



ARTIGO 250

EFFECTS OF THE USE OF PROBIOTICS ON THE IMMUNE RESPONSE AND BLOOD PARAMETERS OF BROILERS

Efeitos do uso de probióticos na resposta imunológica e nos parâmetros sanguíneos das aves

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ABSTRACT: Probiotics are an alternative to the use of antimicrobials as growth promoters for broilers because they do not induce bacterial resistance, promote intestinal microbiota balance, and reduce immune stress. The objective of the present study was to evaluate the effects of probiotics *Lactobacillus acidophilus* (3.5×10^{11} CFU), *Streptococcus faecium* (3.5×10^{11} CFU) and *Bifidobacterium bifidum* (3.5×10^{11} CFU) on the immune and blood parameters of broilers. A total of 1200 one- to 42-d-old broilers were distributed according to a completely randomized experimental design into 3 treatments (antibiotic, probiotic, and control) with 10 replicates of 40 birds each. The following parameters were evaluated: response to a Newcastle vaccine, activity of peritoneal macrophages, skin reaction to phytohemagglutinin, and weight of the lymphoid organs. There was no influence of the immune response, considering macrophages, T lymphocytes and weight of the lymphoid organs. However, a positive response to the probiotic relative to the antibiotic was observed in terms of production of antibodies against Newcastle, which was not different from the control bird.

Key words: Antibiotic, immunity, lymphoid organs, Newcastle

RESUMO: Os probióticos são uma alternativa ao uso de antimicrobianos como promotores de crescimento para frangos de corte, estes não induzem à resistência bacteriana, promove o equilíbrio da microbiota intestinal, e reduz o estresse imunológico. O objetivo do presente estudo foi avaliar os efeitos dos probióticos *Lactobacillus acidophilus* ($3,5 \times 10^{11}$ UFC), *Streptococcus faecium* ($3,5 \times 10^{11}$ UFC) e *Bifidobacterium bifidum* ($3,5 \times 10^{11}$ UFC) sobre os parâmetros imunológicos e sanguíneos de frangos de corte. Um total de 1.200 aves de 1 a 42 dias de idade foram distribuídos em um delineamento experimental inteiramente casualizado, com três tratamentos (antibióticos, probióticos e controle) com 10 repetições de 40 aves cada. Os seguintes parâmetros foram avaliados: resposta a vacina de Newcastle, a atividade de macrófagos peritoneais, reação da pele a fitohemaglutinina, e peso dos órgãos linfóides. Não houve efeito da resposta imune, considerando macrófagos, linfócitos T e peso dos órgãos linfóides. No entanto, uma resposta positiva ao uso do probiótico em relação ao uso do antibiótico foi observado em termos de níveis produção de anticorpos contra Newcastle, resultado este não observado nas aves do grupo controle.

Palavras-chave: antibióticos, imunidade, órgãos linfóides, Newcastle

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INTRODUCTION

Public health safety is a major global concern relative to animal production. Therefore, animal production systems need to focus not only on increasing productivity, but also on the impacts of production on the environment and both on animal and human health (Ferket, 2003).

Feed antibiotics used as growth promoters allow better performance (Dibner & Richards, 2005). However, the possible relationship between in-feed antimicrobials and the increase of bacterial resistance in animals and humans to antibiotics resulted in the adoption of new measures to control this type of chemical compound (Ferket, 2003; Jin, 1997).

Good performance results in broiler homeostasis are directly dependent on the immune system. An efficient immune response requires the presence of immune-modulating nutrients in the diet (Qureshi, 2003). These nutrients reduce the immune stress, preventing the mobilization of nutrients to activities that are not related to animal production, thereby, preventing any performance damage (Ferket, 2003).

The species most commonly included in probiotics for broilers belong to the genera *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces*. Several studies have demonstrated that these products have a beneficial effect on broiler performance (Tortuero, 1973; AwaD et al., 2009) by promoting intestinal microbiota balance (Fuller, 1989), modulation and pathogen inhibition (Samnya & Yamauchi, 2002; Chichlowski et al., 2007), immunomodulation (Matsuzaki & Chin, 2000; Apata, 2008), and improvement of some blood biochemistry parameters (Jin et al., 1998; Ashayerizadeh et al., 2009). In addition, probiotics may improve the

sensorial characteristics (Pelicano et al., 2003) and the microbiological quality of chicken meat (Kabir et al., 2005).

However, there are few studies on the effect of probiotics on broiler immunity and therefore, this study aimed at evaluating their influence on immune response and blood parameters of broilers.

MATERIALS AND METHODS

Broilers were housed in an experimental poultry house in floor pens measuring 4.25m² each and covered with wood-shavings litter. The experimental period was 42 days. The experiment was approved by the Committee of Bioethics, protocol n. 501/2004.

The experimental poultry house is laterally opened with manual curtain management, and no environmental control. Conventional management and equipment were used. Birds were weighed on day 1 of the experiment, and then housed in the pens. Average, minimal and maximal house temperatures were daily recorded from dry-bulb thermometers placed in two different points of the house during the experimental period. Average maximal and minimal temperatures of 28°C and 20°C were recorded.

In this trial, 1200 day-old male AgRoss 308 chicks were reared until 42 days of age. A completely randomized experimental design was applied, with three treatments of 10 replicates each, totaling 30 experimental units with 40 birds each.

The experimental feeds were formulated according to the nutritional levels recommended by Rostagno (2005) for broilers. Treatments consisted of a basal diet (control), the basal diet with an antibiotic growth promoter (avilamycin) or the basal diet with a probiotic. The different treatments were established by the



replacement of the analyzed product (antibiotic or probiotic) by its equivalent weight as inert material (sand). The probiotic composition was: *Lactobacillus acidophilus* (3.5×10^{11} CFU), *Streptococcus faecium* (3.5×10^{11} CFU) e

Bifidobacterium bifidum (3.5×10^{11} CFU). The product was added at 2 kg/ton of feed.

The iso-protein and iso-caloric experimental diets were based on corn and soybean meal (Table 1), and did not contain and anticoccidial or any other drug or additive, except for those under study.

Table 1 – Ingredient composition and calculated analysis of the experimental feeds supplied during the different rearing phases.

Ingredients	Starter feed (%)	Grower feed (%)	Finisher feed (%)
Corn	52.26	57.11	63.70
Soybean meal	40.13	34.00	28.00
Soybean oil	3.52	4.90	4.5
Salt	0.35	0.35	0.35
Limestone	1.24	1.60	1.60
Dicalcium phosphate	1.60	1.14	0.95
Methionine	0.24	0.21	0.18
Vit-min supplement ¹	0.30	0.30	0.30
Antibiotic	0.01	0.01	0.01
Probiotic	0.20	0.20	0.20
Inert material	0.15	0.18	0.21
Total	100	100	100
CALCULATED ANALYSIS			
Metabolizable energy (kcal/kg)	2950	3100	3150
Protein (%)	22.5	20.0	18.0
Methionine (%)	0.35	0.32	0.30
Methionine + cystine (%)	0.71	0.65	0.60
Calcium	0.95	0.95	0.90
Available phosphorus	0.45	0.35	0.30

Birds were vaccinated against coccidiosis in the first week of live with an attenuated live vaccine via drinking water, according to the manufacturer's recommendations.

All birds were vaccinated against Newcastle disease at 14 days of age by eye or nose drop with a freeze-dried vaccine according to the manufacturer's recommendations. Blood samples to determine serum titers of antibodies against

Newcastle disease were collected from 20 birds per treatment (two from each experimental unit) that were individually identified, thereby allowing collecting blood from the same birds on days 14, 28, 35, and 42. Antibodies were tittered by a commercial ELISA (Enzyme-Linked Immunosorbent Assay) kit (Idexx Laboratórios), using the methodology described by Purchase et al. (1989).



On day 21, 1ml for each 200g body weight of a Sephadex solution (Sephadex G-50 Fine at 3% in saline solution at 0.9%) were injected in the peritoneal cavity of 20 birds per treatment (two birds per experimental unit) to attract macrophages. Forty eight hours post-inoculation, birds were sacrificed by neck dislocation. A volume of 20ml of the culture medium RPMI-1640 were inoculated into peritoneal cavity, the region was massaged to detach the cells, the peritoneal liquid was aspirated and transferred to plastic tubes, which were kept on ice. The number of cells in each sample was determined by counting in a Neubauer chamber. Cell viability was determined by the technique of Tripán Blue exclusion, where only non-viable cells are stained blue. Aliquots of these samples were collected for subsequent phagocytosis and oxygenated water production tests.

Phagocytosis was tested counting the number of cells with the inclusion of one or more particles of dead cell wall of *Sacharomyces cerevisiae* out of a total of 200 cells in each cover slip. And the result was expressed as a percentage. Oxygenated water (H_2O_2) release by the macrophages was quantified using the method based on the oxidation of phenol red by H_2O_2 dependent on peroxidase, as described by Pick & Keisari (1980), adapted by Pick & Mizel (1981) and modified by Russo et al. (1989). The results are presented in nmols of H_2O_2 using a regression equation based on the standard curve.

Basophil skin response to phytohemagglutination was measured by intradermal injection of 10 birds per treatment (one per experimental unit) at 36 days of age, between the 3rd and 4th interdigital fold of the right leg with 0.1 ml of phytohemagglutinin (Cultilab). The same volume of saline solution was injected in the

left leg. Skin fold thickness was measured using a pachymeter before the injection. The results were obtained by the difference among measurements made at different times in mm.

At 40 days of age, 10 birds per treatment (one per experimental unit) were sacrificed by neck dislocation for collecting and weighing the following lymphoid organs: Bursa, thymus, and spleen. Results were expressed as organ weight relative to body weight.

Blood parameters (complete hemogram and leukogram) were evaluated by collecting 1.0-ml blood samples by ulnar vein puncture, of 10 birds per treatment (one per experimental unit) on days 14, 28, and 35, totaling 90 samples. Samples were placed in Eppendorf tubes with EDTA (ethylenediaminetetraacetic acid) as anti-clotting agent) at 0.1 ml for each 1.0 ml of blood.

At the Biology Institute, Centro Avançado de Pesquisa Tecnológica do Agronegócio Avícola de Descalvado, state of São Paulo, the following blood tests were carried out: Hematocrit, using the method of micro-hematocrit; plasma protein levels, using the method of refractometry; hemoglobin level, using the method of cyanometahemoglobin; and erythrocyte and leukocyte counts, using a hematocytometer. Differential leukocyte and thrombocyte count was performed in blood smears with no anti-clotting agent and stained with hematoxylin and eosin (Panótipo LB). The relative and absolute values of lymphocytes, heterophils, eosinophils, monocytes, and basophils were determined.

The following Windrobe indexes were calculated using standardized formulas: Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin



concentration (MCHC) and mean corpuscular volume (MCV).

Hemoglobin concentration was determined according to the methodology of Campbell & Dein (1984), whereas the other blood tests were performed according to Charles Noriega (2000).

Before analysis, residue normality was checked using the Shapiro-Wilk test (Proc Univariate) and variances were compared by the test of Hartley (Ott, 1983). Data were then submitted to analysis of variance using the General Linear Model (Proc GLM) of the software package Statistical Analysis System (SAS, 1985) and treatment effects were compared by the test of Tukey. When data did not comply with normality assumptions, non-parametric statistics was used.

The probability of interactions with time was determined by the Greenhouse-Geisse test, using the command REPEATED generated by the GLM procedure (Proc GLM) of SAS. Analyses per time were only performed when the interactions between time and treatment were significant.

RESULTS AND DISCUSSION

One of the mode of action of probiotics in broilers is the stimulation of

the immune system (Huang et al., 2004; Dalloul et al., 2005; Haghighi et al., 2006; Nayeopor et al., 2007; Brisbin et al., 2008). The manipulation of the intestinal microbiota by the administration of probiotics influences the development of the immune response (McCracken & Gaskins, 1999). The precise mode of action of probiotics on immunomodulating activities is not fully understood yet. However, it was demonstrated that they stimulate different cell sets to produced cytokines, which play a role in the induction and regulation of the immune response (Maassen et al., 2000; Christensen et al., 2002; Lammers et al., 2003). Probiotics, particularly may modulate the systemic response of antibodies against antigens in poultry (Koenen et al., 2004; Mathivanan & Kalaiarasi, 2007; Apata, 2008). Moreover, Haghighi et al. (2005) showed that the supply of probiotics increase the blood and intestinal number of antibodies against different poultry antigens. In the present study, the effect of the inclusion of additives in broiler diets in mean antibody titers against Newcastle disease, as obtained by ELISA, was evaluated (Table 2).

Table 2 - Effect of the inclusion of additives in broiler diets on mean antibody titers against Newcastle disease, as obtained by ELISA at different ages (Table 2).

Age	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
14 days	367.75 b	786.70 a	695.70 ab	0.0196	88.62
28 days	1070.45 b	3256.85 a	2173.74 ab	0.0474	154.96
35 days	2219.00 a	3268.53 a	2910.11 a	0.2803	97.39
42 days	2075.30 b	3717.26 a	2860.53 ab	0.0728	101.57

Means followed by different letters in the same row are different by the Chi-Square test ($p < 0.05$). CV(%) – coefficient of variation.

At 14 days of age, before vaccination, the obtained antibody titers were considered

passive (maternal) antibodies. It was observed that the birds fed the probiotic



product present significantly higher antibody titers than those fed the antibiotic ($p < 0.05$), whereas there was no difference between the control and the other groups. After hatching, maternal antibodies gradually decline, but in the present experiment, this decline was delayed in the broilers fed the probiotic. At 28 and 42 days of age, the differences were maintained, whereas no statistical differences among treatments were observed at 35 days of age.

The increase in the synthesis of specific antibodies against the Newcastle disease virus found in the present study was not observed by Balevi et al. (2001), who evaluated the effects of a commercial probiotic on the humoral immune response of layers and did not find any significant differences and attributed this lack of stimulation to the use of non-specific microorganisms in the tested product. On the other hand, Newcastle (2008) evaluated the dietary addition of a prebiotic and a probiotic, obtaining higher antibody titers against Newcastle when the probiotic was used relative to the control, but no

differences relative to the antibiotic and the prebiotic. Khaksefidi & Ghoorchi (2006) also found that the supplementation with a probiotic (*Bacillus subtilis*) promoted higher antibody production against Newcastle disease relative to the control group. Zulkifli et al. (2000) used a probiotic containing *Lactobacillus spp* and also observed higher antibody titers against Newcastle disease compared with the control, but the same titers as the antibiotic-fed group. However, these results were obtained only after a period of exposure to high temperature, supporting the argument that the benefits of probiotics are enhanced under stress situations. Ramarao et al. (2004) also observed higher antibody titers against Newcastle disease and IBD in broilers fed probiotics during the period of 3 to 35 days of age.

The mean percentage of peritoneal macrophages in 21-d-old broilers that phagocytosed one or more particles of yeast cell wall, according to the different treatments is shown in Table 3.

Table 3 – Mean phagocytosis rates in 21-d-old broilers according to the different treatments.

Phagocytosis rate (%)	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
	24.04 a	15.90 a	15.94 a	0.1935	56.00

p - probability CV(%) - coefficient of variation

No statistical differences were observed among treatments for the phagocytic activity of peritoneal macrophages. Nunes (2008) also did not observe any significant difference between the treatments with a probiotic containing *Lactobacillus acidophilus* (3.5×10^8 CFU), *Streptococcus faecium* (3.5×10^8 CFU) and *Bifidobacterium bifidum* (3.5×10^8 CFU) and the control diet.

Methods to evaluate the phagocytic activity of peritoneal, circulating or splenic macrophages are commonly used to measure unspecific or innate immune response. Although these assays are simple, Erickson & Hubbard (2000) believe that, particularly due to the distance of the site of action of the probiotics (gastrointestinal tract) from the sites where the immune system is commonly evaluated (peritoneal



cavity, in the present study), results may not be easy to understand. However, in studies carried out with mice, Perdígón et al. (1991) obtained protection against intestinal pathogens by inducing the increase of phagocytic activity of peritoneal macrophages in mice treated with a probiotic. Perhaps the systemic action of the probiotic occurs by stimulating the release

of mediators, indirectly stimulating the immune system in location distant from the intestinal tract.

Table 4 shows the mean values of oxygenated water released by the activated macrophages in the presence or absence of PMA in 21-d-old broilers, according to the different treatments.

Table 4 – Mean values of oxygenated water released by the activated macrophages in the presence or absence of PMA in 21-d-old broilers, according to the different treatments

H ₂ O ₂	Treatments			P	CV (%)
	Antibiotic	Probiotic	Control		
No PMA	21.65 a	20.08 a	30.94 a	0.3249	83.34
w/PMA	52.40 a	52.15 a	61.88 a	0.5196	45.54

p - probability CV(%) - coefficient of variation

In the present study, the different treatments did not influence macrophage activation, as measures by the release of oxygenated water. Nunes (2008) also did not observe any significant difference in oxygenated water production and release by the macrophages of birds fed a probiotic or a control diet.

Hydrogen peroxide is one of the reactive-oxygen metabolites produced by macrophages after their activation (Oliveira et al., 2003). According to literature data, probiotics activate macrophages through components present in the cell wall of the bacteria present in the product and that are constantly released in the intestinal lumen

(Erickson & Hubbard, 2000). The production of oxygenated water by the macrophages occurs through a process known as “respiratory burst”, which basically consists in an increase in molecular oxygen consumption by the phagocytic cell. The produced amount varies according to the stage of cell activation (Oliveira et al., 2003).

The results of the basophilic skin reaction after the intradermal administration of phytohemagglutinin in the interdigital space of broilers during the intervals before and 8h after inoculation (T8h-T0h) and before and 24h after inoculation (T24-T0h) are in Table 5.

Table 5 - Basophilic skin reaction, in mm of increase, after intradermal inoculation of phytohemagglutinin.

Intervals	Treatments			P	CV (%)
	Antibiotic	Probiotic	Control		
T8h-T0h	0.98 a	0.90 a	1.19 a	0.0835	29.54
T24h-T0h	1.18 a	1.14 a	1.36 a	0.3849	29.56

p - probability CV(%) - coefficient of variation

It is observed that there was a proliferation and differentiation of T



lymphocytes due to the increase in the thickness of the interdigital space after the intradermal inoculation of phytohemagglutinin; however, there was no statistical difference among treatments, showing that, under the conditions of the present experiment, it was not possible to demonstrate the influence of the probiotic on the T lymphocyte response during the evaluated intervals.

In a study using a probiotic (*Lactobacillus acidophilus*, *Lactobacillus*

casei, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and *Torulopsis* spp.) in layers, Panda et al. (2003) found higher basophilic skin reaction after the intradermal administration of phytohemagglutinin in 64-week-old layers fed, but in the other tested periods (24 and 40 weeks), the response was not different from the other experimental groups.

Mean relative weights of the lymphoid organs (spleen, bursa, and thymus), as a percentage of live weight of the broilers are presented in Table 6.

Table 6 - Mean weights of the lymphoid organs (spleen, bursa, and thymus) relative to the live weight of broilers, according to the different treatments.

	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
Spleen	0.123 a	0.121 a	0.118 a	0.8506	17.04
Bursa	0.250 a	0.200 b	0.227 ab	0.0349	18.63
Thymus	0.373 a	0.293 a	0.363 a	0.2306	31.18

p - probability CV(%) - coefficient of variation

Only the relative weight of the bursa was affected by the treatments ($p < 0.05$), with heavier bursas detected in the group fed antibiotic relative to that receiving probiotic, but both were not different from the control group.

In the present study, no relationship between the relative weight of the lymphoid organs and antibody production was detected, similarly to Murarolli (2008) testing a probiotic that contained *Enterococcus* ssp (106 CFU/g) and *Lactobacillus acidophilus* (107 CFU/g).

On the other hand, Kabir et al. (2004) observed higher relative spleen and bursa weight in broilers fed a probiotic containing *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*,

Enterococcus faecium, *Aspergillus oryzae* and *Candida pintolopessi*, relative to the control group, which was also related to the higher antibody titers in the birds fed the probiotic. On the hand, Awad et al. (2009) observed higher relative spleen weight when a probiotic (*Lactobacillus* sp.) was fed relative to the control treatment, but no effect on relative bursa weight.

Tables 7 and 8 show the mean blood parameters measured in 10 birds per treatment. No significant interactions were detected between the parameters hemoglobin, hematocrit, erythrocytes, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, and thrombocytes (Table 7) and time, and therefore, it was not necessary to separate data according to age, which did not affect the results.



Hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and thrombocyte values were not different among treatments. On the other hand, hematocrit values were higher in the control group, lower in the antibiotic group, and similar to the others in the probiotic group ($p < 0.05$). Similar results were obtained with erythrocytes counts, but in this case, the antibiotic group was not different from the others ($p < 0.05$).

Mean corpuscular hemoglobin concentration (MCHC) was higher in the group fed the antibiotic, lower in the probiotic-fed birds, and the control group was similar to the other groups ($p < 0.05$).

Alkhalif et al. (2010) did not find any significant difference between a group fed a probiotic (*Pediococcus acidilactici* (1×10^9 CFU/g)) and the control group in terms of hemoglobin and packed cell volume (PCV) on blood collection days (7, 28 and 42 days of age). Consistent results were found by

Dimcho et al. (2005), who did not observe any effect of probiotic supplementation on broiler blood parameters.

Total protein values (Table 8) were influenced by the factor time at the ages of 28 days ($p < 0.0001$) and 35 days ($p < 0.0005$) and increased as birds aged, which was also detected by Meluzzi et al. (1992), who observed an increase of up to 4.25 g/dl at 45 days of age. In the present study, no differences among treatments were detected at 14 days of age, but at 28 days of age, the group fed antibiotic presented higher total protein values as compared to the other groups ($p < 0.05$), and at 35 days, higher values relative to the probiotic group, whereas the control group was not different ($p < 0.05$). However, Alkhalif et al. (2010) did not find any significant difference between a group fed a probiotic and a control group in terms of total protein concentration when broilers were 7, 28 or 42 days of age.

Table 7 – Broiler blood values: hemoglobin, hematocrit, erythrocytes, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and thrombocytes, according to the different treatments.

Parameters	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
Hemoglobin (g/dl)	8.33 a	8.22 a	8.47 a	0.0504	7.05
Hematocrit (%)	35.53 b	36.07 ab	36.57 a	0.0518	4.61
Erythrocytes ($10^6 \mu\text{L}$)	2.32 ab	2.27 b	2.36 a	0.0485	5.05
MCH (pg)	36.16 a	36.48 a	36.18 a	0.9856	9.07
MCHC (g/dl)	23.42 a	22.56 b	23.15 ab	0.0144	5.77
MCV (fl)	154.15 a	159.88 a	156.25 a	0.2412	7.36
Thrombocytes ($10^3 \mu\text{L}$)	24.04 a	23.47 a	23.89 a	0.7052	5.02



Means followed by different letters in the same row are different by the test of Tukey ($p < 0.05$). CV(%) – coefficient of variation.

Table 8 – Total protein values according to treatment in 14-, 28-, and 35-d-old broilers

Age	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
14 days	2.99 a	2.99 a	3.00 a	0.9721	3.63
28 days	3.51 a	3.09 b	3.13 b	0.0005	7.11
35 days	3.89 a	3.53 b	3.69 ab	0.0061	6.19

Means followed by different letters in the same row are different by the test of Tukey ($p < 0.05$). CV(%) – coefficient of variation.

Dimcho et al. (2005) also did not observe any effect of probiotic supplementation on total blood probiotic concentration compared with the control group.

Although the obtained values are considered normal, the following parameters

were significantly different: hematocrit, erythrocytes, MCHC and total proteins. This may be explained by the wide interval between minimal and maximal values mentioned in literature as normal (Table 9).

Table 9 – Ranges of normal blood values for broilers (*Gallus gallus domesticus*) of different ages

Parameters	Intervals
Erythrocytes (10 ⁶ μ L)	2.5 - 3.5
Hemoglobin (g/dl)	7 - 13
Hematocrit %	29 - 55
MCV (fl)	90 - 140
MCH (pg)	33 - 47
MCHC %	26 - 35
Thrombocytes (10 ³ μ L)	20 - 40
Blood proteins (g/dl)	3 - 6
Leukocytes (10 ³ μ L)	12 - 30
Heterophils (10 ³ μ L)	3 - 6
Lymphocytes (10 ³ μ L)	7 - 17.5



Monocytes (10 ³ µL)	0.15 – 2
Eosinophils (10 ³ µL)	0 – 1
Basophils (10 ³ µL)	Rare

Adapted from JAIN, 1993; CHARLES NORIEGA, 2000; CARDOSO & TESSARI, 2003

The mean corpuscular volume (MCV) found in the present study is somewhat higher than the upper limited mentioned in literature (Charles Noriega, 2000; Jain, 1993), which was also observed by Cardoso & Tessari (2003) when studying normal blood parameter of broilers. Those authors said that these changes may be related to age, as some of the reference literature

values are based on studies with broiler breeders.

Tables 10 and 11 show the mean relative and absolute leukocyte differential counts of 10 birds per treatment. As there was no statistical difference among treatments as a function of time (ages), the parameters are shown as means of the different ages.

Table 10 – Mean leukocyte values and relative lymphocyte, heterophil, monocyte, eosinophil and basophil values, according to treatment.

Parameters	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
Leukocytes (10 ³ µL)	15.96 a	15.93 a	15.99 a	0.9379	7.03
Lymphocyte (%)	59.27 a	57.54 a	58.1 a	0.5303	8.19
Heterophils (%)	38.43 a	40.40 a	40.1 a	0.3411	13.11
Monocytes (%)	1.8 a	1.53 a	1.2 a	0.0715	54.14
Eosinophils (%)	0.43 a	0.47 a	0.5 a	0.9307	112.2
Basophils (%)	0.07 a	0.06 a	0.1 a	0.858	346.27

p - probability CV(%) - coefficient of variation

Table 11 – Mean leukocyte values and absolute lymphocyte, heterophil, monocyte, eosinophil and basophil values, according to treatment.

Parameters	Treatments			p	CV (%)
	Antibiotic	Probiotic	Control		
Leukocytes (10 ³ µL)	15.96 a	15.93 a	15.99 a	0.9379	7.03
Lymphocytes (10 ³ µL)	9.46 a	9.17 a	9.29 a	0.5974	11.32
Heterophils (10 ³ µL)	6.13 a	6.43 a	6.41 a	0.359	14.35
Monocytes (10 ³ µL)	0.29 a	0.24 a	0.195 a	0.078	54.88
Eosinophils (10 ³ µL)	0.069 a	0.075 a	0.079 a	0.8318	112.05
Basophils (10 ³ µL)	0.011 a	0.015 a	0.016 a	0.8505	347.03

p - probability CV(%) - coefficient of variation



There was no influence of the treatments on total or relative leukocyte, lymphocyte, heterophil, monocyte, eosinophil, or basophil counts.

The leukocyte values obtained in the present study are consistent with those reported by Charles Noriega (2000).

The blood parameter values found are within normal ranges and may be considered as reference values due to the lack of studies published on broiler hematology in Brazil.

Differences in age, sex, breed, region, environment, management (Campbell & Dein, 1984), as well as sampling, blood collection and analyses methods, in addition of other factors, may influence blood analysis results (Meluzzi et al., 1992).

Rearing conditions may directly influence the efficiency of growth promoter additives, both in terms of performance and of immune response (Takahashi et al., 1997).

The presence of health challenges, stressful situations and the relationship between the number and type of viable microorganisms in the probiotic product may all influence the efficacy of such products. Therefore, it is difficult to compare the results of different studies, also taking into account the wide variety of probiotic types, administration routes, and experimental conditions adopted in literature reports. Santos (2010) said that the

efficiency or inefficiency of a probiotic product may be related to its composition in terms of microbial species and their livability, method and frequency of application, bird age, hygiene of the facilities, feed composition (cereals and their synergy or antagonism with the product microorganisms), and environmental stress factors.

Although the results are still inconclusive and controversial, many studies report immunostimulating effects related to the use of probiotics in feeds, and this justifies further research on the mode of action of this feed additive.

CONCLUSIONS

The study of the effect of probiotics on immune response and blood parameters of broilers did not show any influence of the probiotic on the immune response of macrophages, T lymphocytes, or change in lymphoid organ weight at the evaluated ages and according to the methods used. However, a positive response to the probiotic relative to the antibiotic was observed in terms of production of antibodies against Newcastle vaccine virus, which, however, was not different from the control birds. Also, the studied probiotic did not cause any changes in the evaluated blood parameters.

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