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Original Article

Performance and Economic Viability of Broiler Chickens Fed with Probiotic and Organic Acids in an Attempt to Replace Growth-Promoting Antibiotics

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ABSTRACT

The aim of this study was to evaluate diets supplemented with probiotic (*Bacillus amyloliquefaciens)* and organic acids (lactic, acetic and butyric acid) in attempt to replace the antibiotics and anticoccidial (avilamycin + sodium monensin) growth promoters on performance and economic viability of broilers challenged by *Eimeria acervulina, E. maxima* and *E. tenella*. A total of 900 male Cobb® chickens, with a mean weight of 39.90g, were distributed in a completely randomized design in a 2 × 2 + 1 factorial arrangement: supplementation or not of probiotic and organic acids and a treatment with inclusion of antibiotics, comprising five treatments with six replicates. For the studied performance variables, there was no effect of the isolated additives and no interaction between them (p>0.05). Only the antibiotics promoted better results for weight gain (WG), feed intake (FI) and feed conversion rate (FCR). Therefore, the use of organic acids and probiotic, isolated or associated, provided lower performance to those receiving antibiotics, not improving the performance of chickens under the imposed challenge conditions. The highest revenues were generated with the use of antibiotics, providing greater profits.

INTRODUCTION

In broiler production, the main aim is to obtain productivity with satisfactory results of zootechnical performance. It is always followed by an economic analysis as food occupies a prominent place for being one of the factors that most demands costs, representing around 80% of the capital spent (Rufino *et al.* 2015). Thus, increasing feed efficiency using additives can help reduce costs by increasing the profitability of this production system.

The use of antibiotics as a growth promoter shows that their use in broiler nutrition is extremely important to maintain the types and quantity of bacteria in the digestive tract, as well as to form adequate protection to the wall of the intestinal mucosa, thus, to which enables a better utilization of the nutrients (Gonzales, Mello, Café, 2012; Shawkat *et al.* 2015). Their use has been pointed out as one of the possible causes of the increase in bacterial resistance, intensifying discussions based on the possibility of the presence of residues of animal origin in food that could harm the consumer's health. There is also the likelihood of an increase in microbial resistance, which can lead to problems in antibiotic treatments in humans, raising the attention of research to overcome such issues (Kelley *et al.* 1998; Sorum & Sunde, 2001; Roe & Pillai, 2003; Silva *et al.* 2010).

In 2006, the European Union chose to ban antibiotic use in animal feed (Langhout, 2000; Huyghebaert *et al.* 2011) and, this way, some diseases related to enteritis, such as coccidiosis, worsened on farms causing the broilers to have performance loss (Kipper *et al.* 2013).

Probiotics can be used to replace antibiotics, since they are dietary supplements composed of live and non-pathogenic microbial agents that benefit host health through intestinal balance (Loddi *et al.* 2000a; Kaur *et al.* 2002; Mountzouris *et al.* 2007; Sanders, 2008; Kabir, 2009; Ayasan, 2013). The organic acids, on the other hand, show specific antimicrobial activity with additional effects, including the pH reduction at the moment of digestion, trophic effects on the gastrointestinal tract mucosa, and increase of pancreatic secretion (Dibner & Buttin, 2002; Van Immerseel *et al.* 2006; Kim & Kim, 2015). Thus, it is greatly important that the animals' performance be quantified in order to evaluate the effects of eventual substitutions; however, the comparison among additives must be followed by an economic analysis to clarify the feasibility of using each one in the production systems.

Therefore, the aim of this study was to evaluate diets supplemented with probiotics and/or organic acids in an attempt to replace growth-promoting antibiotics on zootechnical performance and economic viability in broiler production.

MATERIAL AND METHODS

The experiment was conducted in accordance with the principles and regulations of the ethics committee on animal use – CEUA – of São Paulo State University, UNESP, Dracena Campus (Protocol n° 26, 2013).

A total of 900 one-day-old male *Cobb*® chicks with an initial weight of 39.99 g were housed in thirty floor pens (2.5 m^2) with first used wood-shavings beddings where they remained until 42 days of age. The chicks were distributed in a completely randomized design in a 2 × 2 + 1 factorial design: probiotic supplementation or not, organic acid supplementation or not, and a positive control treatment with inclusion of avilamycin + sodium monensin, comprising five treatments and six replicates of 30 broilers per experimental unit. The treatments were: diet without additives, diet with probiotic inclusion, diet with organic acid inclusion, diet with probiotic and organic acid inclusion, and diet with avilamycin + sodium monensin inclusion.

The probiotic used was *Bacillus amyloliquefaciens* (1 x 10^9 CFU/g), 1 kg/t addition. The organic acid blend consisted of lactic acid (40%), acetic acid (7%), and butyric acid (1%), 8 kg/t addition. The antibiotic used was 20% avilamicin with 50 g/t addition, and the anticoccidian was 40% sodium monensin 300 g/t addition, allowing 10 to 120 ppm of the active principle.

The water and feed supply were *ad libitum*. The feeding program was divided into four phases: pre-starter (1 to 7 days), starter (8 to 21 days), grower (22 to 34 days), and final (35 to 42 days). The rations were isoenergetic, isoproteic, and based on corn and soybean meal according to the recommendations of Rostagno *et al.* (2011) (Table 1).

Williams (1999, 2005), coccidiosis has caused economic losses in the poultry industry. Thus, three species of eimerias (*E. Maxima, Tenella* and *Acervulina*) were chosen because they are the ones that most affect the broiler production (Shirley *et al.* 2004). At 10 days of age, each broiler individually was orally inoculated with 1 ml solution of $2×10^5$ sporulated oocysts/ml *Eimeria acervulina* and $2×10^4$ sporulated oocysts/ml *E. maxima* and *E. tenella*.

Lighting was constant, and temperature and ventilation were manually controlled by moving the side curtains. Temperature monitoring was performed daily, three times a day (at 8:00am, 12:00pm, and 5:00pm), by using a set of maximum-minimum thermometers.

At day 42, the following performance variables were analyzed: weight gain (calculated by the difference between the weight of the broilers at the end of each period and the weight at housing), feed intake (obtained by the difference between the total feed supplied and the remainder collected at the end of each period), feed conversion ratio (calculated as the ratio of total feed intake and the weight gain, corrected for dead weight), and viability (expressed as percentage, 100 - mortality).

In the economic study, the feed cost, gross income, operating profit, profitability index, and the final cost/broiler were evaluated according to the method described by Lana (2000).

The profitability indicators used in this study were those considered by Martin *et al.* (1997): gross income, which is the total kilogram obtained by the treatment multiplied by the average unit price of broiler paid to producers; operating profit, which refers to the difference between the gross income and the total cost of the production; and profitability index, which is total profit divided by the total income, then multiplied by 100.

The cost of each experimental diet was calculated with the prices of the ingredients based on the performance data of the animals throughout the experimental period and based on the price of January 2018, during which the economic analysis was

Table 1 – Composition and calculated values of the experimental diets.

Ingredients, %	Diets[1]			
	Pre-starter	Starter	Grower	Final
Corn	53.61	57.67	62.12	66.94
Soybean meal (46 CP%)	38.43	35.03	31.51	27.25
Soybean oil	2.687	2.682	3.049	2.837
Choline chloride 60	0.072	0.064	0.058	0.043
Salt	0.508	0.482	0.457	0.444
Dicalcium phosphate	1.902	1.533	1.334	1.069
Limestone	0.917	0.907	0.825	0.777
L-lysine	0.283	0.210	0.193	0.234
DL-methionine	0.357	0.285	0.253	0.237
L-Threonine	0.106	0.058	0.039	0.048
L-Valine	0.075	0.024	0.015	0.030
Mineral Premix[2]	0.050	0.050	0.050	0.050
Vitamin Premix[3]	0.100	0.100	0.100	0.050
Kaolin[4]	0.900	0.900	-	-
Total	100.0	100.0	100.0	100.0
Calculated Values				
ME (kcal/kg)	2,950	3,000	3,100	3,150
CP (%)	22.20	20.80	19.50	18.00
Methionine + Cystine[5] (%)	0.944	0.846	0.787	0.737
Lysine[5] (%)	1.310	1.174	1.078	1.010
Threonine[5] (%)	0.852	0.763	0.701	0.656
Valine[5] (%)	1.009	0.904	0.841	0.788
Calcium (%)	0.920	0.819	0.732	0.638
Phosphorus[5] (%)	0.395	0.343	0.313	0.273
Sodium (%)	0.220	0.210	0.200	0.195
Choline (mg/kg)	375.0	330.0	300.0	225.0
Linoleic acid (%)	2.72	2.77	3.07	3.01

[1]Pre-starter, 1 to 7 days of age; starter, 8 to 21 days of age; grower, 22 to 34 days of age; and final, 35 to 42 days of age.

[2]Mineral Premix (per feed kg): Cu, 9 mg; I, 1 mg; Zn, 60 mg; Fe, 30 mg; Mn, 60 mg.

[3]Vitamin Premix (per feed kg) for phase 1 to 21 days: vitamin A, 11,000 UI; vitamin D3, 2,000 UI; vitamin E, 16 UI; vitamin K3, 1.5 mg; vitamin B1, 1.2 mg; vitamin B2, 4.5 mg; vitamin B6, 2 mg; vitamin B12, 16 mcg; folic acid, 0.4 mg; pantothenic acid, 9.2 mg; biotin, 0.06 mg; niacin, 0.035 mg; Se, 0.25mg.

[3]Vitamin Premix (per feed kg) for phase 22 to 33 days: vitamin A, 9,000 UI; vitamin D3, 1,600 UI; vitamin E, 14 UI; vitamin K3, 1.5 mg; vitamin B1, 1 mg; vitamin B2, 4 mg; vitamin B6, 18 mg; vitamin B12, 12 mcg; folic acid, 0.3 mg; pantothenic acid, 8.28 mg; biotin, 0.05 mg; niacin, 0.03 mg; Se, 0.25mg.

[3]Vitamin Premix (per feed kg) for phase 34 to 42 days: vitamin A, 6,000 UI; vitamin D3, 1,000 UI; vitamin E, 10 UI; vitamin K3, 1 mg; vitamin B1, 0.6 mg; vitamin B2, 2 mg; vitamin B6, 0.8 mg; vitamin B12, 6 mcg; pantothenic acid, 7.36 mg; biotin, 0.03 mg; niacin, 0.010 mg; Se, 0,40mg.

[4]The treatments were obtained by replacing kaolin with additives: diet without additives, 0.9% kaolin. Diet with probiotics, 0.1% probiotics + 0.8% kaolin. Diet with organic acids, 0.8% organic acids + 0.1% kaolin. Diet with probiotics + organic acids, 0.1% probiotics + 0.8% organic acids. Diet with antibiotics, 0.005% avilamycin + 0.03% monensin sodium + 0.865% kaolin.

[5] Digestible values.

performed. The prices of the ingredients/kg used to elaborate the feed costs were: corn, \$0.34; soybean meal, \$0.33; soybean oil, \$1.03; choline chloride 60, \$1.60; dicalcium phosphate, \$0.68; calcitic limestone, \$0.15; L-lysine, \$2.05; DL-methionine, \$3.76; salt, \$0.15; threonine \$6.06; valine \$12.16; probiotics \$2.16; organic acid, \$0.46; vitamin and mineral supplement for the pre-starter stage, \$2.80; starter stage, \$3.48; grower stage, \$3.56, and final stage, \$1.80. The feed cost was determined from the total feed intake per broiler multiplied by the cost of the diet used.

The price paid in the day-old chick was \$0.47 and the final value obtained in the chicken was \$0.82, whose value was obtained by means of the product between the final gross weight of the bird and the average price/kg of the broiler, as practiced in the southeastern Brazil in January 2018.

Statistical Analysis System software (SAS Institute, 2012) was used to data analysis, accepting 5% of error. Residue normality was verified through the Shapiro-Wilk test in the UNIVARIATE procedure. The analysis of variance was calculated by the GLM procedure using orthogonal contrasts. The first contrast was designed to evaluate the main effect of probiotic: diets with probiotic against diets without probiotic (contrast 1), the second contrast evaluated the main effect of organic acids: diets with organic acids against diets without organic acids (contrast 2), and the third was used to test the effect of their interaction (contrast 1 * contrast 2). Last contrast was utilized to study the effect of the antibiotics against all the other groups.

RESULTS AND DISCUSSION

From 1 to 42 days of age, it was possible to observe that, for the performance variables studied, there was only antibiotic effect ($p<0.05$) on the sanitary challenge imposed. The broilers showed increased weight gain (WG) and feed intake (FI), as well as better feed conversion ratio (FCR) in the presence of antibiotics ($p<0.05$) (Table 2).

The lack of effect of the additives added to the feed can be explained by the adequate experimental environment, the good management conditions, and the nutritional quality of the feed provided (Santos *et al.* 2005; Paz *et al.* 2010).

Among the treatments, there was no difference ($p>0.05$) for viability in the period from 1 to 42 days of age. This suggests that even with the *Eimerias* inoculation, the broilers have been able to react to the microbiological challenge imposed.

Studies with organic acids and probiotics have been conducted to evaluate broiler performance in an attempt to reduce or replace antibiotic growth promoters (Waldroup *et al.* 1995; Denli *et al.* 2003; Jamroz *et al.* 2004; Gunal *et al.* 2006; Lorençon *et al.*

2007; Willis *et al.* 2007; Yalcinkayal *et al.* 2008; Silva *et al.* 2011; Ramos *et al.* 2014; Barbieri *et al.*, 2015).

Differently from the findings in this study, Abdel-Fattah *et al.* (2008) added citric acid, acetic acid and lactic acid in diets for broilers in the period from 1 to 42 days and noticed an improvement in their weight gain compared to the feed without additives. Likewise, Godoi *et al.* (2008), adding prebiotics, symbiotics and antibiotics in broilers feed in the period from 1 to 42 days, observed improvement of up to 3.3% in weight gain ($p<0.05$) in relation to the negative control treatment with no additives.

Research by Chowdhury *et al.* (2009) showed that the inclusion of 0.5% citric acid resulted in an improvement in broiler performance in the period from 1 to 35 days. Ashayerizadeh *et al.* (2009) also showed satisfactory results of weight gain of broilers supplemented with prebiotics, probiotics and antibiotics in relation to the negative control in the period from 1 to 42 days.

Khosravi *et al.* (2010), when testing the inclusion of probiotics and organic acids in broiler diets from 1 to 42 days, did not observe beneficial effects for weight gain and feed intake, which is similar to the present study, but their interaction showed a significant effect ($p<0.05$) for feed conversion ratio. Dalólio *et al.* (2015), when supplementing broiler diets with enzyme complex, garlic powder and probiotics as an alternative to conventional antimicrobials, found no significant differences ($p>0.05$) in the variables of productive performance, which is similar to the findings in this study.

Rocha *et al.* (2010) found a significant effect ($p<0.05$) of the addition of prebiotics, probiotics, organic acids and antibiotics in comparison to the control diet on the performance variables of broilers in the total period of breeding. Studies with organic acids mixtures in an attempt to improve the performance of broilers, claim to be more efficient than the antibiotic growth promoter (Enramycin) Hassan *et al.* (2010).

Barbieri *et al.* (2015) tested probiotics, organic acids and their interaction in broiler diets in the period from 1 to 21 days but did not find a satisfactory effect of these additives on performance parameters, as neither did the current research.

The economic analysis of this study shows that there was no significant effect on the profitability index (Table 3). Broilers receiving diets containing antibiotics showed higher costs, revenue, and profits. The higher costs came mainly from higher feed intake, which resulted in higher body weight of broilers fed antibiotics. In addition, it is important to mention that

Table 2 – Performance of broilers fed diets supplemented or not with probiotics, organic acids and antibiotic in the period from 1 to 42 days of age.

Performance at 42 days of age				
Effects[1]	AWG[2]	AFI	FCR	VB
Prob				
With	2734.3	4447.1	1.8046	97.50
Without	2782.6	4540.1	1.8058	98.05
AO				
+	2754.5	4509.4	1.8052	97.77
-	2762.4	4477.7	1.8052	97.79
Prob x AO				
With +	2729.1	4469.7	1.7970	97.78
Without +	2779.9	4549.2	1.8134	97.76
With -	2739.4	4424.5	1.8122	97.22
Without -	2785.4	4531.0	1.7981	98.35
Antib				
Presence	2913.2	4673.7	1.7750	96.67
Absence	2758.5	4493.6	1.8052	97.78
SEM[3]	17.5566	24.5312	0.0050	0.4209
Source of Variation	Probability			
Prob	0.1245	0.0510	0.9100	0.5778
OA	0.7978	0.4915	0.9968	0.9839
Prob x OA	0.9376	0.7694	0.1492	0.5643
Antib	0.0001	0.0016	0.0145	0.3206

[1]Prob, probiotics; OA, organic acids; Antib, antibiotic; with, presence of probiotics; without, absence of probiotics; +, presence of organic acids; -, absence of organic acids.

[2]AWG, average weight gain; AFI, average feed intake; FCR, feed conversion ratio; VB, viability.

[3]SEM, standard error of the mean.

feed costs are quite significant in the total production cost, since about 70 to 80% of production is related to food (Teixeira *et al.* 2005).

Despite the highest cost, the income generated from the broilers that consumed antibiotics was quite significant and superior to the income from broilers that did not, surpassing expenses and leading to higher profits, making antibiotic treatment the most economically viable.

Chowdhury *et al.* (2009) demonstrated that the lowest production cost of broilers was found in those fed with organic acids, followed by antibiotics, negative control (without additives), and the interaction among them.

When evaluating broiler productive performance using enzymatic complexes with nutritional matrices in their diets, Pasquali *et al.* (2017) observed that they did not favor the economic viability of broilers in the period from 1 to 42 days.

According to Garcia & Ferreira Filho (2005), the large poultry farms are located in the central-west and south of Brazil, and it would be easier to reduce costs and expand the Brazilian economy more and more in the poultry sector. This is because a large part of the animal nutrition basis, such as corn and soybean meal, comes from these regions, which are the largest domestic producers.

As the price of commodities always varies, the interesting thing is to pay attention to the forms that allow a monitoring of production costs (Melo *et al.*, 2008), so that the producer has a better financial return of his production system.

CONCLUSION

Under the challenge conditions imposed in this research, organic acids and probiotic, isolated or associated, do not present satisfactory effects on broiler performance and economic viability to be characterized as potential substitutes for antibiotic growth promoters.

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Table 3 – Economic analysis of breeding broilers fed diets supplemented or not with probiotic, organic acids, and antibiotic.

Effects[1]		Economic analysis[2]								
		Costs ($)				Revenue ($)		Profit ($)		
		Feed	Other	Bird	Total	Bird	Total	Bird	Total	Index
Prob	with	128.1	22.0	6.6	196.6	7.5	219,1	0.94	22.4	10.2
	without	131.2	22.5	6.7	200.0	7.6	224.2	0.95	24.2	10.8
OA	+	130.6	22.4	6.7	199.4	7.6	221.3	0.90	21.9	9.9
	-	128.7	22.1	6.6	197.3	7.6	221.9	0.99	24.7	11.1
Prob×OA	with +	130.4	22.3	6.6	199.2	7.5	219.3	0.83	20.0	9.1
	without +	130.7	22.4	6.7	199.5	7.6	223.3	0.96	23.8	10.6
	with -	125.8	21.7	6.5	194.0	7.5	218.8	1.04	24.8	11.3
	without -	131.6	22.5	6.7	200.5	7.6	225.0	0.94	24.5	10.9
Antib	presence	134.9	22.9	6.8	204.2	8.0	231.3	1.20	27.0	11.6
	absence	129.6	22.2	6.6	198.3	7.6	221.6	0.95	23.3	10.5
SEM[3]		1.0124	0.1276	0.0378	1.1397	0.0475	1.4684	0.0379	1.0381	0.4245
Probability										
Prob		0.1582	0.1555	0.1608	0.1584	0.1216	0.0914	0.8135	0.4599	0.5588
OA		0.3719	0.3698	0.3735	0.3715	0.7788	0.8199	0.2470	0.2346	0.2075
Prob × OA		0.1910	0.1914	0.1931	0.1910	0.9520	0.7071	0.1623	0.3846	0.3117
Antib		0.0317	0.0318	0.0323	0.0318	0.0001	0.0063	0.0175	0.1535	0.2877

[1]Prob, probiotic; OA, organic acids; Antib, antibiotic.

[2]Costs: Feed (total feed cost), Other (other costs), Bird (costs per bird), Total (total costs). Revenue: Bird (revenue per bird), Total (total revenue). Profit: Bird (profit per bird), Total (total profit). Index (profitability index).

[3]SEM, standard error of the mean.

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