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Evaluation of the use of probiotics in diets with or without growth promoters for broiler chicks

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ABSTRACT - This work was carried out to evaluate the use of *Bacillus subtilis* probiotic C-3102 (10^{10}cfu/g) in diets with or without growth promoters on the performance of broilers in the period from 1 to 42 days of age. It was used 1,200 Cobb line broilers, distributed in a complete randomized block with five diets: negative controller (without promoters); *Bacillus subtilis* (30 g/t ration); *Bacillus subtilis* (50 g/t ration); *Bacillus subtilis* (30 g/t ration) + colistin (10 ppm); avilamycin (10 ppm) + colistin (10 ppm). Each diet was evaluated in 10 repetitions with 24 birds. From 21 to 42 days of age, feed intake, weight gain and feed conversion were evaluated, and at 42 days of age, productive efficiency index was evaluated. At the end of the experimental period, it was observed an increase in the consumption of diet with the lowest dose of *Bacillus subtilis* (30 g) in relation to that one with *Bacillus subtillis* (30 g) + colistin (10 ppm). The values of weight gain obtained with the diet with the lowest dose of *Bacillus subtillis* (30 g) and with avilamycin (10 ppm) + colistine (10 ppm) were higher than those of the birds fed control diet (without promoters). For feed conversion, the best results were obtained by supplying diets containing 50 g of *Bacillus subtillis*, diet with 30 g of *Bacillus subtillis* + 10 ppm of colistin; and diet with 10 ppm of avilamycin + 10 ppm colistin. For the analysis of productive efficiency index, the best results were obtained with diets containing additives (probiotics and/or antibiotics) in comparison to the control diet. *Bacillus subtillis* C-3102 probiotic, at concentration 10^{10} cfu/g, is an efficient substitute of antibiotics.

Key Words: additives, alternative products, antibiotics, performance

Avaliação do uso de probiótico em dietas para frangos de corte com ou sem promotores de crescimento

RESUMO - Este trabalho foi realizado para avaliar o uso do probiótico *Bacillus subtilis* C-3102 (10^{10} ufc/g) em dietas contendo ou não promotores de crescimento sobre o desempenho de frangos de corte no período de 1 a 42 dias de idade. Foram utilizados 1.200 frangos de corte da linhagem Cobb, distribuídos em delineamento inteiramente ao acaso com cinco dietas: controle negativo (sem promotores); *Bacillus subtilis* (30 g/t de ração); *Bacillus subtilis* (50 g/t de ração); *Bacillus subtilis* (30 g/t de ração) + de colistina (10 ppm); avilamicina (10 ppm) + colistina (10 ppm). Cada dieta foi avaliada em 10 repetições de 24 aves. Foram avaliados aos 21 e 42 dias o consumo de ração, o ganho de peso e a conversão alimentar e, aos 42 dias, o índice de eficiência produtiva. Ao final do período experimental, observou-se aumento no consumo para a dieta com a menor dose de *Bacillus subtilis* (30 g) em relação àquela com *Bacillus subtilis* (30 g) + colistina (10 ppm). Os valores de ganho de peso obtidos com a dieta com menor dose de *Bacillus subtilis* (30 g) e com avilamicina (10 ppm) + colistina (10 ppm) foram superiores aos das aves alimentadas com a dieta controle (sem promotores). Para conversão alimentar, os melhores resultados foram obtidos com o fornecimento das dietas com 50 g de *Bacillus subtilis*; com 30 g de *Bacillus subtilis* + 10 ppm de colistina; e com 10 ppm de avilamicina + 10 ppm de colistina. Na análise do índice de eficiência produtiva, os melhores resultados foram obtidos com as dietas contendo aditivos (probióticos e/ou antibióticos) em comparação à dieta controle. O probiótico *Bacillus subtilis* C-3102, na concentração 10^{10} ufc/g, é um substituto eficiente de antibióticos.

Palavras-chave: aditivos, antibióticos, desempenho, produtos alternativos

Introduction

The use of antibiotics as growth promoter has been a common practice in poultry production since the 1950s (Dibner & Richards, 2005). These compounds may improve animal performance by excluding microorganisms which compete with them for food nutrients in the gastrointestinal tract (Macari & Furlan, 2005), and by favoring the

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microorganisms considered as beneficial (Flemming & Freitas, 2005). However, the indiscriminate use of antibiotic growth promoters (AGP) led to emergence of resistance in pathogenic bacteria, and as result, the European Union has banned the addition of several antibiotics in broiler feeds since 2006, and other countries should follow this trend soon (Palermo, 2006).

Considering the inevitable ban of AGP, and aiming at maintaining the productivity levels achieved by modern poultry production, research has been developed to find efficient alternatives to the use of AGPs (Rostagno et al., 2003). In this context, microbial products, such as probiotics, may be one of such alternatives.

Probiotics are defined as a feed supplement composed of live microorganisms that benefit their host by balancing its intestinal microbiota (Fuller, 1989). Their mode of action is related to the competition for binding sites or competitive exclusion, that is, probiotic bacteria occupy the binding sites (receptors or attachment sites) of the cells of the intestinal mucosa, thereby posing a physical barrier to pathogenic bacteria. This means that these pathogens are excluded due to competition for space (Furlan et al., 2004). Aiming at improving the efficacy of probiotics, several research studies have been carried out to develop products with different bacterial species and strains (Albino et al., 2007).

The objective of the present study was to evaluate the effects of the prebiotic *Bacillus subtilis* C-3102 added to diets containing or not antibiotic growth promoters at a concentration of 10^{10} CFU/g on the performance of broilers at 42 days of age.

Material and Methods

The experiment was carried out at the Setor de Avicultura do Centro de Estações Experimentais do Canguiri, Universidade Federal do Paraná, Brasil. A total number of 1,200 male Cobb broilers at one day of age were used. Birds were housed in an experimental house divided in pens with the floor covered with wood-shaving litter, and equipped with tube feeders, bell drinkers, and electrical brooder. It was used a completely randomized experimental design consisting of five dietary treatments: negative control (with no growth promoters); *Bacillus subtilis* (30 g/t of ration); *Bacillus subtilis* (50 g/t of ration); *Bacillus subtilis* (30 g/t of ration) + colistin (10 ppm); avilamycin (10 ppm) + colistin (10 ppm). Each treatment had 10 replicates with 24 birds each.

Birds were submitted to standard management practiced in the poultry industry. Water and feed were supplied *ad libitum*. The experimental diets (Table 1) were formulated using practical levels applied in the Brazilian broiler industry for rations containing animal and vegetable ingredients.

Table 1 - Composition of the experimental diets

Ingredient (%)	Rearing phase		
	Starter	Grower	Finisher
Corn	56.60	65.51	71.30
Soybean meal	34.00	22.00	14.53
Meat meal	5.80	4.50	4.00
Feather meal	-	2.00	3.00
Poultry offal meal	-	2.00	3.00
Vegetable oil	2.00	2.40	2.70
Limestone	0.35	0.40	0.34
NaCl	0.40	0.32	0.30
L-lyisine	0.10	0.23	0.30
DL-methionine	0.30	0.20	0.10
Choline chloride	0.05	0.04	0.03
Premix*	0.40	0.40	0.40
Nutritional composition (calculated)			
Crude protein (%)	22.71	20.26	18.50
Metabolizable energy (kcal/kg)	3,001	3,152	3,249
Calcium (%)	0.88	0.84	0.80
Available phosphorus (%)	0.44	0.42	0.40
Sodium (%)	0.21	0.20	0.19
Digestible lysine (%)	1.15	1.05	0.95
Digestibility methionine+cystine (%)	0.92	0.74	0.70

* Guaranteed levels/kg product: folic acid - 250 mg; pantothenic acid – 2,750 mg; biotin - 15 mg; copper – 30,000 mg; choline – 120,000 mg; iron – 12,500 mg; zinc – 15,000 mg; iodine - 250 mg; manganese – 17,500 mg; niacin – 8,750 mg; pyridoxine- 650 mg; riboflavin – 1,500 mg; selenium - 75 mg; thiamin - 450 mg; vitamin A – 2,000,000 IU; vitamin B12 – 3,250 mcg; vitamin D3 – 500,000 IU; vitamin E – 4,250 mg; vitamin K - 500 mg. Antibiotic growth promoters were added from day 1 to 35.

The prebiotics and the antibiotics were premixed with soybean meal according to their respective treatments before being added to the mixer in order to prevent any mixing problems. Birds and rations were weighed when birds were one (placement), 21 and 42 days of age. Mortality was daily recorded, and dead birds were weighed to correct the measured parameters for mortality. Rations samples were collected to recover *Bacillus subtilis* and to check its proper dosage in the ration.

The following parameters were evaluated: feed intake, weight gain, and feed conversion ratio. The collected data were submitted to analysis of variance and means were compared by the test of Tukey at 5% of significance.

Results and Discussion

Analyses of *Bacillus subtilis* recovery were carried out to verify if the dosages were correct and if diets were cross-contaminated. The obtained values (Table 2) were as it was expected; however, the diet containing only antibiotics was cross-contaminated.

This cross contamination was probably caused by the fact that the mixture containing only antibiotics was performed after all other feeds were mixed, allowing probiotic residues to contaminate that ration.

The performance results obtained for the starter phase (Table 3) did not show significant differences (P>0.05) in feed intake or weight gain among treatments; however, feed conversion ratio of the birds fed rations containing probiotics and or antibiotics was better as compared to the birds receiving the diet with no additives. Moreover, the diet containing the highest *Bacillus subtilis* (50 g/t of ration) level promoted the best feed conversion ratio.

The results of the present study are consistent with the findings by Flemming & Freitas (2005), Cardozo (2006), and Da Silva (2008), who also tested a probiotic composed of *Bacillus subtilis*, and concluded that birds submitted to the diet without promoters presented the worst feed conversion ratio. Conversely, Corrêa et al. (2003) and Lorençon et al. (2007) showed that the performance of broilers in the starter phase was not affected by the use of probiotics or antibiotics.

When the results of the finisher phase were analyzed (Table 4), birds fed diet with *Bacillus subtilis* (30 g/t of ration) presented feed intake lower than those fed diet containing *Bacillus subtilis* (30 g/ t of ration) + colistin (10 ppm).

In a similar study, Rigobelo et al. (2008) observed lower feed intake in broilers fed diets with antibiotics and prebiotics compared to those fed a diet with no additives. In terms of weight gain, the diets containing prebiotics promoted the best results relative to the negative control diet. The diets containing antibiotics and 30 g *Bacillus subtilis*/t/ration also promoted higher weight gain, probably due to the higher feed intake.

The results of the present experiment are in agreement with the findings by Flemming & Freitas (2005), who also evaluated the prebiotic *Bacillus subtilis* and obtained better broiler performance with the diets with that probiotic as compared to the diet without promoters.

Consistently with the other parameters, feed conversion ratio was better in the groups that were fed diets containing probiotics and/or antibiotics; those containing only probiotics – *Bacillus subtilis* (30 g/t ration) + colistin (10 ppm) and *Bacillus subtilis* (50 g/t ration) – promoted the best results. In this context, in the present study, it was clearly shown that the antibiotic could have been replaced by probiotics, which have the additional benefit of excluding undesirable bacteria and not stimulating bacterial resistance.

Table 2 - Results of the recovery of *Bacillus subtilis* in the diets

Diet	Analyzed result	Expected result
Negative control(with no growth promoters)	Not identified	Not identified
Bacillus subtilis (30 g/t of ration)	3.4E + 0.5	3.0
Bacillus subtilis (50 g/t of ration)	4.9E + 0.5	5.0
Bacillus subtilis (30 g/t of ration) + colistin (10 ppm)	3.3E + 0.5	3.0
Avilamycin (10 ppm) + colistin (10 ppm)	1.7E + 0.3	Not identified

Table 3 - Feed intake, weight gain, and feed conversion ratio of broilers at 21 days of age

Diet	Feed intake (g)	Weight gain (g)	Feed conversion ratio (g/g)
Negative control (with no growth promoters)	1.478	1.052	1.405a
Bacillus subtilis (30 g/t of ration)	1.462	1.053	1.388ab
Bacillus subtilis (50 g/t of ration)	1.442	1.057	1.364b
Bacillus subtilis (30 g/t of ration) + colistin (10 ppm)	1.456	1.060	1.374ab
Avilamycin + colistin (10 ppm)	1.451	1.041	1.393ab
Probability	0.428	0.742	0.009
Coefficient of variation	2.961	3.017	2.091

Means followed by different letters in the same column differ by Tukey test at 5% probability level.

Table 4 - Feed intake, weight gain, and feed conversion ratio of broilers at 42 days of age

Diet	Feed intake (g)	Weight gain (g)	Feed conversion ratio (g/g)
Negative control (with no growth promoters)	5.114ab	2.742b	1.864a
Bacillus subtilis (30 g/t of ration)	5.188a	2.831a	1.832b
Bacillus subtilis (50 g/t of ration)	5.063ab	2.816ab	1.798c
Bacillus subtilis (30 g/t of ration) + colistin (10 ppm)	5.040b	2.811ab	1.793c
Avilamycin (10 ppm) + colistin (10 ppm)	5.074ab	2.835a	1.790c
Probability	0.027	0.015	0.001
Coefficient of variation	2.227	2.485	2.026

Means followed by different letters in the same column differ by Tukey test at 5% probability level.

Cardozo (2006) and Opalinski et al. (2007) also observed better performance in broilers fed diets supplemented with probiotics and/or antibiotics. Moreover, Rigobelo et al. (2008) showed that the use of probiotics or antibiotics presented better feed conversion ratio when compared to the control diet.

On the other hand, Silva et al. (2000), at the end of an experimental period of 42 days, verified that the use of probiotics did not influence broiler performance. Santos et al. (2004) argue that, under good health and adequate environmental conditions, the effects of probiotics may not be detected. According to Corrêa et al. (2003), as experimental conditions, where stress is minimal, are different from commercial rearing environments, it may be difficult to observe any benefits promoted by probiotics when birds are not challenged by environmental or health stressors.

Production efficiency values (Table 5) were calculated according to the following formula: [((live weight (kg) × feasibility (%)) / (age (days) × feed conversion ratio)) × 100], in which higher values indicate better performance.

In the present study, the diets containing additives (probiotics and/or antibiotics) promote better production efficiency results than the control diet (with no additives). These results are consistent with those of Lorençon et al. (2007), who observed that the use of probiotics in broiler diets resulted in similar production efficiency index as that obtained with the diets containing antibiotics. On the other hand, Da Silva (2008) did not observe any influence of the addition of the probiotic *Bacillus subtilis* in the feed on production efficiency.

Table 5 - Production efficiency index results of broilers at 42 days of age

Diet	Production efficiency index
Negative control (with no growth promoters)	338.4b
Bacillus subtilis (30 g/t of ration)	362.1a
Bacillus subtilis (50 g/t of ration)	363.5a
Bacillus subtilis (30 g/t of ration) + colistin (10 ppm)	365.4a
Avilamycin (10 ppm) + colistin (10 ppm)	369.1a
Probability	0.0001
Coefficient of variation	4.7660

Means followed by different letters in the same column differ by Tukey test at 5% probability level.

Conclusions

The use of probiotics – with or with no antibiotics – promotes higher weight gain and better feed conversion ratio and production efficiency in broilers from one to 42 days of age. The probiotic *Bacillus subtilis* at a dose of 50 g/t of ration improves broiler feed conversion ratio and may be used to replace antibiotic growth promoters in rations for broiler.

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