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Original Article

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

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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 synergistic 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), phytogenics and prebiotics used 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 & Laksmi, 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 phytogenic 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 - UFRRJ, 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 (%)	Phases		
	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
Calcitic 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 [1]	0.10	0.10	0.10
Mineral mixture [2]	0.05	0.05	0.05
Choline chloride	0.05	0.04	0.04
Butylated hydroxytoluene	0.01	0.01	0.01
Kaolin	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
Sodium (%)	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; niancine 35 g / kg; Calcium pantothenate (min) 10 g / kg; biotin (min) 67 mg / kg; [2]Iron (min) 60 g / kg; copper (min) 13 g / kg; manganese (min) 120 g / kg; zinc (min) 100 g / kg; iodine (min) 2500 mg / kg; selenium (min) 500 mg / kg.

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 ileocecocolic 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 avilamicyn.

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
Initial phase (days 9 to 21)						
	Treatments [1]					
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	100	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.

[a, b] Means with different letters in the same row differ statistically (p<0.05), SNK test.

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 Fabrícius (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 Fabrícius 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.

Regarding the total coliform counts, there was influence in the addition of the additives (p=0.001), the birds that consumed the CWSc + piperine ration showed the lowest count of these microorganisms in the ileal content when compared to the content counts of the birds that consumed the ration containing avilamycin and CWSc and the control ration (Table 4) .

Based on the results it is possible to propose that the possible antimicrobial mechanisms of piperine and CWSc found in the literature, have acted synergistically or were potentiated. Regarding piperine, Mitsch *et al.* (2004), reports that the possible mechanism by which the component may lead to a decrease in pathogenic

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

	Treatments [1]					
Variables	CR	AV	CWSc	PIP	CWSc + PIP	Probability
Yield (%)						
Carcass	69.50 ± 2.47	69.14 ± 2.10	69.77 ± 2.32	69.37 ± 2.21	69.17 ± 2.89	0.312
Thigh	16.07 ± 0.47	15.93 ± 0.87	15.48 ± 0.54	16.09 ± 0.69	16.25 ± 0.52	0.125
Thigh and Drumstick	15.78 ± 0,71	15.99 ± 0,79	15.95 ± 67	16.25 ± 0,96	15.90 ± 0,65	0.432
Wing	10.87 ± 0,67	11.47 ± 0,47	10.89 ± 0,66	11.46 ± 0,41	11.07 ± 0,70	0.165
Breast	38.72 ± 1.56	37.93 ± 1.73	38.99 ± 1.26	37.75 ± 1.72	32.23 ± 1.74	0.101
Back	18.27 ± 0.74	18.37 ± 1.01	17.76± 0.44	18.05 ± 1.08	17.79 ± 0.43	0.218
Abdominal fat	2.28 ± 0.48	2.31 ± 0.61	2.55 ± 0.51	2.26 ± 0.42	2.32 ± 0.48	0.375
Relative Weight (%)						
Liver	2.39± 0.23	2.54± 0.19	2.40 ± 0.29	2.31 ± 0.22	2.39± 0.27	0.243
Gizzard	1.68± 0.13b	1.79± 0.14ab	1.68± 0.12b	1.76± 0.12ab	1.89± 0.15 a	0.032
Heart	0.66 ± 0.09	0.69 ± 0.07	0.66 ± 0.08	0.64 ± 0.07	0.65 ± 0.08	0.127
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	0.164

[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.

[a, b] Means with different letters in the same row differ statistically (p<0.05), SNK test.

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

	Treatments[1]					
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

[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.

[a, b] 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 in (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 5 – Biochemical profile of broiler chickens fed rations containing different zootechnical additives in the diet.

	Treatments[1]					
Variables	CR	AV	CWSc	PIP	CWSc + PIP	Probability
AST[2] (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[4] (UI/ L)	1773 ± 362.2b	2479 ± 272.2a	1750 ± 312.7b	1887 ± 523.8b	1815 ± 443.5b	0.001
ALT[3](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[5] (UI /L)	6.31 ± 1.60c	13,16 ± 2.43a	7.73 ± 1.17c	7.46 ± 1.50c	11.23 ± 3.06b	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	0.221
Urea (mg / dL)	0.57 ± 0.14b	0.73 ± 0.09a	0.45 ± 0.09c	0.55 ± 0.12b	0.67 ± 0.10a	0.025
Creatinine (mg dL)	4.58 ± 1.08b	6.42 ± 0.90a	5.50 ± 1.38b	4.33 ± 0.78b	5.00 ± 1.21b	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; [2]AST - aspartate aminotransferase; [3]ALT- alanine aminotransferase; [4]ALP - alkaline phosphatase; [5]GGT-gamma glutamyl transferase.

[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 (Schmidit *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 d / L (Schmidit *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.

	Treatments[1]					
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)	4.50 ± 0.52a	3.50 ± 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
MGHC[2] (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[3] (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 µ / L)	30.58 ± 1.68b	25.25 ± 1.87c	32.42 ± 2.68b	31.08 ± 1.73b	35.67 ± 2.23 a	0.012
Lymphocytes (x10^{3}µ / L)	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 µ / L)	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]MGHC - mean globular hemoglobin concentration; [3]MGV - mean globular volume.

[a, b] Means with different letters in the same row differ statistically (p<0.05), SNK test.

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 leucometry increase. An elevation of lymphocytes and leukocytes may be associated with infections, traumas, intoxications, and hemorrhages (Schimidt *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).

	Treatments[1]					
Variables	CR	AV	CWSc	PIP	CWSc + PIP	Probability
CMDM[2] (%)	70,27 ± 2.90	70,76 ± 2.63	71,07 ± 3.02	69,11 ± 2.76	72,36 ± 3.12	0.343
CNM[3] (%)	60,52 ± 4.89	61,45 ± 5.01	60,53 ± 4.70	60,55 ± 4.81	61,22 ± 4.90	0.540
AME[4] (kcal/kg)	3.370 ± 104	3.415 ± 121	3.392 ± 114	3.318 ± 128	3.432 ± 110	0.210
AMEn[5] (kcal/kg)	3.352 ± 89	3.395 ± 97	3.374 ± 108	3.298 ± 92	3.414 ± 112	0.312

[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]Coefficient of metabolism of dry matter. [3]Coefficient of nitrogen metabolism. [4]Apparent metabolizable energy; [5]Apparent metabolizable energy corrected by nitrogen balance.

[a, b] Means with different letters in the same row differ statistically (p<0.05), SNK test.

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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