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Performance of Broilers Fed Diets Containing Natural Growth Promoters

■ Author(s)

Pelicano ERL
Souza PA
Souza HBA
Oba A
Norkus EA
Kodawara LM
Lima TMA

Departamento de Tecnologia
Faculdade de Ciências Agrárias e Veterinárias
Unesp

■ Mail Address

Elizabete Regina Leone Pelicano
Departamento de Tecnologia
Faculdade de Ciências Agrárias e Veterinárias
FCAV/Unesp
Via de Acesso Prof. Paulo Donato Castellane, km 5 - Jaboticabal, SP, Brazil
Telephone: (55) 16 32092675, ext. 245
Fax: (55) 16 32092675

E-mail: erlpelicano@yahoo.com.br

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ABSTRACT

The present study evaluated the effect of different probiotics on the performance of broiler chickens. A thousand and fifty one-day-old male Cobb chicks were distributed in a completely randomized design in a 3 *x* 2 + 1 factorial arrangement (3 probiotics sources in the diet, 2 probiotics concentrations in drinking water and 1 control group), with 5 repetitions of 30 birds per parcel. The results showed better feed conversion ($p<0.01$) (1-21, 22-35 and 1-45 days) and weight gain ($p<0.05$) (22-35 and 1-45 days) in the control group in relation to the groups receiving probiotics. The use of *Bacillus subtilis* in the diet improved ($p<0.05$) feed conversion during the growing phase, but this was not seen in the following period. Thus, it was concluded that probiotics supplementation had no beneficial effects on the performance.

INTRODUCTION

The aims of the modern broiler industry are to decrease production costs with high productivity by means of adequate genetics, nutrition and management procedures. Thus, the poultry industry has used for many years some tools that resulted in improved growth and higher yield, among which the use of growth promoter additives (Pelicano *et al.*, 2002).

Antibiotic utilization dates from the 50s' and positive results of production indexes were rapidly achieved, which lead to an indiscriminate and abusive use of antibiotics, and consequently the presence of residues in the meat and meat products. There are strong microbiological and clinical evidences for a possible relationship between the use of antibiotics in animal production and the increasing number of resistant bacteria in humans (Padilha, 2000).

Therefore, the indiscriminate use of such drugs was questioned. In addition to the problems already mentioned, there was the supposition that such additives caused the destruction not only of pathogenic bacteria, but also of beneficial bacteria. This could result in unbalanced symbiosis between the desirable microbiota and the host (Mulder, 1991).

International health organisms and authorities such as the Food and Drug Administration (FDA) are more concerned about animal diets containing antibiotics since the 70s' and the 80s'. Rigorous guidelines on the use of these substances in feed formulations for animals were established in developed countries. In Brazil, the Ministry of Agriculture has prohibited the use of many antibiotics in animal diets since the 90s' (Menten, 2002).

In face of such problems and regulations, researchers began to evaluate potential alternatives to antibiotics worldwide; if antibiotics were simply to be taken out of diets, the production of animal protein might be seriously affected as a consequence of poorer animal

performance. The interest was then focused on one of the natural defense mechanisms of animals, to which little attention had been given until then: the so-called probiotics (Biotecnal, s.d.). Probiotics are comprised of the populations of non-pathogenic microorganisms that reside in the digestive tract of all domestic animals and men.

Probiotics are classified as GRAS (Generally Recognized as Safe) by the FDA. The concept of their use relates to maintaining the equilibrium of the intestinal microflora by the addition of beneficial microorganisms (Goldin, 1998).

Many studies have reported the benefits of probiotics utilization on productive indexes (Wolke *et al.*, 1996; Cavazzoni *et al.*, 1998; Jin *et al.*, 1998, Sogaard & Suhr-Jessen, 1999; Besnard *et al.*, 2000; Campos *et al.*, 2002). On the other hand, Barrow (1992) and Loddi *et al.* (2000) found no beneficial effects.

Since the efficacy of such products was still not confirmed by consistent data, further studies should be carried out aiming to assure future utilization of probiotics as an alternative to traditional growth promoters. The present study evaluated the utilization of different probiotics in the diets and drinking water and the effects on the performance of broilers.

MATERIAL AND METHODS

Experimental design and treatments

The experiment was conducted at the poultry experimental facility from Faculdade de Ciências Agrárias e Veterinárias from UNESP, Campus Jaboticabal, SP, Brazil. One thousand and fifty one-day-old male chicks from Cobb strain were used. Birds were vaccinated against Marek's disease and fowl pox at the hatchery. Chicks were assigned to 35 pens (2.75m *x* 1.4m) in the experimental poultry house. There were 30 birds/pen, in a final density of 8 birds/m^2. Infrared lamps were used to provide initial heating. After the second week of age, initial drinkers and feeders were replaced by automatic drinkers and tubular feeders with capacity for 20 kg.

Ambient temperature and relative humidity was recorded daily and adequate curtain and fan management was performed to assure adequate environment conditions to the birds. Feed and water were given *ad libitum*.

In order to prevent cross-contamination of diets with microorganisms, diets were handled one at a time and with separate scoops. Besides, separate cleaning material was used for the drinkers of different treatments and disposable plastic booties were used when entering each pen, so as to prevent microbial contamination between treatments.

The broilers were distributed in a randomized design in a 3 *x* 2 + 1 factorial arrangement. There were three probiotics sources added to the diet (*Bacillus subtilis*; *Bacillus subtilis* and *Bacillus licheniformis*; and *Saccharomyces cerevisiae*), two concentrations of probiotics in drinking water (with and without probiotics) and one control group (no probiotics added), with a total of 7 treatments and 5 replications with 30 birds.

The treatments were as follows: Control (no antibiotic or probiotics added); addition of *Bacillus subtilis*-based probiotics to the diet (10^{10} colony forming units (CFU)/g product) and no probiotics added to the drinking water; addition of *Bacillus subtilis*-based probiotics to the diet (10^{10} CFU/g product) and *Lactobacillus*-based probiotics to the drinking water (*Lactobacillus reuteri*, 6.6 *x* 10^9 CFU/g product; *Lactobacillus johnsonii*, 3.3 *x* 10^9 CFU/g product); addition of *Bacillus*-based probiotics to the diet (*Bacillus subtilis*, 1.6 *x* 10^9 CFU/g product; *Bacillus licheniformis*, 1.6 *x* 10^9 CFU/g product) and no probiotics added to the drinking water; addition of *Bacillus*-based probiotics to the diet *(Bacillus subtilis*, 1.6 *x* 10^9 CFU/g product, *Bacillus licheniformis*, 1.6 *x* 10^9 CFU/g product) and *Lactobacillus*-based probiotics to the drinking water (*Lactobacillus reuteri*, 6.6 *x* 10^9 CFU/g product; *Lactobacillus johnsonii*, 3.3 *x* 10^9 CFU/g product); addition of *Saccharomyces*-based probiotics to the diet (*Saccharomyces cerevisiae*, 8 *x* 10^9 CFU/g product) and no probiotics added to the drinking water; and finally addition of *Saccharomyces*-based probiotics to the diet (*Saccharomyces cerevisiae*, 8 *x* 10^9 CFU/g product) and *Lactobacillus*-based probiotics to the drinking water (*Lactobacillus reuteri*, 6.6 *x* 10^9 CFU/g product; *Lactobacillus johnsonii*, 3.3 *x* 10^9 CFU/g product).

The commercial products containing the microorganisms were added to the diet following the instructions given by the manufacturers:

- ***Bacillus subtilis* -** based probiotics added to the diet at 300 g of product per ton of diet, throughout the rearing period (1-45 days of age);

- Probiotics based on a mixture of *Bacillus subtilis* and *Bacillus licheniformis* added to the diet at 1,000 g of product per ton of starter diet (1-21 days of age) and 400 g of product per ton of diet until slaughter age (22-45 days of age);

- ***Saccharomyces cerevisiae*** - based probiotics added to the diet at 2,000 g of product per ton of starter diet (1-21 days of age), 1,000 g product per ton of grower diet (22-35 days of age) and 800 g product per ton of finisher diet (36-45 days of age);

- ***Lactobacillus reuteri*** **and** ***Lactobacillus johnsonii*** - based probiotics added to drinking water at 25 g for each 5,000 chicks, at the first day of age.

Experimental Diets

Experimental feeds (Table 1) were based on corn, soybean meal, soybean oil, dicalcium phosphate, calcitic limestone, salt, synthetic amino acid and vitamin-mineral supplement. The nutritional levels for the three phases of rearing were as recommended by NRC (1994).

Table 1 - Composition of experimental diets.

Ingredients (%)	Initial (1-21 days)	Growing (22-35 days)	Final (36-45 days)
Corn	52.94	60.77	66.25
Soybean meal	40.10	32.20	27.00
Soybean oil	2.40	3.30	3.70
Dicalcium phosphate	1.95	1.43	1.18
Calcitic limestone	1.03	1.19	1.10
Salt	0.40	0.34	0.25
Vitamin-mineral supplement[1]	0.80	0.60	0.40
Inert	0.20	0.10	0.08
Methionine	0.18	0.07	0.04
Total	100.00	100.00	100.00
Nutritional Levels			
ME (kcal/kg)	2,944	3,100	3,200
CP (%)	23.00	20.00	18.00
Methionine (%)	0.537	0.388	0.333
Methionine + Cystine (%)	0.909	0.720	0.639
Lysine (%)	1.285	1.074	0.935
Calcium (%)	1.001	0.913	0.803
P available (%)	0.481	0.377	0.327

1 - Vitamin-mineral supplement - Composition (kg/product): Initial: Vit. A 2,160,000 IU; Vit. D_3 396 IU; Vit. E 4,500 mg; Vit. K 540 mg; Vit. B_1 360 mg; Vit. B_2 900 mg; Vit. B_6 540 mg; Vit. B_{12} 4,500 mcg; Biotin 16 mg; Niacin 4,500 mg; Pantothenic Acid 2,700 mg; Folic Acid 180 mg; Choline 80,000 mg; I 200 mg; Se 60 mg; Fe 10,000 mg; Cu 2,400 mg; Zn 12,000 mg; Mn 14,000 mg; Coccidiostat 16,000 mg; Antioxidant 10,000 mg. Growing: Vit. A 1,680 IU; Vit. D_3 308 IU; Vit. E 3,500 mg; Vit. K_3 420 mg; Vit. B_1 280 mg; Vit. B_2 700 mg; Vit. B_6 420 mg; Vit. B_{12} 3,500 mcg; Biotin 12 mg; Niacin 3,500 mg; Pantothenic Acid 2,100 mg; Folic Acid 140 mg; Choline 60,000 mg; I 200 mg; Se 60 mg; Fe 10,000 mg; Cu 2,400 mg; Zn 12,000 mg; Mn 14,000 mg; Coccidiostat 12,000 mg; Antioxidant 10,000 mg. Final: Vit. A 960 IU; Vit. D_3 176 IU; Vit. E 5,000 mg; Vit. K_3 240 mg; Vit. B_1 160 mg; Vit. B_2 400 mg; Vit. B_6 240 mg; Vit. B_{12} 2,000 mcg; Biotin 8 mg; Niacin 2,000 mg; Pantothenic Acid 1,200 mg; Folic Acid 80 mg; Choline 40,000 mg; I 200 mg; Se 60 mg; Fe 10,000 mg; Cu 2,400 mg; Zn 12,000 mg; Mn 14,000 mg; Antioxidant 10,000 mg.

Statistical Analysis

Statistical analysis was performed using the software Estat 2.0 (1992), and differences between treatment means were evaluated by Tukey's test. Significance levels ($p<0.05$ and $p<0.01$) are indicated.

Evaluated Parameters

Performance data were recorded in the periods from 1 to 21, 22 to 35, 36 to 45 and 1 to 45 days of age. Feed intake was determined for each repetition as the difference between the amount of feed supplied and the remaining feed at the end of each experimental period, and weight gain was calculated as the difference between the final and initial bird weight. Feed conversion was determined as the ratio between feed intake and weight gain at each phase of the experimental period and viability was determined as the number of birds produced at 45 days of age divided by the initial number of chicks x 100.

RESULTS AND DISCUSSION

There was no significant interaction between the two factors, which indicates that probiotics utilization in the diet or in the drinking water had independent effects on the evaluated characteristics.

Feed intake was similar in the groups receiving probiotics and the control group (Table 2) in all rearing periods that were evaluated, corroborating previous results reported for feed intake at 21 days (Sato *et al.*, 2002; Pelicano *et al.*, 2004) and at 42 days of age (Mohan *et al.*, 1996; Loddi, 2003).

There were also no differences among the groups fed different probiotics in the diets, and no differences when the groups fed probiotics only in the diet were compared with the groups given an association of products in the water and feed. Nevertheless, feed intake was slightly higher when an association of probiotics was administered. Maybe if probiotics based on *Lactobacillus reuteri* and *Lactobacillus johnsonii* had been added to the drinking water for longer periods, significant differences in feed intake from 1 to 45 days of age would have been seen.

Table 3 shows that there were no differences in weight gain for birds receiving probiotics and the control group in the starter phase (1-21 days). These findings are similar to the results reported by Fethiere & Miles (1987), Maiorka *et al.* (2001) and Sato *et al.* (2002). On the other hand, results were better in the control group during the growing period (22-35 days). Birds fed probiotics had lower feed intake ($p<0.05$)

Table 2 - Feed intake of broilers fed probiotics in the diet and drinking water at different rearing phases.

Evaluated Parameter	Feed Intake (g)			
	1-21 d	22-35 d	36-45 d	1-45 d
Probiotics in Diet (A)				
Bacillus subtilis	897	1,944	1,785	4,626
B. subtilis + B. Licheniformis	922	1,951	1,740	
Saccharomyces cerevisiae	925	1,972	1,765	4,662
Test F	1.40 ns	0.36 ns	0.59 ns	0.19 ns
LSD	46.10	86.18	104.57	211.56
Probiotics in the drinking water (B)				
No Probiotics	905	1,957	1,755	4,617
L. reuteri + L. johnsonii	924	1,955	1,771	4,650
Test F	1.63 ns	0.01 ns	0.22 ns	0.22 ns
LSD	31.16	58.25	70.68	143.00
Control *vs* Factorial				
Control	893	1,976	1,781	4,650
Factorial	915	1,956	1,763	4,634
Test F	1.17 ns	0.29 ns	0.15 ns	0.03 ns
A x B	0.74 ns	3.12 ns	0.19 ns	0.76 ns
CV (%)	4.57	3.97	5.35	4.12

For each independent factor, means followed by the same letters within the column are not different (p>0.05) by Tukey's test. Test F: ns, non-significant. LSD - Least significant difference.

Table 3 – Weight gain of broilers fed probiotics in the diet and drinking water at different rearing phases.

Evaluated Parameter	Weight gain (g)			
	1-21 d	22-35 d	36-45 d	1-45 d
Probiotics in Diet (A)				
Bacillus subtilis	640	977	672	2,289
B. subtilis + B. Licheniformis	646	947	671	2,264
Saccharomyces cerevisiae	651	967	656	2,274
Test F	0.58 ns	0.88 ns	0.31 ns	0.14 ns
LSD	26.78	58.16	56.22	114.63
Probiotics in the drinking water (B)				
No Probiotics	643	964	659	2,266
L. reuteri + L. johnsonii	649	963	674	2,286
Test F	0.50 ns	0.00 ns	0.67 ns	0.28 ns
LSD	18.10	39.31	38.00	77.48
Control *vs* Factorial				
Control	654	1021[a]	702	2,377 [a]
Factorial	646	964 [b]	666	2,276 [b]
Test F	0.46 ns	5.19 *	2.15 ns	4.13 *
A x B	0.79 ns	2.10 ns	0.04 ns	0.74 ns
CV (%)	3.74	5.40	7.56	4.52

a,b - For each independent factor, means followed by different letters within the column are different (p<0.05) by Tukey's test. Test F: ns, non-significant; * - p<0.05. LSD - Least significant difference.

associated to poor feed conversion in almost all evaluated periods (p<0.01), which were decisive to result in the lower weight gain (p<0.05) seen in these birds. Although no significant differences in performance were observed between these groups in the finisher phase (36-45 days), the decrease (p<0.05) in the growing period was enough to negatively influence the performance of birds fed probiotics in the total period of rearing (1-45 days). Such results corroborate the findings of Buenrostro & Kratzer (1983) and Sugeta *et al*. (2004), but are nevertheless opposite to those reported by Santoso *et al*. (1995), Yeo & Kim (1997), Cavazzoni *et al*. (1998) and Moreira *et al*. (2001).

According to Buenrostro & Kratzer (1983), the decrease that was seen in performance when probiotics were given to birds might have resulted from a series of factors, among those, inadequate dosing of microorganisms, lack of sanitary challenge, as well as competition with the host for nutrients.

It was also observed that the utilization of the association of probiotics (water and feed) resulted in a slight increase of weight gain in the period from 1 to 45 days of age (2,286 g), when compared to the use in the diet only (2,266 g).

The groups fed the probiotics based on *Bacillus subtilis* in the diet had better feed conversion (p<0.05) from 22 to 35 days (Table 4) compared to the other groups. However, the difference was not seen at the finisher phase (36 to 45 days) or at the total period of evaluation (1 to 45 days). Feed conversion was better (p<0.05) in the control group compared to the other treatments in the periods from 1 to 21, 22 to 35 and 1 to 45 days of age. The poorer feed conversion seen in the groups fed probiotics if compared to the control group evidences the reason for the lower weight gain indexes, since all treatments had similar feed intake. These findings are different from the results described by Jin *et a*l. (1998) and Besnard *et al*. (2000). The authors reported worse feed conversion in the control group when compared to groups of broilers and turkeys fed probiotics based on *Lactobacillus sp* and *Saccharomyces cerevisiae* in the diets, respectively.

It is interesting to note that the negative performance results that were observed in the total period for birds fed probiotics may be due to the fact that the birds were reared in an environment with all measures needed to prevent diseases, and therefore with low challenge. According to some studies, results of probiotics utilization may not be so evident in conditions of minimal stress (Fox, 1988; Dale, 1992; Maruta, 1993). Therefore, it is supposed that an unbalance in the intestinal microbiota might have occurred as a consequence of the higher quantities of different microorganisms that were supplemented in the probiotics when compared to the normal levels found in the digestive tract. The microorganisms might have impaired the metabolization and absorption of the nutrients somehow and, consequently, might have had a negative effect on bird performance. According

to Visek (1978), the microflora has diverse beneficial functions, such as increasing starch digestion, recovering endogenous nitrogen, facilitating mineral absorption and being involved in vitamin synthesis. Nevertheless, some other negative functions will decrease the nutrient absorption, such as increase in intestinal thickness and increased food passage rate (Visek, 1978). Besides, they compete with the host for nutrients and accelerate cellular turnover.

Table 4 - Feed conversion of broilers fed probiotics in the diet and drinking water at different rearing phases.

Evaluated Parameter	Feed conversion			
	1-21 d	22-35 d	36-45 d	1-45 d
Probiotics in Diet (A)				
Bacillus subtilis	1.40	1.99 [b]	2.66	2.02
B. subtilis + B. Licheniformis	1.43	2.06 [a]	2.60	2.04
Saccharomyces cerevisiae	1.42	2.04 [ab]	2.69	2.05
Test F	1.24 ns	3.78 *	1.22 ns	1.37 ns
LSD	0.04	0.07	0.15	0.05
Probiotics in the drinking water (B)				
No Probiotics	1.41	2.03	2.67	2.04
L. reuteri + L. johnsonii	1.43	2.03	2.63	2.03
Test F	1.48 ns	0.00 ns	0.62 ns	0.02 ns
LSD	0.03	0.04	0.10	0.03
Control *vs* Factorial				
Control	1.37 [a]	1.94 [a]	2.54	1.96 [a]
Factorial	1.42 [b]	2.03 [b]	2.65	2.04 [b]
Test F	7.90 **	11.05 **	2.56 ns	15.20 **
A x B	0.02 ns	0.28 ns	0.01 ns	0.28 ns
CV (%)	2.70	2.97	5.27	2.14

a,b - For each independent factor, means followed by different letters within the column are different (p<0.05) by Tukey's test. Test F: ns, non-significant; * - p<0.05; ** p<0.01. LSD - Least significant difference.

Viability was not significantly different among treatments. Nevertheless, it was better in the groups of birds fed probiotics and, among those, in the groups fed microorganisms based on *Bacillus subtilis* and *Bacillus licheniformis* (Table 5). Therefore, it seems that the microorganisms have stimulated the immune system of the birds, resulting in higher resistance against pathogens and consequently better viability. These findings corroborate the results reported by Leedle (2000) and Silva (2000), who suggested that the immunological status of the host is directly related to the intestinal microbiota, since the antigenic load resulting from these bacteria induce stimulation of the immune system. Some studies reported lower mortality indexes when probiotics were used (Henrique *et al.*, 1998; Campos *et al.*, 2002).

Table 5 - Viability of broilers fed probiotics in the diet and drinking water from 1 to 45 days of age.

Evaluated Parameter	Viability (%)
Probiotics in Diet (A)	
Bacillus subtilis	91.33
B. subtilis + B. Licheniformis	93.00
Saccharomyces cerevisiae	90.33
Test F	0.56 ns
LSD	6.30
Probiotics in the drinking water (B)	
No Probiotics	92.00
L. reuteri + L. johnsonii	91.11
Test F	0.18 ns
LSD	4.26
Control *vs* Factorial	
Control	86.67
Factorial	91.56
Test F	3.16 ns
A x B	0.35 ns
CV (%)	6.26

For each independent factor, means followed by the same letters within the column are not different (p>0.05) by Tukey's test. Test F: ns, non-significant. LSD - Least significant difference.

CONCLUSIONS

It was concluded that no beneficial effects of probiotics supplementation were seen on the performance.

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