Research Note

Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase

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ABSTRACT A study was conducted to evaluate the effects of increased levels of a β-xylanase on energy and nutrient utilization of broiler chickens fed corn-soy diets. A total of 480 slow feathering Cobb × Cobb 500 male broilers were randomly distributed to 10 treatments having 8 replicates of 6 birds each. Birds were fed a common starter diet to d 14 post hatch (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% nPP). The experimental diets were provided afterwards until 25 d. Two experimental diets, a conventional corn/soy-based basal diet (CS) and the basal diet in which 40% of the diet was displaced by corn (CN), were fed as-is or supplemented with 50, 100, 150, or 200 fungal β-xylanase units (FXU)/kg. Dietary treatments were distributed factorially as a 2 × 5 arrangement. Samples of feed, excreta, and ileal digesta were analyzed for determination of ileal digestible energy (IDE), metabolizable energy, and total tract retention of protein and lipid. No interactions between diet and xylanase were observed. The CS diets had higher ($P < 0.05$) energy utilization and nutrient digestibility when compared to the CN diets. AME_n and IDE were improved ($P < 0.05$) by 192 and 145 kcal/kg, respectively, when diets were supplemented with 100 FXU/kg xylanase. The xylanase added to the CN diet led to quadratic increases ($P < 0.05$) in IDE ($Y = -\ 0.014x^2 + 2.570x + 3{,}155$; $r^2 = 0.60$) and in AME_n ($Y = -\ 0.016x^2 + 3.982x + 3{,}155$; $r^2 = 0.68$). Crude protein digestibility and AME_n were linearly increased ($P < 0.05$) when xylanase was added to the CN diet. In conclusion, energy utilization and digestibility of crude protein and dry matter increased with xylanase supplementation in corn/soy-based diets. When xylanase was tested in the CS diet, 92 and 124 FXU/kg maximized the energy release effect; however, the maximum energy response in the CN diet or corn was not achieved until 200 FXU/kg.

Key words: broiler, corn, digestibility, metabolizable energy, xylanase

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INTRODUCTION

Corn and soybean meal (**SBM**) are incumbent ingredients used in the majority of commercial poultry diets worldwide and contain varying levels of non-starch polysaccharides (**NSP**). Non-starch polysaccharides are carbohydrates that can interfere with nutrient utilization by poultry (Bach Knudsen, 1997; Caffall and Mohnen, 2009). This is in part because nutrients such as starch, fat, and protein are trapped within the insoluble cell wall matrix, which acts as a physical barrier that limits access for the endogenous enzyme array (Theander et al., 1989; Slominski et al., 1993). Soluble fiber also can form viscous gels within the gut and slow digestion and feed passage rate (Bedford et al., 1991).

The concentration of NSP in corn and SBM ranges from 6.8 to 9.4% and 17 to 30%, respectively (Smits and Annison, 1996; Choct, 1997; Kocher et al., 2003). The total amount of arabinoxylan is variable among ingredients but has been reported to be around 5.2% in corn (Choct, 1997) and 3.3% in SBM (Back Knudsen, 1997). Carbohydrate composition is also important to determine energy and nutrient utilization of ingredients for broilers. Thus, different substitution methods have been described to determine energy and nutrient digestibility of cereals and by-products. These methods use either one substitution level of a tested ingredient or multiple substitution inclusions by the regression method (Matterson et al., 1965; Villamide, 1996; Adeola, 2001; Adeola et al., 2010).

Adding exogenous enzymes targeting insoluble and soluble fibers may facilitate the release of nutrients encapsulated in cell walls or incorporated into the cell wall itself, resulting in improved access for digestive enzymes (Cowieson, 2005). Exogenous xylanases may hydrolyze cell wall arabinoxylans, improving the access of endogenous enzymes to cell contents (Meng et al., 2005; Francesch and Geraert, 2009) and decrease endogenous

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amino acid (**AA**) losses, particularly through changes on pancreatic amylase and mucin secretion (Jiang et al., 2008; Cowieson and Bedford, 2009).

Improvements in broiler performance are often associated with increased nutrient digestibility and energy utilization (Olukosi et al., 2008). Accurate estimations of improvements in energy digestibility due to exogenous xylanase is relevant to account for the effects of these enzymes in diet formulation according to the inclusion of different ingredients. This is important also because the efficiency of energy utilization from NSP is lower than the efficiency of use of energy from fat, starch, or protein for growth, though NSP digestibility does contribute to the measured metabolizable energy in response to xylanase supplementation in some diets or ages in broiler chickens (Savory, 1992; Chwalibog, 2002).

Xylo-oligosaccharides released during the degradation of NSP by exogenous xylanase in the small intestine are fermented by the intestinal microbiota and the end products (various volatile fatty acids) are subsequently used as energy yielding substrates for broilers (Choct et al., 1996). As suggested by Cowieson and O'Neill (2013), the fatty acids have some energetic value for the host animal but perhaps more importantly, the lower pH may restrain the proliferation of putrefactive organisms, encourage the proliferation of enterocytes, and may directly mediate gastric emptying, perhaps via the same infrastructure involved in the ileal brake mechanism. Additionally, if the micro biome has a central role in the effect of exogenous xylanase, it is possible that these mechanisms will be cumulative as the microflora adapt to substrate provision.

Though there are several reports in the literature on the efficacy of xylanase (or xylanase-based enzyme admixtures) on the nutritional value of corn-based diets, there are rather few that explore the relative effect on corn and SBM independently. More studies are therefore needed to evaluate if xylanase acts specifically on corn fiber or on arabinoxylan-containing carbohydrates in SBM. Furthermore, while SBM contains very low concentrations of arabinoxylan, it is possible that hydrolysis of arabinoxylan in corn would indirectly influence the digestibility of SBM via gross changes to intestinal pH, passage rate, and so on.

The objective of the present study was to evaluate the effects of various concentrations of an exogenous β-xylanase on energy utilization and nutrient digestibility of corn-SBM-based diets for broiler chickens. These effects were assessed both in a conventional corn-soybean meal diet and in a diet in which 40% of this diet was displaced with corn to allow estimation of the interactive effects of xylanase and diet proportionality. The displacement of the corn/soy diet with pure corn facilitated extrapolation to evaluate corn independently from the rest of the diet in order to explore the possibility that xylanase efficacy may be influenced by diet composition.

MATERIALS AND METHODS

All procedures used in this study were approved by the Ethics and Research Committee of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Birds and Experimental Diets

A total of 480 one-day-old, slow-feathering Cobb × Cobb 500 male broiler chicks, vaccinated for Marek's disease at the hatchery, with an average BW of 48 g were randomly placed into 80 wire battery cages (0.9 × 0.4 m^2). Each cage was equipped with one feeder and one drinker. Birds had ad libitum access to water and mash feeds. Average temperature was 32°C at placement and was reduced by 1°C every 2 d until 23°C to provide comfort throughout the study. Lighting was continuous until d 25 post hatch.

Birds were allocated to 10 experimental diets with 8 replications of 6 birds each in a completely randomized design. A standard corn-SBM-based broiler starter diet was fed from one to 14 d (3,050 kcal/kg AME_n, 21.7% CP, 1.05% Ca, and 0.53% non-phytate P). From 14 to 25 d, broilers were fed 2 basal diets, an industry-standard corn-soybean meal basal diet (**CS**) or the same basal diet in which 40% of the diet was displaced with corn (**CN**) as presented in Table 1. Both basal diets were supplemented with 0, 50, 100, 150, and 200 fungal β-xylanase units (**FXU**)/kg [Ronozyme WX (CT); Novozymes A/S, Bagsvaerd, Denmark]. A 2 × 5 factorial arrangement of 2 control diets and 5 xylanase supplements was used. The xylanase was a granulated heat-stable endo-xylanase from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism containing 1,000 FXU/g. One FXU is the amount of endo-1,4-β-xylanase, which liberates 7.8 micromoles of reducing sugars (xylose equivalents) per min from azo-wheat arabinoxylans at pH 6.0 and 50°C. The CS diet had 1% Celite as indigestible marker (Celite, Celite Corp., Lompoc, CA).

Experimental Procedures

Excreta were collected twice daily on wax paper from 21 to 24 d being immediately mixed and pooled by cage and stored at −20°C until analysis. Previous to calorimetry, excreta were dried in a forced-air oven at 55°C (DeLeo, Porto Alegre, Brazil) and ground to pass a 0.5-mm screen. Ileal digesta were collected from all birds at 25 d after euthanasia by electrical stunning using 45 V for 3 s. Ileal digesta were collected from a section of intestine between Meckel's diverticulum to approximately 2 cm cranial to the ileo-cecal junction. Digesta were flushed with distilled water into plastic containers, pooled by cage, immediately frozen in liquid nitrogen, and stored in a freezer at −20°C

Table 1. Ingredient and nutrient composition of the experimental diets (as-is basis).

Item	Corn-soy-based diet (CS)	Corn-based diet (CN)[1]
Ingredients, %		
Corn	53.61	72.17
Soybean meal	36.30	21.78
Soybean oil	5.09	3.05
Dicalcium phosphate	1.83	1.10
Limestone	0.97	0.58
Salt	0.51	0.31
DL-Methionine 99%	0.29	0.17
L-Lysine HCl 76%	0.16	0.10
L-Threonine 98.5%	0.04	0.02
Choline chloride 60%	0.05	0.03
Vitamin and mineral mix[2]	0.15	0.09
Celite[3]	1.00	0.60
Calculated nutrient composition, % unless noted		
AME_n, kcal/kg	3,100	3,214
CP	21.00	15.75
Ca	0.90	0.55
Non-phytate P	0.45	0.29
Total P	0.68	0.51
Na	0.22	0.14
Choline, mg/kg	1,500	1,100
Dig. Lys[4]	1.15	0.77
Dig. TSAA	0.86	0.39
Dig. Thr	0.75	0.56
Dig. Trp	0.23	0.16
Dig. Arg	1.34	0.94
Dig. Val	0.89	1.34
Dig. Ile	0.82	0.59

[1]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

[2]Composition per kg of feed: vitamin A, 8,000 UI; vitamin D_3, 2,000 UI; vitamin E, 30 UI; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.

[3]Indigestible marker (Celite, Celite Corp., Lompoc, CA).

[4]Ratios of digestible amino acids to digestible Lys were maintained at TSAA: 0.75; Thr: 0.65; Val: 0.70; Trp: 0.17; Arg: 1.08; Ile: 0.67 (Rostagno et al., 2011).

until lyophilized (Christ Alpha 2–4 LD Freeze Dryer, Newtown, UK).

Chemical Analysis and Calculations

Diet and freeze-dried samples of ileal digesta were ground to pass a 0.5-mm screen in a grinder (Tecnal, TE-631/2, São Paulo, Brazil). Dry matter (**DM**) analysis of samples was performed after oven drying the samples at 105°C for 16 h (method 934.01; AOAC International, 2006). Ileal digesta, excreta, and diet samples were analyzed for gross energy using a calorimeter calibrated with benzoic acid as a standard (IKA Werke, Parr Instruments, Staufen, Germany). Calculations of ileal digestible energy (**IDE**) and AME_n were done afterwards. Crude protein (N × 6.25) was determined by the combustion method (method 968.06; AOAC International, 2006). The calculated AME was corrected to zero N retention (AME_n) using a factor of 8.22 kcal/g (Hill and Anderson, 1958). Acid insoluble ash concentration in the diets, excreta, and ileum samples was determined using the method described by Vogtmann et al. (1975), and Choct and Annison (1992). Ether extract (**EE**) in the diets and excreta samples was determined by extracting in petroleum ether using a Soxhlet apparatus for approximately 8 h (method 934.01; AOAC International, 2000).

Energy utilization and nutrient digestibility of corn were calculated by the substitution method described by Matterson et al. (1965) and Sakomura and Rostagno (2007). In this method, a corn-soybean meal-based diet was used as the reference diet, and the test diet was the diet in which corn (40%) was used to dilute this reference diet. This substitution method allowed the determination of nutrient digestibility of a tested ingredient that was used to displace the reference diet. Calculations were used to determine the digestibility of the corn-soy-based diet and corn-based diet, and then extrapolated to 100% corn.

Apparent ileal digestibility, total tract utilization, and AME_n were calculated using the following equations (Kong and Adeola, 2014):

$$\text{Digestibility}\,(\%) = [1 - (M_i/M_o) \times (E_o/E_i)] \times 100,$$

$$AME_n\,(\text{kcal/kg}) = GE_i - [GE_o \times (M_i/M_o)] - 8.22 \times \{N_i - [N_o \times M_i/M_o)]\},$$

where M_i represents the concentration of acid insoluble ash in the diet in grams per kilogram of DM; M_o represents the concentration of acid insoluble ash in the excreta and ileal digesta in grams per kilogram of DM output; E_i represents the concentration of DM, CP, energy, or EE in the diet in milligrams per kilogram of DM; and E_o represents the concentration of DM, CP, energy, or EE in the excreta and ileal digesta in milligrams per kilogram of DM. GE_i is gross energy (kcal/kg) in the diet; GE_o is the gross energy (kcal/kg) in the excreta; N_i represents nitrogen concentration in the diet; and N_o represents nitrogen concentration in the excreta in g/kg DM.

Statistical Analysis

The experimental design was a completely randomized factorial arrangement of 2 control diets (CS or CN) and 5 xylanase supplementations. Data were submitted to a 2-way ANOVA using the GLM procedure of SAS Institute (SAS Institute, 2009). Significance was accepted at $P < 0.05$. Linear and quadratic regression equations were estimated with the increased levels of β-xylanase supplementation on corn-soy-based diet, corn-based diet, and corn. A regression analysis also was conducted with data in which corn values were extrapolated according to increasing levels of xylanase.

RESULTS AND DISCUSSION

The corn used in this study was analyzed to contain 88.6% DM, 3,776 kcal of GE/kg, 8.1% CP/kg, 0.94%

Table 2. Declared and analyzed activity of β-xylanase in the experimental diets.

	Xylanase, FXU/kg[1]	
Treatment	Declared	Analyzed
Corn-soy-based diet (CS)	0	<LOD[2]
CS + 50 FXU/kg	50	64
CS + 100 FXU/kg	100	113
CS + 150 FXU/kg	150	167
CS + 200 FXU/kg	200	206
Corn-based diet (CN)[3]	0	<LOD
CN + 50 FXU/kg	50	54
CN + 100 FXU/kg	100	109
CN + 150 FXU/kg	150	172
CN + 200 FXU/kg	200	208

[1]FXU = fungal β-xylanase units per kg of feed.
[2]LOD = limit of detection.
[3]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

crude fiber, 3.55% EE, 0.04% Ca, and 0.25% of total P. Analysis of β-xylanase in the experimental diets showed that the supplemental xylanase had in-feed activity in agreement with the expected values (Table 2). Nutrient digestibility and energy values of the CS diet, CN diet, and extrapolated values for pure corn are shown in Tables 3 and 4. Ileal digestible energy was 3,278, 3,201, and 3,132 kcal/kg, for CS, CN, and corn, respectively. When diet effect was evaluated, CS diets had higher ($P < 0.01$) AME, IDE, and EE and CP digestibilities compared to the CN diet. Differences in AME and ileal digestibility of CP were found between CS and CN diets ($P < 0.01$) with values, respectively, of 3,544 kcal/kg and 69.3% for CS and 3,432 kcal/kg and 62.2% for CN.

An explanation regarding the highest energy and nutrient utilization observed when broilers were fed a complete diet compared to the corn-based diet may be related to supplying all required nutrients in the CS diet in contrast with an imbalance in the CN diets. Based on calculated composition of the corn-based diet, most nutrients including calcium, phosphorus, amino acids and electrolyte balance were poorly balanced, and this may have affected the digestion process, absorption of nutrients, and gastrointestinal (**GI**) flow (Cowieson and Bedford, 2009). The extrapolated calculation for corn allowed estimation of energy values, DM, CP and EE digestibilities of corn for broilers. Digestibility of CP and EE of corn without xylanase were 63.5 and 83.4%, respectively. The IDE, AME, and AME_n values of corn were 3,132, 3,366, and 3,178 kcal/kg, respectively, which compares well with the 3,381 kcal/kg AME reported by Rostagno et al. (2011), 3,340 kcal/kg AME in Lopez and Leeson (2008), and 3,3500 kcal/kg AME_n in NRC (1994). Energy utilization of corn may be influenced by the substitution method (Matterson et al., 1965; Adeola, 2001; Sakomura and Rostagno, 2007; Kong and Adeola, 2014), because the CN diet was imbalanced. However, the main objective of this study was to evaluate the effects of various concentrations of an exogenous β-xylanase in diets with different corn proportions and considering 100% corn.

Evaluation of the effects of xylanase supplementation on CS diets, CN diets, and corn was another objective of this study. The effects of dietary treatments on total tract retention and ileal digestibility of nutrients by broilers are presented in Tables 3 and 4 and showed no interactions between diet and xylanase. In the present study, AME_n and IDE were improved ($P < 0.05$) by 192 and 145 kcal/kg, respectively, when diets were supplemented with 100 FXU/kg xylanase. Xylanase

Table 3. Apparent ileal digestibility and total tract retention responses of broilers fed corn-soy-based diets and corn-based diets supplemented with β-xylanase (on DM basis).[1]

	Ileal digestibility		Total tract retention				
Item	DM, %	IDE[2], kcal/kg	DM, %	AME, kcal/kg	AME_n, kcal/kg	CP[3], %	EE[4], %
Diet							
Corn-soy-based	67.9	3,278	74.2	3,544	3,324	69.3	86.7
Corn-based[5]	64.6	3,201	72.9	3,432	3,295	62.2	83.4
Xylanase, FXU/kg[6]							
0	65.1[b]	3,176[b]	71.5[b]	3,359[b]	3,179[b]	62.7[b]	83.1[b]
50	67.9[a]	3,312[a]	72.6[a,b]	3,451[a,b]	3,271[a,b]	64.2[a,b]	83.7[b]
100	67.4[a]	3,321[a]	75.6[a]	3,551[a]	3,371[a]	67.7[a]	85.7[a,b]
150	65.7[a,b]	3,196[a,b]	74.8[a,b]	3,548[a]	3,364[a]	67.3[a]	86.9[a]
200	65.5[a,b]	3,195[a,b]	74.2[a,b]	3,530[a]	3,362[a]	66.8[a]	86.6[a]
SEM	0.49	19.36	0.40	15.36	13.61	0.60	0.44
Main effect *P*-value							
Diet	0.001	0.041	0.102	0.001	0.155	0.001	0.001
Xylanase	0.027	0.025	0.030	0.001	0.001	0.003	0.007
Diet × xylanase	0.821	0.765	0.753	0.228	0.112	0.262	0.345

[a,b]Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.
[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage at the start of the experiment.
[2]IDE = ileal digestible energy.
[3]Crude protein.
[4]Ether extract.
[5]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.
[6]FXU = fungal β-xylanase units per kg of feed.

Table 4. Energy and nutrient utilization of corn supplemented with β-xylanase (on DM basis).[1]

	Ileal digestibility		Total tract retention				
Item	DM, %	IDE[2], kcal/kg	DM, %	AME, kcal/kg	AME_n, kcal/kg	CP[3], %	EE[4], %
Xylanase, FXU/kg[5]							
0	64.7	3,132	71.5	3,366	3,178	63.5	83.5
50	65.0	3,169	72.9	3,457	3,269	65.1	83.5
100	67.6	3,187	74.6	3,538	3,359	67.6	85.9
150	65.5	3,303	74.6	3,562	3,372	67.7	86.9
200	66.9	3,322	74.8	3,572	3,386	68.6	87.0
SEM	0.38	21.94	0.43	17.17	16.56	0.45	0.43
P-value[6]							
L	0.389	0.004	0.008	0.001	0.001	0.001	0.005
Q	0.920	0.704	0.219	0.038	0.019	0.037	0.099

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage at the start of the experiment.
[2]IDE = ileal digestible energy.
[3]Crude protein.
[4]Ether extract.
[5]FXU = fungal β-xylanase units per kg of feed.
[6]Linear (L) or quadratic (Q) effect.

supplementations provided an increase in all evaluated parameters when compared to the diet without xylanase ($P < 0.05$).

In the present study, xylanase supplementation at 100 FXU/kg in the corn-soy diet provided an increase ($P < 0.05$) of 145 kcal/kg, 5%, and 2.6% on IDE, CP digestibility, and EE digestibility, respectively, when compared to the diet without xylanase. This response is in agreement with findings by Kalmendal and Tauson (2012) who used the same enzyme product and observed an increase of 114 kcal/kg in AME_n when 34-day-old chickens were fed diets supplemented with 200 FXU/kg. Cowieson et al. (2010) also observed an increase of 100 kcal/kg in IDE after supplementing corn-soy diets with xylanase (8,000 β-xylanase units/kg) in 21-day-old broilers.

Regression equations of increased β-xylanase supplementation on total tract retention and ileal digestibility of CS diets, CN diets, and corn for broilers are shown in Table 5. Xylanase added to the CS diet resulted in quadratic increases ($P < 0.05$) for IDE, AME, AME_n, and CP digestibility. A maximum AME_n and IDE release was obtained with 92 and 124 FXU/kg, respectively. A maximum CP digestibility was obtained with 122 FXU/kg corresponding to 71%. However, xylanase supplementation to the CN diet led to linear increases ($P < 0.05$) in DM digestibility, AME_n, CP digestibility, and EE digestibility. Energy values and digestibility of

Table 5. Regression equations of apparent ileal digestibility and total tract retention of nutrients and energy of corn-soy-based diets, corn-based diets, and corn supplemented with β-xylanase.

Item	Regression equations[1]	*P*-value[2]	r^2	SD
Corn-soy-based diet (CS)				
IDE[3], kcal/kg DM	$Y = -0.014x^2 + 2.570x + 3{,}155$	0.001	0.60	89
Dry matter, %	$Y = -0.0002x^2 + 0.055x + 71.84$	0.042	0.61	1.5
AME, kcal/kg DM	$Y = -0.015x^2 + 3.687x + 3{,}178$	0.001	0.70	106
AME_n, kcal/kg DM	$Y = -0.016x^2 + 3.982x + 3{,}155$	0.001	0.68	109
Crude protein, % DM	$Y = -0.0003x^2 + 0.073x + 66.36$	0.006	0.71	1.9
Corn-based diet (CN)[4]				
Dry matter, %	$Y = 0.020x + 70.96$	0.026	0.22	4.0
AME, kcal/kg DM	$Y = 0.778x + 3{,}354$	0.002	0.33	116
AME_n, kcal/kg DM	$Y = 0.911x + 3{,}204$	0.001	0.47	107
Crude protein, % DM	$Y = 0.035x + 58.64$	0.005	0.38	4.8
Ether extract, % DM	$Y = 0.032x + 80.22$	0.001	0.46	3.8
Corn				
IDE, kcal/kg DM	$Y = 1.027x + 3{,}120$	0.004	0.28	139
Dry matter, %	$Y = 1.016x + 72.11$	0.007	0.17	2.7
AME, kcal/kg DM	$Y = 1.033x + 3{,}396$	0.001	0.47	108
AME_n, kcal/kg DM	$Y = 1.036x + 3{,}209$	0.001	0.51	105
Crude protein, % DM	$Y = 0.023x + 64.16$	0.001	0.34	2.8
Ether extract, % DM	$Y = 0.017x + 83.73$	0.005	0.19	2.7

[1]Regression equations for xylanase levels (0, 50, 100, 150, and 200 fungal β-xylanase units per kg of feed). The coefficient of determination (r^2) was obtained using all data.
[2]Linear (L) or quadratic (Q) effect ($P < 0.05$).
[3]IDE = ileal digestible energy.
[4]Corn-based diet was composed by 60% of corn-soybean meal basal diet + 40% of corn.

CP and EE increased linearly ($P < 0.05$) with xylanase supplementation, and a maximum energy release was not achieved until 200 FXU/kg when broilers were fed corn-based diets. As reported by Choct (1997) and Back Knudsen (1997) the total amount of arabinoxylans is variable among ingredients, and it was reported as 5.2% in corn and 3.3% in SBM. Then, based on corn and SBM inclusion, the amount of arabinoxylans was 4.0% in the CS diet and 4.5% in the CN diet. Regression analysis of data using the calculation extrapolated for corn suggested no significant effects on digestibility of DM when xylanase was used. However, energy values and digestibility of CP and EE of corn supplemented with xylanase increased linearly ($P < 0.05$).

As Table 5 shows, it seems that the improvement in energy from the CN diet with xylanase supplementation may be masked when the energy value of an ingredient is high. We also could observe based on linear equations that the amount of xylanase needed to improve digestibility and energy responses was higher in CS diets compared to CN diets. Also, there is a more optimal ME:AA ratio in the CS diet. Adding xylanase to the CN diet would only compound this problem by increasing metabolizable energy/digestible energy further while the diet under-supplies AA. Additionally, there may be more room for improvement in the digestibility of energy in SBM than for corn. Perhaps displacing SBM with corn resulted in a reduction in the opportunity for energy digestibility improvement.

Horvatovic et al. (2015) reported that the supplementation of diets with xylanase increased diet digestibility possibly because it promoted an increase in the activity of endogenous enzymes by increasing the availability of substrates. Xylanase increases access of encapsulated nutrients to endogenous enzymes due to disruption of cell wall arabinoxylans and establishes more beneficial bacterial in the lower GI tract through the production of xylo-oligomers (Kocher et al., 2003; Meng et al., 2005; Francesch and Geraert, 2009; O'Neill et al., 2012). Supplemental xylanase increased nutrient digestibility and also was shown to increase volatile fatty acid concentration linked to the increased flow of fermentable xylo-oligomers into the ceca (Choct et al., 1996). Furthermore, exogenous xylanases also have been related to increased nutrient digestibility via reduction in digesta viscosity and cell wall integrity, generating fermentable disaccharides and low-molecular weight polysaccharides and oligosaccharides; improving protein solubility; and decreasing endogenous losses and overcoming anti-nutritional factors (Cowieson and Ravindran, 2008).

The reason for the observed increase in energy utilization using xylanase may be associated with increased utilization of starch and fat from corn; protein from corn and soybean; and other carbohydrates from dietary components (Batal and Parsons, 2002). Broiler chickens fed diets that are essentially adequate in all nutrients often still respond to exogenous enzyme addition (Stefanello et al., 2015, Vieira et al., 2015), suggesting that enzyme benefits may result from changes in less tangible metrics such as appetite control, digestive physiology, immunology, or microbiology. However, if the diet is not able to provide balanced nutrients composition, energy utilization and nutrient digestibility can be affected (Bao et al., 2013).

In conclusion, the substitution method used in this study showed that AME_n, IDE, crude protein, and ether extract digestibility was higher in broilers fed a complete corn-soy-based diet than an "artificial" corn-based ration. However, the digestibility of CP and energy of corn-soy-based diets, corn-based diets, and corn increased with xylanase supplementation. When β-xylanase was tested in a corn-soy-based diet, 92 FXU/kg and 124 FXU/kg maximized its IDE and AME_n release effect; however, this maximum energy response in the corn-based diet or 100% corn was not achieved until 200 FXU/kg.

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REFERENCES

Adeola, O. 2001. Digestion and balance techniques in pigs. Pages 903–916 in Swine Nutrition. A. J. Lewis, and L. L. Southern, 2nd ed. CRC Press, Washington, DC.

Adeola, O., J. A. Jendza, L. L. Southern, S. Powell, and A. Owusu-Asiedu. 2010. Contribution of exogenous dietary carbohydrases to the metabolizable energy value of corn distillers grains for broiler chickens. Poult. Sci. 89:1947–1954.

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC Int., Arlington, VA.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC Int., Gaithersburg, MD.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338.

Bao, Y. M., L. F. Romero, and A. J. Cowieson. 2013. Functional patterns of exogenous enzymes in different feed ingredients. World's Poult. Sci. J. 69:759–774.

Batal, A. B., and C. M. Parsons. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81:400–407.

Bedford, M. R., H. L. Classen, and G. L. Campbell. 1991. The effect of pelleting, salt and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. Poult. Sci. 70:1571–1577.

Caffall, K. H., and D. Mohnen. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr. Res. 344:1879–1900.

Choct, M. 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. Feed Mill International. 13–26.

Choct, M., and G. Annison. 1992. Anti-nutritive effect of wheat pentosans in broiler chickens: Roles of viscosity and gut microflora. Br. Poult. Sci. 33:821–834.

Choct, M., R. J. Hughes, J. Wang, M. R. Bedford, A. J. Morgan, and G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br. Poult. Sci. 37:609–621.

Chwalibog, A. 2002. Substrate oxidation and retention in pigs and poultry. Anim. Sci. J. 73:95–104.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Technol. 119:293–305.

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

Cowieson, A. J., and M. R. Bedford. 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: Complementary mode of action? World's Poult. Sci. J. 65:609–624.

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

Cowieson, A. J., and H. V. M. O'Neill. 2013. Effects of exogenous xylanase on performance, nutrient digestibility and caecal thermal profiles of broilers given wheat-based diets. Br. Poult. Sci. 54:346–354.

Francesch, M., and P. A. Geraert. 2009. Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. Poult. Sci. 88:1915–1924.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive determinations with growing chicks. J. Nutr. 64:587–603.

Horvatovic, M. P., D. Glamocic, D. Zikic, and T. D. Hadnadjev. 2015. Performance and some intestinal functions of broilers fed diets with different inclusion levels of sunflower meal and supplemented or not with enzymes. Braz. J. Poult. Sci. 17:25–30.

Jiang, Z., Y. Zhou, F. Lu, Z. Han, and T. Wang. 2008. Effects of different levels of supplementary alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of young broilers. Asian-australas. J. Anim. Sci. 21:97–102.

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

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

Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-australas. J. Anim. Sci. 27:917–925.

Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Matterson, L. D., L. M. Potter, and M. W. Stutz. 1965. The metabolizable energy of feed ingredients for chickens. Agric. Exp. Stn. Res. 7:3–11.

Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell, and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. Poult. Sci. 84:37–47.

NRC, National Research Council, 1994. Nutrient Requirements for Poultry. 9th rev. ed. National Academy Press, Washington, DC.

Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2008. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. Br. Poult. Sci. 99:682–690.

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

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Lopes, A. S. Ferreira, S. L. T. Barreto, and P. F. Euclides. 2011. Tabelas brasileiras para aves e suínos. Composição de alimentos e exigências nutricionais. 3rd ed. UFV, Viçosa, MG, Brazil.

Sakomura, N. K., and H. S. Rostagno. 2007. Métodos de pesquisa em nutrição de monogástricos. Funep, Jaboticabal, SP, Brazil.

SAS User's Guide. 2009. Version 9.2 ed. SAS Inst. Inc., Cary, NC.

Savory, C. J. 1992. Metabolic fates of U-^{14}C-labelled monosaccharides and an enzyme-treated cell wall substrate in the fowl. Br. J. Nutr. 67:103–114.

Slominski, B. A., W. Guenter, and L. D. Campbell. 1993. New approach to water-soluble carbohydrate determination as a tool for evaluation of plant cell wall degrading enzymes. J. Agric. Food Chem. 41:2304–2308.

Smits, C. H. M., and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. World's Poult. Sci. J. 52:203–221.

Stefanello, C., S. L. Vieira, G. O. Santiago, L. Kindlein, J. O. B. Sorbara, and A. J. Cowieson. 2015. Starch digestibility, energy utilization and growth performance of broilers fed corn-soybean basal diets supplemented with enzymes. Poult. Sci. 94:2472–2479.

Theander, O., E. Westerlund, P. Åman, and H. Graham. 1989. Plant cell walls and monogastric diets. Anim. Feed Sci. Technol. 23:205–225.

Vieira, S. L., C. Stefanello, H. V. Rios, N. C. Serafini, R. G. Hermes, and J. O. B. Sorbara. 2015. Efficacy and metabolizable energy equivalence of an α-amylase-β-glucanase complex for broilers. Braz. J. Poult. Sci. 17:227–236.

Villamide, M. J. 1996. Methods of energy evaluation of feed ingredients for rabbits and their accuracy. Anim. Feed Sci. Technol. 57:211–223.

Vogtmann, H., P. Frirter, and A. L. Prabuck. 1975. A new method of determining metabolizability of energy and digestibility of fatty acids in broiler diets. Br. Poult. Sci. 16:531–534.