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Short Communication

Evaluation of the use of probiotic (*Bacillus subtilis* C-3102) as additive to improve performance in broiler chicken diets

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ABSTRACT - The objective of this experiment was to evaluate the use of probiotic based upon *Bacillus subtilis* C-3102 (BS) in broiler diets containing or not conventional additive growth-promoter (AGP). A total of 1,824 one-day-old chicks of the Cobb-500 strain were used and distributed into 48 plots (1.50 × 2.00 m). A completely randomized design with four treatments and 12 repetitions was adopted. The treatments evaluated were: 1- Negative Control - AGP-free diet (NC); 2 - Positive Control, with AGP halquinol 30 mg/kg diet (PC); 3 - NC + BS ($3x10^5$ cfu/g diet); and 4 - PC + BS ($3x10^5$ cfu/g diet). The diets were on corn, soybean meal and meat and bone meal basis, formulated according to the nutrient allowances of the Strain Cobb Guide, following a feed program with three diets (starter, grower and finisher). At 21 and 42 days of age, feed intake, weight gain and feed conversion were evaluated, and carcass yield characteristics were determined at 42 days of age. Throughout the trial period (starter and total phases), better feed conversion was found for broilers fed diet with both additives (AGP and BS) combined. At the end of the experiment (1 to 42 days), the highest weight gain also occurred in broilers fed diets with a combination of the two additives. In present study, the use of BS or AGP alone in diets did not affect the performance of broilers. The carcass yield was not influenced by the use of the additives and beneficial effects of supplementation of BS and AGP combined in the diet on broiler performance were observed.

Key Words: antimicrobial, meat poultry production, microbiota, nutrition

Introduction

The achievement of high productivity associated with the meat quality and low costs are the main objectives of the meat poultry production. For this purpose, the use of feed additives is essential and it is also necessary for commercial poultry production. Among the additives used in the feed, antimicrobial products or antibiotic growth-promoter (AGP) are included in diets in low dosages (under the minimum inhibitory concentration – MIC), supporting the intestinal microbiota balance and consequently improving poultry performance and health. In spite of the known and testified efficiency of the AGP in poultry production, currently, its use has been broadly questioned.

According to Rostagno et al. (2003), the safety of antibiotics has been questioned, mainly due to the routine use in poultry feeding. The possibility of selecting pathogenic microorganisms more resistant to the antibiotics themselves by the continuous addition of sub-therapeutic AGP doses in diets is one considerable problem in the use of antimicrobials in broiler production.

Therefore, the use of probiotics has received special attention, since an occasional replacement of AGP in particular situations or even acting jointly might also decrease the supplementation of the AGP, since the legislation has become more and more restrictive to the use of conventional products. Recently, many experiments have been conduced to develop different products with different bacterial species and strains as an attempt to improve the efficacy of probiotics (Albino et al., 2007).

According to Loddi et al. (2000), probiotics can be used in substitution of AGP because they constitute feed additives, composed of live or viable non-pathogenic microbial agents, which act beneficially in broiler chickens by improving the balance of the gastrointestinal microbiota. In accordance with Sato et al. (2002), through pathogenic bacteria in the intestine, probiotics promote better balance between beneficial microorganisms and pathogenic microorganisms in the gastrointestinal system of broilers and consequently improve their performance. Experimental trials conducted by Cuevas et al. (2000) showed greater weight gain and rearing feasibility of the broiler fed probiotics on the basis of *Bacillus subtilis*.

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Thus, the objective of this experiment was to evaluate the effects of the probiotic *Bacillus subtilis* strain C-3102 in diets with and without addition of conventional AGP upon the performance and carcass yield of broilers at 42 days of age.

Material and Methods

The experiment was carried out at the poultry production sector of the Department of Animal Science of Universidade Federal de Lavras. A total of 1,824 one-day-old male Cobb broilers from commercial hatchery were used. Broilers were housed in 48 floor pens (1.50 × 2.00 m); each pen was covered with wood-shaving litter re-utilized by one rearing cycle, and equipped with tube feeders, bell drinkers and electrical brooder.

A completely randomized experimental design was adopted, consisting of four dietary treatments and 12 replicates. The treatments evaluated were: 1 - negative control - AGP-free diet (NC); 2 - positive control, with AGP halquinol 30 mg/kg diet (PC); 3 - NC + BS ($3x10^5$ cfu/g diet); and 4 - PC + BS ($3x10^5$ cfu/g diet). Water and feed were supplied *ad libitum* and the management followed standards of the poultry industry and academic knowledge.

In order to obtain the dosages of the main actives evaluated (experimental treatments), a source of commercial halquinol at the concentration of 60% and *Bacillus subtilis* strain C-3102 with minimum count of $1x10^{10}$cfu/g in the replacement of kaolin (inert material) were used so that the diets of the different treatments in each phase were equal in nutrients (Table 1).

Table 1 - Formulation (g/kg) and nutritional composition of the experimental diets

Ingredients	Phases		
	Starter	Grower	Finisher
Corn	572.56	623.72	635.53
Soybean meal	334.43	272.30	251.86
Bone and meat meal (40%)	55.48	51.79	49.00
Soybean oil	20.62	34.57	47.56
Limestone	3.56	4.54	4.49
Common salt	3.19	2.49	2.28
DL-methionine (99%)	2.82	2.76	2.67
L-lysine HCl (78%)	1.34	1.65	1.32
L-threonine (98%)	0.73	1.01	0.71
Vitamin premix[1]	1.00	1.00	1.00
Trace mineral premix[2]	1.00	1.00	1.00
Choline chloride (60%)	0.77	0.67	0.58
Sodium bicarbonate	1.00	1.00	1.00
Salinomycin (12%)	0.50	0.50	-
Probiotic/halquinol/kaolin[3]	1.00	1.00	1.00
Calculated nutritional composition			
Metabolizable energy (kcal/kg)	3,000	3,150	3,250
Crude protein (g/kg)	225.0	200.0	190.0
Digestible lysine (g/kg)	11.7	10.4	9.6
Digest. methionine + cystine (g/kg)	8.7	8.1	7.8
Digestible threonine (g/kg)	8.0	7.4	6.8
Calcium (g/kg)	9.0	8.8	8.4
Available phosphorus (g/kg)	4.5	4.2	4.0
Sodium (g/kg)	2.0	1.7	1.6
Analyzed nutritional composition			
Crude protein (g/kg)	224.4	199.4	181.8
Calcium (g/kg)	10.3	9.3	8.8
Total phosphorus (g/kg)	6.4	5.6	5.4

[1] Enrichment per kg diet: vit. A - 12,000 IU; vit. D - 2,200 IU; vit. E - 30 mg; vit. K - 2.5 mg; niacin - 53 mg; folic acid - 1.0 mg; pantothenic acid - 13 mg; biotin - 110 µg; vit. B1 - 2.2 mg; vit. B2 - 6 mg; vit. B6 - 3.3 mg; vit. B12 -16 µg; selenium - 0.25 mg.
[2] Enrichment per kg diet: Iron - 50 mg; Copper - 8.5 mg; Zinc - 70 mg; Manganese - 75 mg; Iodine -1.5 mg; Cobalt - 0.2 mg.
[3] Obtained by inclusion of sources of halquinol and *Bacillus subtilis* in replacement of kaolin (inert material).

The experimental diets (Table 1) were on the basis of corn, soybean meal and bone and meat meal, formulated according to the nutrient matrix of ingredients described by Rostagno et al. (2005) and nutrient requirements of the Cobb Guide (Broiler Nutrition Supplement, Cobb Vantress 2004). The feeding program was composed of three diets (starter, of 1-21 days; grower, of 22-35 days; and finisher, of 36-42 days).

Analysis of crude protein, calcium and phosphorus in experimental samples feed were carried out. The analyses of the nitrogen content in diets were performed according to the methodology proposed by methodology from the AOAC (1990), while calcium and phosphorus were analyzed in accordance with the methodological procedures reported by Silva & Queiroz (2002).

In order to calculate and/or determine the performance, broilers and diets (and/or residual feed) were weighed at 1, 21 and 42 days of age. The difference between the total number of chickens and dead birds (recorded daily) was used for calculating feasibility, and mortality was used to correct feed intake and feed conversion. The carcass yield evaluation was performed by means of individual weighing of the broilers (live weight) and after 4 hours of fast, followed by slaughter procedures and cuts. The carcass yield was obtained by the ratio between carcass weight (eviscerated carcass without neck and feet) and live weight, while breast, thigh/drumstick yield and percentage of abdominal fat were obtained by the ratio between their respective weight and the weight of the eviscerated carcass.

The collected data were submitted to analysis of variance following procedures of software Sisvar in agreement with Ferreira (2000) and when the significance in the F test was 0.05 (5%), the means of treatments were compared by the SNK test and additional orthogonal contrasts were considered up to 0.10 (10%) of significance in the F test in function of specificity and singularity of the comparisons and degrees of freedom.

Results and Discussion

There was no significant effect (P>0.05) from the feed additives used (halquinol and BS) on feed intake and weight gain at the starter phase; however, feed conversion was affected (P<0.05) by the treatments evaluated (Table 2).

At 21 days of age, the feed conversion of the broilers fed BS alone (NC+BS) and in combination with halquinol (PC+BS) presented markedly better results. Thus, broilers fed NC presented intermediary value for this variable among the diets evaluated. Broilers fed the diet containing conventional AGP (PC) presented the worst value for this measure. This result draws the attention to the logic of the use of AGP, which are basically used to improve performance, once there was no reasonable hypothesis to explain this result, in spite of there not being any clear distinction between broilers on PC and those on NC by test of means adopted.

Three orthogonal contrasts were used for comparison with more detail between treatments aiming to clarify the effects of AGP and BS on the measurements evaluated. The first contrast [NC *versus* PC + (NC+BS)] evaluated intended to measure possible differences between characteristics of broilers fed diets without additives (NC) and broilers fed AGP or BS in the diet, in other words, to measure the effects of additives singly in the diet. The results at the starter phase showed (P>0.10) no differences for performance characteristics.

The second contrast [NC *versus* PC+BS] was proposed to remark possible differences between broilers fed diets without additives (NC) and the ones which were given diets with both additives (AGP e BS). In the phase evaluated, no positive effects (P>0.10) were found from the use of additives in combination.

The objective of the third contrast, PC+(NC+BS) *versus* (PC+BS), was to determine whether the use of the additives evaluated in combination could generate some positive effect upon the performance over the use alone. The results did show better feed conversion (P = 0.085) of the broilers fed two additives combined in the diet compared with the use separately. It is important to stress the response to feed conversion from the use of AGP only (halquinol), i.e., broilers from group PC presented worse value and this might have contributed towards the significance of the contrast.

The results found at the starter phase were contradictory when compared with those obtained by Hooge et al. (2004), Flemming & Freitas (2005) and Meurer et al. (2010), who found, in a generalized manner, improvement in feed conversion in both broilers from the AGP group and those fed diet containing BS.

In the evaluation of the whole experimental period (Table 3), there were no significant differences (P>0.05) for feed intake, weight gain and feed conversion. However, the weight gain (P = 0.083) and feed conversion (P = 0.053) presented significance probabilities liable to evaluation by orthogonal contrasts.

The use of additives alone (AGP or BS) did not improve the performance of broilers (P>0.10) in comparison with the broilers in the negative control group [NC *versus* PC+(NC+BS)]. There were significant differences (P<0.10) for the second and third contrasts evaluated, which may show the efficacy of the combined use of two additives (AGP and BS) in the improvement of broiler performance.

In the present experiment, it was shown that association of probiotic composed of BS and conventional AGP (Haquinol) should be utilized to obtain better weight gain and improved feed conversion. These results were partly

Table 2 - Performance of broiler chickens in the starter phase, from one to 21 days of age

Diet	Feed intake (g/bird)	Weight gain (g/bird)	Feed conversion (g/g)
Negative control (NC)	1,129.0	784.5	1.441ab
Positive control (PC)	1,136.8	776.7	1.464b
NC + BS	1,133.5	800.7	1.416a
PC + BS	1,140.3	805.2	1.417a
Mean	1,134.9	791.8	1.434
CV (%)	3.20	4.01	2.55
Probability	0.889	0.109	0.007
Contrasts			
NC *versus* PC+(NC+BS)	0.637	0.706	0.924
NC *versus* (PC+BS)	0.452	0.117	0.115
PC+(NC+BS) *versus* (PC+BS)	0.691	0.150	0.085

Means followed by different letters in the column are different by the SNK test (P<0.05).
BS - *Bacillus subtilis*; CV - coefficient of variation.

Table 3 - Performance of broilers in the whole phase, from 1 to 42 days of age

Diet	Feed intake (g/bird)	Weight gain (g/bird)	Feed conversion (g/g)
Negative control (NC)	4,277.7	2,459.4	1.741
Positive control (PC)	4,244.3	2,452.6	1.731
NC+ BS	4,296.0	2,467.2	1.742
PC+ BS	4,286.0	2,520.1	1.701
Mean	4,276.0	2,474.8	1.729
CV (%)	1.94	2.79	2.28
Probability	0.459	0.083	0.053
Contrasts			
NC versus PC+(NC+BS)	0.798	0.983	0.228
NC versus (PC+BS)	0.808	0.037	0.018
PC+(NC+BS) versus (PC+BS)	0.592	0.018	0.015

Means with different letters in the column are different by the SNK test (P<0.10).
BS - *Bacillus subtilis*; CV - coefficient of variation.

different from those found by Brito et al. (2005), Opalinski et al. (2007) and Meurer et al. (2010), who observed better performance in broilers fed both diets supplemented with the additives (AGP or BS) alone and combined. Rigobelo et al. (2008) found better feed conversion in broilers fed probiotic or AGP-supplemented diets when compared with the broilers in the negative control group.

The performance results obtained in the present study may be related to the management conditions of the rearing, health status and cleaning of the facilities. In accordance with Gonzales (2004), the benefits from the use of probiotics are greater in contaminated or challenged environment, in broilers with low resistance to diseases and higher rearing density. Santos et al. (2004) claim that under adequate environmental or facility conditions and good health, the effects of the use of probiotics such as a performance-improving additive for broilers may not be detected.

There were no clear differences (P>0.10) according to treatment or additive evaluated in the diets on the carcass yield, cuts (breast, thigh+drumstick) yield and percentage of abdominal fat. Therefore, neither BS nor Halquinol-AGP (alone or combined) affected the carcass characteristics of the broilers (Table 4).

Table 4 - Measurements of carcass and cuts from broilers at 42 days of age

Diet	Carcass yield (%)	Breast yield (%)	Thigh+drumstick yield (%)	Abdominal fat (%)
Negative control (NC)	75.23	35.21	30.70	2.17
Positive control (PC)	75.10	35.96	30.39	2.41
NC+ BS	75.14	36.48	30.16	2.36
PC+ BS	75.22	36.06	30.17	2.21
Mean	75.17	35.93	30.35	2.29
CV (%)	2.02	5.04	4.18	24.71
Probability	0.990	0.114	0.423	0.415

CV - coefficient of variation; BS - *Bacillus subtilis*.

The results obtained from the evaluation of the carcass and cuts yield of the broilers with and without the use of additives were similar to those found by Corrêa et al. (2003), who also found no significant effect on carcass and cuts yield when probiotics were used in broiler diets. Similar response was obtained by Maiorka et al. (2001), who found no differences on carcass measurements with the use of probiotic, prebiotic or AGP in experimental diets.

In different studies with probiotics, AGP and other alternative growth promoters, still there are no clear explanations as to the association of theses products in the diet with positive effects on carcass and cuts yield.

Conclusions

The probiotic on the basis of *Bacillus subtilis* strain 3102 associated with halquinol (conventional AGP) enables better feed conversion throughout the rearing cycle. The weight of broilers at 42 days of age is increased from the use of the two additives in association. Regardless of the use of the additives evaluated (alone or combined), the carcass yield characteristics are not affected.

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