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Effect of hydrolyzed copra meal separately or in combination with *Bacillus cereus* var. *toyoi* on growth performance of broiler chickens

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ABSTRACT. The effects of mannanase-hydrolyzed copra meal (MCM) and MCM + probiotic *Bacillus cereus* var. *toyoi* (TY) on growth performance and gut morphometry of broiler chickens were investigated. A total of 1120-one-day-old Cobb chicks were distributed in a completely randomized design with 4 diet treatment groups. Dietary treatments were (1) negative control; (2) positive control (avilamycin 10 ppm); (3) 0.1% MCM for basal diets (4) 0.1% MCM + 0.05% TY. The best feed conversion ratio (FCR), body weight (BW), productivity index (PI) was obtained with 0.1% MCM + 0.05% TY at 42 days of age. With regard to productivity index, every supplementation group had a better rate when compared to that in negative group. Although 0.1% MCM supplemented alone is worse than positive group, it reveals a significantly better value than when it is combined with 0.1% MCM and 0.05% TY. The combination of mannanase-hydrolyzed copra meal with *Bacillus cereus* var. *toyoi* improved broiler performance and duodenum and jejunum mucous morphometry, when compared to the negative, positive and 0.1% MCM alone supplementation groups. The combination of MCM and TY probiotics is capable of improving intestinal morphology by behaving like a good growth promoter, with possibility of being an alternative to antibiotics.

Keywords: avilamycin, mannanase-hydrolyzed copra meal, prebiotics, probiotics.

Efeito da farinha hidrolisada da torta de coco, isoladamente ou em combinação com *Bacillus cereus* var. *toyoi,* sobre o desempenho de frangos de corte

RESUMO. Foram investigados os efeitos da farinha hidrolisada da torta de coco (MCM) e MCM + probiótico *Bacillus cereus* var. *toyoi* (TY) sobre o desempenho e morfometria intestinal de frangos de corte . Foram utilizados 1.120 pintos de corte da linhagem Cobb 500, distribuídos em delineamento inteiramente casualizado, com quatro tratamentos e dez repetições de 28 aves cada. Os tratamentos foram: (1) controle negativo, (2) controle positivo (avilamicina 10 ppm), (3) 0,1% MCM na dieta controle negativo, (4) 0,1% MCM + 0,05% TY na dieta controle negativo. A melhor conversão alimentar (CA), o peso corporal (PC) e o índice de produtividade (IP) foram obtidos no tratamento com 0,1% MCM + 0,05% TY aos 42 dias de idade. A combinação da farinha hidrolisada da torta de coco com o *Bacillus cereus* var. *toyoi* melhorou o desempenho e a morfometria da mucosa do duodeno e do jejuno, quando comparado com os grupos controle negativo, positivo e 0,1 % MCM isoladamente. A combinação do MCM com o TY tem potencial para melhorar a morfologia intestinal, comportando-se como um bom promotor de crescimento com possibilidade de alternativa aos antibióticos.

Palavras-chave: avilamicina, mannanase-hidrolisada da torta de coco, prebiótico, probiótico.

Introduction

Antibiotics are used worldwide in the poultry industry not only to prevent poultry pathogens and disease but also to improve meat and egg production. However, contemporary biosecurity threats from pathogens´ increasing resistance to antibiotics (SORUM; SUNDE, 2001), imbalances of normal microflora (ANDREMONT, 2000) and the accumulation of antibiotic residues in animal products and the environment (BARTON, 2000; VAN DEN BOGAARD; STOBBERINGH, 2000; SNEL et al., 2002) have resulted in a claim for a worldwide ban on antimicrobial growth promoters (AGP). Consequently, the development of alternatives to AGP using either beneficial microorganisms or non-digestible ingredients that enhance microbial growth becomes necessary.

It had been demonstrated that β-1,4-mannobiose (MNB) could act as an immune-modulating agent *in vivo*, preventing *Salmonella enteritidis* infection in broilers by increasing IgA production and improving *Salmonella enteritidis* clearance (AGUNOS et al., 2007), as well as by up-regulating the local expression of genes involved in host defense and innate immunity (IBUKI et al., 2010). These authors have also found that MNB enhances *Salmonella*-killing activity and activates innate immune responses in chicken macrophages (IBUKI et al., 2011). Recently, mannanase-hydrolyzed copra meal (MCM), which contains 10% MNB, was reported effective for improving intestinal morphology in broiler chickens (IBUKI et al., 2014) and for increasing breast muscle in growing chickens without affecting muscle proteolysis (IBUKI et al., 2013). Results indicate that MCM, which includes MNB, may be an alternative to antibiotics. However, no studies have been undertaken comparing antibiotics to MCM or MNB.

Therefore, within the context of finding an alternative to antibiotics, current investigation was designed to determine the effect of MCM, or its combination with the probiotic *Bacillus cereus* var. *toyoi* on growth performance, carcass yield and intestinal mucosal morphometry of broiler chickens between 1 and 42 days of age.

Material and methods

Animals and experimental procedures

A total of 1120 one-day-old Cobb chickens were distributed in a completely randomized design into four diet treatments groups of 28 broilers in each group, with ten replications. The housing and experimental procedures reported herein were approved by the Institutional Animal Care and Use Committee (CEBEA 001771-09) of the State University of São Paulo, São Paulo State, Brazil.

The broilers were vaccinated against Marek disease, Newcastle disease, and infectious bronchitis at the hatchery. Chicks were immunized against Gumboro and Newcastle diseases on the 7th day of age and received reinforcement immunization against Gumboro disease on the 14th day of age. Registered room temperature during the experimental period was 27 ± 2.8 ºC and the relative humidity was 78 ± 8.7%.

The feeding program was divided into 3 phases, an initial phase (1–21 days of age), growth phase (22–35 days of age), and final phase (36–42 days of age). Table 1 shows the experimental treatments.

Table 1. Experimental treatments.

Treatments	Supplementation
Negative control	No antibiotics and no MCM
Positive control	10 ppm of avilamycin for basal diets
0.1% MCM	0.1% of MCM for basal diets
0.1% MCM + 0.05% TY	0.1% of MCM + 0.05% TY for basal diets

MCM: prebiotic, mannanase-hydrolyzed copra meal contained 11.4% β-1,4-mannobiose, Fuji Oil, Ltd., Osaka, Japan. Y: probiotic, containing 1010 viable spores of *Bacillus cereus* var. *toyoi* per gram, Toyocerin, Rubinum Animal Health, Barcelona, Spain.

Formulated experimental diets (Table 2) were based on corn and soybean meal, following recommendations by Rostagno et al. (2005). Water and experimental diets were provided *ad libitum*. The prebiotics consisted of MCM with 11.4% β-1,4-mannobiose (Fuji Oil Ltd., Osaka, Japan). The probiotic microorganism contained Toyocerin (powder feed additive EC no. 1701, Rubinum Animal Health, Barcelona, Spain), with 1010 viable spores of *Bacillus cereus* var. *toyoi* (NCIMB 40112/CNCM I-1012) per gram.

Table 2. Composition of basal diets.

Ingredients (g kg^{-1} as feed)	Initial (1 – 21 day) 21% CP	Growth (22 – 35 day) 19% CP	Final (36 – 42 day) 17% CP
Corn	58.20	63.22	65.28
Soybean meal	35.20	29.70	27.40
Soy oil	2.20	2.80	3.45
Dicalcium phosphate	1.90	1.80	1.60
Limestone	1.00	1.00	0.90
Common salt (NaCl)	0.40	0.38	0.35
Premix*	0.20	0.20	0.20
DL-methionine	0.30	0.25	0.20
L- lysine	0.14	0.25	0.25
L- threonine	0.09	0.07	0.07
Choline chloride (60%)	0.12	0.08	0.05
Variable portion**	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated composition			
ME (kcal kg^{-1})	3,000	3,100	3,170
PB (%)	21.00	19.00	17.00
Ca (%)	0.96	0.90	0.85
Available Phosphorus (%)	0.45	0.42	0.39
Total Phosphorus (%)	0.68	0.64	0.59
Sodium (%)	0.20	0.19	0.18
Digestible Lysine (%)	1.12	1.08	1.04
Digestible Methionine + Cystine (%)	0.89	0.79	0.75
Digestible Arginine (%)	1.32	1.16	1.10
Digestible Threonine (%)	0.81	0.71	0.68
Digestible Tryptophan (%)	0.24	0.21	0.20
Choline (mg kg^{-1})	700	500	300
($Na^{+}K$)-Cl (mEq kg^{-1})	220	212	198

*Premix mineral provides (mg kg^{-1} of feed): Mn, 80; Zn, 60; Fe, 35; Cu, 9; I, 0.80; Se, 0.30. Vitaminic Premix provides (per kg feed^{-1}): vitamin A, 11,000 UI, vitamin D3, 2200 UI, vitamin E, 22 mg; vitamin K, 2.3 mg; riboflavin, 8,0 mg; calcium pantothenate, 12 mg; nicotinic acid, 60 mg; vitamin B12, 25 μg; vitamin B6, 40 mg; folic acid, 1.0 mg; biotin, 0.10 mg; thiamine, 2.7 mg. **Variable portion consisting of different growth promoters tested.

Growth performance and carcass yields

Mortality was checked daily, and data on death and body weights (BW) were recorded. Growth performance parameters, such as BW, body weight gain (G), feed intake (FI), feed conversion ratio (FCR; defined as FI:G (g:g), and livability (L,%) were determined for broilers aged 1–42 days. The production index [PI = ((BW, g age^{-1}, day) × L, %) / (FCR × 10] was calculated at 42 days of age. Livability (L) was obtained from the total number of birds housed minus dead that died or were removed from the experimental unit, divided by the total number of birds housed (multiplied by 100).

On the 42nd day, 16 broilers that had BWs near plot average were selected per treatment, identified and submitted to a fasting period of 8h. All birds were then weighed individually, euthanized by cervical dislocation, manually exsanguinated, plucked, and eviscerated, after weighing the carcasses, cuts were submitted for evaluation of carcass yield (excluding head, neck, and feet), breast yield, thigh + drumstick yield, wing yield and back yield (MENDES; KOMIYAMA, 2011).

Intestinal morphometry analyses

Intestinal morphometry was determined at 42 days of age by anesthetizing 4 birds from each treatment (2 males and 2 females) with zoletil® and ketamin and killed by cervical dislocation. Approximately 5-cm-long fragments were obtained from the duodenum, from the pylorus to the distal portion of the duodenal loop, and the jejunum, from the distal portion of the duodenal loop to Meckel's diverticulum. Segments were then placed on polystyrene sheets, opened longitudinally, washed with saline solution, fixed in Bouin's solution for 24h, and processed until paraffin embedding, according to method described by Beçak and Paulete (1976). Each fragment was submitted to semi-seriate cuts (5 mm thick) and stained following hematoxylin-eosin method.

For the morphometric study (villus height and crypt depth), images were captured using a light microscope and a computerized image analysis system (Image Pro-Plus 5.2, Média Cibernética, São Paulo, São Paulo State, Brazil). Villus length was measured from the top of the villus to the top of the lamina propria, whereas the villus:crypt ratio was defined as the ratio of villus height to crypt depth. The height of 30 villi and the depth of 30 crypts were measured in each segment and the average rates were used for statistical analysis.

Statistical analysis

Data were submitted to ANOVA using the GLM program implemented by Statistical Analysis System (SAS, 2000). Mean rates of treatment groups were compared by Student-Newman-Keuls tests with $p < 0.05$ as statistically significant.

Results and discussion

Growth performance

There was a significant effect ($p < 0.05$) of the treatment on body weight and feed conversion ratio. Birds receiving both MCM and TY had significantly higher body weight than those with other treatments (Table 3). Feed conversion ratio was also significantly better for birds that received 0.1% MCM with TY probiotic than others. There was no effect ($p > 0.05$) of treatment on feed intake, livability or production index.

Table 3. Effect of diets with different growth promoters on feed intake (FI), body weight (BW), feed conversion ratio (FCR), livability (L) and production index (PI) of broiler chickens between 1 and 42 days of age.

Treatments (T)	FI	BW (g)	FCR ($g\ g^{-1}$)	L (%)	PI
Negative control	4669	2601 A	1.82 A	97.14	331
Positive control (avilamycin 10 ppm)	4689	2630 A	1.81 A	98.57	341
0.1% MCM	4615	2621 A	1.79 A	97.14	339
0.1%MCM + 0.05% TY	4676	2663 B	1.78 B	97.85	349
p-rate					
Treatments	0.8695	0.0025	0.0013	0.2466	0.1320
CV (%)	3.56	2.83	2.05	3.04	7.18

Averages in each column followed by different letters are significantly different ($p < 0.05$) by SNK test at 5% probability.

When compared to results from negative control, the positive control tended to improve the production index, although MCM treatment alone was not so effective. Results suggested that since combinations, such as MCM+TY, had a positive effect on growth performance, they may be an alternative to antibiotics. In fact, results corroborate those by Awad et al. (2009), who investigated symbiotic BIOMIN IMBO (a combination of *Enterococcus faecium* and a prebiotic derived from chicory) and found significant differences in body weight between control, symbiotic and probiotic groups of birds at 35 days of age.

It has been reported that β 1.4-Mannobiose (MNB) used as a type of prebiotics may act as an immune-modulating agent *in vivo*, by preventing *Salmonella enteritidis* infection in broilers through an increase in IgA production, improvement of *Salmonella enteritidis* clearance (AGUNOS et al., 2007) and up-regulating the local expression of genes involved in host defense and innate immunity (IBUKI et al., 2010). The combination of MCM that includes MNB and probiotics may accelerate immune defenses and improve the intestinal environment. The combination of MCM and probiotics may have better growth-promoting properties than either MCM or probiotics alone. Although the reason is not clear, activated host immune system may increase survival of the probiotic in the intestine and contribute to the enhancement of probiotic effects. MCM activates the immune system in the intestine and then TY might survive easily. Further investigation is required to clarify the mechanism in growth performance.

Treatment effects on chicken parts

There were no significant differences between treatments ($p > 0.05$) in carcass yield, breast yield, thigh + drumstick yield, wing yield, or back yield of broiler chickens at 42 days of age (Table 4). These results were inconsistent with those of Ibuki et al.

(2013) who found that MNB effectively increased breast muscles in growing chickens. In their experiment, these authors supplied purified MNB (99%) to chickens. Presumably the purification of MNB might be more effective on the growth performance of the chickens in the experiment. Nonetheless, MCM, which included MNB, might increase the growth performance of chickens and, combining MCM with probiotics, increase more the above effect.

Table 4. Effect of diets containing different growth promoters on carcass yield (CY), breast yield (BY), thigh + drumstick yield (T+DTY), wings yield (WY) and back yield (BKY) of broilers at 42 days of age.

Treatments	CY* (%)	BY (%)	T+DTY (%)	WY (%)	BKY (%)
Negative control	74.74	39.44	28.26	21.82	10.14
Positive control (avilamycin 10 ppm)	73.90	39.54	29.33	21.48	10.35
0.1% MCM	74.75	39.26	28.29	21.74	10.17
0.1%MCM + 0.05% TY	75.30	39.63	28.30	21.17	9.87
p-rate					
Treatments	0.3673	0.9687	0.2741	0.5271	0.1277
CV (%)	1.94	3.08	6.58	4.19	3.50

Averages in each column followed by different letters are significantly different ($p < 0.05$) by the SNK test at 5% probability.

Morphology of the intestine

Treatment significantly affected villus length, crypt depth and VL / CD ratio of duodenum (Table 5; $p < 0.01$). With regard to the duodenum, villus length in birds receiving MCM plus TY, MCM alone and positive control were significantly better than those for birds receiving negative control treatment, but there was no significant difference in villus length ($p > 0.01$) among the groups of each treatment. Neither crypt depth nor the villus:crypt ratio differed between the treatments (Table 5).

Table 5. Effect of diets containing different growth promoters on duodenum villus length (VL), crypt depth (CD), and villus:crypt ratio (VL:CD) of broilers at 42 days of age.

Treatment	VL (μm)	CD (μm)	VL:CD(μm)
Negative control	1098 C	228 B	4.83 C
Positive control (avilamycin 10 ppm)	1193 B	220 B	5.41 B
0.1% MCM	1190 B	252 A	4.73 C
0.1% MCM + 0.05% TY	1310 A	211 B	6.19 A
p-rate			
Treatments	< 0.0001	< 0.0001	0.0052
CV (%)	4.85	11.90	10.04

Averages in each column followed by different letters are significantly different ($p < 0.05$) by the SNK test at 5% probability.

In the case of the jejunum (Table 6), significantly better villus length results were obtained for broilers receiving 0.1% MCM or 0.1% MCM + 0.05% TY than negative control and positive control. Although crypt depth did not differ between treatments, the best villus:crypt ratio occurred when receiving 0.1% MCM + 0.05% TY treatment, not differing from negative control (Table 6).

Table 6. Effect of diets containing different growth promoters on jejunum villus length (VL), crypt depth (CD), and villus:crypt ratio (VL:CD) of broilers at 42 days of age.

Treatment	VL (μm)	CD (μm)	VL:CD(μm)
Negative control	794 B	170	4.66 A
Positive control (avilamycin 10 ppm)	809 B	214	3.78 B
0.1% MCM	843 A	205	4.11B
0.1%MCM +0.05% TY	869 A	170	5.11 A
p-rate			
Treatments	< 0.0001	0.1009 NS	0.0063
CV (%)	4.32	13.83	13.32

Averages in each column followed by different letters are significantly different ($p < 0.05$) by the SNK test at 5% probability.

It may be presumed that increased villus height is paralleled by increased digestive and absorptive function of the intestine due to increased absorptive area, expression of brush border enzymes and transport systems (PLUSKE et al., 1996).

Results in current study differed from those of Loddi et al. (2000), Sato et al. (2002), and Chiquieri et al. (2007), who did not report any difference in the morphometry of the villus between broilers receiving control diet and those receiving probiotics. However, Pedroso et al. (1999) administered a probiotic containing *Bacillus subtilis* in the feed of battery hens and observed that the greatest villus length occurred when the administration of the probiotic was continuous, and that villi were shorter in birds that did not receive the probiotic. Similarly, Pelicano et al. (2003) registered that the addition of a probiotic containing a mixture of *Lactobacillus* sp. to drinking water resulted in greater villus height in the duodenum and greater villus perimeters in the duodenum and jejunum. Prebiotics also have several effects on intestinal villi.

Increased values in light microscopy parameters and hypertrophied epithelial cells in chickens fed on MCM were previously reported (IBUKI et al., 2014). The authors suggested that increases indicated that MCM could stimulate intestinal function, thereby improving growth performance in chickens. The results of the present study support the above suggestion. Furthermore, in current study, the combination of MCM and probiotics tended to have a greater effect on intestinal function than negative control groups.

In general terms, the combination of MCM with probiotic TY improved the growth performance of birds and mucosal morphometry of the duodenum and jejunum when compared with no additives or with the addition of the antibiotic avilamycin at a dose of 10 ppm in the feed.

Conclusion

The avilamycin antibiotic failed to influence broilers' performance. The combination of mannanase-hydrolyzed copra meal (MCM) and probiotic *Bacillus cereus* var. *toyoi* (TY) may be an alternative to avilamycin antibiotic as a growth promoter for broiler chickens between 1 and 42 days old and does not affect the carcass yields of the birds.

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