those reported by Liu *et al.* (2011), Francesch *et al.* (2012) and Guo *et al.* (2014). Xylanase supplementation decreases the digesta viscosity of wheat-based diets (Francesch *et al.* 2012, Zhang *et al.* 2014, Liu and Kim 2017), reduces arabinose and xylose concentration and total non-starch polysaccharides ileal digestion (Barekatain *et al.* 2013, Zhang *et al.* 2014), increases chymotrypsin and lipase activities (Engberg *et al.* 2004), and raises peptide YY and insulin levels (Singh *et al.* 2012). Xylanase supplementation improves the apparent digestibility of proteins, lipids and DM, and dietary AME (Francesch *et al.* 2012). However, xylanase supplementation in DDGS did not improve energy digestibility and masked xylanase effects (Yanez *et al.* 2011).

Even though there were no significant differences among treatments (P>0.05), DM, GE and AME reached maximum values at 300 mg/kg xylanase. At xylanase concentration of 500 mg/kg, the studied parameters did not improve (P > 0.05), consistent with the findings of a previous report (Zhang *et al.* 2012). Furthermore, regression analysis showed significant linear (P<0.05) and quadratic (P<0.05) responses of DM, GE and AME in wheat-maize-soybean meal diets with increasing xylanase concentrations. Similarly, Nitrayova *et al.* (2009) reported a significant linear regression between enzyme level and total tract energy digestibility, and Barrera *et al.* (2004) observed that nutrient digestibility increased initially and subsequently decreased with increasing xylanase concentration.

To achieve the maximum benefit of dietary supplements, it is important to derive a mathematical model and predict the optimal concentration of the enzyme in the diet based on the relationship between enzyme addition and nutrient digestibility. A prediction model from the curve-fitting analysis was constructed to determine the optimal concentration of xylanase in the wheat-maize-soybean meal diet. Even though Liu and Kim (2017) found that linear model better describes the dose-response relationship of xylanase in broilers, our experimental data revealed a significant lack of fit for a linear model (P<0.05).

Table 2. Effect of xylanase supplementation on total tract energy (DM and GE) and AME in 18-week-old yellow-feather male broilers.

| Xylanase (mg/kg) | Total tract digestibility | | AME (cal/kg) |
|---|---|---|---|
| | DM (%) | GE (%) | |
| 0 | 74.04** | 76.22** | 3,334** |
| 100 | 76.89* | 78.82* | 3,447* |
| 200 | 78.42* | 80.57* | 3,524* |
| 300 | 78.97* | 81.10* | 3,547* |
| 400 | 77.60* | 80.23* | 3,509* |
| 500 | 77.66* | 79.93* | 3,495* |
| SEM | 0.91 | 0.82 | 35.55 |
| *p*-value | 0.008 | 0.002 | 0.002 |
| Linear | 0.003 | 0.001 | 0.001 |
| Quadratic | 0.001 | 0.002 | 0.002 |
| Cubic | 0.376 | 0.556 | 0.551 |

*P<0.05, **P<0.01.

Table 3. Lack-of-fit analysis for the linear model between xylanase concentration and dry matter (DM), gross energy (GE), and apparent metabolizable energy (AME).

| Variable | Source | SS[a] | Df[b] | MS[c] | F | Pr > F |
|---|---|---|---|---|---|---|
| DM | Lack of fit | 71.05 | 4 | 17.76 | 3.82 | 0.011 |
| | Pure error | 162.71 | 35 | 4.64 | | |
| GE | Lack of fit | 63.08 | 4 | 15.77 | 3.10 | 0.028 |
| | Pure error | 177.76 | 35 | 5.08 | | |
| AME | Lack of fit | 120,455 | 4 | 30,113 | 3.12 | 0.027 |
| | Pure error | 338,132 | 35 | 9,660 | | |

[a]Sum of squares; [b]Degrees of freedom; [c]Mean square.

Table 4. Estimates of dry matter (DM), gross energy (GE) and apparent metabolizable energy (AME) for different concentrations of xylanase in broiler diets, using quadratic and segmented models.

| Parameter | Model | Model parameters[a] | $R^2$ | MSE[b] | *P* value |
|---|---|---|---|---|---|
| DM | Quadratic | a = 74.26 | | | |
| | | b = 2.87 | 0.528 | 6.37 | 0.001 |
| | | c = -0.46 | | | |
| | Segmented | a = 75.14 | | | |
| | | b = -0.03 | | | |
| | | c = $-9 \times 10^{-5}$ | 0.913 | 0.435 | 0.026 |
| | | Plateau = 78.15 | | | |
| | | $x_0^3$ at plateau = 215.62 | | | |
| GE | Quadratic | a = 76.33 | | | |
| | | b = 2.90 | 0.587 | 5.13 | < 0.0001 |
| | | c = -0.45 | | | |
| | Segmented | a = 78.72 | | | |
| | | b = -0.02 | | | |
| | | c = $-7 \times 10^{-5}$ | 0.947 | 0.276 | 0.012 |
| | | Plateau = 80.47 | | | |
| | | $x_0^3$ at plateau = 244.23 | | | |
| AME | Quadratic | a = 3338 | | | |
| | | b = 126.55 | 0.591 | 9,591 | < 0.0001 |
| | | c = -19.50 | | | |
| | Segmented | a = 3442 | | | |
| | | b = -0.98 | | | |
| | | c = $-3.15 \times 10^{-3}$ | 0.947 | 531.2 | 0.012 |
| | | Plateau = 35.19 | | | |
| | | $x_0^3$ at plateau = 244.02 | | | |

[a]The model is $a + bx + cx^2$. [b]MSE, mean square of errors; [c]$x_0$, optimum xylanase concentration.

Consequently, we concluded that a linear model did not adequately describe the dose-response relationship of xylanase in broilers.

Quadratic and segmented models were constructed (Table 4). Lamberson and Firman (2002) found that segmented regression procedures resulted in more precise estimates of nutrient requirements, were less likely to suffer from bias, and required less a priori knowledge of the true requirement than did quadratic regression procedures. In this study, the quadratic model had higher MSE and lower $R^2$ values than the segmented model. The segmented model revealed that nutrient digestibility and AME were positively correlated to xylanase concentration in a quadratic manner when all dietary substrates were combined with xylanase. We did not observe any further improvement in these two parameters with increasing xylanase concentrations, revealing a saturation effect. Maximum digestibility values were 78.15% DM, 80.47% GE and 3,519 cal/kg AME at 215.62, 244.23 and 244.02 mg/kg xylanase, respectively. This effect follows the "law of diminishing returns" for biological data. However, further studies are needed to verify that the segmented model may be used to describe the dose-response relationship of other supplemental enzymes in broilers.

Some reports have shown that enzyme supplementation significantly improves broiler total tract nitrogen-corrected AME (Stefanello *et al.* 2016), AME (Zeng *et al.* 2015, Abdollahi *et al.* 2016) and apparent energy digestibility (Mahmood *et al.* 2017). However, Olukosi *et al.* (2007) found that enzyme supplementation had no effect on ileal digestible energy. Yanez *et al.* (2011) concluded that xylanase supplementation improved both total tract and ileal apparent energy digestibility in barrows (37.1±0.8 kg of body weight). Nortey *et al.* (2008) compared tract digestibility experiment and ileal digesta digestibility experiment and reported that xylanase had no effect on ileal DE and only significantly improved total tract DE. Based on the results obtained from the total tract and ileal digesta collection methods in this study, xylanase supplementation improved DM, GE and AME. However, only DM was improved by the ileal digesta collection method ($P<0.05$), indicating that the ileal digesta collection method might be better for evaluating the effect of xylanase on DM (Table 5). Additionally, we found that xylanase had no effect on GE and AME by any detection method. However, when xylanase was not added, the results from the total excreta collection method were significantly higher than those obtained from the ileal digesta collection method ($P<0.05$).

Compared to the ileal digesta collection method, the total tract collection method had higher AME and GE ($P<0.05$; Table 5). The xylanase-supplemented groups had higher DM ($P<0.05$) than the control group. The interaction between methods and xylanase concentrations did not affect DM, GE, or AME ($P>0.05$; Table 5). GE and AME did not differ significantly between the two metabolic methods in the xylanase-supplemented groups. However, in the control group, GE and AME were significantly higher from the total tract collection method than from the ileal digesta collection method. The coefficients of variation (CVs) for DM, GE and AME in the ileal digesta collection method were about 10%, whereas those in the total tract collection method were only about 1.7%.

There were no obvious differences among the groups obtained from the total tract collection method in that microorganisms in the ceca or hindgut play important roles in digesting nutrients of various feeds; however, the corresponding microorganisms could not affect the results from the ileal digesta collection method. Therefore, xylanase could impair the cage-effect of non-starch polysaccharides, affect nutrient release, and improve starch

Table 5. Effect of xylanase on dry matter (DM), gross energy (GE) and apparent metabolizable energy (AME) in 18-week-old Chinese yellow-feather male broilers using total tract excreta (TE) and ileal digesta (ID) collection methods.

| Methods | Xylanase (mg/kg) | n | Digestibility (%) | | | | AME (cal/kg) | CV[a] (%) |
|---|---|---|---|---|---|---|---|---|
| | | | DM | CV[1] | GE | CV | | |
| TE | 0 | 16 | 75.35±0.35 | 1.88 | 78.60±0.34 | 1.75 | 3,446±15.08 | 1.75 |
| | 244.23 | 16 | 76.25±0.27 | 1.40 | 79.29±0.30 | 1.51 | 3,477±13.13 | 1.51 |
| ID | 0 | 16 | 73.65±1.90 | 10.33 | 74.57±1.97 | 10.59 | 3,253±86.14 | 10.59 |
| | 244.23 | 16 | 78.14±1.57 | 8.02 | 78.38±1.79 | 9.15 | 3,419±78.23 | 9.15 |
| TE | 0 | 32 | 75.80** | 1.73 | 78.94** | 1.67 | 3,462** | 1.67 |
| | 244.23 | 32 | 75.90** | 9.53 | 76.47* | 10.03 | 3,336* | 10.03 |
| ID | 0 | 32 | 74.50** | 7.32 | 76.58* | 7.76 | 3,350** | 7.76 |
| | 244.23 | 32 | 77.20* | 5.87 | 78.83** | 6.44 | 3,448** | 6.44 |
| *P* value | | | | | | | | |
| Method | | | 0.937 | | 0.073 | | 0.037 | |
| Xylanase concentration | | | 0.036 | | 0.102 | | 0.102 | |
| Method × Xylanase concentration | | | 0.156 | | 0.254 | | 0.254 | |
| TE vs. ID (0 mg/kg) | | | 0.341 | | 0.039 | | 0.023 | |
| TE vs. ID (244.23 mg/kg) | | | 0.288 | | 0.636 | | 0.490 | |

*P<0.05, **P<0.01; [a]CV, coefficient of variation.

digestibility in the ileum. Nevertheless, in the ileal digesta collection method, CVs of relevant parameters were higher than the ones obtained from the total tract excreta collection method. These results might be attributed to the weight of the ileal digesta collected from broilers. According to reports, the ileal digesta-to-feed ratio is 5%–7% at 4 h post-feeding (Kim *et al.* 2011) and the DM-to-feed ratio is 16%–20% (Engberg *et al.* 2004). These reports showed that it is difficult to collect a sufficient amount of digesta.

In conclusion, xylanase efficiently improved DM, GE and AME of a wheat-maize-soybean meal diet in 18-week old Chinese yellow-feather broilers. Furthermore, the dose-response relationship between xylanase supplementation and nutrient digestibility, and AME was described by a segmented model. The total tract excreta collection method appeared to be a better approach for evaluating xylanase effects than the ileal digesta collection method based on the lower CV values. However, 18-week old Chinese yellow-feather broilers were chosen as the experimental animal with the purpose of collection adequate ileal digesta for test analysis in this study. Further investigation about the repeatability of total tract excreta collection method on younger broilers or the xylanase–hindgut microorganism interaction is required.

## ACKNOWLEDGEMENT

We would like to thank Minghao Xia, Yan Ma, Jiandong Wu (College of Animal Science, South China Agricultural University, Guangzhou, Guangdong) and Songbai Liu, Yugang Yin (Wen's Foodstuffs Group Corporation Co. Ltd., Yunfu, Guangdong) for their assistance with sample collection. This work was supported by grants from the National Key Research and Development Program of China (2016YFD0501308), the Key Project in the National Science and Technology Pillar Program during the Twelfth Five-Year Plan Period (2013BAD10B01), the Guangdong Province Natural Science Foundation (S2013010012040).

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