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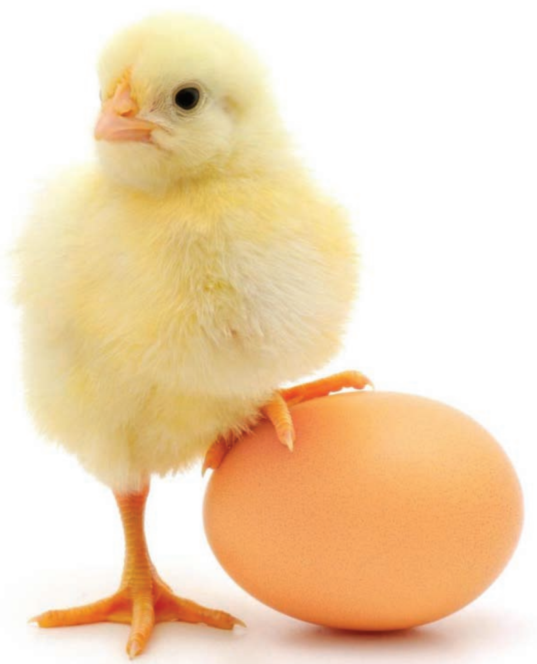

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Research Article

Immunonutrition of Broilers Challenged with *Eimeria* spp. and Fed with Organic Acids as an Alternative to Chemotherapeutics

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Abstract

Objective: This study was designed to evaluated the effect of organic acids as an alternative to chemotherapeutics in diets of broilers challenged with *Eimeria* spp. on their performance and immune system. **Materials and Methods:** A total of 840 male Cobb broiler chicks were utilized. The treatments were: basal diet, without additive (BD)-unchallenged birds; BD-challenged birds; BD+organic acids-challenged birds; BD+antibiotic and anticoccidial-challenged birds. The organic acids were a blend consisting of lactic acid (40%), propionic acid (5%) and butyric acid (1%). The birds were challenged by oral inoculation at the age of 11 days with *E. acervulina*, *E. maxima* and *E. tenella*. The performance was evaluated in the periods of 1-7, 1-14, 1-21 and 1-42 day. Blood from previously identified birds were collected on day zero, at three and at 10 days post-inoculation for immunological analyses. **Results:** The organic acids did not increase the performance of the challenged birds. They only showed a small improvement in the viability (VB), which provided an intermediate result between the control diet and the diet supplemented with the antibiotics. On day zero, it was observed from hemogram of the birds that the treatment had no effect on erythrocytes, hematocrit and hemoglobin. The microbiological challenge decreased the number of erythrocytes and the additives did not provide any improvement. These results evidenced an increase in the immune system response with the use of antibiotics, which fight against pathogenic microorganisms in the gastrointestinal tract (GIT). Although *Eimeria* challenge affects performance, even then, it was not possible to demonstrate a difference between the unchallenged and the challenged control in the immunological study. **Conclusion:** Diet supplemented with organic acids or antibiotics did not improve the broiler performance and few immunological changes were observed when inoculated with *Eimerias*.

Key words: Antimicrobial additives, broilers, butyric acid, immunology, lactic acid, propionic acid

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics are widely used in poultry diet as growth promotor and to treat infections. However, the consumer market and public agencies have been incessantly questioning about the human health hazards due to over use of antibiotics as growth promotor in animals feed. In 2006, the European Union decided to ban the use of antibiotics in animal feed not only based on concern but also taking into account the emergence of bacteria and other microbes resistant to antibiotics which are used to treat humans[1,2]. On the other hand, banning the use of antibiotics in poultry feed poses risk of enteritis caused by opportunistic pathogens. Coccidiosis, for example, is a disease that causes significant losses in birds growth performance[3]. Therefore, it is through that the administration of organic acids may influence the gut and its residing microorganisms in broiler.

Organic acids present a mechanism of antibacterial action that varies according to the organism and the environment[4,5]. In the birds, acidifiers are being used for antimicrobial effects[6]. Recently, it has been shown that organic acid supplementation promotes an improvement in cecal short-chain fatty acids (SCFAs) and SCFA-producing microbiota[7].

The hematology and blood chemistry is a useful tool to establish a diagnosis, allowing a deeper understanding of pathophysiological conditions that affect birds. The blood count in an animal may be influenced not only by its nutritional status but also by its sex, age, habitat, reproductive status, breeding, some trauma, the season and stress caused by the environment[8]. The interaction between immunology and nutrition in poultry comprises an area of knowledge that has received great importance by nutritionists in order to use nutrition as a tool to modulate the immune system[9].

Thus, the objective of this study was to evaluate the effect of organic acids as an alternative to antibiotics on the growth performance and the immune system of broilers.

MATERIALS AND METHODS

The experiment was performed in accordance with the principles and regulations of the Ethics Committee on Animal Use-CEUA, São Paulo State University-UNESP, Dracena campus, SP (protocol number 30/2014).

Birds, design and experimental diets: A total of 840 male chicks Cobb were distributed in a completely randomized design, housed in 28 boxes with reused wood-shavings bedding and raised up to 42 days old. These birds were divided into seven replicates with 30 birds per box and distributed according to the following treatments:

- Basal diet (BD) without additive-unchallenged birds
- Basal diet (BD) without additive-challenged birds
- BD+organic acids-challenged birds
- BD+antibiotics and anticoccidials-challenged birds

The blend of organic acids was composed of lactic acid (40%), propionic acid (5%) and butyric acid (1%), with 8 kg t^{-1} inclusion. The antibiotic used was 20% avilamycin with 50 g t^{-1} inclusion and the anticoccidials used was 40% monensin sodium with 300 g t^{-1} inclusion, with 10 and 120 ppm of active ingredient, respectively.

The water and feed were provided *ad libitum*. The feeding program was divided into four stages: pre-starter (1-7 day), starter (8-21 day), grower (22-33 day) and finisher (34-42 day). The feed was isoenergetic and iso amino acid, formulated based on corn and soybean meal according to the recommendations of Rostagno *et al.*[10] (Table 1). The additive inclusion was done in order to replace the inert material (kaolin). Antibiotics and coccidiostats were withdrawn from the vitamin-mineral supplement so as not to interfere with the effect of the treatments.

***Eimeria* spp. challenge:** To challenge the birds, 1 mL solution containing *E. acervulina*, *E. maxima* and *E. tenella* was inoculated orally at 11 days old[11]. The concentrations used were 2×10^5 sporulated oocysts mL^{-1} of *Eimeria acervulina* and 2×10^4 sporulated oocysts mL^{-1} of *E. maxima* and *E. tenella*. The solution was put in a glass Becker and diluted in distilled water[12]. The process was performed individually, with each broiler retained manually. With the help of an automatic pipette, the solution containing the inocula was given orally. The unchallenged treatment received 1 mL saline solution (placebo). The three species were chosen because of the importance they represent in broiler production due to their high incidence and the economic losses they can cause[13,14].

Evaluated variables

Performance: The following performance variables were analyzed:

- **Weight gain (WG):** Body weight gain is obtained by subtracting the initial weight from the final weight
- **Feed intake (FI):** Feed intake is measured by the difference between the feed supplied and the feed consumed, corrected by the average number of birds

Table 1: Centesimal composition and values calculated of experimental diets

	Diets[1]			
Feed stuffs (%)	Pre-starter	Starter	Grower	Finisher
Corn	53.820	57.880	60.880	66.110
Soybean meal	38.390	34.990	31.730	27.400
Soybean oil	2.616	2.611	3.472	3.119
Choline chloride 60	0.072	0.064	0.058	0.043
Salt	0.508	0.482	0.457	0.444
Dicalcium phosphate	1.901	1.532	1.340	1.073
Calcitic limestone	0.918	0.908	0.821	0.768
L-lysine	0.283	0.211	0.188	0.231
DL-methionine	0.357	0.285	0.254	0.238
L-threonine	0.106	0.058	0.039	0.048
L-valine	0.075	0.024	0.015	0.030
Mineral supplement[2]	0.050	0.050	0.050	0.050
Supplement				
Vitamin[3]	0.100	0.100	0.100	0.050
Inert (kaolin)[4]	0.800	0.800	0.600	0.400
Total	100.000	100.000	100.000	100.000
Calculated values				
Metabolizable energy (kcal kg^{-1})	2.950	3.000	3.100	3.150
Crude protein (%)	22.200	20.800	19.500	18.000
Methionine (%)	0.646	0.562	0.518	0.486
Methionine+cystine (%)	0.944	0.846	0.787	0.737
Lysine (%)	1.310	1.174	1.078	1.010
Threonine (%)	0.852	0.763	0.701	0.656
Tryptophan (%)	0.250	0.232	0.215	0.192
Valine (%)	1.009	0.904	0.841	0.788
Calcium (%)	0.920	0.819	0.732	0.638
Phosphorus (%)	0.395	0.343	0.313	0.273
Sodium (%)	0.220	0.210	0.200	0.195
Choline (mg kg^{-1})	375.000	330.000	300.000	225.000
Linoleic acid (%)	2.745	2.790	3.270	3.146

[1]Pre-Starter, 1-7 days old (d.o.); Starter: 8-21 d.o.; Grower: 22-33 d.o.; Finisher: 34-42 d.o. [2]Mineral supplement provided per kg of feed; Copper: 9 mg, Iodine: 1 mg, Zinc: 60 mg, Iron: 30 mg, Manganese: 60 mg. [3]Vitamin supplement provided per kg of feed in the pre-starter and starter stages; Vitamin A: 11,000.00IU, Vitamin D3: 2,000.00IU, Vitamin E: 16.00IU, Vitamin K3: 1.50 mg, Vitamin B1: 1.20 mg; Vitamin B2: 4.50 mg, Vitamin B6: 2.00 mg, Vitamin B12: 16.00 mcg, Folic acid: 0.40 mg, Pantothenic acid: 9.20 mg, Biotin: 0.06 mg, Niacin: 35mg and Selenium: 0.25 mg. Guarantee levels per kg of feed in the grower phase; Vitamin A: 9,000.00IU, Vitamin D3: 1,600.00IU, Vitamin E: 14.00IU, Vitamin K3: 1.5 mg, Vitamin B1: 1.00 mg, Vitamin B2: 4.00 mg, Vitamin B6: 1.80 mg, Vitamin B12: 12.00 mcg, Folic acid: 0.3 mg, Pantothenic acid: 8.28 mg, Biotin: 0.05 mg, Niacin: 30 mg and Selenium: 0.25 mg. Guarantee levels per kg of feed in the finisher stage; Vitamin A: 3,000.00IU, Vitamin D3: 500.00IU, Vitamin E: 5.00IU, Vitamin K3: 0.50 mg, Vitamin B1: 0.30 mg, Vitamin B2: 1.00 mg, Vitamin B6: 0.40 mg, Vitamin B12: 3.00 mcg, Pantothenic acid: 3.68 mg, Biotin: 0.015 mg, Niacin: 5.00 mg and Selenium: 0.20 mg. [4]The treatments were obtained by adding the additives as a replacement to kaolin. Diet with organic acids, 0.8% in the pre-starter and starter stages, 0.6% in the grower stage and 0.4% in the finisher stage. Antibiotic diet, 0.005% avilamycin+0.03% monensin sodium in the pre-starter, starter and grower stages. [5]Digestible values

- **Feed conversion ratio (FCR):** FCR is the total amount of feed consumed by the bird, divided by the final body weight, corrected by the weight of dead birds.
- **Mortality:** Mortality in each experimental unit was obtained by the ratio between the initial number of live birds and the number of dead birds, which was measured daily between 7:00 a.m. and 7:00 p.m. and birds that died after 7:00 p.m. were counted the following day. Mortality results were converted to viability with the following equation:

100-mortality

Productive efficiency index (PEI): The productive efficiency index was calculated only at the end of the experiment by the following formula:

$$PEI = \frac{(VIAB. \times ADWG)}{(FC)/10}$$

Where:

PEI : Productivity efficiency index
VB : Viability (%)
ADWG : Average daily weight gain (g) = average weight gain divided by the number of days of the experiment
FCR : Feed conversion ratio

Hematological and biochemical variables: On day zero (before inoculation), on the third and on the tenth day after inoculation, 2 mL blood sample of two birds per box were collected through cardiac puncture, totaling 56 birds per collection. The cardiac puncture method is indicated for collection in chicks and young chickens. The blood samples obtained were conditioned in plastic tubes with lids containing Glistab Vet anticoagulant (Labtest Diagnostic, Lagoa Santa, Minas Gerais, Brazil). The samples were kept in refrigerator according to the method recommended by Hawkey *et al.*[15].

In the immunity assessment, the erythrocyte (red cell) and leukocyte (white cell) hematological profile of the broilers was evaluated. The total concentrations of circulating erythrocytes and leukocytes, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were obtained. As for the biochemical variables, the total protein and glucose concentrations were evaluated. In the total blood, the hematocrit was determined by the microhematocrit technique, in which the capillary was used. It was centrifuged at 11,500 rpm for 5 min and the results were presented in percentage by means of specific tables. The hemoglobin concentration was determined by using the cyanmethemoglobin method [16]. The total erythrocyte count was performed in a Neubauer chamber in blood samples containing anticoagulant at 1:200 dilution[17]. The count of total leukocytes was performed on blood extensions on glass slides stained with hematoxylin-eosin, quick panoptic Laborclin (Laborclin Products for Laboratory, Pinhais, Paraná, Brazil). Total proteins, glucose and hemoglobin were determined by photocolorimetric techniques using Labtest reagent kits (Labtest Diagnostic, Lagoa Santa, Minas Gerais, Brazil).

Statistical analysis: All data generated were subjected to a one-way analysis of variance (ANOVA), with the help of the General Linear Model procedure of the Statistical Analysis System (SAS)[18] followed by Tukey test for comparisons among means with a significance level of 5%.

RESULTS AND DISCUSSION

The broiler performance is presented in Table 2. Birds fed diet with antibiotics showed small improvements in FCR and VB at the age of 1-7 and 1-14 days respectively. From 1-21 days, it is observed that the birds challenged with *Eimerias* showed overall poorer performance compared to the unchallenged one. In such period, 31.53, 22.76, 6.90 and 12.03% reduction in WG, FI, VB and FCR respectively is caused by *Eimerias* spp. It is known that *Eimerias* impair the digestion and nutrient absorption in the intestine of birds. This harmful effect caused by *Eimerias* spp. was clear in this research, validating the challenge protocol used. Dibner and Richards[19] reported that healthy birds have an increased

Table 2: Performance of broilers fed diets with or without organic acids, with or without sanitary challenge in different breeding stages

	Diets[2]					
Variables[1]	NC	NC+C	OA+C	PEA+C	MSE[3]	p≤0.05
1-7 days old						
AWG (g)	125.83	125.03	131.88	134.25	1.6236	0.1161
AFI (g)	144.44	141.16	146.08	143.52	1.8840	0.8370
FC	1.16[a]	1.14[ab]	1.11[ab]	1.07[b]	0.0122	0.0456
VB (%)	98.10	97.14	99.05	99.05	0.5285	0.5491
1-14 days old						
AWG (g)	423.05	412.66	425.63	429.51	4.4550	0.5983
AFI (g)	516.46	492.31	510.54	517.15	5.4095	0.3366
FC	1.23	1.23	1.22	1.21	0.0039	0.2771
VB (%)	97.14[ab]	91.43[b]	97.14[ab]	98.57[a]	0.9707	0.0354
1-21 days old						
AWG (g)	898.81[a]	615.41[b]	635.83[b]	869.22[a]	27.0479	<0.0001
AFI (g)	1170.86[a]	904.36[b]	931.90[b]	1131.05[a]	25.0081	<0.0001
FC	1.33[a]	1.49[b]	1.50[b]	1.32[a]	0.0176	<0.0001
VB (%)	96.67[a]	90.00[b]	94.76[ab]	98.10[a]	0.9922	0.0141
1-42 days old						
AWG (g)	2732.89[a]	2434.47[b]	2506.07[b]	2755.95[a]	31.2983	<0.0001
AFI (g)	4515.09[a]	4019.10[b]	4062.10[b]	4492.95[a]	53.8436	<0.0001
FC	1.71[a]	1.73[a]	1.72[a]	1.67[b]	0.0058	0.0001
VB (%)	95.24[ab]	89.05[b]	91.43[ab]	97.62[a]	1.1112	0.0204
PEI	362.38[a]	299.75[b]	312.99[b]	380.15[a]	7.4630	<0.0001

[1]AWG: Average weight gain (g), AFI: Average feed intake (g), FC: Feed conversion, VB: Viability (%), PEI: Productive efficiency index. [2]NC: Negative control diet (no additive), C: Sanitary challenge (inoculation of sporulated oocysts of *Eimeria acervulina*, *E. maxima* and *E. tenella*), OA: Organic acids, PEA: Performance-enhancing antibiotics (avilamycin + monensin sodium). [3]MSE: Mean standard error

Table 3: Hematological variables of broilers fed with organic acids as an alternative to antibiotics on day zero (prior to inoculation with *Eimeria* spp.), at three days after inoculation and at 10 days post-inoculation

	Diets[1]					
Variables	NC	NC+C	OA+C	PEA+C	MSE[2]	$p \leq 0.05$
Day zero (before inoculation)						
Erythrocytes ($\times 10^6$ μL^{-1})	2.350	2.100	2.180	2.320	0.0654	0.1502
Hematocrit (%)	19.710	20.860	17.430	21.290	0.8068	0.3427
Hemoglobin (g dL^{-1})	14.570	12.000	12.380	14.660	0.6055	0.2573
Total Leukocytes ($\times 10^3$/mm^3)	9.604^b	9.774^b	5.338^b	26.061^a	2069.8100	0.0016
Third Day (after inoculation)						
Erythrocytes ($\times 10^6$ μL^{-1})	2.920^a	2.400^b	2.240^b	2.380^{ab}	0.0580	0.0026
Hematocrit (%)	27.000	24.860	24.430	24.000	0.4332	0.0585
Hemoglobin (g dL^{-1})	17.770	15.590	16.340	16.220	0.4142	0.3075
Total Leukocytes ($\times 10^3$/mm^3)	33.971^a	20.808^a	20.071^a	33.535^a	1937.2200	0.0289
Tenth Day (after inoculation)						
Erythrocytes ($\times 10^6$ μL^{-1})	2.920	2.400	2.240	2.380	0.0960	0.8642
Hematocrit (%)	25.290	23.140	23.710	24.430	0.3439	0.1385
Hemoglobin (g dL^{-1})	16.860	14.890	14.330	15.310	0.4218	0.1569
Total leukocytes ($\times 10^3$/mm^3)	31.092	38.101	31.805	28.861	1928.9200	0.3886

[1]NC: Negative control diet (no additive), C: Sanitary challenge (inoculation of sporulated oocysts of *Eimeria acervulina*, *E. maxima* and *E. tenella*); OA: Organic acids, PEA: Performance-enhancing antibiotics (avilamycin+monensin sodium). [2]MSE: Mean standard error

appetite and can utilize more nutrients from feed. Moreover, reduction in cellular epithelial scaling avoid unnecessary energy expenditure in physiological functions that do not increase breeding.

The microbial challenge is very important in evaluating the effectiveness of antimicrobial additives. In a meta-analytical study, Polycarpo *et al.*[20] presented the effect of interaction between microbial challenge and organic acids on the FCR and VB[20]. In non-challenged experiments, authors observed that organic acids improved FCR and showed results similar to antibiotics; however, organic acids were not as effective as antibiotics in challenged birds. Therefore, it is essential to consider the situation in which organic acids are evaluated, because from a known challenge context, the better response and clear conclusion can be obtained.

In the period of 1-21 days, antibiotics improved all the performance variables of the challenged broilers. Organic acids did not improve the performance of birds, except for VB, in which organic acids provided an intermediate result between control diet and the diet supplemented with antibiotics. Barbieri *et al.*[21] reported that broilers challenged with *Eimeria* spp. showed non-satisfactory performance when the mixture of organic acids (lactic, acetic and butyric) was used in their diet; only the use of antibiotic as growth promoters was found to be effective[21]. However, Abbas *et al.*[22] reported a contradictory results and stated that increasing doses of acetic acid control the suppressive effect of coccidiosis[22]. This contradiction raises the question that may the coccidiosis be controlled more effectively by using acetic acid alone (in larger doses). Experiments could also be conducted to understand whether the improvement in the performance of broilers is related to the acid type or the dose used.

The performance results obtained in the initial period (1-21 days) were more evident in the total breeding period (1-42 days) as the organic acids did not increase the performance of the challenged birds. They showed only a small improvement in the VB, which provided an intermediate result between the control diet and the diet supplemented with the antibiotics. Antibiotics, on the other hand, have improved all performance variables, including productive efficiency index (PEI).

In the evaluation of alternative additives, the effects were expected to be similar or close to those of antibiotics. However, in case of the broiler performance, this was not observed in this study. In the literature, it is reported that organic acids have broader effects than antibiotics in the avian organism. Antibiotics improve performance through antibacterial action but organic acids show more pronounced effects like modification of the intestinal microbiota, gut mucosa and increased activity of digestive enzymes and pancreatic secretions[23]. Organic acids also increased the digestibility of minerals in diet[24,25] but despite several beneficial effects reported, supplementation of organic acid did not increase the broiler performance in this study. It might be possible that the intensity of the *Eimeria* challenge was higher than the microbial control supplemented with organic acids. Polycarpo *et al.*[20] reported that organic acids provided poorer FCR than antibiotics only in broiler chickens under microbiological challenge. In unchallenged situations, organic acids presented results comparable to the antibiotic ones.

Table 4: Biochemical variables of broilers fed with organic acids as an alternative to antibiotics on day zero (prior to inoculation with *Eimeria* spp.), at three days after inoculation and at 10 days post-inoculation

	Diets[1]					
Variables	NC	NC+C	OA+C	PEA+C	MSE[2]	p≤0.05
Day zero (before inoculation)						
Total protein (g dL^{-1})	3.66[ab]	3.42[ab]	4.90[a]	3.26[b]	0.2282	0.0422
Glucose (g dL^{-1})	123.99	123.88	102.61	112.25	9.5087	0.8408
Third day (after inoculation)						
Total protein (g dL^{-1})	2.96	2.64	1.30	2.13	0.3354	0.3353
Glucose (g dL^{-1})	254.80[ab]	329.86[a]	243.75[b]	289.53[ab]	11.8417	0.0337
Tenth day (after inoculation)						
Total Protein (g dL^{-1})	1.64[ab]	3.60[a]	3.57[a]	1.37[b]	0.3174	0.0058
Glucose (g dL^{-1})	249.06	260.38	262.53	246.59	8.0489	0.8783

[1]NC: Negative control diet (no additive), C: Sanitary challenge (inoculation of sporulated oocysts of *Eimeria acervulina*, *E. maxima* and *E. tenella*), OA: Organic acids, PEA: Performance-enhancing antibiotics (avilamycin+monensin sodium). [2]MSE: Mean standard error

On day zero (Table 3), it was observed that the treatment had no effect on erythrocytes, hematocrit and hemoglobin. The red blood cell count (erythrocytes) found in this study was similar to those reported by Tessari *et al.*[26] (2.5×10^6 erythrocytes μL^{-1}) as well as reference values reported by Lima[17] *et al.* for hemoglobin ranged from 7.0-15.0 g dL^{-1}. On the other hand, hematocrit values observed in this study were lower than the average levels (43.5%) reported by Tessari *et al.*[26] for broilers. In most bird species, normal hematocrit values are between 35-55%[16,27]. Lower values indicate anemia, the values between 25-35% are considered as moderate anemia whereas less than 20% are considered as severe anemia. The low levels can also be due to the fact that in the birds' first weeks of life, their immune system is still underdeveloped, so the immunity transmitted by the mother is predominant in this period. Over the first few weeks, the defensive mechanisms of body begin to be activated, , led to increase in concentration of immune cells and consequently accumulation of beneficial microorganisms to destroy pathogens in GIT and thus benefit the immune system[28].

The total leukocyte values were higher in broilers fed diet containing antibiotics compared to control and organic acids. These results showed an increase in the response of immune system with the use of antibiotics, which fight the pathogenic microorganisms in the GIT. The microbiota improves bowel health and nutrient digestion, which in turn improve the nutritional status of broilers by modulating the immune response. Changes in leukocyte values prior to *Eimeiras* spp. inoculation confirm that leukocytosis in birds can occur even in the absence of a persistent infectious agent[29-31].

On the third day, after inoculation, effect of treatment was observed on the total leukocytes; however, no difference was observed when Tukey test was used to compare means. The microbiological challenge decreased the number of erythrocytes and the additives did not provide any improvement. There was no difference between treatments with respect to the hematological variables on the third day after inoculation. On the tenth day post-inoculation, treatment has no effect on hematological variables also.

The biochemical variables are presented in Table 4. There was a smaller amount of total protein with the use of antibiotics as compared to organic acids before and after inoculation. Protein deficiency is associated with malnutrition, acute infections and hemorrhages[31]. However, in the present study, it can be seen that, even with low protein concentrations, the birds did not show any of the characteristics mentioned above; on the contrary, performance data at this age show that birds, regardless of treatment, maintained a good zootechnical index. On the third day after inoculation, the use of organic acids reduced the glucose levels, which indicates that this additive can reduce the stress from challenged birds. As for probiotics, prebiotics and symbiotics, these changes were not observed in the hematological and biochemical variables of broilers[32].

There was no effect of treatments on the Wintrobe indices (Table 5). Wintrobe indices[33], which are: mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) indicate the presence of anemia and evaluate the bone marrow's ability to produce red blood cells of normal size and metabolic capacity, as well as hemoglobin content. In the present study, the MCH and MCHC values were higher than the reference values (27.4 pg and 15.7-29.52 g dL^{-1} respectively). As for the MCV, the values detected were 155.43-174.70 fL[26,34,35], which are lower than the reference values. The higher values found in these variables indicate that the birds had to utilize more energy to protect themselves from the effect of caloric stress and produce more red blood cells. It is noteworthy that during the blood collection period (11-21 days old), the room temperature underwent great oscillations, registering 35.5°C maximum temperature.

Table 5: Wintrobe indices of broilers fed with organic acids as an alternative to antibiotics on day zero (prior to inoculation with *Eimeria* spp.), at three days after inoculation and at 10 days post-inoculation

	Diets[2]					
Variables[1]	NC	NC+C	OA+C	PEA+C	MSE[3]	p≤0.05
Day zero (before inoculation)						
MCH	62.64	67.95	59.61	65.67	5.2410	0.7007
MCHC	78.42	64.89	71.02	76.62	7.1461	0.5459
MCV	80.66	110.98	89.18	86.93	9.9032	0.1841
Third day (after inoculation)						
MCH	66.47	72.77	71.88	63.94	3.9722	0.3549
MCHC	66.54	64.66	68.68	68.32	2.7714	0.7243
MCV	100.26	113.16	104.39	93.76	4.9735	0.0754
Tenth day (after inoculation)						
MCH	60.82	67.80	63.78	63.15	3.4739	0.5642
MCHC	66.16	68.36	62.88	62.58	2.5792	0.3526
MCV	91.45	99.48	101.81	101.42	4.6498	0.3793

[1]MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume. [2]NC: Negative control diet (no additive), C: Sanitary challenge (inoculation of sporulated oocysts of *Eimeria acervulina*, *E. maxima* and *E. tenella*), OA: Organic acids, PEA: Performance-enhancing antibiotics (avilamycin+monensin sodium). [3]MSE: Mean standard error

It was not possible to demonstrate a difference between the performances of birds (unchallenged and the challenged with *Eimeria* spp.) in the immunological study. Because the immunological variables present a greater natural variability than the performance variables, new experiments are suggested so that conclusions on the subject could be elaborated with more robustness.

CONCLUSION

Dietary supplementation of organic acids does not improve the performance of broilers challenged with *E. acervulina*, *E. maxima* or *E. tenella*. Only antibiotics are able to control the harmful effects of coccidiosis on broiler performance.

Few immunological changes are observed due to inoculation of *Eimeria* spp. and supplementation of organic acids or antibiotics. More studies are suggested to evaluate the effects of organic acids on the immunological responses of broiler.

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