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Use of Prebiotics and Probiotics of Bacterial and Yeast Origin for Free-Range Broiler Chickens*

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ABSTRACT

This study aimed to evaluate the effects of probiotics and prebiotics of bacterial and yeast origin on the performance, development of the digestive system, carcass yield and meat quality of free-range broiler chickens.

Five hundred and sixty male chicks of the strain ISA S757-N were reared from one to 84 days old. The birds were distributed in four treatments according to a completely randomized block design: T1 = Control, T2 = Probiotics and Prebiotics of bacterial origin, T3 = Probiotics and prebiotics of yeast origin, T4 = Probiotics and prebiotics of bacterial origin + probiotics and prebiotics of yeast origin. There were four repetitions with 35 birds per repetition, and the birds had access to a pasture area after 35 days of age. Characteristics evaluated were performance, development of the digestive system, carcass and parts yield, abdominal fat, breast meat physical measurements (length, width and height) and meat quality parameters (pH from breast and leg meat, cooking loss and shearing force from breast meat).

Lower mortality ($p<0.05$) and higher weight gain from 64 to 77 and 64 to 84 days of age were seen in birds supplemented with probiotics and prebiotics of bacterial origin compared to the non-supplemented birds (control). There were significant differences ($p<0.05$) among treatments for carcass yield. Birds supplemented with both probiotics and prebiotics of microbial and yeast origin (T4) showed higher carcass yield than control birds. Supplementation with probiotics and prebiotics of bacterial origin (T2) or the supplementation of these together with those of yeast origin (T4) reduced mortality and increased the carcass yield in free-range broiler chickens.

INTRODUCTION

The criteria for the production of organic foodstuffs in Brazil are described by two regulations issued by Ministério da Agricultura (Ministry of Agriculture; Brasil, 1999): Portaria MA Nº 505 from 16/10/1998 and Instrução Normativa Nº 007 from 17/05/1999. Furthermore, regulations for free-range broiler chikens production are in the Oficio Circular Nº 007 from 19/05/99 (Brasil, 1999). Specific strains must be used and diets should not contain ingredients of animal origin, chemical growth promoters and coccidiostats. Birds must be inside poultry houses up to 28 days of age and afterwards should have access to a pasture area with at least 3 m^2 per bird. The rearing period should be of at least 85 days.

The association of probiotics and prebiotics is an alternative for the use of chemical additives in the production of free-range broiler chickens. Probiotics are "food supplements that act as growth promoters" according to Fuller (1989), and they compete with pathogenic bacteria in the colonization of the intestinal environment (Vanbelle *et al.*, 1990;

Silva, 2000). Prebiotics are defined as non-digestible food ingredients such as carbohydrates, peptides, lipids, proteins, oligosaccharides and polysaccharides, that are favorable to a limited number of beneficial bacteria or probiotics (Bradley & Savage, 1994; Gibson & Roberfroid, 1995; Andreatti Filho & Sampaio, 1999).

The naked-neck free-range broiler chickens from the strain ISA S 757-N carries the gene *Na*, and therefore birds from such strain lack feathers, mainly in the region of the neck (Cahaner *et al.*, 1987; Mérat, 1990).

There are few studies that report the effects of probiotics and prebiotics on the production parameters and meat quality of free-range chickens. Therefore, the present study evaluated the use of different probiotics associated to prebiotics on the performance parameters, development of the digestive system, carcass and part yields and meat quality characteristics of free-range broiler chickens.

MATERIAL AND METHODS

This experiment lasted 84 days and was conducted at the Research and Development Unity of Agência Paulista dos Agronegócios (DDD-APTA-SAA), Brotas, SP, Brazil, from July to September 2003. One-day-old male free-range broiler chickens from the strain ISA S757-N Label Rouge were used. Five hundred and sixty birds were distributed in two poultry houses according to a completely randomized block design. There were four treatments (T1= Control, T2= Probiotics and prebiotics of bacterial origin, T3= Probiotics and prebiotics of yeast origin, and T4= Probiotics and prebiotics of bacterial and yeast origin), and four replicates with 35 birds each. The birds had access to open areas after 35 days of age. The probiotics and prebiotics of bacterial origin were, respectively, the commercial products "Colostrum avis", given at the first day of age (2g per bird), and "Simbiotico plus", given from 1 to 77 days of age (2 kg per ton feed). The compounds contained 10^6 CFU of Enterococcus sp per gram (probiotics) and 85% of mannanoligosaccharides obtained from the cellular wall of *Saccharomyces cerevisiae* (prebiotics). The probiotics of yeast origin associated with the prebiotics present on the cell wall of such yeast was provided by the commercial product "Levucell SB 20", given from 1 to 77 days of age at 100g/ton diet. It contains $2x10^{10}$ CFU/g of *Saccharomyces cerevisiae* (probiotics and prebiotics).

Diets were given ad libitum and nutritional levels are in accordance with the requirements for the strain in each of the phases (Table 1). Birds were weighed at the beginning of each phase, from 1 to 35, 36 to 63, and 64 to 84 days of age. Mortality was recorded daily and percentages were transformed before statistical analysis using the formula (Steel & Torrie, 1980):

$$\sqrt{x + 0.5}$$

Table 1 – Percentage composition of experimental diets in the different rearing phases of free-range broiler chickens.

Ingredient	Days		
	1-35	36-63	64-84
Corn	61.117	66.770	73.020
Soybean meal	34.547	29.084	23.494
Bicalcium phosphate	1.869	1.738	1.497
Limestone	1.062	1.187	1.254
Soybean oil	0.763	0.572	0.100
Sodium chloride	0.350	0.350	0.350
Methionine	0.093	0.099	0.084
Vitamin supplement*	0.100	0.100	0.100
Mineral supplement**	0.100	0.100	0.100
Total	100.00	100.00	100.00
Calculated analysis			
ME kcal/kg	2,900	2,950	3,000
CP %	21.0	19.0	17.0
Ca %	1.0	1.0	0.950
Available phosphorus%	0.46	0.43	0.380
Lysine %	1.05	0.93	0.800
Methionine %	0.42	0.40	0.360
Met + Cist %	0.735	0.685	0.618

*Levels per kg product: Vit. A – 1,500,000 IU; Vit. D3 – 500,000 IU; Vit. E – 3,000 mg; Vit. K3 – 200 g; Thiamin – 250 mg; Riboflavin – 1125 mg; Pyridoxine – 375 mg; Vit. B12 – 3,000 µg; Niacin – 7,500 mg; Calcium Pantothenate – 2,500 mg; Folic acid – 1,375.5 mg; Biotin – 12,5 mg; Choline chloride – 81,250 mg; Methionine – 325,000 mg; Antioxidant – 5,000 mg.**Levels per kg product: Fe – 5,000mg; Cu – 70,000 mg; Mn – 60,000 mg; Zn – 50,000 mg; I – 1,250 mg; Se – 200 mg.

At 85 days old, five birds were randomly taken from each experimental parcel to evaluate the digestive system, yield of carcass and parts, and breast meat quality. The 80 birds were fasted for 12 hours and slaughtered at the experimental slaughterhouse of FMVZ/UNESP, Botucatu, SP, Brazil. After evisceration, the components of the digestive system were separated. The proventriculus, gizzard, liver, pancreas, duodenum, jejunum and ileum were weighed, and the length of the duodenum, jejunum and ileum was recorded. Besides, the yield of carcass and parts (breast with and without bone and skin; thigh and drumstick with and without bone and skin; wings and back) and abdominal fat were evaluated. The carcass yield, abdominal fat and organs of the digestive system were expressed as percentage of the live weight (weight*

100)/live weight). Parts were expressed as percentages of the carcass (part weight*100/carcass weight).

Yield data were evaluated according to Mendes (1990). Percentage data (x) were transformed to arc sen $(x/100)^{1/2}$ previously to the statistical analysis (Steel & Torrie, 1980).

Meat quality analysis was also performed. The length, width and height of the breast meat were evaluated, besides the pH of breast and leg meat, cooking loss and shearing force of the breast meat.

The pH of the breast and leg meat was measured 24 hours *post mortem* with a pointed-tip electrode (Model 1001, SENTRON) coupled to a LanceFET probe (Model 1074-001, SENTRON). In order to perform physical evaluations of the breast meat, the pectoralis major muscles were dissected, weighed and measured (length, width and height). Measurements were carried out with a pachymeter and the height was measured at the thickest part of the breast. Cooking loss was evaluated in the fillet taken from the left side of each breast. Fillets were weighed, wraped with aluminium foil and both sides were cooked on a grill at an internal temperature of approximately 82 ºC. After cooking, the fillets were placed onto an absorbent paper, left at room temperature until their temperature was approximately 25 ºC and re-weighed. The cooking loss was calculated as the difference between the raw and the cooked fillets (Honikel, 1987).

The shearing force was evaluated in the samples used for cooking loss assessment. Cubic samples (2x2x1.13 cm) were cut with the fibers placed perpendicularly to the Warner-Bratzler device according to Froning *et al.* (1978).

Statistical analysis were carried out using the GLM procedure of SAS (2000).

RESULTS AND DISCUSSION

During the experimental period, mean ambient temperature was 20.27 ºC ± 0.142, with a minimum temperature of 14.69 ºC ± 0.103 and maximum temperature of 27.22 ºC ± 0.179.

Performance data (live weight, weight gain, feed intake, feed conversion and mortality) are shown in Tables 2 and 3.

The treatments had no effect (p>0.05) on the evaluated performance parameters, except for mortality in the initial phase (1 to 35 days). Afterwards, there was no mortality recorded until the end of the experiment. Birds supplemented with bacterial probiotics and prebiotics showed lower mortality than the non-supplemented birds, but there was no difference (p>0.05) compared to the other treatments. The difference was seen in the first rearing phase, a phase in which the birds are more sensitive. Indeed, the naked neck strain used in this study has shown low mortality indexes, as reported by Takahashi *et al.* (2004), who described lower mortality for the naked neck strain when many free-range broiler chickens and conventional strains were compared in confinement and semi-confinement conditions. They also reported concentration of mortality in the initial phase.

Performance results in the present study corroborate findings reported by Dionizio *et al.* (2002), who observed no effects of the addition of different prebiotic sources in broiler diets on the weight gain,

Table 2 – Performance of free-range broiler chickens in different rearing phases (1-35, 36-63, 1-63 d) supplemented with different additives in the diet.

Days	Treatment	Live weight (g)	Weight gain (g)	Food intake (g)	Feed conversion	Mortality (%)
1-35	T1	823	780	1,426	1.83	8.51 a
	T2	819	776	1,499	1.93	2.23 b
	T3	810	766	1,383	1.81	5.50 ab
	T4	832	782	1,481	1.89	4.88 ab
	CV (%)	2.6	2.6	8.6	7.4	28.5
36-63	T1	1,960	1,137	3,172	2.79	0
	T2	2,008	1,189	3,166	2.66	0
	T3	1,992	1,182	3,219	2.72	0
	T4	1,957	1,125	3,172	2.82	0
	CV (%)	2.5	3.5	4.1	4.1	37.4
221-63	T1	1,960	1,916	4,598	2.40	8.51 a
	T2	2,008	1,965	4,665	2.37	2.23 b
	T3	1,992	1,948	4,602	2.36	5.50 ab
	T4	1,957	1,907	4,653	2.44	4.88 ab
	CV (%)	2.5	2.6	5.0	4.2	30.0

Means followed by different letters in the column are different by Tukey's test (p<0.05). T1 = control, T2 = probiotics and prebiotics of bacterial origin, T3 = probiotics and prebiotics of yeast origin, T4 = probiotics and prebiotics of bacterial origin + probiotics and prebiotics of yeast origin.

Table 3 – Performance of free-range broiler chickens in the periods of 64-77, 78-84, 64-84 and 1-84days of age supplemented with different additives in the diets.

Days	Treatment	Live weight (g)	Weight gain (g)	Food intake (g)	Feed conversion	Mortality (%)
	T1	2,396	437 b	1,866	4.27	0
	T2	2,535	527 a	1,967	3.73	0
64-77	T3	2,486	494 ab	1,640	3.32	0
	T4	2,466	510 ab	1,633	3.20	0
	CV (%)	3.0	7.6	16.2	16.1	0
	T1	2,567	171	1,121	6.56	0
	T2	2,699	165	1,067	6.47	0
78-84	T3	2,627	142	1,073	7.56	0
	T4	2,603	136	1,093	8.04	0
	CV (%)	2.8	19.1	8.8	23.8	0
	T1	2,567	607 b	2,988	4.92	0
	T2	2,699	692 a	3,034	4.38	0
2264-84	T3	2,627	635 ab	2,712	4.27	0
	T4	2,603	646 ab	2,726	4.22	0
	CV (%)	2.8	6.0	12.7	13.3	0
	T1	2,567	2,523	7,586	3.01	8.51 a
	T2	2,699	2,656	7,699	2.90	2.23 b
21-84(dias)	T3	2,627	2,584	7,314	2.83	5.50 ab
	T4	2,603	2,560	7379	2.88	4.88 ab
	CV (%)	2.8	2.9	5.7	4.7	30.0

Means followed by different letters in the column are different by Tukey's test (p<0.05). T1 = control, T2 = probiotics and prebiotics of bacterial origin, T3 = probiotics and prebiotics of yeast origin, T4 = probiotics and prebiotics of bacterial origin + probiotics and prebiotics of yeast origin.

feed intake and feed conversion. Similarly, no effects on feed conversion, feed intake and live weight were seen in birds challenged with different salmonellas (*S.* Typhimurium, *S.* Enteritidis, *S.* Gallinarum and *S.* Pullorum) and different probiotics sources, although lower mortality was seen in birds fed probiotics compared to the birds fed the control diet without additives (Gusils, 2001). Therefore, it can be assumed that the addition of probiotics in the diets of free-range broiler chickens reduces mortality in comparison to birds that are not fed additive supplements in the diets. This is due to the colonization of the intestinal tract by probiotic bacteria, which confers higher resistance to the birds, according to the mechanism of action of probiotics reported by Andreatti Filho & Sampaio (1999).

Although no performance differences were seen among treatments, increased weight gain would be expected in chickens fed with mannanoligosaccharide-based prebiotics (MOS), since previous studies reported better development of the intestinal mucosa when such additive was used (Sell, 1996; Blikslager & Roberts, 1997; Macari & Maiorka, 2000). As stated by some authors, a better development of the intestinal mucosa results in higher weight gain because less energy is used in order to reduce the effects of cells loss in the intestinal lumen, which is caused by pathogenic bacteria when no prebiotics are used. McBride & Kelly (1990), estimated that the maintenance of the intestinal epithelium and other supporting structures consume 20% of the crude energy ingested by the animal. Therefore, part of the ingested energy in chickens is destined to the mucosa maintenance, and a greater need of mucosa repair will result in less available energy for weight gain. In the present study, in the later phases, and more specifically, in the periods from 64 to 77 and 64 to 84 days of age, the addition of bacterial probiotics and prebiotics increased weight gain (p<0.05) compared to the control group. Although no differences (p>0.05) were seen compared to the control group, the other treatments showed numerically higher weight gain from 1 to 84 days. Ofinade & Babatunde (1996), observed higher weight gain in broilers supplemented with yeast-based probiotics and prebiotics.

The development data of the digestive system (liver, proventriculus, gizzard, pancreas, duodenum, jejunum, ileum and cecum) are shown in Table 4. There were no differences (p>0.05) among treatments on the evaluated parameters. The addition of probiotics and prebiotics of different origins had no effect on the parts of the digestive tract probably because the intestinal microflora is balanced, and thus the different additives are not expressed in such situation.

A study with chemical growth promoters and biological growth promoters (prebiotics associated with probiotics) in the diet of free-range broiler chickens reared in semi-confinement and confinement has also not shown effects on the intestine length, but there was higher intestine percentage (Pelícia, 2004). On the other hand, Sato *et al.* (2002) did not observe effects of probiotics addition to the diet of broilers on the percentage and length of the intestine. Birds fed probiotics and prebiotics in the diet and challenged with *Salmonella* Enteritidis had also no differences in the length and percentage of cecum, duodenum and jejunum (Takahashi *et al.*, 2004). The addition of probiotics in the diets of broilers had no effect on the percentages of liver and pancreas, although higher gizzard percentage was seen (Loddi *et al.*, 2000).

Table 4 – Development of the components of the digestive system in free-range broiler chickens supplemented with different additives in the diet from 1 to 84 days of age.

Variables	Treatments				CV(%)
	T1	T2	T3	T4	
Proventriculus (%)	0.31	0.31	0.30	0.31	13.24
Gizzard (%)	2.08	1.88	1.90	1.78	13.49
Liver (%)	1.58	1.51	1.58	1.57	7.74
Pancreas (%)	0.18	0.16	0.17	0.17	13.49
Duodenum (%)	0.45	0.43	0.54	0.47	15.84
Duodenum (cm)	24.50	24.12	24.75	25.3	8.69
Jejunum (%)	0.90	0.86	0.87	0.92	16.88
Jejunum (cm)	57.84	56.92	52.63	57.08	8.94
Ileum (%)	0.86	0.72	0.74	0.84	10.75
Ileum (cm)	57.08	57.42	57.29	56.75	9.24
Cecum (%)	0.77	0.76	0.71	0.74	11.30
Cecum (cm)	44.58	41.92	42.63	43.17	6.32

T1 = control, T2 = probiotics and prebiotics of bacterial origin, T3 = probiotics and prebiotics of yeast origin, T4 = probiotics and prebiotics of bacterial origin + probiotics and prebiotics of yeast origin.

Yield results of the carcass and parts (breast, breast meat, leg, leg meat, wings and back) and abdominal fat results are shown in Table 5. Birds fed both bacterial- and yeast-based probiotics and prebiotics (T4) had higher carcass yield ($p<0.05$) compared to the control group. No effects ($p>0.05$) of treatments were seen on the yields of breast, breast meat, leg, leg meat, wings and back. Although there were differences among treatments for the carcass yield, this finding was not expected and must be better investigated, since the birds showed no differences in performance, digestive system parameters and other factors that could explain such result. These results are different from a previous study in which probiotics were added to the diet of broilers and had no effect on the carcass, leg and breast yields (Maiorka *et al.*, 2001). Dionizio *et al.* (2002) also evaluated different prebiotic sources in broiler diets and reported no effects on carcass and breast yield. Pelícia (2004) evaluated chemical additives and probiotics associated to prebiotics in the diet of free-range broiler chickens reared in two different rearing systems (confined and semi-confined) and reported no effects on the carcass and part yields (breast, breast meat, leg, breast meat, wings and back).

Abdominal fat percentage was not affected ($p>0.05$) by the addition of probiotics and prebiotics. Such finding corroborates previous findings reported for broilers (Loddi *et al.*, 2000; Dionizio *et al.*, 2002) and free-range broiler chickens reared in two different systems, confined and semi-confined (Pelicia, 2004).

A possible explanation for the abdominal fat and parts yield results from the present study and other previously reported results is that there were no unbalance in the intestinal microflora and that the different chemical and biological promoters are similar in the control of the intestinal flora in the digestive tract. Once parasites injure the digestive tract, feed efficiency is directly affected and, consequently, there is a decrease in meat transformation and fat deposition.

Table 5 – Carcass and part yields, abdominal fat, physical measurements of the breast meat (length, width and height) and characteristics of meat quality (pH of breast and leg meat, cooking loss and shearing force) of free-range broiler chickens supplemented with different additives in the diet from 1 to 84 days of age.

Variables	Treatments				CV(%)
	T1	T2	T3	T4	
Carcass (%)	67.49 b	68.16 ab	67.92 ab	69.01 a	2.56
Breast (%)	28.72	29.74	29.76	28.88	5.33
Leg (%)	33.26	32.56	32.25	33.08	4.02
Breast meat (%)	19.47	19.94	20.36	19.58	6.77
Leg meat (%)	21.47	20.80	21.08	21.46	4.99
Back (%)	24.69	24.47	25.07	24.84	5.39
Wings (%)	13.34	13.44	13.57	13.42	6.87
Abdominal fat (%)	1.63	1.59	1.99	1.78	21.31
Breast length (cm)	16.96	17.64	17.56	17.41	5.50
Breast width (cm)	14.75	14.78	14.75	14.54	7.33
Breast height (cm)	2.09	2.15	2.12	2.07	10.59
Breast meat pH	5.74	5.71	5.73	5.76	2.15
Leg meat pH	6.01	5.95	5.98	5.95	2.08
Cooking loss (g)	19.49	20.12	20.86	20.39	19.66
Shearing force (kgf/kg)	1.80	2.14	1.92	1.97	25.44

Means followed by different letters in the line are different by Tukey's test ($p<0.05$). T1 = control, T2 = probiotics and prebiotics of bacterial origin, T3 = probiotics and prebiotics of yeast origin, T4 = probiotics and prebiotics of bacterial origin + probiotics and prebiotics of yeast origin.

The physical measurements and quality of meat in free-range broiler chickens are shown in Table 5. There were no differences ($p>0.05$) among treatments for the evaluated variables. These results are similar to a previous study that evaluated the use of growth promoters of chemical and biological (prebiotics associated to probiotics) origin and two rearing systems (confined and semi-confinement) on the physical measurements and meat quality characteristics in free-range broiler chickens (Pelícia, 2004). A similar rationale as the one for yield and abdominal fat can be used, i.e., there was no unbalance of the intestinal microflora. The debilitation of the intestinal system results in poorer feed efficiency, and consequently in lower meat transformation and effects on the physical characteristics of the breast meat. It is known that there is a relationship between nutrients and organism, making it clear that the poorer feed efficiency is related to a lower nutrient absorption. Therefore, it can also have influence on the meat qualitative characteristics, since the nutrient balance is directly related to the normal physiological funtions in birds.

CONCLUSIONS

The use of probiotics and prebiotics of bacterial origin or the association with probiotics and prebiotics of yeast origin are alternatives for free-range broiler chickens, since they not only reduce mortality indexes but also increase carcass yield.

Further studies about the use of probiotics and prebiotics should be performed and should include microorganism counts in the digestive tract, because some studies reported that the balance of the intestinal microflora is reflected in better usage of the diet by the birds, which consequently affects the performance indexes.

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