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Effect of Xylanase Supplementation on the Net Energy for Production, Performance and Gut Microflora of Broilers Fed Corn/Soy-based Diet

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ABSTRACT : The objective of this study was to assess the effect of xylanase on net energy for production, performance, nutrient digestion and gut microflora of broilers fed corn/soy-based diet. Eighty-four day-old male broiler chicks were allocated to two groups receiving two treatments, respectively. Each treatment had six replicate cages with seven broilers per cage. The diets were based on corn and soybean. The treatments were: i) basal diet reduced in apparent metabolizable energy (-0.63 MJ/kg compared to commercial diet specifications); ii) basal diet supplemented xylanase at 4,000 u/kg feed. The experiment used the auto-control, open circuit respiration calorimetry apparatus to examine the heat production and net energy for production. The results revealed that xylanase supplementation did not affect growth performance and diet AME value, but increased NE_p value by 18.2% ($p<0.05$) and decreased daily heat production per $kg^{0.75}$ by 31.7% ($p<0.05$). There was no effect ($p>0.05$) of xylanase supplementation on the ileal digestibility of N and hemicelluloses, but the ileum digestibility of energy was increased by 2% by xylanase supplementation ($p<0.05$). Xylanase supplementation increased ($p<0.05$) the count of lactobacillus and bifidobacterial in the caecum. (**Key Words :** Xylanase, Corn/Soybean Diet, Growth, Net Energy for Production, Heat Production, Bacteria, Broiler)

INTRODUCTION

In the recent years, research has shown that the nutritional value of corn for poultry can be almost as variable as for wheat. This can be related to the variability in starch quality as well as the presence of storage protein (Péron and Gilbert, 2010). The application of multiple enzyme activities including amylase, protease and xylanase can result in improvement of broiler performance, subject to the quality of the corn used in the diet. More importantly, the arabinoxylans are proven one of the most important anti-nutritional factors in corn, accounting for 5.8%, which is the substrate for xylanase. It has been well documented that application of xylanase in corn based diets can improve nutrient utilization and performance of broilers (Wyatt et al., 1997, 1999; Jin, 2001; Iji et al., 2003), however the amount of energy that can be released with xylanase addition in corn/soy-based diets is still being debated.

While most nutritionists are still using apparent metabolisable energy for feed formulation in broilers, the net energy was used to assess the effect of xylanase on energy utilization by broilers. Choct et al. (2010) examined the effect of xylanase on energy utilization of broilers fed wheat based diet. In this trial, broiler chickens were fed wheat based diet (76%) and the net energy was measured using closed circuit respiration chambers. The result revealed that xylanase supplementation did not affected RQ and daily heat production per kg body weight (0.91 vs. 0.81), but AME was increased by 29.1% and net energy by 37.3%. The energy loss as VFAs was reduced by over 60% when xylanase was supplemented. In another study where wheat was included at 75% (Nian et al., 2011), the inclusion of 4,000 u xylanase/kg feed increased diet AME by 4.2% and net energy for production by 26.1%, but decreased daily heat production per kg metabolisable weight by 26.2%. Xylanase supplementation numerically increased the ileal

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digestibility of protein and energy by 3 and 6 percentage units, respectively. This study further confirmed that xylanase is working effectively on its substrate indicated by the significant improvement in ileal digestibility of hemicelluloses. However, the effect of xylanase on energy utilization for production has not been assessed for corn/soy based diet which has less non-starch polysaccharides than wheat based diet. The objective of this study was to assess the effect of xylanase on net energy for production, performance, nutrient digestion and gut microflora of broilers fed corn/soy based diet.

MATERIALS AND METHODS

Enzyme

A commercial xylanase (Porzyme® 9302; Danisco Animal Nutrition, Marlborough, UK) was used for this experiment. The xylanase (0.5 kg/tonne of feed) was added in diets to provide a guaranteed minimum of 4,000 u xylanase per kg feed. The definition of the xylanase unit is that 1 unit is the amount of enzyme which liberates 0.5 μmol of reducing sugar (expressed as xylose equivalents) from a cross-linked oat spelt xylan substrate at pH 5.5 and 50°C in one minute.

Diet : The experiment included 2 dietary treatments. The basal diet was formulated to the commercial diet specifications used in China (NY/T33-2004), however, the AME content was reduced by 0.63 MJ/kg (down to 11.91 MJ/kg). The NE/AME was 0.72. The NE levels of the raw materials were adapted from de Groote (1974). In the second dietary treatment, the xylanase was added to the basal diet to provide a guaranteed minimum of 4,000 u xylanase per kg feed. Titanium oxide (4 g/kg) was added to all diets as an external marker. The diets were fed in mash form. The basal diet formulation is shown in Table 1.

A total of 84, day-old, male Arbor Acre chickens were purchased from a commercial hatchery (Beijing Arbor Acre Hatchery, Beijing, China). The chickens were randomly allocated into 2 treatments, with 6 replicates per treatment and 7 chickens per replicate. All birds were housed in 3-deck wire floored 90×70×45 cm (length×width×height) cages and exposed to light for 24 h/d. The environment temperature was maintained at 35°C during the first week, 33°C in the second week, 29°C in the third week and 25°C in the fourth week. All chickens offered feed and water *ad libitum* during the experimental period.

Sampling : On day 30, all birds were euthanized by CO_2 asphyxiation for collection of small intestine and caecal digesta samples. The small intestine was divided into three segments: duodenum, jejunum and ileum. The content of the distal of the ileum from 4 birds per replicate was collected by flushing with distilled water. Digesta were pooled for each replicate and frozen immediately. They were subsequently lyophilized, dried and milled through a 0.4-mm sieve for chemical analysis (Mathlouthi and Lalles, 2002). The ileum and caecum of 3 other birds per replicate were frozen in liquid nitrogen and stored at -80°C until the enumeration of bacteria (Cao et al., 2003).

Table 1. Composition and nutrient levels of the basal diet

Ingredients (g/kg)	Basal diet
Corn	526.50
Soybean meal	206.00
Soybean oil	30.00
Corn gluten meal	111.86
Limestone	11.20
Dicalcium phosphate	24.00
Salt	3.60
L-lysine HCl	4.57
DL-methionine	1.17
Threonine	0.20
Tryptophan	0.00
Vitamin premix*	0.20
Mineral premix**	2.00
Chloride choline	1.60
Bacitracin zinc	0.20
Antioxidant	0.20
Medical stone***	72.70
Titanium oxide	4.00
Calculated nutrient level	
Crude protein (%)	20.00
NE (MJ/kg)	8.63
AME (MJ/kg)	11.91
NE/AME	0.72
Calcium (%)	1.00
Total phosphorus (%)	0.68
Lysine (%)	1.15
Methionine (%)	0.50
Methionine and cystine (%)	0.86
Tryptophan (%)	0.21
Threonine (%)	0.81
Total xylans (%)	3.22
Soluble xylans (%)	0.13

* Vitamin premix provided per kilogram of diet with: vitamin A, 10,800 IU; vitamin D_3, 2,160 IU; vitamin E, 151 IU; vitamin K_3, 1.0 mg; vitamin B_1, 4 mg; riboflavin, 5 mg; pantothenic acid, 10 mg; niacin, 25 mg; vitamin B_6, 8 mg; folic acid, 0.4 mg; vitamin B_{12}, 0.08 mg; biotin, 0.15 mg.

** Mineral premix provided one kilogram of diet with: I, 0.35 mg; Se, 0.15 mg; Zn, 40 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg.

*** Medical stone: a natural mineral additive, mainly composed of Al_2O_3 and SiO_2.

Measurement

Growth performance : Chickens were weighted on day 1 and 26, feed intake was recorded on a cage basis, and the

corresponding feed conversion ratio (FCR) was calculated. Any bird that died was weighed and the weight was recorded to adjust the feed conversion ratio (FCR).

Respiratory chambers and heat production : The trial was conducted using the auto-control, open circuit, respiration calorimetry apparatus (KB-2, National Animal Nutrition Research Institute, China Agricultural University, China). The apparatus consists of two chambers (A and B), a ventilation system, a temperature control system, a flow and gas analysis system (including a O×OR610 O_2 analyzer, a QGS-08 CH_4 analyzer and a QGS-08B CO_2 analyzer), and a recorder. The chamber dimensions were 380×240×200 cm (length×width×height). Each chamber can hold 6 single-bird cages (85 cm length×80 cm width×95 cm height) fitted with individual galvanized iron food troughs and small adjustable scoop channels. Plastic trays (90 cm length× 80 cm width) were placed under each wire-mesh cage for the collection of excreta.

At 22 days of age, all birds of each replicates of each treatment (7 birds/replicate) were placed in the chambers in order to adapt to the chamber environment. Chamber A contained the control treatment and chamber B contained the xylanase treatment. Feed and water were offered *ad libitum*. The chambers remained sealed at all time, except during excreta collection (between 8:00 and 10:00 am every day). Atmospheric pressure, temperature and humidity were recorded for each chamber. Room temperature was controlled by an air conditioning unit at 26±1°C to maintain a constant temperature within the chamber.

The respiration calorimetry apparatus collected the gas sample and assayed the gas flow rate in every 3 minutes. The respiratory quotient (RQ) during each run (3 minutes) refers to the ratio of CO_2 produced by the birds to the volume of O_2 consumption (RQ = CO_2 produced/O_2 consumption). Heat production was calculated using the Brouwer equation (Brouwer, 1965) incorporated into the computer program. Observations of HP were made over 3 days but were suspended for 2 hours each day while the feed and water containers were replenished and the excreta collected. The HP was calculated based on every 3 minute gas flow rate and then converted to a 24 h heat production per kg metabolisable weight.

Apparent metabolisable energy and net energy : An AME trial was conducted between 23 and 26 day of age. Feed intake and body weigh were recorded for each replicate during the 3-day period and excreta were collected. Excreta were then dried for 48 hours at 65°C, weighed and ground. The gross energy (GE) content of the diets and dried excreta samples was determined using an oxygen bomb calorimeter (WZR-IA, Changsha, China). The AME value for each diet was calculated as follows: AME = GE-FUE (Faecal and Urinary Energy). The NE_p value for each diets was calculated as follows: NE_p = AME-HP (heat production).

Chemical analysis

Gross energy (GE) was determined using an oxygen bomb calorimeter (WZR-IA, Changsha, China), standardized with benzoic acid. Nitrogen (N) content was determined by the Kjeldahl apparatus (KDY-9380, Shanghai, China). Titanium oxide content was measured using a UV spectrophotometer (Short, 1996). All diet samples were analyzed for xylanase activity (Danisco Animal Nutrition, Marlborought, UK) and xylanase activity of the treatment diet was 4,500 u/kg feed.

Enumeration of bacteria

The contents from ileum and caecum (right section, 1 g) were homogenized with 99 ml of a sterile solution of 0.9% (w/v) NaCl and shaken vigorously for 5 min. Further 10-fold serial dilutions were made. Lactobacilli were cultured on LBS agar placed into an anaerobic cabinet at 37°C for 48 h (GB/T 4789.35-2008, China). Bifidobacteria were cultured on BBL agar incubated anaerobically at 37°C for 48 h (GB/T 4789.34-2008, China). Coliform bacteria were cultured on MacConkey agar incubated aerobically at 37°C for 24 h (GB/T 4789.38-2008, China). Salmonellas were cultured on S.S agar incubated aerobically at 37°C for 24 h (GB/T 4789.4-2008, China). The counts were reported as $\log_{10}$ cfu/g of digesta.

Statistical analysis

Trial data were subjected to analysis of variance using the independent-sample *t*-test of SAS 8.2. Differences were considered significant at $p<0.05$.

RESULTS

Over the 26-day growth period, feeding xylanase did

Table 2. Effect of xylanase on growth performance of broilers fed corn/soy-based diets over a 26 day period

	Corn/soy (-xylanase)	Corn/soy (+xylanase)	Pooled SEM	p
Body weight gain (g/bird)	696.4	695.9	12.5	0.986
Feed intake (g/bird)	1,090.3	1,063.4	20.1	0.534
FCR (g/g)	1.566	1.535	0.023	0.518

SE = Standard error; FCR = Feed conversion ratio.

Table 3. Effect of xylanase on energy utilization by broilers fed corn/soy-based diet

	Corn/soy (-xylanase)	Corn/soy (+xylanase)	Pooled SEM	p
AME (MJ/kg DM)	12.47	12.89	0.12	0.069
HP (MJ/kg DM)	5.45	4.22	0.36	0.078
HP (MJ/d/kg$^{0.75}$)	0.514^{a}	0.350^{b}	0.04	0.027
NEp (MJ/kg DM)	7.27^{b}	8.59^{a}	0.23	0.000
Vco_2 (L/d/kg$^{0.75}$)	23.71	16.50	2.03	0.059
Vo_2 (L/d/kg$^{0.75}$)	24.40^{a}	17.11^{b}	1.88	0.026
RQ	0.973	0.968	0.015	0.887

AME = Apparent metabolisable energy; HP = Heat production; NEp = Net energy for production; Vco_2, the production of CO_2; Vo_2, the consumption of O_2; RQ, respiratory quotient; SE = Standard error.
^{a,b} Means with different superscripts are significantly different ($p<0.05$).

not affect ($p>0.05$) bodyweight gain, feed intake and FCR for broilers fed corn/soy-based diets (Table 2). However, xylanase supplementation resulted in a numerical improvement of FCR (by 2%).

Xylanase supplementation did not significantly affect diet AME values or the RQ of broilers (Table 3). However, diet AME value was numerically higher in presence of xylanase (+3.9%, p = 0.069). Xylanase increased NE_p values by 18.2% ($p<0.05$) and reduced daily heat production per kg $W^{0.75}$ by 31.7% ($p<0.05$).

Addition of xylanase in corn/soy-based diet did not significantly affect ileal N and hemicellulose digestibility ($p>0.05$). However, ileal N and hemicelluloses digestibility values were numerically increased by 1.4 and 6.6 percentage units respectively. The inclusion of xylanase in corn-soy based diet improved ($p<0.05$) the ileum digestibility of gross energy from 78.90 to 80.97% (Table 4).

Xylanase supplementation increased ($p<0.05$) the count of lactobacilli and bifidobacteria in caecal contents. It did not affect the counts of coliforms and salmonellas in caecal contents, or the counts of lactobacilli and bifidobacteria in the ileum ($p>0.05$), under this experiment conditions, the counts of coliforms and salmonellas in ileum were not detected (Table 5).

DISCUSSION

The present study showed that the addition of xylanase to a corn/soy-based diet significantly improved the feed net energy for production and numerically improved broiler FCR by 2% (with a 27 g feed intake savings for the same body weight gain). The FCR improvement observed in this study was consistent with the results of Cowieson (2005), where FCR was improved from 1.68 to 1.64 when broiler chicks were fed corn/soy-based diets supplemented with xylanase. It has been well documented that xylanase hydrolyses polysaccharides which are involved in encapsulation of starch or protein in cereal grains, reducing

Table 4. Effect of xylanase on ileal digestibility of nutrients by broilers fed corn/soy-based diet

	Corn/soy (-xylanase)	Corn/soy (+xylanase)	Pooled SEM	p
N digestibility	83.67	84.83	0.44	0.198
GE digestibility	78.90^{b}	80.97^{a}	0.46	0.013
Hemicellulose digestibility	38.72	41.26	2.23	0.594

^{a,b} Means with different superscripts are significantly different ($p<0.05$); N = Nitrogen; GE = Gross energy.

Table 5. Effect of xylanase on the counts of bacteria in the ileum and caecum (log_{10} cfu/g) of broilers fed corn/soy-based diet

	Caecum				Ileum			
	Corn/soy (-xylanase)	Corn/soy (+xylanase)	Pooled SEM	p	Corn/soy (-xylanase)	Corn/soy (+xylanase)	Pooled SEM	p
Coliforms	6.04	7.26	0.39	0.117				
Salmonella	5.60	4.79	0.32	0.244				
Lactobacillus	9.00^{b}	10.45^{a}	0.31	0.012	6.45	6.46	0.12	0.957
Bifidobacteria	9.86^{b}	10.69^{a}	0.18	0.018	8.61	9.60	0.26	0.060

^{a,b} Means with different superscripts are significantly different ($p<0.05$).

the barriers to nutrient digestion and utilization. Such an effect is reflected in this trial where hemicellulose's digestibility was improved by 2.5% units and the ileal energy digestibility was increased by 2.1% units following xylanase addition, and resulted in a numerical improvement of FCR.

In this study, xylanase supplementation improved the net energy for production by 18.2%. While there is no previous report about the effect of xylanase supplementation on the NEp value of corn/soy-based diets for broilers, a similar study with wheat-based diets demonstrated that xylanase addition increased NEp value by 26.1% and reduced daily heat production per kg metabolisable weight by 26.2% (Nian et al., 2011). The reduction of heat loss and subsequent improvement of net energy for production following xylanase supplementation may be explained by a reduction of the weight and proportion of metabolically active organs which accounts for >50% of the heat increment and total energy requirement for maintenance purposes, even though these organs usually make up less than 10% of the total animal bodyweight (Esteve-Garcia et al., 1997; Wang et al., 2005). Gao (2008) also reported that the addition of xylanase to a wheat-based diet significantly reduced the relative weights of the duodenum, jejunum, colon and pancreas in 21-day-old broiler chickens. This should also be the case for broilers fed corn/soy based diet although very limited information is available.

The greater positive effect of xylanase addition in wheat vs. corn diets is most likely related to the higher amount of soluble NSP in wheat compared to corn. Corn contains only l g/kg water soluble NSP (primarily arabinoxylan) compared with 24 g/kg for wheat (Choct, 1997). So the extent of improvement on NEp by supplementing xylanase alone for corn/soy-based diet is probably limited by the lower amount of substrate.

In this trial, the improvement in NEp for corn/soy-based diet of broilers was not fully reflected in the performance. The xylanase supplementation in corn/soy-based diet improved the net energy for production by 18.2%, but did not significantly improve the performance of broilers. This could be explained by the fact that broiler performance is also affected by environmental conditions, especially temperature. The NEp was measured with birds housed in an ideal environment in the respiratory chamber. The effect of xylanase supplementation on energy utilization can be sensitively detected while the birds' growth performance were measured in an animal house where the temperature and humidity were not as ideal as in the respiratory chamber (the temperature of animal house and respiratory chamber was maintained at 25±2°C and 26±1°C, the humidity was 50%-60% and 40%-45%, respectively). Obviously, the environment temperature and humidity of the growth trial were fluctuated within a certain range, causing less amount of energy for production and more energy for maintenance. So, the effectiveness of xylanase was not sensitively detected in growth performance. Another important factor for such unmatched results is the ratio of protein (especially lysine) and NE. According to the feeding standard for broilers at 0-3 week, the ratio of CP and NEp (calculated using the relationship between AME and NEp by Pirgozliev and Rose, 1999) is 26.4 while the ratio of lysine and NEp is 1.41. In the current study, the ratio of CP: NEp and lysine: NEp were 27.5 and 1.58 respectively for the control group while the corresponding values for the xylanase treatment group were 23.3 and 1.34 respectively. This suggests that the protein and/or lysine are lower for xylanase treatment group, which might also limit the expression of full growth potential of broilers in this group. This further demonstrated the importance of adjusting the CP and lysine levels with the xylanase addition for improved broiler production.

Exogenous enzymes can alter microbial populations indirectly within the GI tract through their action on the substrates that bacteria use as a carbon source (Choct et al., 1999; Bedford, 2000; Cowieson et al., 2000). Gao et al. (2008) and Engberg et al. (2004) reported that xylanase addition in wheat-based diets had no significant effect on bifidobacterium, lactobacillus and *E. coli* in the ileum and caecum of broilers. Nian et al. (2011) showed that adding xylanase in wheat-based diet reduced the number of coliform and salmonellas, and increased the number of lactobacillus in the ileum, increased the counts of all bacteria in the caecum, did not cause any negative effect on health and performance of broilers. In the present study, xylanase supplementation increased the count of lactobacillus and bifidobacterial in the caecal contents. This result was consistent with the previous trial. Multiplication of beneficial lactobacillus and bifidobacterial indicated additing xylanase could inhibit the growth of pathogenic bacteria, with a beneficial effect on broilers with the healthy gut microflora.

In conclusion, the application of xylanase in corn-soybean based diet significantly reduced heat production, leading to greater NE_p. The application of xylanase also increased the number of beneficial bacteria (lactobacilli and bifidobacteria) in the caecum.

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