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■ Keywords

Carbohydrases, energy, enzymes, intestine, quail production.



Effects of the Dietary Inclusion of Xylanase on the Performance and Jejunum Morphometry of Meat-Type Quails

ABSTRACT

The objective of this study was to evaluate the effects of the supplementation of two xylanase products to diets with reduced metabolizable energy fed to meat-type quails during the starter phase (1-14 days). A completely randomized experimental design in a 2 x 3 + 1 factorial arrangement two reduced metabolizable energy (ME) diets, inclusion or not of xylanase, and a control diet with no enzyme addition) was applied, totaling seven treatments with five replicates of 45 quails each. At 14 days of age, jejunum segments were collected for morphometry evaluation. No interaction between the studied factors were detected for performance and jejunal morphometry parameters. Body weight, weight gain, feed intake and feed conversion were not influenced by enzyme inclusion. Reduced ME diets (-70 or -140 kcal/kg) did not affect performance, except for feed intake. Xylanase inclusion increased villus height and villus:crypt ratio. Therefore, xylanase supplementation can be effective in corn and soybean meal-based diets, without causing any impairment in the performance of 1- to 14-day-old quails. Xylanases A and B were more efficient when dietary energy level was reduced in 140 kcal ME/kg, and were also shown to effectively improve the jejunal morphometry of starter meat-type quails.

INTRODUCTION

Poultry production has exponentially grown in the last decades. Nutrition plays a prominent role in this expansion, particularly due to the increasing knowledge on the nutritional potential of nutrients and nutritional requirements. Nutritionists have researched diets that meet the nutritional requirements of quails, since their rapid growth and high productivity require quality feeds and the use of additives in order to promote better utilization of dietary nutrients (Lima *et al.*, 2011). One strategy is the dietary inclusion of exogenous enzymes, which helps to improve the performance of the birds by increasing nutrient digestibility, in addition of reducing the excretion of minerals, such as phosphorus (Fischer *et al.*, 2002). In addition, enzymes are alternative natural components allow reducing the use of in-feed antibiotics, addressing growing public concerns (Teixeira, 2007). In general, these natural components not only improve the condition of the intestinal epithelium, but also act as immune modulators.

The types of enzymes currently used in animal feed are those that breakdown fiber, protein, starch, and phytate, and are classified according to the substrates on which they act as cellulases, carbohydrases, proteases, amylases (Barletta, 2011), and phytases. Carbohydrases act on non-starch polysaccharides (NSPs), which are harmful to animals as they increase intestinal viscosity, interfering with the availability of nutrients and energy.



Among the carbohydrases, xylanase has gained prominence and shown satisfactory results, whether isolated or complexed with other enzymes. That enzyme is capable of breaking down the arabinoxylans present in grains. Arabinoxylan are hydrolyzed mainly by the activity of an endo-1,4- β -xylanase, which breaks down the (1,4) bonds of the xylan central chain, because the xylanases are specific for β -1,4 internal bonds of xylan polymers (Classen, 1996; Bhat & Hazlewood, 2001).

Although xylanases are typically used in viscous grain diets (Mathlouthi *et al.*, 2002; Schoulten *et al.*, 2003; Tekeli *et al.*, 2014), studies have also investigated its use with non-viscous or low viscosity grains (Iwahashi *et al.*, 2011; Barbosa *et al.*, 2012; Souza *et al.*, 2012; Khoramabadi *et al.*, 2014). In general, the inclusion of exogenous enzymes in poultry diets promotes a more efficient digestion, reducing maintenance energy requirements, as well as the amount of substrate that reaches the large intestine by improving substrate utilization in the small intestine, thereby altering the microbial population in the terminal ileum (Bedford & Apajalahti, 2001).

The objective of this study was to evaluate the live performance and intestinal morphometry of 1 to 14-day-old (starter phase) quails fed diets based on corn and soybean meal and supplemented with two xylanases in diets with reduced metabolizable energy content.

MATERIALS AND METHODS

The experiment was conducted at the quail production sector of the Experimental Farm of Iguatemi, of the State University of Maringá (UEM), state of Paraná, Brazil. The experimental protocol was approved by the Committee for Ethical Conduct on the Use of Animals in Experimentation of UEM (protocol n° 6841070515).

A total of 1575 unsexed meat-type quails (*Coturnix coturnix* sp) were housed in a conventional shed, built in the east-west direction, which was divided into 35 pens (2.5-m² each). The open-sided shed had a clay-tiled roof, dirt floor with rice strawlitter, and masonry side walls with wire screens up to the roof, fitted with side curtains.

A completely randomized experimental design in a 2 x 3 + 1 factorial arrangement (two reduced metabolizable energy (ME) diets, inclusion or not of xylanase, and a control diet with no enzyme addition) was applied, totaling seven treatments with five replicates of 45 quails each. The treatments consisted

of a control diet, formulated to meet the quails' nutritional requirements, with no enzyme inclusion (T1); control diet with xylanase A inclusion (T2); control diet with xylanase B (T3); diet with 70 kcal ME/kg and 0.35% crude protein (CP) reductions and no enzyme inclusion (T4); diet with 70 kcal ME/kg and 0.35% CP reductions with xylanase A inclusion (T5); diet with 70 kcal ME/kg and 0.35% crude protein (CP) reductions with xylanase B inclusion (T6); diet with 140 kcal ME/kg and 0.35% CP reduction and no xylanase inclusion (T7). The diets of T5 and T6 were formulated based on the diets of T 1-4, and the T7 diet was formulated based on the control treatment diet.

Xylanases were included at 100 g per ton of feed, with dietary titrations from the nutritional matrix of the enzymes, according to the manufacturer's recommendations. Xylanase A derives from the fungus *Trichoderma longibrachiatum*, with a minimum activity of 15000 EPU/g, and secondary activity of cellulase, β -glucanase, α -amylase and protease. Xylanase B derives from the fungus *Trichoderma reesei*, with a minimum activity of 160000 BXU/g. Both enzymes have primary endo-1,4 β -xylanase enzymatic activity.

The experimental diets (Table 1) were based on corn and soybean meal and their chemical and energy composition were calculated based on the values proposed by Rostagno *et al.* (2011).

In order to meet the nutritional requirements of quails, we followed the recommendations of Scherer *et al.* (2011) for ME requirements, Furlan *et al.* (2011) for digestible lysine requirements, Otutumi *et al.* (2009) for CP requirements, and Silva *et al.* (2009) for calcium and phosphorus requirements. Throughout the experimental period, feed and water were supplied *ad libitum*.

For the evaluation of live performance, birds and feed offer were weekly weighed to determine feed intake (FI; g/bird), body weight (BW; g), weight gain (WG; g) and feed conversion ratio (FCR; g/g).

At 14 days of age, one bird per experimental unit, representative of the average body weight of the pen (\pm 10%), was intravenously desensitized using barbiturate thiopental and then sacrificed by cervical dislocation.

Fragments of the jejunum were collected for morphometric analysis by light microscopy. Jejunal samples were washed in saline solution, fixed in buffered formalin solution (10%), dehydrated in graded ethanol series, cleared in xylol and embedded in paraffin, according to the methodology of Beçak & Paulete (1976). The 7- μ m thick longitudinal and



Table 1 – Ingredients and calculated nutritional composition of the experimental diets fed to 1- to 14-day-old meat-type quails.

Treatments	Control treatment	Xylanase A	Xylanase B	Reductions of ME ³ 70 kcal/kg	Reductions of ME + Xylanase A	Reductions of ME + Xylanase B	Reductions of ME 140 kcal/kg
Ingredients (%)							
Soybean meal 46%	51.10	49.80	49.80	49.80	49.50	49.50	49.50
Corn	41.44	44.29	44.29	44.29	45.96	45.96	45.96
Soybean oil	4.300	2.700	2.700	2.700	1.300	1.300	1.300
Dicalcium phosphate	0.920	0.920	0.920	0.920	0.920	0.920	0.920
Limestone 38% Ca	0.572	0.600	0.600	0.600	0.626	0.626	0.636
Mineral and vitamin supplement ¹	0.600	0.600	0.600	0.600	0.600	0.600	0.600
Salt	0.400	0.400	0.400	0.400	0.400	0.400	0.400
DL-Methionine 99%	0.270	0.265	0.265	0.265	0.263	0.263	0.263
L-Lysine 98%	0.234	0.253	0.253	0.253	0.259	0.259	0.259
L-Threonine	0.148	0.146	0.146	0.146	0.146	0.146	0.146
Xylanase A	-	0.010	-	-	0.010	-	-
Xylanase B	-	-	0.010	-	-	0.010	-
Phytase	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Antioxidant ²	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Total weight	100	100	100	100	100	100	100
Nutritional Levels							
Metabolizable energy (kcal/kg)	2.995	2.925	2.925	2.925	2.854	2.854	2.854
Crude protein (%)	27.489	27.139	27.139	27.139	27.142	27.142	27.142
Ether extract (%)	6.639	5.165	5.165	5.165	3.855	3.855	3.855
Calcium (%)	0.743	0.755	0.755	0.755	0.765	0.765	0.765
Available phosphorus (%)	0.425	0.425	0.425	0.425	0.426	0.426	0.426
Sodium (%)	0.181	0.181	0.181	0.181	0.181	0.181	0.181
Digestible Lysine (%)	1.599	1.585	1.585	1.585	1.585	1.585	1.585
Digestible Met+Cys (%)	1.150	1.139	1.139	1.139	1.139	1.139	1.139
Digestible Threonine (%)	1.039	1.025	1.025	1.025	1.025	1.025	1.025

¹Vitamin/mineral supplement (guaranteed levels per kilogram of diet): retinol acetate – 18,000 IU; cholecalciferol – 5000 IU; dl- α -tocopherylacetate – 16 mg; thiamine hydrochloride – 1.12 mg; riboflavin – 8 mg; pyridoxine hydrochloride – 2.1 mg; cyanocobalamin – 20 mcg; menadione nicotinamide bisulphite – 4.028 mg; D-calcium pantothenate – 16 mg; niacin acid – 40 mg; choline chloride – 560 mg; zinc oxide – 126 mg; ferrous sulphate – 98 mg; manganese sulphate – 155 mg; copper sulphate – 30.624 mg; cobaltous sulfate heptahydrate – 0.4 mg; potassium iodate – 1.936 mg; sodium selenite – 0.508 mg; butylated hydroxytoluene – 0.02 mg.

²BHT (butyl hydroxy toluene).

³Metabolizable energy.

semi-serial histological sections were stained by the hematoxylin-eosin method. Image capture of the slides was performed using a Leica optical microscope with an image capture system (Moticam 5MP). Ten villi and ten crypts per replicate were measured, at 4x magnification with Motic Images Plus software (version 2.0), and average jejunal villus height, crypt depth, and villus: crypt ratio were calculated.

Statistical analysis was performed using the software System of Statistical Analysis and Genetics - SAEG (version 9.1), of the State University of Viçosa - MG, according to the statistical models presented below:

$$Y_{ijk} = \mu + ME_i + ENZ_j + MEENZ_{ij} + e_{ijk} \quad (1)$$

and

$$Y_{ij} = \mu + T_i + e_{ij} \quad (2)$$

Where: Y_{ijk} is the response variable related to the level of metabolizable energy reduction ($i = 70$ kcal/

kg and 140 kcal/kg) inclusion or not of enzymes ($j =$ xylanase A, xylanase B, and no xylanase) in each replicate ($k = 1, 2, 3, 4$ and 5); μ is the general average; ME_i is the effect of metabolizable energy reductions ($ME_1 = 70$ kcal/kg and $ME_2 = 140$ kcal/kg); ENZ_j is the effect of enzyme inclusion ($ENZ_1 =$ xylanase A; $ENZ_2 =$ xylanase B and $ENZ_3 =$ no xylanase); $MEENZ_{ij}$ is the effect of the interaction of metabolizable energy with enzymes; e_{ijk} is the random error associated with each observation Y_{ijk} ; Y_{ij} is the response variable obtained in subject j , receiving treatment i ; T_i is the effect of the additional treatment; e_{ij} is the experimental error associated with the additional treatment.

For model 1, the data were submitted to an analysis of variance. There was a significant interaction ($p < 0.05$) between ME reductions and enzyme inclusion. Therefore, data were deployed and means were compared by Tukey's test ($p < 0.05$).



When the interaction was not significant, the effects of the factors were individually analyzed, with ME reductions subjected to analysis of variance and F test ($p < 0.05$) and enzyme inclusions subjected to an analysis of variance and Tukey's test ($p < 0.05$). For model 2, data were submitted to analysis of variance and means were compared by Dunnett's test ($p < 0.05$). All data were analyzed according to both statistical models.

RESULTS AND DISCUSSION

Table 2 shows the live performance results of 1 to 14-day-old meat-type quails fed the control treatment and the treatments with ME reductions of 70 kcal/kg and 140 kcal/kg and supplemented with xylanase A, B or not.

There was no interaction ($p > 0.05$) between ME reductions and xylanase inclusion for live performance.

Table 2 – Body weight (BW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) of 1- to 14-day-old meat-type quails fed diets with reduced metabolizable energy (ME) and supplemented or not with xylanase (X).

Variables	X (100g/t)	Reductions of ME (kcal/kg)		Mean	Control	Probabilities			CV (%) ¹
		70	140			ME	X	ME*X	
BW (g)	A	88.29	89.14	88.71					5.01
	B	87.71	88.86	88.28	87.00	1.0000	0.8447	0.6429	
	No	88.70	86.45	87.57					
	Mean	88.23	88.15						
WG (g)	A	79.94	80.73	80.33					5.48
	B	79.33	80.52	79.92	78.64	1.0000	0.8530	0.6306	
	No	80.38	78.10	79.24					
	Mean	79.88	79.79						
FI (g)	A	152.42	155.85*	154.1					2.95
	B	153.90	153.20	153.6	147.88	0.0782	0.5785	0.2342	
	No	148.87	155.25*	152.1					
	Mean	151.73 ^b	154.77 ^a						
FCR	A	1.90	1.93	1.91					4.89
	B	1.94	1.90	1.92	1.88	0.213	1.0000	0.1561	
	No	1.85	1.99	1.92					
	Mean	1.90	1.94						

¹Coefficient of variation.

*Different from the control treatment, according to Dunnett's test ($p < 0.05$).

^{a,b}Means followed by different lowercase letters in the same row significantly differ, according to the F test ($p < 0.10$).

Xylanase inclusion did not influence ($p > 0.05$) body weight, weight gain, feed intake, and feed conversion ratio were. Dietary ME reductions (70 or 140 kcal/kg) also did not affect ($p > 0.05$) live performance parameters, except for feed intake ($p = 0.0782$).

The birds fed the diet with 70 kcal ME/kg reduction presented 2% lower FI compared with 140 kcal ME/kg reduction, independently of xylanase supplementation. When comparing average FI of each treatment was compared with the control treatment, which diet supplied the birds' nutritional requirements and did not include xylanase, by Dunnett's test, the birds fed the diets with 70 kcal ME/kg reduction showed similar FI as those fed the control treatment ($p > 0.05$), independently of xylanase inclusion. On the other hand, higher FI was determined in the birds fed the 140 kcal ME/kg reduced diets compared with the control birds ($p < 0.05$), except for those fed the diet with xylanase B, which the FI was similar to that of the control birds ($p > 0.05$).

The reduction of 70 kcal ME/kg reduction did not significantly affect FI, even when xylanase was not supplemented. On the other hand, the diets with reduction of 140 kcal/kg caused an increase in feed intake. Iwahashi *et al.* (2011) also found higher FI in quails fed corn and soybean meal-based diets with a reduction of 120 kcal ME/kg on dry matter basis and supplemented with an enzyme complex. However, evaluating enzyme supplementation to reduced-energy broiler diets, Barbosa *et al.* (2012) determined lower FI in the birds fed the reduced-energy diet with enzyme inclusion compared with those fed the diet with adequate energy levels not supplemented with enzymes.

The dietary treatments did not influence ($p > 0.05$) quail body weight, weight gain, or FCR. The average values of these parameters in birds fed reduced-ME diets with enzymes or not were similar to those obtained in the control birds.



The inclusion of xylanases A and B, independently of ME level, yielded numerically better performance results compared with the control diet slightly better in relation to the control diet ($p>0.05$), except for FCR. This may be attributed to a possible release of dietary nutrients by the xylanases, which were not considered when the diets were formulated. According to Barbosa *et al.* (2008), the inclusion of enzymes is recommended in starter diets due to the immaturity of the birds' enzymatic system.

The supplementation of exogenous enzymes is known to produce variable responses, even when added to similar diets and fed to animals of the same age (Officer, 2000). Evaluating the effects of protease supplementation on the performance of meat-type quails, Torres *et al.* (2014) did not observe any differences ($p>0.05$) in the feed intake, weight gain, or FCR of quails in the starter phase.

The birds fed xylanase A, regardless of ME reduction, showed 2.1 and 4% improvements in weight gain and feed intake, respectively, compared with those fed the control diet; however, this was not significant ($p>0.05$). The FCR of quails fed reduced-ME diets was 1.5% higher, although not statistically significant ($p>0.05$), than that of the control birds, which is a result of their higher FI to try to supply dietary energy deficiency.

Quail performance is still low, with high FI and FCR (Oliveira, 2001), especially when compared with broilers, which may be attributed to lower selection pressure of meat-type quails relative to broilers.

Although xylanase A also has secondary cellulase, β -glucanase, α -amylase and protease activities, they may have not effectively improved the digestibility of feed nutrients. This may be due to the lack of substrates, since enzymes are substrate-dependent. Another hypothesis is that the dosage 100 g/t may not be sufficient to yield any significant effects. On the other hand, xylanase B led to 1.6 and 3% improvements in weight gain and feed intake, respectively, when compared to the control diet. Evaluating four xylanase inclusion levels (0, 200, 400 and 600 g/ton), Schoulten *et al.* (2003) found that xylanase, at an adequate dose, is effective in reducing the negative effects caused by NSPs present in highly-viscous diets. Xylanase also has positive effects on low viscosity diets (Iwahashi *et al.*, 2011; Souza *et al.*, 2012; Khoramabadi *et al.*, 2014).

The inconsistency of performance results of the quails supplemented with exogenous enzymes in the present study may be attributed to the levels of soluble NSPs present in corn and soybean meal, which are relatively low compared with other cereals with high NSP content (Cowieson *et al.*, 2006). The values of soluble NSPs in corn vary between 0.8 and 1 g/kg DM (Mathlouthi *et al.*, 2002; Choct, 2006), with a predominance of arabinoxylans. On the other hand, soybean meal has between 13.4 and 27 g/kg DM (Choct, 2006).

Table 3 shows mean jejunal morphometric values of 14-day-old quails fed the control diet, and the diets with ME reductions of 70 kcal/kg and 140 kcal/kg and supplemented or not with xylanases A or B.

Table 3 – Mean villus height (VH), crypt depth (CD) and villus:crypt (V:C) ratio of the jejunum of 14-day-old meat-type quails fed diets with reduced metabolizable energy (ME) and supplemented or not xylanases (X).

Variables	X (100g/t)	ME reduction (kcal/kg)		Mean	Control	Probabilities			CV (%) ¹
		70	140			ME	X	ME*X	
VH (μ m)	A	446.14	532.21	489.17 A					
	B	468.88	504.42	486.65 A	412.51	0.1152	0.0013	0.6633	16.28
	No	337.93	362.27	350.10 B					
	Mean	417.65	466.3						
CD (μ m)	A	78.66	82.04	80.35					
	B	80.34	83.38	81.86	80.02	0.1434	0.8450	0.4204	13.84
	No	71.42	85.83	78.62					
	No	76.81	83.75						
V:C (μ m)	A	5.65	6.47	6.06 A					
	B	5.94	6.08	6.01 A	5.38	0.7440	0.0022	0.2652	15.07
	No	4.82	4.22	4.52 B					
	Mean	5.47	5.59						

¹Coefficient of variation.

*Different from the control treatment, according to Dunnett's test ($p<0.05$).



There was no interaction ($p>0.05$) between ME reductions and xylanase supplementation for the intestinal morphometric parameters. Dietary ME reduction had no influence on the measured parameters ($p>0.05$). Xylanase inclusion did not affect villus height or crypt depth; however, it significantly influenced villus:crypt ratio ($p<0.05$). In addition, no differences were detected by Dunnett's test ($p>0.05$) relative to the control treatment.

Although there was no significant difference between xylanases A and B according to Tukey's tests, the villi of the birds fed the diets with no inclusion of xylanase were much lower than those of birds fed the diets containing xylanase. In this case, the enzymes allowed the villi to be exposed at a greater height. Exposure increased when metabolizable energy was reduced, although this difference was not statistically significant ($p>0.05$).

The diet with reduction of 70 kcal ME/kg and no xylanase inclusion resulted in numerically lower crypt depth values than when xylanase was included, while the opposite was observed with the reduction of 140 kcal ME/kg ($p>0.05$).

The villus height:crypt depth ratio was influenced by the inclusion of xylanases ($p<0.05$). The villus:crypt ratio of the birds fed no xylanase was statistically lower compared with those obtained with the inclusion of xylanases A and B. However, mean V:C values were not statistically different between xylanases A and B ($p>0.05$).

The gastrointestinal tract (GIT) undergoes major changes post-hatching, such as functional maturation of the intestine, which involves morphological and physiological changes that allow for an increase in the surface area for digestion and absorption (Maiorka *et al.*, 2002). Intestinal changes intestine due to the presence of dietary exogenous enzymes tend to be small, but they are frequently observed, not only in terms of a reduction of intestinal size and/or the release of endogenous enzymes, but also of an increase in villi (Yang *et al.*, 2008). One response to the improved nutrient absorption of ingested food is the greater development of the small intestine, that is, the longer its length, the more extensive the area of exposure of the absorptive cells to the nutrients, resulting in better nutrient utilization for muscle accretion (Gomes *et al.*, 2007).

The development of the intestinal mucosa consists of increasing the height or density of the villi, which corresponds to a larger number of epithelial cells (enterocytes, and goblet and enteroendocrine cells)

and, consequently, an increase in the digestive and absorptive capacity of the intestine (Uni *et al.*, 2000). Therefore, the higher the villi, the greater the nutrient absorption capacity. Villus height may decrease, however, when the proliferation rate is reduced and/or when extrusion rate is increased (Macari, 1995).

The presence of nutrients in the GIT lumen stimulated villus and crypt growth (Maiorka *et al.*, 2002). This may explain the mean villus height values found in the present experiment. Despite the lack of statistical significance, the highest villi were measured in the presence of xylanases, which suggests greater nutrient availability, and consequently, an improvement of intestinal digestive and absorptive capacities. Therefore, the higher FI of the birds fed xylanase may have influenced the observed changes in jejunal morphometry.

Crypt depth values accompanied villus height values, that is, villus:crypt ratio also decreased or increased accordingly. Therefore, higher V:C ratios increase nutrient absorption and reduced cell turnover energy losses. The highest V:C ratios were determined with the diets with xylanase inclusion, independently of xylanase type, indicating the efficacy of this enzyme to improve epithelial conditions.

Overall, the obtained performance results indicate that both xylanases evaluated were efficient to maintain quail performance, and had no detrimental effects. Jejunal morphometry showed improvement in villus height and villus:crypt ratio in 14-day-old quails. Therefore, xylanase supplementation can be effective in corn and soybean meal-based diets, without causing any impairment in the performance of 1- to 14-day-old quail. Xylanases A and B were more efficient when dietary energy level was reduced in 140 kcal ME/kg, and were also shown to effectively improve the jejunal morphometry of starter meat-type quails.

ACKNOWLEDGMENTS

The authors thank the National Council for Scientific and Technological Development (CNPq), for the granting of the scholarship and Br Nova Sistemas Nutricionais, for their support and for supplying the xylanase products.

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