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Performance and morphology of intestinal mucosa of broilers fed mannan-oligosaccharides and enzymes

[*Desempenho e morfologia da mucosa intestinal de frangos de corte alimentados com mananoligossacarídeos e enzimas*]

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ABSTRACT

The performance and the morphology of intestinal mucosa of broilers fed mannan-oligosaccharides (MOS) and enzymes (E) from one to 21-day-old were evaluated using 750 one-day-old chicks, assigned to a 2 x 2 + 1 factorial design - two levels of MOS (0 and 0.1%), two levels of E (0 and 0.05%) plus an antibiotic positive control diet – performing five treatments of five replications each one. MOS x E interaction was significant for both duodenal (P<0.002 and P<0.002) and ileal (P<0.04 and P<0.05) perimeters and heights of villi, being the values lower in the mucosa of birds fed non-supplemented diets. MOS based-diet determined an increase on perimeter of jejunal villi (P<0.05). Compared with antibiotic treatment group, villi perimeter (P<0.02) and height (P<0.005), and crypt depth (P<0.02) of duodenum of broiler fed MOS were higher. Broilers fed MOS and/or E did not perform better, but higher villi perimeter and height were observed in the intestinal mucosa of those birds.

Keywords: broiler, nutrition, enzymes, prebiotic

RESUMO

Avaliaram-se o desempenho e a morfologia da mucosa intestinal de frangos de corte alimentados com mananoligossacarídeos (MOS) e enzimas (E) até os 21 dias de idade. Utilizaram-se 750 pintainhos de um dia em delineamento experimental inteiramente ao acaso, em esquema fatorial 2 x 2 + 1 (dois níveis de MOS - 0 e 0,1%, dois níveis de E - 0 e 0,05% e uma dieta controle positivo com antibióticos) totalizando cinco tratamentos com cinco repetições cada. A interação MOS x E foi significativa para o perímetro de altura de vilos no duodeno (P<0,02 e P<0,02) e no íleo (P<0,04 e P<0,05), sendo os valores menores observados na mucosa das aves alimentadas com dietas não-suplementadas. A dieta contendo MOS determinou aumento no perímetro dos vilos no jejuno (P<0,05). Comparado com o grupo controle positivo, o perímetro (P<0,02) e a altura (P<0,005) dos vilos e a profundidade de cripta (P<0,02) no duodeno das aves do tratamento com MOS foram maiores. As aves que consumiram dietas com MOS e/ou E não tiveram melhor desempenho, mas maiores perímetros e alturas de vilos foram observados na mucosa intestinal dessas aves.

Palavras-chave: frangos de corte, enzimas, nutrição, prebiótico

INTRODUCTION

Among substances quoted as prebiotic, the mannan-oligosaccharides (MOS) has emerged. They are fragments of cellular wall of *Saccharomyces cerevisiae*, containing in their structure mannose, glucose, and protein (Spring et al., 2000). They have been associated to better performance of broilers (Flemming et al., 2004). Although corn and soybean-based diets are considered to be of low viscosity, these feedstuffs have non-starch polysaccharides

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(NSPs) in their structures (Kocher et al., 2003). Dietary exogenous enzymes diminish the anti-nutritional effects of NSPs while furnishing extra quantity of endogenous enzymes synthesized by the gastrointestinal tract (GIT) of the poultry. Improvement of body weight gain and feed conversion of 18-day-old broiler chickens were obtained with the use of dietary cellulase (Meng et al., 2005).

Feeding 0.1 to 0.5% of MOS to broilers, some authors registered deeper jejunal and ileal crypts (Iji et al., 2001) and augment of perimeter and height of duodenal villi (Loddi, 2003); however, the same amount of MOS furnished to turkeys resulted in smaller villi height as compared to groups fed antibiotics-based diets (Sims et al., 2004).

The use of exogenous enzymes that promote hydrolysis of NSPs can increase the height of villi and the proportion height-to-depth of the crypts, and lower the bacterial activity of the GIT as well (Mathlouthi et al., 2002a). Very high bacterial growth in the GIT can negatively interfere with the digestibility of both nitrogen and fat of the diet (Mathlouthi et al., 2002b).

This study aimed to evaluate the effects of the diet inclusion of MOS and enzymes (E) upon the performance and morphology of intestinal mucosa of broilers at 21 days of age.

MATERIAL AND METHODS

Seven hundred and fifty one-day-old Cobb chicks averaging 41.51 ± 0.59g were randomly assigned to a 2 x 2 + 1 factorial design - two levels of MOS[1] (0 and 0.1%) *vs* two levels of enzymes[1] (0 and 0.05%) plus an antibiotic positive control diet – performing five treatments of five replicates each. Positive control diet contained colistin sulphate (125ppm) and virginiamycin (10ppm) as growth promoters, and salinomycin (51ppm) as anticoccidial**.** The diet with no MOS or enzymes was considered as negative control.

The enzyme phytase[3] with minimal activity of 250U/g was added to all diets, formulated to have 0.38% of available phosphorus that was correspondent to 85% of the broiler chicken requirement on starter phase. Experimental mashed diets showed in the Table 1 were isonutritives and formulated to meet requirements proposed by Rostagno et al. (2000) for feeding broiler chickens until 21 days of age, except for metabolizable energy (ME) and crude protein (CP) that were 98% of the level recommended by the latter authors. Water and feed were provided *ad libitum*. Liquid enzyme, a blend of cellulase, protease, and α-amylase was premixed in 500g of soybean meal before inclusion to the diets.

At 21 days of age, body weight gain, feed intake, feed conversion, and liability of the broilers were evaluated. After 12h of fasting, four birds per treatment were submitted to euthanasia, and two samples (2cm each one) were taken from the midpoint of the duodenum, from the midpoint between the point of entry of the bile duct and vitelline diverticulum (jejunum), and midway between Meckel's diverticulum and the ileocecal junction (ileum) from each broiler, one for light and other for scanning electron microscopy techniques.

For light microscopy, the segments were opened and rinsed with phosphate buffer (0.1M, pH 7.4) and fixed in Bouin`s solution for 24 hours. Afterward, they were dehydrated in a graded series of ethanol, diaphanized in xylene and paraffin embedded. Cross sections of the intestine were made at 5µm and they were stained with hematoxylin and eosin. Forty readings per sample for villi perimeter and height, and crypt depth were done. The counting of the number of goblet cells was done in an area of 1mm square.

For scanning electron microscopy, the samples of each intestinal segment were fixed in glutaraldeyde (3%) for 48 hours and then rinsed with phosphate buffer (0.1M, pH 7.4). The samples were post-fixed in 2% osmium tetra oxide for two hours and rinsed again with the same buffer solution. Afterward, they were dehydrated in a graded series of ethanol and dried in CO_2 critical point dryer. The dried samples were metalized with gold/palladium and examined with a Jeol-JSM 5410 scanning electron microscope (operated at 15 kV) from three areas (1mm square each one) of each sample for the villi density.

[1]Bio-Mos and Allzyme Liquid Vegpro. Alltech do Brasil Agroindustrial Ltda. - Araucária, Brazil.
[3]Allzyme Phytase 2X. Alltech Inc. - Nicholasville, USA.

Table 1. Ingredient and chemical composition of experimental diets containing mannan oligosacharides (MOS) or enzymes (E) fed to broiler chickens from one to 21-day-old

Ingredient	Treatment				
	Positive control	Negative control	E	MOS	MOS+E
Corn	59.74	59.74	59.74	59.74	59.74
Soybean meal	34.34	34.34	34.34	34.34	34.34
Soybean oil	1.58	1.58	1.58	1.58	1.58
Dicalcium phosphate	1.45	1.45	1.45	1.45	1.45
Limestone	1.22	1.22	1.22	1.22	1.22
DL-Metionine 99%	0.04	0.04	0.04	0.04	0.04
L-Lysine 78.8%	0.20	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45	0.45
Phytase	0.01	0.01	0.01	0.01	0.01
MOS[1]	-	-	-	0.10	0.10
Enzymes[1]	-	-	0.05	-	0.05
Caulim	0.15	0.15	0.10	0.05	-
Antioxidant	0.02	0.02	0.02	0.02	0.02
Mineral and vitamin premix	0.80[2]	0.80[3]	0.80[3]	0.80[3]	0.80[3]
			Calculated nutrients		
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
ME (Kcal/kg)	2,940	2,940	2,940	2,940	2,940
Calcium (%)	0.96	0.96	0.96	0.96	0.96
Available phosphorus (%)	0.38	0.38	0.38	0.38	0.38
Lysine (%)	1.26	1.26	1.26	1.26	1.26
Methionine (%)	0.49	0.49	0.49	0.49	0.49

[1]Per kg: phosphorilated mannan oligosaccharides 30%.
[2]Per kg of premix: 1,500,000UI vit. A; 285,000UI vit.D_3, 1350mg vit.E, 230mg vit.K_3, 115mg vit.B_1, 1150mg vit.B_2, 2,000mcg vit.B_{12}, 4,800mg nicotinic acid, 1,240mg pantothenic acid, 230mg pyridoxine, 12mg biotin, 115mg folic acid, 85g choline, 170g methionine, 6,300mg Fe, 9,400mg Cu, 9,400mg Mn, 7,819mg Zn, 160mg I, 23mg Se, 20g antioxidant, 5.4g growth promoter, 6.4g anticoccidial.
[3]The same as in 2 but with no growth promoter and anticoccidial.

The data obtained of MOS x enzyme factorial were submitted to the two-way ANOVA test and the means were compared by the Tukey test. The Dunnett test was used to check significant differences among the means of positive control treatment and MOS x enzymes factorial.

RESULTS AND DISCUSSION

Performance (data not shown) was not influenced by treatments (P>0.05). Average body weight (862g), weight gain (821g), feed intake (1,185g), feed conversion (1.44) and liability (98.80%) were comparable to those obtained for Cobb broilers at 21 days of age.

Although MOS and enzymes are considered promoters of better digestibility of nutrient and could improve performance of broilers as consequence, the use of these additives, either isolated or associated, did not determine any beneficial effects in this study; but similar performance results of broiler chickens compared to antibiotic use were achieved. It is possible that the non-effective action of both MOS and enzymes reflected the good housing conditions provided to the birds. On the other hand, reduced ME and CP levels of diet without enzymes did not sufficiently depress the performance of chickens (data not shown).

Hooge et al. (2003) and Flemming et al. (2004) obtained similar results of non-improvement on performance of 21-day-old broiler chickens fed 0.01% to 0.10% of MOS. Also, Iji et al. (2003) and Pinheiro et al. (2004) did not find any difference upon the performance of chickens fed cellulase, protease, and α-amylase supplemented diets. Kirkpinar et al. (2004) tested prebiotic and E (protease, amylase, cellulase, xylanase, lipase,

phytase, and β-glucanase), and no increase on broiler performance was achieved.

A MOS x E interaction was observed for perimeter (P<0.002) and height (P<0.002) of duodenum villi (Table 2) that were larger compared to those of negative-control chickens. In jejunal segment (Table 3), addition of MOS to the diet increased (P<0.05) the perimeter of the villi. On the other hand, perimeter (P<0.04) and height (P<0.05) of ileum (Table 4) villi from chickens fed non-additive diets were both smaller. Compared to chickens fed antibiotic-treated diet, duodenal villi from birds of MOS group had larger perimeter (P<0.02), larger height (P<0.005), and deeper crypts (P<0.02).

Probably, the high proliferation of all types of bacteria in duodenum and ileum of broiler chicken fed diets without antibiotic, MOS and/or E damaged the intestinal mucosa. This effect could justify the lower villi perimeter and height in duodenum and ileum segments obtained from non-supplemented birds, as suggested by Juskiewicz et al. (2004) and Santos Jr. et al. (2004).

On the other hand, the increased size of the villi and the deeper crypts observed in the gut mucosa of broiler chickens fed MOS, as compared to those fed antibiotic, seems to be due to the limited bacterial capacity of proliferation and colonization determined by the use of antibiotic. The reduction of crypt depth coupled with lower villi height and perimeter are indicative that the epithelial turnover diminished as consequence of the suppression of beneficial bacteria in the gut, as *Lactobacillus* and bifidobacteria. Such effect diminishes the production of short chain fatty acids (SCFA) and increases the intestinal pH, supporting the development of pathogenic bacteria (Edens et al., 1997) that damage the mucosa of the intestine (Ferket, 2004).

Table 2. Perimeter (PV) and height (HV) of villi, crypt depth (CD), and goblet cell (GCD), and villi densities (VD) of duodenum of 21-day-old broilers fed according to dietary treatments

Positive control [1]	MOS	Enzymes	PV (μm)	HV (μm)	CD (μm)	GCD/mm^2	VD/mm^2
+	-	-	2,361	1,161	126	736	22.92
-	-	-	2,332b	1,167b	155	770	18.70
-	-	+	2,807a	1,408a	164	806	15.93
-	+	-	2,972a*	1,513a*	189*	837	17.68
-	+	+	2,759a	1,391a	167	591	21.81
	-		2,570	1,288	159	788	17.32
	+		2,865	1,452	178	714	19.75
		-	2,652	1,340	172	803	18.19
		+	2,783	1,399	165	699	18.87
					Probability (P<)		
Positive control *vs* factorial			0.02	0.006	0.02	NS	NS
MOS			0.05	0.003	NS	NS	NS
E			NS	NS	NS	NS	NS
MOS *vs* E			0.002	0.002	NS	NS	NS
SE			78.86	41.52	6.50	58.70	1.03

+ = with; - = without. NS = non significant.
Means within the same column followed by distinct letters differ by Tukey test (P<0.05).
* Different from positive control by Dunnett test at P<0.05.
[1]Growth promoter (colistin sulphate, 125ppm and virginiamycin, 10ppm) and anticoccidial (salinomycin, 51ppm).

Table 3. Perimeter (PV) and height (HV) of villi, crypt depth (CD), and goblet cell (GCD), and villi densities (VD) of jejunum of 21-day-old broilers fed according to dietary treatments

Positive control [1]	MOS	Enzymes	PV (μm)	HV (μm)	CD (μm)	GCD/mm^2	VD/mm^2
+	-	-	1,734	852	126	1,145	26.97
-	-	-	1,443	692	134	902	26.33
-	-	+	1,750	894	123	1,116	24.71
-	+	-	1,844	914	137	1,291	23.95
-	+	+	1,809	890	147	1,369	25.65
	-		1,596b	793	128	1,009	25.52
	+		1,827a	902	142	1,330	24.80
		-	1,644	803	135	1,097	25.14
		+	1,780	892	135	1,242	25.18
					Probability (P<)		
Positive control *vs* factorial			NS	NS	NS	NS	NS
MOS			0.05	NS	NS	NS	NS
E			NS	NS	NS	NS	NS
MOS *vs* E			NS	NS	NS	NS	NS
SE			60.50	33.70	6.40	97.33	0.55

+ = with; - = without. NS = non significant.
Means within the same column followed by distinct letters differ by Tukey test (P<0.05).
[1]Growth promoter (colistin sulphate, 125ppm and virginiamycin, 10ppm) and anticoccidial (salinomycin, 51ppm).

Table 4. Villi perimeter (PV) and height (HV), crypt depth (CD), goblet cell (GCD), and villi densities (VD) of ileum of 21-day-old broilers fed according to dietary treatments

Positive control [1]	MOS	Enzymes	PV (μm)	HV (μm)	CD (μm)	GCD/mm^2	VD/mm^2
+	-	-	1,475	668	116	1,183	35.97
-	-	-	1,211b	584b	113	1,212	35.19
-	-	+	1436a	699a	120	1133	36.30
-	+	-	1,392ab	679ab	120	982	36.45
-	+	+	1,310ab	657ab	106	1,251	33.04
	-		1,323	641	116	1,172	35.74
	+		1,351	668	113	1,116	34.74
		-	1,301	632	116	1,097	35.82
		+	1,373	678	113	1,192	34.67
					Probability (P<)		
Positive control *vs* factorial			NS	NS	NS	NS	NS
MOS			NS	NS	NS	NS	NS
E			NS	NS	NS	NS	NS
MOS *vs* E			0.04	0.05	NS	NS	NS
SE			39.06	18.90	4.95	110.86	1.14

+ = with; - = without.
Means within the same column followed by distinct letters differ by Tukey test (P<0.05).
[1]Growth promoter (colistin sulphate. 125ppm and virginiamycin, 10ppm) and anticoccidial (salinomycin, 51ppm).

In the jejunum, the inclusion of MOS to broiler diet increased villi perimeter probably enlarging the nutritive absorptive area. The stimulatory effects of MOS may be attributed to the production of SCFA after fermentation of digesta in the distal intestine. It was suggested that some SCFA induce cell proliferation of the intestinal mucosa (Lan, 2004).

It appears that the enzymes-based diets caused a higher effect upon the perimeter area and villi height of ileal mucosa than the use of MOS. This fact possibly occurred because the inclusion of the enzymes to the diet quantitatively lowered the available substrate to the bacteria growth, usually high in the lower segment of the small intestine. Besides, an increased retention time of the digesta in the gut could determine a higher hydrolysis by endogenous enzymes. According to Camiruaga et al. (2001), even non-viscous NSP can diminish the retention time of intestinal contents and impair the action of the enzymes present in the gut.

The increase of villi height and superficial area of birds subject to rich viscous cereal, protease, α-amylase, and xylanase or β-glucanase diets was supposed to be a consequence of the lower concentration of intestinal bacteria (Gilbert et al., 2001; Mathlouthi et al., 2002a). Loddi (2003) and Sims et al. (2004) also observed higher height and perimeter of duodenal villi determined by the inclusion of MOS to broiler diet.

CONCLUSIONS

The present study showed that the use of MOS and/or E to diets of one to 21-day-old broilers did not improve their productive performance, despite the fact that higher perimeter and height of villi were observed in duodenum and ileum, increasing the absorption surface of these intestinal segments.

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