Performance, Carcass Traits, Biochemical and Hematological Profile, Ileal Microbiota and Nutrient Metabolizability in Broilers Fed Diets Containing Cell Wall of Saccharomyces Cerevisiae and Piperine

ABSTRACT

The objective of this study was to evaluate the inclusion of cell wall of Saccharomyces cerevisiae (CWSc) and piperine in broiler rations and their effects on performance, carcass traits, blood parameters, ileal microbiota and nutrient digestibility. A randomized block design with five treatments and six replicates of 10 birds was used, totaling 300 chickens. The treatments consisted of: control ration (CR); CR + avilamycin (10 mg / kg); CR + CWSc (2.0 g / kg); CR + piperine (60 mg / kg); and CR + CWSc (2.0 g / kg) + piperine (60 mg / kg). The use of isolated piperine resulted in greater weight gain from 9 to 40 days of age (2505g). The additives CWSc and piperine conjugates influenced the lower coliform count in the ceca (4.45 CFU / g) and caused significant alterations in the biochemical serum and hepatic renal profile. The treatments had no effect on the nutrient metabolizable coefficients or on the carcass traits. There was no positive synergic effect of the combined use of CWSc and piperine on broiler performance. The cell wall of Saccharomyces cerevisiae and piperine are effective at guaranteeing productivity, intestinal microbiota dynamics and hematological parameters; and as zootechnical additives, especially in broiler feeds free of antimicrobial performance enhancers.

INTRODUCTION

The use of antimicrobials in broiler feeds has contributed to the increase of bacterial resistance, which is a worldwide concern (Garcia-Migura et al., 2014). The restrictions to the addition of antimicrobials in animal feed as growth promoters has led to an increased interest in functional ingredients that can be used to ensure the intestinal health of the birds via their feed. In this sense, the use of phytogenics (Murugesan et al., 2015) and prebiotics (Yadav et al., 2016) can be highlighted, with the aim of improving the intestinal health and, consequently, broiler performance due to positive changes in their intestinal microbiota and stimulating the immune system.

According to Normative Instruction 13 of 01/12/2004 (Brazil, 2004), the use of phytogenics and prebiotics in animal nutrition are classified as zootechnical additives. Phylogenetics are plant-derived substances that are used in animal feed to improve their performance and comprise a variety of compounds derived from herbs, spices, essential oils and resins (Bobko et al., 2016). Among the phytogenic compounds, one compound that stands out is piperine, the main active compound found in peppers of the genus Piper sp. (Jang et al., 2007), which has several effects, such as: being antimicrobial (Karsha & Laksimi, 2010), acting as a stimulant in the secretion of pancreatic enzymes (Jang et al., 2007), and having pepper (Piper nigrum L.)
anti-inflammatory effects (Guidetti et al., 2016). Cardoso et al. (2012), who studied the effects of piperine as a phytochemical supplement in the broiler diet, concluded that 60 mg / kg of dietary piperine increased the weight gain in broilers and improved the feed conversion rate.

Prebiotics are ingredients that are not digested by the digestive enzymes of the host, but are fermented by the microbiota of the digestive tract of animals, contributing to their equilibrium (Comendio, 2009). The cell wall of Saccharomyces cerevisiae is a prebiotic rich in mannanoligosaccharides (MOS) that has stimulatory effects on the immune system of birds (Jacob & Pescatore et al., 2014), aiding in the competitive exclusion and manipulation of the microbiota, thereby preventing the colonization of pathogens in the intestine (Koc et al., 2010). Barroso et al. (2013), who studied the addition of yeast cell wall (Saccharomyces cerevisiae) in broilers' diet, concluded that amounts of up to 0.2% could be used as an additive in antimicrobial-free diets as performance-enhancers without compromising performance.

The objective of this study was to evaluate the effects of the inclusion of the cell wall of Saccharomyces cerevisiae and piperine, in the individual or associated form, on the performance, carcass traits, ileal microbiota composition, biochemical profile, and hematological parameters of antimicrobial-free broilers, as well as on the metabolizable coefficient of the ration.

MATERIALS AND METHODS

Birds and housing

All procedures performed in this research were approved by the Animal Use Ethics Committee - CEUA, Federal Rural University of Rio de Janeiro - UFRJR, under the number 23083.010042 / 2017-38.

A total of 300 male broiler chickens of the Cobb 500 strain from 9 to 42 days of age were used. The birds were vaccinated in the hatchery against Marek, New Castle, Gumboro and Avian Bouba disease.

At 9 days of age, the chicks were housed in metal cages of 0.90 x 0.85 x 0.40 m and arranged on three floors. The chicks were weighed individually and separated into groups of 10 to equalize the mean weight (205 g) between all experimental units. In the initial phase, screens with 5/8-inch aperture mesh were placed on the floor of the cages to make it difficult to drop the excreta to the trays and increase the time of contact with the birds, causing a challenge condition.

Diets and experimental design

The experimental design used was in randomized blocks, with the block represented by the position of the metallic batteries (upper, intermediate and lower), with 6 replicates per treatment and 10 chicks per experimental unit. The experimental rations were formulated to meet the minimum nutritional requirements for each stage according to Rostagno et al. (2011) and provided at will (Table 1); they consisted of: 1 - control ration (CR), without inclusion of zootechnical additives; 2 - CR + avilamycin performance enhancer (10 mg / kg); 3 - CR + cell wall of Saccharomyces cerevisiae - CWSc (2.0 g / kg); 4 - CR + piperine (60 mg / kg) and 5 - CR + CWSc (2.0 g / kg) + piperine (60 mg / kg). Additives were included in the diet instead of the inert (kaolin).

Table 1 – Percent composition of the experimental rations used in each experimental phase.

| Ingredients (%) | Initial (days 9 to 21) | Growth (days 22 to 33) | Final (days 34 to 40) |
|-----------------|------------------------|------------------------|------------------------|
|                |                        |                        |                        |
| Corn (7.49% CP) | 60.31                  | 62.83                  | 66.56                  |
| Soybean meal (47.10% CP) | 33.74                  | 30.39                  | 26.76                  |
| Soybean oil    | 1.88                   | 2.92                   | 2.99                   |
| Dicalcium phosphate | 1.75                   | 1.61                   | 1.46                   |
| Calcium limestone | 0.89                   | 0.85                   | 0.81                   |
| Salt            | 0.49                   | 0.47                   | 0.44                   |
| DL-methionine   | 0.22                   | 0.21                   | 0.20                   |
| L-lysine HCl    | 0.17                   | 0.18                   | 0.23                   |
| L-Threonine     | 0.04                   | 0.04                   | 0.05                   |
| Vitamin mixture | 0.10                   | 0.10                   | 0.10                   |
| Mineral mixture | 0.06                   | 0.05                   | 0.05                   |
| Choline chloride | 0.05                   | 0.04                   | 0.04                   |
| Butylated hydroxytoluene | 0.01                   | 0.01                   | 0.01                   |
| Kailoin         | 0.30                   | 0.30                   | 0.30                   |
| Total           | 100.00                 | 100.00                 | 100.00                 |

Nutritional composition

| Metabolisable energy (Mcal kg⁻¹) | 3.00 | 3.10 | 3.15 |
| Crude Protein (%)                | 20.79| 19.41| 18.03 |
| Digestible lysine (%)            | 1.168| 1.094| 1.038 |
| Digestible methionine (%)        | 0.514| 0.490| 0.465 |
| Digestible methionine+cysteine (%)| 0.822 | 0.782 | 0.742 |
| Digestible threonine (%)         | 0.758| 0.708| 0.670 |
| Digestible tryptophan (%)        | 0.231| 0.214| 0.195 |
| Calcium(%)                       | 0.884| 0.824| 0.763 |
| Total phosphorus (%)             | 0.687| 0.645| 0.604 |
| Available phosphorus (%)         | 0.442| 0.411| 0.380 |
| Sodiuim(%)                       | 0.214| 0.205| 0.194 |

1 Vitamin A (min) 7,500,000 IU / kg; vitamin D3 (min) 2,500,000 IU / kg; vitamin E (min) 1,200 mg / kg; vitamin K3 (min) 1,200 mg / kg; thiamine (min) 1,500 mg / kg; riboflavin (min) 5,500 mg / kg; pyridoxine (min) 2000 mg / kg; vitamin B12 (min) 12,000 mcg / kg; niacin (min) 35 g / kg; Calcium pantothenate (min) 10 g / kg; biotin (min) 67 mg / kg; Fe (min) 60 g / kg; copper (min) 13 g / kg; manganese (min) 120 mg / kg; zinc (min) 100 mg / kg; iodine (min) 2500 mg / kg; selenium (min) 500 mg / kg.
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The antimicrobial used in the experiment was avilamycin, according to the experimental model for research and development of alternative additives for broiler chickens described by Bellaver et al. (2002). The cell wall of Saccharomyces cerevisiae (SafMannan®, SAF do Brasil Produtos Alimentícios Ltda, Brazil) was used in the formulation of the diet in the amount of 2.0 g / kg of feed. Piperine (Piperine, piperine, Ambe Phytoexctracts, UK) was manufactured in India, extracted from the dried fruits of the black pepper (Piper nigrum), and used at a concentration of 60 mg / kg of feed.

The breeding period was divided into initial (9-21 days), growth (22-33 days) and final (34-40 days) and at the end of each period, the birds of each experimental unit were weighed to obtain the average weight and determination of weight gain, feed intake, feed conversion and viability (%), the latter being calculated by the ratio between the number of live birds at the end and at the beginning of each stage.

Measurements

For the evaluation of the carcass parameters, two chickens per experimental unit were slaughtered at 42 days of age, totaling 12 birds per treatment. To determine the carcass yield, the weight of the carcass was considered clean and eviscerated in relation to the post-fast weight. The yields of the cuts were calculated from the cut weights on the carcass weight. The edible viscera (gizzard, liver and heart), abdominal fat and viscera linked to the immune system (Fabricius bursa and spleen) were also weighed to obtain the relative weights, calculated in relation to the carcass weight.

Total coliform count

To evaluate the total coliform count of the ileal microbiota, at the slaughtering stage during evisceration, the ileum of two broilers from each experimental unit was removed, totaling 12 birds per treatment, with a section of Meckel’s diverticulum to ileoceccolic junction placed in identified plastic bags, packed in ice and sent immediately to the laboratory; the analyzes were carried out following the methods of Barroso et al. (2013).

Biochemical and hematological profile

To determine the serum biochemical profile and hematological parameters, blood was collected from all slaughtered animals in tubes with and without anticoagulant (EDTA) at slaughter during bleeding. Laboratory procedures were performed as described by Cardoso et al. (2012). Total plasma proteins (g / dL), hemoglobin concentration (g / dL), hematocrit (%), red blood cell count (x106 μ / L), total and differential leukocyte counts (x103 μ / L), mean globular volume (MVM) (f / L) and mean globular hemoglobin concentration (CHGM) (g / dL). Aspartate aminotransferase (AST) (IU / L), alanine aminotransferase (ALT) (IU / L), gamma glutamyltransferase (GGT) (IU / L), alkaline phosphatase (ALP) (IU / L), creatinine (mg / dL), urea (mg / dL) and uric acid (mg / dL).

Digestibility assay

At 22 days of age, in the growing period, the digestibility test was started by the traditional method of total collection. Total fecal samples were taken from each experimental unit twice a day for five days. The feces were stored in identified plastic bags and stored in a freezer until the end of the collection period. Samples of feces and experimental rations were sent to the bromatology laboratory for determination of dry matter, crude energy and nitrogen according to the techniques described by AOAC (Association ..., 1990). The metabolizable coefficients of dry matter (%) and nitrogen (%) and apparent metabolizable energy (kcal / kg) were using the equations proposed by Matterson et al. (1965).

Statistical analysis

Data were analyzed with an analysis of variance using the SISVAR (Ferreira, 2002) version 5.1 statistical program, and the means, when they had a verified significant effect by the F test, were evaluated by a Student Newman-Keuls (SNK) test with significance of 5%.

RESULTS AND DISCUSSION

Significant effects were observed among the treatments evaluated for weight gain in the initial phase (p=0.003), during which the birds fed feed without additives and containing piperine presented a higher weight result than that of the birds that consumed feed with avilamycin (Table 2). As for the growing period (22 to 33 days), birds fed a diet supplemented with piperine presented a higher weight gain and better feed conversion than those that received CWSc + piperine in the diet, but the broilers that consumed the ration containing CWSc alone showed similar results to those obtained by broilers fed avilamycin.
In the final phase (34 to 40 days of age), effects were observed only in the weight gain \((p=0.001)\) and feed conversion \((p=0.001)\) parameters, and the best feed conversion results were obtained for the avilamycin and CWSc + piperine treatments. When the whole breeding period was studied (9 to 42 days of age), the additives influenced the weight gain \((p=0.010)\) and feed conversion \((p=0.006)\), with a greater amount of weight gain in the chickens that consumed the ration with piperine. The viability was not influenced by the additives tested in the phases as well as in the experimental period.

In general, the addition of piperine and the cell wall of Saccharomyces cerevisiae resulted in improvements in zootechnical parameters, with the treatment with piperine alone having a better result than the treatments containing avilamycin, results that resemble what was found by Cardoso et al. (2009). This improvement in performance caused by the use of the alternative additives may be due to the influence of these compounds on the chickens’ organism, with the CWSc being rich in protein, minerals and vitamins of the B complex (Hassanein & Soliman, 2010) and piperine acting in the digestive processes (Srinivasan, 2007), stimulating the secretion of pancreatic enzymes such as lipases, amylases and proteases, thus affecting digestion processes and leading to improved weight gain (Shahverdi et al., 2013).

Regarding the carcass traits, treatments did not influence carcass and cut yields (Table 3), showing effects on the relative weight of gizzards \((p=0.032)\) and bursa of Fabricius \((p=0.038)\). No significant effects on the carcasses of broilers fed with phytogenics or prebiotics were reported by Cardoso et al. (2012) and Zhang et al. (2017). Changes in the size of the bursa of Fabricius can mean an increase or suppression of the activity of this organ and may be related to a stimulus promoted by the evaluated substance or by an infectious or inflammatory process.

In the initial phase (days 9 to 21), variables including feed intake, weight gain, feed conversion ratio, and viability were observed. The results are presented in Table 2, showing the effect of different zootechnical additives on these parameters. The table indicates significant differences \((p<0.05)\) in weight gain and feed conversion, with treatments containing CWSc + piperine showing better results.

### Table 2 – Feed intake, weight gain, feed conversion ratio and viability of broilers fed diets containing different zootechnical additives.

| Variables | CR   | AV  | CWSc | PIP  | CWSc + PIP | Probability |
|-----------|------|-----|------|------|------------|-------------|
| FI (g)    | 1169 ± 30.3 | 1133 ± 32.8 | 1128 ± 30.8 | 1153 ± 29.4 | 1160 ± 37.2 | 0.200 |
| WG (g)    | 696 ± 20.91a | 661 ± 18.3b | 671 ± 26.2ab | 695 ± 30.3a | 678 ± 28.4ab | 0.003 |
| FCR       | 1.68 ± 0.03 | 1.72 ± 0.05 | 1.68 ± 0.04 | 1.66 ± 0.03 | 1.71 ± 0.03 | 0.124 |
| Viability,% | 100  | 100 | 100 | 100 | 98.33 | 0.210 |
| Growth phase (days 22 to 33) |      |     |      |      |      |             |
| FI (g)    | 1862 ± 41.1a | 1820 ± 40.5ab | 1797 ± 41.1b | 1882 ± 43.2a | 1825 ± 42.6ab | 0.002 |
| WG (g)    | 1163 ± 30.1b | 1131 ± 32.1bc | 1134 ± 28.6bc | 1206 ± 35.2a | 1097 ± 32.2c | 0.001 |
| FCR       | 1.60 ± 0.05ab | 1.61 ± 0.04ab | 1.59 ± 0.05ab | 1.56 ± 0.04a | 1.66 ± 0.04b | 0.023 |
| Viability,% | 98.33 | 96.67 | 95  | 96.67 | 98.33 | 0.281 |
| Final phase (days 34 to 40) |      |     |      |      |      |             |
| FI (g)    | 1275 ± 40.2 | 1256 ± 42.3a | 1272 ± 40.1a | 1273 ± 41.8a | 1226 ± 38.4a | 0.109 |
| WG (g)    | 559 ± 18.3d | 661 ± 22.1a | 591 ± 19.5c | 604 ± 19.8bc | 616 ± 21.6b | 0.001 |
| FCR       | 2.28 ± 0.08c | 1.90 ± 0.09a | 2.15 ± 0.08b | 2.11 ± 0.08b | 1.99 ± 0.09a | 0.001 |
| Viability,% | 95  | 98.33 | 95  | 98.33 | 98.33 | 0.123 |
| Days 09 to 40 |      |     |      |      |      |             |
| FI (g)    | 4307 ± 98.2a | 4209 ± 112a | 4197 ± 97.3a | 4308 ± 108a | 4212 ± 98.2a | 0.163 |
| WG (g)    | 2418 ± 33.4bc | 2453 ± 44.8b | 2397 ± 50.4c | 2505 ± 48.1a | 2391 ± 37.8c | 0.001 |
| FCR       | 1.78 ± 0.03b | 1.72 ± 0.04a | 1.75 ± 0.04ab | 1.72 ± 0.05a | 1.76 ± 0.04ab | 0.006 |
| Viability,% | 95  | 95  | 95  | 95  | 93.33 | 6.26  |

\(^{1}\) CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell Wall of Saccharomyces cerevisiae + Piperine.

\(^{2}\) Means with different letters in the same row differ statistically \((p<0.05)\), SNK test.
bacterial growth is partially explained by the direct inhibition of bacteria or favoring the stabilization of the microbiota. As regards the effect of CWSc, it is necessary to remember that it is rich in MOS (Jacob & Pescatore, 2014), and MOSs may exert antimicrobial effects by reducing the binding of gram-negative bacteria, such as coliforms, to the intestinal mucosa, intervening (Chacher et al., 2017), that CWSc is capable of positively affecting the composition of ileal and cecal microbiota, significantly reducing the coliform population (Ozduven et al., 2009).

The results found for the chickens that consumed the control diet highlighted the importance of including additives in the feed that are able to control the proliferation of potentially dangerous microorganisms to the health of the birds.

In the analysis of the hepatic and renal serum biochemical profile, the additives did not influence the uric acid concentration alone ($p = 0.221$) (Table 5). The concentration of the enzymes AST and ALP was higher in poultry that consumed rations with avilamycin, suggesting that the antimicrobial may have caused some hepatic alteration. ALT was also higher in broilers fed avilamycin feed, but similar to broiler chickens fed piperine.

### Table 3 – Carcass traits of broilers at 42 days of age fed diets containing different zootechnical additives.

| Variables | CR | AV | CWSc | PIP | CWSc + PIP | Probability |
|-----------|----|----|------|-----|------------|-------------|
| Yield (%) |    |    |      |     |            | 0.312       |
| Carcass   | 69.50 ± 2.47 | 69.14 ± 2.10 | 69.77 ± 2.32 | 69.37 ± 2.21 | 69.17 ± 2.89 | 0.125       |
| Thigh     | 16.07 ± 0.47 | 15.93 ± 0.87 | 15.48 ± 0.54 | 16.09 ± 0.69 | 16.25 ± 0.52 | 0.432       |
| Thigh and Drumstick | 15.78 ± 0.71 | 15.99 ± 0.79 | 15.95 ± 0.67 | 16.25 ± 0.96 | 15.90 ± 0.65 | 0.165       |
| Wing      | 10.87 ± 0.67 | 11.47 ± 0.47 | 10.89 ± 0.66 | 11.46 ± 0.41 | 11.07 ± 0.70 | 0.101       |
| Breast    | 38.72 ± 1.56 | 37.93 ± 1.73 | 38.99 ± 1.26 | 37.75 ± 1.72 | 32.23 ± 1.74 | 0.375       |
| Back      | 18.27 ± 0.74 | 18.37 ± 1.01 | 17.76 ± 0.44 | 18.05 ± 1.08 | 17.79 ± 0.43 | 0.164       |
| Abdominal fat | 2.28 ± 0.48 | 2.31 ± 0.61 | 2.55 ± 0.51 | 2.26 ± 0.42 | 2.32 ± 0.48 | 0.243       |
| Relative Weight (%) |    |    |      |     |            | 0.032       |
| Liver     | 2.39 ± 0.23 | 2.54 ± 0.19 | 2.40 ± 0.29 | 2.31 ± 0.22 | 2.39 ± 0.27 | 0.127       |
| Gizzard   | 1.68 ± 0.13b | 1.79 ± 0.14ab | 1.68 ± 0.12b | 1.76 ± 0.12ab | 1.89 ± 0.15a | 0.001       |
| Heart     | 0.66 ± 0.09 | 0.69 ± 0.07 | 0.66 ± 0.08 | 0.64 ± 0.07 | 0.65 ± 0.08 | 0.028       |
| Bursa     | 0.11 ± 0.02ab | 0.12 ± 0.01a | 0.09 ± 0.02b | 0.11 ± 0.02ab | 0.10 ± 0.02ab | 0.038       |
| Spleen    | 0.17 ± 0.02 | 0.18 ± 0.02 | 0.17 ± 0.34 | 0.19 ± 0.04 | 0.21 ± 0.04 | 0.013       |

1CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell Wall of Saccharomyces cerevisiae + Piperine.

### Table 4 – Total coliform counts (log ufc / g ileal content) of broilers fed diets containing different zootechnical additives at 42 days.

| Variables | CR | AV | CWSc | PIP | CWSc + PIP | Probability |
|-----------|----|----|------|-----|------------|-------------|
| Total coliforms | 7.02 ± 1.90a | 6.49 ± 1.81a | 6.22 ± 1.72a | 5.19 ± 1.53ab | 4.45 ± 1.50 b | 0.001       |

1CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell Wall of Saccharomyces cerevisiae + Piperine.

### Table 5 – Biochemical profile of broiler chickens fed rations containing different zootechnical additives in the diet.

| Variables        | CR | AV | CWSc | PIP | CWSc + PIP | Probability |
|------------------|----|----|------|-----|------------|-------------|
| AST (UI / L)     | 368.6 ± 53.69b | 522.7 ± 149.6a | 313.8 ± 44.79b | 327.7 ± 63.59b | 346.3 ± 89.83b | 0.001       |
| ALP (UI / L)     | 1773 ± 362.2b | 2479 ± 272.2a | 1750 ± 312.7b | 1887 ± 523.8b | 1815 ± 443.5b | 0.001       |
| ALT (UI / L)     | 28.81 ± 5.89b | 36.28 ± 4.84a | 30.09 ± 3.70b | 32.59 ± 8.03ab | 29.96 ± 3.75b | 0.013       |
| GGT (UI / L)    | 6.31 ± 1.60c | 13.16 ± 2.43a | 7.73 ± 1.17c | 7.46 ± 1.50c | 11.23 ± 3.60b | 0.001       |
| Uric acid (mg / dL) | 16.18 ± 2.30 | 18.33 ± 0.99 | 15.14 ± 3.25 | 15.49 ± 3.70 | 15.39 ± 3.86 | 0.025       |
| Plasma creatinine (mg / dL) | 0.57 ± 0.01b | 0.73 ± 0.09a | 0.45 ± 0.09c | 0.55 ± 0.01b | 0.67 ± 0.10a | 0.001       |

1CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell Wall of Saccharomyces cerevisiae + Piperine; 2AST - aspartate aminotransferase; 3ALT- alanine aminotransferase; 4ALP - alkaline phosphatase; 5GGT-gamma glutamyl transferase.

CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell Wall of Saccharomyces cerevisiae + Piperine. 

a, b Means with different letters in the same row differ statistically ($p < 0.05$), SNK test.
ALT and AST are indicators of hepatic lesions or dysfunctions, and in pathological manifestations, these enzymes are released by the liver into the bloodstream (Toghyani et al., 2011); i.e., higher levels of this enzyme in the serum of birds fed with avilamycin indicates a negative effect on liver health. Absence of differences in the ALT concentration of chickens that consumed piperine alone compared to broilers fed with feeds containing avilamycin may indicate that the percentage of piperine added to the diet leads to liver problems. According to Kaneko (1989), hepatocyte lesions may be caused by changes in cell membrane permeability.

The concentration of GGT and urea present in the blood of birds consuming avilamycin and CWSc + piperine presented higher values than the birds fed the other treatments. Increased values for the above treatments may indicate cholestasis and bile duct hyperplasia (Tennant, 1997) or may also signal hepatic injury in hepatocytes, even with GGT not being liver specific (Schmidt et al., 2007) and with lesions that could only be confirmed by liver biopsies.

The concentration of blood urea may be influenced by liver activity, since the liver is the main organ of its synthesis (Dourado et al., 2017) and its concentration in non-carnivorous birds is 0 to 5 mg/dL (Schmidt et al., 2007); the broilers fed the ration containing avilamycin showed levels above the mentioned range, which may characterize a bird’s renal overload.

On the hematological profile, the broilers that consumed the avilamycin ration presented values for red blood cells, hematocrit, plasma proteins and MVM lower than the other treatments, which did not differ among them (Table 6). For hemoglobin and CHGM, birds consuming the ration with CWSc and piperine alone or associated had the highest values. Analyzing these results, it can be observed that there was a favoring of hematopoiesis, the effect of which was also reported by Toghyani et al. (2010), who studied the inclusion of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*) in rations for broiler chickens. This stimulus to hematopoiesis may be associated with the antioxidant effect of piperine, such as decreased lipid peroxidation and restoration of activities of antioxidant enzymes and GSH (Vijayakumar et al., 2004), considering that oxidative stress is potentially damaging to cells. Similar observation was reported by Arslan et al. (2005), who evaluated the protective effect of thymoquinone on ethanol-induced acute gastric damage rats, suggested that the antioxidant effect of the active components of thymus and thyroquinone found in the *Nigella sativa* plant were responsible for the stimulation of hematopoiesis.

**Table 6 – Hematological parameters of broilers that consumed rations containing different zootechnical additives.**

| Variables                  | CR | AV | CWSc | PIP | CWSc + PIP | Probability |
|----------------------------|----|----|------|-----|------------|-------------|
| Blood cells (x10^6 μ/L)    | 2.35 ± 0.16a | 2.22 ± 0.17b | 2.67 ± 0.20a | 2.68 ± 0.36a | 2.71 ± 0.21a | 0.021       |
| Hematocrit (%)             | 32.58 ± 1.83a | 24.67 ± 3.37b | 33.25 ± 1.87a | 33.17 ± 2.98a | 32.67 ± 1.82a | 0.001       |
| Plasma protein (g/DL)      | 4.60 ± 0.52a | 3.90 ± 0.22b | 4.48 ± 0.54a | 4.72 ± 0.94a | 4.25 ± 0.33a | 0.001       |
| Hemoglobin (g/DL)          | 7.62 ± 0.63b | 6.40 ± 0.75c | 8.81 ± 0.59a | 8.67 ± 0.38a | 8.25 ± 0.44a | 0.001       |
| MGHChC (g/DL)              | 29.62 ± 3.22ab | 27.07 ± 3.52b | 33.09 ± 2.77a | 32.98 ± 5.29a | 30.59 ± 2.52ab | 0.001       |
| MGV (f/L)                  | 126.5 ± 8.44a | 105.2 ± 21.48b | 125.3 ± 13.46a | 125.6 ± 18.81a | 121.1 ± 9.32a | 0.023       |
| Leukocytes (x10^3 μL)      | 30.58 ± 1.68b | 25.25 ± 1.87c | 32.42 ± 2.68b | 31.08 ± 1.73b | 35.67 ± 2.23a | 0.012       |
| Lymphocytes (x10^3 μL)     | 19.53 ± 1.23b | 14.09 ± 1.16c | 19.10 ± 1.45b | 18.89 ± 0.95b | 21.80 ± 1.28a | 0.001       |
| Heterophiles (x10^3 μL)    | 8.96 ± 1.25c | 9.45 ± 0.91bc | 10.97 ± 1.12a | 10.08 ± 0.89b | 11.63 ± 1.07a | 0.001       |
| Monocytes (x10^3 μl/L)     | 2.07 ± 0.27a | 1.60 ± 0.38b | 2.20 ± 0.44a | 2.05 ± 0.36a | 2.20 ± 0.58a | 0.032       |

^1 CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell wall of *Saccharomyces cerevisiae* + Piperine. ^2 MGHChC = mean globular hemoglobin concentration; ^3 MGV = mean globular volume.

The concentration of plasma proteins in birds that consumed avilamycin was lower than that found in birds of the other treatments. The values found in this research are higher than those found by Cardoso et al. (2009). These proteins make up 20% of the blood and help maintain osmotic pressure, regulate the acid-base mechanism of the blood, and provide immunoglobulins (Dourado et al., 2017), with an increased concentration in the bloodstream. The birds that consumed the ration with CWSc + piperine showed total and differential leucocytometry increase. An elevation of lymphocytes and leukocytes may be associated with infections, traumas, intoxications, and hemorrhages (Schmidt et al., 2007). However, as the performance of birds in this treatment were satisfactory and had the lowest total coliform count, the increase in defense cells should not be related to an infectious or inflammatory reaction. With
this reduction in total coliform counts, the intestinal environment may have favored the development of beneficial intestinal microbiota (Lactobacilli and Bifidobacteria), which, with its antigenic load, induced nonspecific stimulation of the immune system (Filho & Silva, 2005).

The additives had no effect on metabolizable coefficients and metabolizable energy (Table 7). No significant effects on nutrient digestibility were reported by Barroso et al. (2013) when studying the effects of different yeast cell wall supplements added to broiler diets. Through this digestibility assay, it was possible to show that the alternative additives had no influence on the metabolizable energy values, even though these treatments had effects on the performance parameters, microbiota and blood parameters, suggesting the importance of further studies on the use of prebiotics and phytogenics in the digestibility of nutrients and the interaction of these factors in the gene expression of the animal.

### Table 7 – Apparent metabolizable coefficient and apparent metabolizable energy values of feed for broilers fed different zootechnical additives (values expressed as% DM).

| Variables       | CR (kcal/kg) | AV (kcal/kg) | CWSc (kcal/kg) | PIP (kcal/kg) | CWSc + PIP (kcal/kg) | Probability |
|-----------------|--------------|--------------|----------------|---------------|----------------------|-------------|
| CMM3 (%)        | 70.36 ± 3.21 | 70.45 ± 3.42 | 70.37 ± 3.18   | 70.38 ± 3.29  | 70.39 ± 3.30         | 0.395       |
| CNM5 (%)        | 60.23 ± 4.56 | 60.34 ± 5.06 | 60.25 ± 4.67   | 60.26 ± 5.18  | 60.27 ± 5.29         | 0.210       |
| AME (%)         | 3.40 ± 0.99  | 3.41 ± 1.00  | 3.39 ± 0.98    | 3.38 ± 0.97   | 3.37 ± 0.96          | 0.010       |
| AMEn (%)        | 3.53 ± 0.89  | 3.54 ± 0.90  | 3.52 ± 0.89    | 3.51 ± 0.88   | 3.50 ± 0.87          | 0.001       |

* *CR = Control Ration; AV = CR + Avilamycin; CWSc = CR + Cell wall of Saccharomyces cerevisiae; PIP = CR + Piperine; CWSc + PIP = CR + Cell wall of Saccharomyces cerevisiae + Piperine. *Coefficient of metabolism of dry matter. 3Coefficient of nitrogen metabolism. 4Apparent metabolizable energy; 5Apparent metabolizable energy corrected by nitrogen balance.

**CONCLUSION**

The cell wall of Saccharomyces cerevisiae and piperine are effective at guaranteeing productivity; acting positively on intestinal microbiota dynamics and hematological parameters; and as zootechnical additives, especially in broiler feeds free of antimicrobial performance enhancers.

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