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Probiotic and Prebiotic Utilization in Diets for Free-Range Broiler Chickens*

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ABSTRACT

This work aimed to evaluate the effects of probiotic associated to prebiotic on performance, carcass and cut yields, qualitative traits of meat, development and score of lesions due to coccidiosis in digestive tract of broiler chickens raised in a free-range system during 85 days. One thousand, six hundred and ninety-six day-old male broiler chicks from naked-neck ISA S757-N Label Rouge line were used in a randomized block design with factorial scheme of 4x2 and four repetitions. The effect of four additive (1 - biologic promoter + coccidiosis vaccine; 2 - biologic promoter + anti-coccidiosis; 3 - chemical promoter + coccidiosis vaccine; 4 - chemical promoter + anti-coccidiosis) and two breeding systems from 35th day of age (one with no-access to pasture or confined and the other with free-access to pasture or semi-confined) on performance parameters, carcass and cut yields, qualitative meat traits, coccidiosis development and lesions in digestive tract were evaluated. There was effect ($p<0.05$) of additives only on sensorial analysis (meat quality) and percentage of large intestine. Breeding systems affected ($p<0.05$) live weight (LW) and LW gains, being the better results observed in semi-confined birds.

INTRODUCTION

Normative instruction nº 007, published in May 17, 1999 by Agriculture and Supply State Department (Brasil, 1999), and Report MA nº 505, from October 16, 1998, established the criteria for agro-ecological production in Brazil. In complement, the Official Circular Letter nº 007, from May 19, 1999 (Brasil, 1999), normalized free-range chicken production. Lines must be specific and birds must be raised in chicken houses until 28 days of age. After, they must have free access to pastures with at least 3 m^2 per bird. Feeding must be made with non-animal ingredients, non-chemical promoters and breeding period must have at least 85 days.

Since them, free-range broiler chicken production has been increasing in Brazil due to its low initial investment and an alternative for small farmers. Besides, there are consumers that prefer meat from birds raised in nature. This kind of producers is common in Europe, where the broiler chickens has access to pasture, the so-called free-range systems, and have been transformed in a very important economical activity (Cothenet, 1998).

However, this kind of system requires some handling and feeding adaptations, mainly in relation to additive utilization. So, an alternative for replacing chemical promoter might be the use of probiotic associated to probiotic and coccidiosis vaccine instead of anti-coccidiosis.

This study was carried out aiming to evaluate the utilization of probiotic associated to prebiotic and coccidiosis vaccine on performance

parameters, carcass and cut yields, meat qualitative traits and lesions scores caused by coccidiosis in digestive tract of broiler colonial chickens raised in free-range conditions.

MATERIAL AND METHODS

The experiment was carried out in the facilities of Agência Paulista de Tecnologia dos Agronegócios, Brotas City, São Paulo State, Brazil, during 85 days. One thousand, six hundred and ninety-six one-day-old male broiler chicks from naked neck ISA S757-N Label Rouge line were used in a randomized block design experiment arranged in a 4x2 factorial schedule. The effect of four additive groups (1 - biologic promoter + coccidiosis vaccine; 2 - biologic promoter + anti-coccidiosis; 3 - chemical promoter + coccidiosis vaccine; 4 - chemical promoter + anti- coccidiosis) and two breeding systems (one with no-access to pasture or confinement and the other with free-access to pasture or semi-confinement) were evaluated with four repetitions per treatment. Pasture of *Brachiaria decumbens* was used and according Brazilian legislation the area was $3m^2$ per bird. Birds from semi-confinement system had access to pasture from 35^{th} of age. Percentual and calculated compositions of ingredients in the diets offered per breeding phase are shown in Table 1. Nutritional levels were those recommended by Embrapa for colonial broiler chickens with modifications (Embrapa, 2004). In Table 2 are shown the group of additives used according to breeding phases. Ration were prepared with ground ingredients and mixed at food facilities.

Birds were weighted and distributed among treatment. The following parameters were evaluated: weight gain, feed intake and feed conversion at 28^{th}, 63^{rd}, 77^{th} and 84^{th} of age. Feed conversion in confinement and semi-confinement was calculated dividing total feed intake by total live weight plus weight of dead birds.

After 12 hours of fasting, slaughter and intestine evaluation were done at 85 days of age. Small and large intestines were separated for individual length measuring and weighting. Carcass yield, cuts (breast, meat breast, legs, leg meat, wings and back), abdominal fat and quantitative analysis of meat (length, width, and height of breast meat, pH of breast meat and leg, weight loss by cooking and shearing force of breast meat) were evaluated. Carcass yield, abdominal fat and intestine were calculated using the formula: [%Y= (Weight of the specific portion*100)/ Live Weight]. For calculation of cuts yields, the formula was: [%Y cut=(Weight of the specific cut*100)/Carcass weight]. The meat pH was measured by inserting an electrode directly on breast and leg muscles 24 hours *post mortem*. For breast physical determinations, *pectoralis major* was dissected, weighted and measured in relation to length, width and height. These measurements were done using a caliper, being the height measured at the thicker part of muscle. For estimating weight loss by cooking, the left steak of each breast was taken, weighted, packaged in aluminum paper and cooked. After cooking, it was weighted again and the weight difference between cooked and raw steak was considered the cooking loss. Shearing force was measured in these same muscles samples using a Warner-Bratzler equipment.

Table 1 - Percentual and calculated composition of experimental diets of colonial broiler chickens at different phases of breeding.

Ingredients	Phases (days)		
	1 – 28	29 – 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
Calcitic Calcarium	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
MethVitamin supplement[1]	0.100	0.100	0.100
Mineral supplement[2]	0.100	0.100	0.100
Total	100.00	100.00	100.00
Calculated nutrient composition			
Metabolizable energy Kcal/kg	2,900	2,950	3,000
Crude Protein	21.0	19.0	17.0
Calcium	1.00	1.00	0.95
Available Phosphorus	0.46	0.43	0.38
Lisine	1.05	0.93	0.80
Methionine	0.42	0.40	0.36
Methionine + Cisteine	0.735	0.685	0.618

1 - Vitamin supplement: Vit. A – 1.500,000 UI; Vit. D3 – 500,000 UI; Vit. E – 3,000 mg; Vit. K3 – 200 g; Thiamin – 250 mg; Riboflavin – 1,125 mg; Piridoxin – 375 mg – Vit. B12 – 3,000 µg; Niacin – 7,500 mg; Calcium Panthotenate – 2,500 mg; Folic Acid – 1,375.5 mg; Biotin – 12.5 mg; Colin chloride – 81,250 mg; Methionine – 325,000; Anti-oxidant – 5,000 mg; Guarantee levels per kg of product. 2 - Mineral supplement: Fe – 5,000mg; Cu – 70,000 mg; Mn – 60,000 mg; Zn – 50,000 mg; I – 1,250 mg; Se – 200 mg; Guarantee levels per kg of product.

Samples for sensorial analysis were without salt and packaged in aluminum paper for heating until 200ºC in an electric surface with double resistance, during 6 minutes, being revolved every three minute and their internal final temperature was around 85ºC. The samples were placed in Petri plates, heated until 45 or 50ºC in microwave per 25 seconds and offered to tasters. This test was done by a panel of 40 tasters in

individual cabins under red light. Also, consumer preference was tested using a linear non-structured scale from 0 to 10 points for the parameters of softness, taste and global impression, where zero corresponded to a sample with a hard consistence, non-characteristic taste and few acceptable, and ten corresponded to a sample very soft, with a very characteristic taste and very acceptable.

Table 2 - Additives used in the experimental diets of colonial broiler chickens at different phases of breeding.

Additives	Phases (days)			
	1 – 28	29 – 63	64 – 77	78 – 84
Chemical Promoters				
Virgimicin 50% (10 ppm)	20 g.ton^{-1}	-	-	-
Olaquidox 99% (50 ppm)	50 g.ton^{-1}	-	-	-
Olaquidox 99% (30 ppm)	-	30 g.ton^{-1}	30 g.ton^{-1}	-
Biological Promoters				
"Colostrum Avis®"[1]	2 g per bird			
probiotic + prebiotic	at 1st day	-	-	-
"Symbiotic Plus®" *	2 kg.	2 kg.	2 kg.	
prebiotic + prebiotic	ton^{-1}	ton^{-1}	ton^{-1}	-
Anti-coccidiosis				
Nicarbazin 25% (50ppm)	200 g.ton^{-1}	-	-	-
Monensin 20% (60 ppm)	300 g.ton^{-1}	-	-	-
Salinomicin 12% (60ppm)	-	500 g.ton^{-1}	500 g.ton^{-1}	-
Coccidiosis Vaccine				
Coccivac-B	1 dose per bird at 1st day	-	-	-

1 - "Colostrum avis" and Simbiotic plus® - 10^6 UFC of *Enterococcus* sp per gram of product and 85% of mananooligosaccharides, produced by Biocamp.

Birds in confinement system showed higher ($p<0.05$) live weight and better weight gain as compared to birds of semi-confinement, findings that are contrary to those reported from Takahashi (2003).

Authors have reported that Na gene improve resistance of birds (Mérat, 1990; Cahaner *et al.,* 1987); thus, the findings of this study in which low mortality was found could related with the presence of this gene in the naked neck chickens.

Table 3 - Performance (live weight, gain of live weight, feed intake, feed conversion and mortality) of colonial broiler chickens, in confinement or semi-confinement systems, fed diets supplemented with different additives from 1 to 84 days of age.

Variables	LW (g)	LW gain (g)	Feed intake (g)	Feed conversion	Mort. (%)
Additives					
Group 1[2]	2330	2289	6466	2.82	1.50
Group 2	2331	2290	6267	2.74	0.64
Group 3	2389	2348	6536	2.78	0.88
Group 4	2367	2326	6456	2.78	1.25
System					
Confinement	2381 a[1]	2340 a	6538	2.79	1.49
Semi-confinement	2327 b	2287 b	6324	2.77	1.15
CV (%)[3]	2.35	2.42	4.80	3.71	136.35

1 - Mean followed by the same letter within column are similar by Tukey test ($p<0.05$) 2 - Group 1: biological promoter + coccidiosis vaccine; Group 2: biological promoter + anti-coccidiosis; Group 3: chemical promoter + coccidiosis vaccine; Group 4: chemical promoter + anti-coccidiosis. 3 - Coefficient of variation.

Lesion caused by coccidiosis in duodenum, jejunum, ileum and cecum were evaluated according to lesion score from 0 to 5 points (0 – no lesion; 1 and 2 – very few lesions; 3 – few lesions; 4 – regular lesions and 5 – lesions).

Statistic analysis were done using GLM proccedings of SAS (2000), and means were compared by Tukey test at 5% of probability.

RESULTS AND DISCUSSION

Mean, minimum and maximum temperatures during experimental period were 24.4ºC ± 0.1, 22.0ºC ± 0.1 and 29.8ºC ± 0.2, respectively.

Since there was no interaction between groups of additives and breeding system, these both factors were individually analyzed.

In the period from 1 to 84 days (Table 3), additive groups did not affect bird performance, but the efficiency of the additive seems to be dependent of amount and quality of the microorganism used in probiotic production as suggested by Tournt (1998).

The different groups of additives in the diets did not affect ($p>0.05$) yield of carcass, cuts and abdominal fat of birds (Table 4). These results are similar to those found by Sartori *et al.* (2003), Gonçalves *et al.* (2002), Maiorka *et al.* (2001), Loddi (1998), Owings *et al.* (1990) and Merkley (1985). Anyway, Moreira *et al.* (2001) reported differences in breast yield, which was higher in birds supplemented with chemical promoter. Jin *et al.* (1998) in their study reported lower fat deposition in birds treated with probiotic, suggesting that this product could interfere in the availability of fat for lipogenesis in the birds. Breeding system did not affect ($p>0.05$) carcass yield, cuts and abdominal fat, corroborating the findings of Sartori *et al.* (2003).

Additives in diet affected meat quality, especially sensorial analysis (Table 5). Diet supplemented with biologic promoter associated to anti-coccidiosis resulted in meat with higher global impression score ($p<0.05$) when it was compared to diet with chemical promoter associated to anti-coccidiosis. In relation to meat taste, groups of additives with biologic promoter were different among themselves; when associated to anti-

Table 4 - Live weight (g), yields (%) of carcass, breast, leg and abdominal fat of colonial broiler chickens raised in confinement or semi-confinement and fed diets with different additives from 1 to 84 days of age.

Variables	Additives				System		CV (%)[3]
	Group 1[2]	Group 2	Group 3	Group 4	Conf.	Semi-conf.	
Live Weight (g)	2223	2249	2241	2269	2273 a[1]	2218 b	2.33
Carcass (%)	70.17	70.93	70.63	70.50	70.16	70.95	1.95
Breast (%)	29.78	30.16	29.54	30.10	29.85	29.94	2.25
Leg (%)	33.43	33.60	33.40	33.38	33.48	33.44	2.28
Breast meat(%)	21.19	21.51	21.27	21.56	21.31	21.45	2.99
Leg meat (%)	22.03	22.27	22.02	22.50	22.28	22.14	3.69
Back (%)	23.23	23.02	23.44	22.89	23.27	23.02	3.69
Wings (%)	12.45	12.64	12.60	12.32	12.50	12.51	2.52
Abdominal fat (%)	2.44	2.26	2.30	2.38	2.42	2.27	14.78

1- Mean followed by the same letter within line are similar by Tukey test (p<0.05). 2 - Group 1: biological promoter + coccidiosis vaccine; Group 2: biological promoter + anti-coccidiosis; Group 3: chemical promoter + coccidiosis vaccine; Group 4: chemical promoter + anti-coccidiosis. 3 - Coefficient of variation.

coccidiosis, they provoked meat with higher score (p<0.05) in relation to those associated to coccidiosis vaccine.

Considering meat softness, there were differences among groups of additives with chemical promoter, being that associated to coccidiosis vaccine responsible by meat with higher score (p<0.05) than that associated to anti-coccidiosis. The other qualitative parameters (Table 5) were not affected (p>0.05) by additives. Also, breeding system did not affect (p>0.05) meat quality.

In Table 6 and Figure 1 are shown results of performance and score of lesion due to coccidiosis in digestive tract, respectively. Percentage of small intestine and length of small and large intestine (Table 6) were not affected by additives. But, in relation to percentage of large intestine, were observed differences (p<0.05) among groups of additive. Anti-coccidiosis associated to biologic promoter induced large intestine development and when it was associated to chemical promoter the effect was the opposite. Other authors showed differences between chemical and biological promoters also in relation to small intestine development, with heavier small intestine in birds fed diets with biological additives (Fethiere & Miles, 1987).

In relation to coccidiosis lesions, diet with chemical promoter and anti-coccidiosis caused higher lesions in duodenum (Figure 1). Owings *et al.* (1990) found more lesions by coccidiosis in jejunum, with fewer incidences in birds fed diets with chemical additives. In this study similar findings were found, being observed higher lesion in jejunum than in duodenum and smaller lesions were related to diets supplemented with chemical promoter and coccidiosis vaccine.

Table 5 - Length, width and height of breast meat (cm), breast meat pH, leg meat pH, weight loss by cooking (g) of breast meat (WLBM), shearing force (kgf.kg^{-1}) and sensorial analysis (taste, softness and global impression) of colonial broiler chicken raised in confinement or semi-confinement with diets supplemented with different additives from 1 to 84 days of age.

Variables	Additives				System		CV (%)[3]
	Group 1[2]	Group 2	Group 3	Group 4	Conf.	Semi-conf.	
Breast length (cm)	17.09	17.12	17.16	17.21	17.25	17.04	2.36
Breast width (cm)	12.56	12.90	12.84	12.95	12.69	12.93	3.60
Breast height (cm)	1.54	1.50	1.50	1.54	1.50	1.49	4.44
Breast meat pH	5.97	5.99	6.00	6.00	5.98	5.99	0.70
Leg meat pH	6.09	6.14	6.12	6.10	6.13	6.09	0.92
WLBM (g)	20.24	21.49	20.97	21.18	20.74	21.19	11.63
Shearing force (kgf/kg)	2.92	3.31	3.07	3.06	3.01	3.18	11.39
Sensorial Analysis							
Taste	4.69 b[1]	5.82 a	5.34 ab	4.94 ab	——-	——	31.03
Softness	5.35 ab	5.68 ab	6.10 a	5.00 b	——-	——	30.51
Global Impression	4.92 ab	5.87 a	5.68 ab	4.90 b	——-	——	31.30

1 - Mean followed by the same letter within line are similar by Tukey test (p<0.05). 2 - Group 1: biological promoter + coccidiosis vaccine; Group 2: biological promoter + anti-coccidiosis; Group 3: chemical promoter + coccidiosis vaccine; Group 4: chemical promoter + anti-coccidiosis.3 - Coefficient of variation.

Table 6 - Intestine development of colonial broiler chicken raised in confinement or semi-confinement with diets supplemented with different additives from 1 to 84 days of age.

Variable	Small Intestine		Large Intestine	
	Length (cm)	Percentage (%)	Length (cm)	Percentage (%)
		Additives		
Group 1[2]	117.08	1.80	37.75	0.55 ab[1]
Group 2	122.50	1.63	39.21	0.57 a
Group 3	120.88	1.84	37.17	0.52 ab
Group 4	111.42	1.57	35.79	0.51 b
		System		
Confinement	118.19	1.69	38.40	0.55
Semi-Confinement	117.75	1.74	36.56	0.53
CV[3] (%)	6.58	11.29	7.59	12.74

1 - Mean followed by the same letter within column are similar by Tukey test (p<0.05). 2 - Group 1: biological promoter + coccidiosis vaccine; Group 2: biological promoter + anti-coccidiosis; Group 3: chemical promoter + coccidiosis vaccine; Group 4: chemical promoter + anti-coccidiosis. 3 - Coefficient of variation.

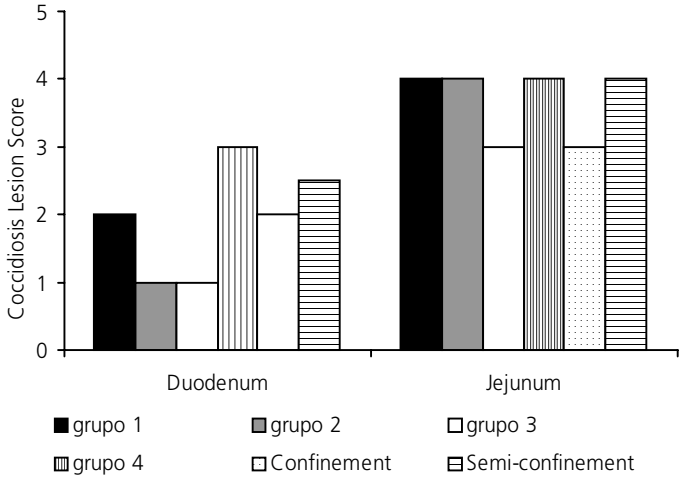

Figure 1 – Score of coccidiosis lesions in digestive tract.
Coccidiosis lesion score: where 0 is not considered, 1 and 2 are very few, 3 is few, 4 is regular and 5 is considered; Group 1: biologic promoter + coccidiosis vaccine; Group 2: biologic promoter + anti-coccidiosis; Group 3: chemical promoter + coccidiosis vaccine; Group 4: chemical promoter + anti-coccidiosis.

CONCLUSIONS

Breeding system and different growth promoter resulted in coccidiosis lesion score in digestive tract below that considered harmful to birds.

Substitution of chemical by biological promoters did not affect performance, carcass and cut yields, and meat quality traits, such as length, width and height of breast meat, pH of leg and breast meat, weight loss by cooking and shearing force of breast meat.

Confinement system provoked better bird live weight and live weight gain, but semi-confinement should be an adequate alternative since it was responsible by good indexes of feed conversion, mortality rate, quality and yield of meat; and it did not affect the incidence of lesions in digestive tract due to coccidiosis, besides it proportionate better environmental comfort.

Also, it is possible to use biological growth promoter in diets for colonial broiler chickens because it was efficient for controlling coccidiosis lesion in digestive tract, besides to provide good performance indexes and meat yield and quality.

Considering breeding system, higher incidence of lesions in jejunum was observed in birds from semi-confinement than those from confinement system. In ileum and cecum no lesions were found, but Owings *et al.* (1990) observed higher coccidiosis lesion in ileum and cecum than in jejunum and duodenum.

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