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Addition of a blend of exogenous enzymes to broiler chickens diets: impacts on performance and production costs

Adição de uma mistura de enzimas exógenas a dietas de frangos de corte: impactos no desempenho e custos de produção

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ABSTRACT

Broiler diets are based on corn, soybean and wheat production; however, some protein ingredients have many antinutritional factors and low digestibility. The objective of this study was to add a blend of exogenous enzymes to the feed with low nutritional value for broilers to reduce production costs and improve digestibility while maintaining good zootechnical performance. The experimental design was completely randomized, including three treatments with five replications (n=15) each: a) positive control (PC), diet calculated for males with average performance; b) negative control + exogenous enzymes (NC+EE), minimum nutrient diet according to the requirements of the production phase, and the enzymatic blend was added; and c) negative control (NC), minimum levels of nutrients for each phase. At 21 days, the PC group showed greater weight gain and lower feed conversion than the NC ($P<0.05$). At 42 days, PC had lower feed intake than NC ($P=0.040$), while lower feed conversion was observed in groups PC and NC+EE than NC ($P=0.001$). The production efficiency index was higher in the PC treatment, but the NC+EE treatment was higher than the NC ($P=0.001$). Considering production costs and body weight, we found that NC+EE birds had greater profitability. Therefore, we conclude that the blend of exogenous enzymes added to a diet with minimal nutritional levels has practical application in the broiler production system.

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Keywords: Carbohydrases, non-starch polysaccharides, proteases.

RESUMO

As dietas de frangos de corte são baseadas na produção de milho, soja e trigo; no entanto, alguns ingredientes proteicos possuem muitos fatores antinutricionais e baixa digestibilidade. O objetivo deste estudo foi adicionar à ração uma mistura de enzimas exógenas com baixo valor nutricional para frangos de corte para reduzir os custos de produção e melhorar a digestibilidade, mantendo o bom desempenho zootécnico. O delineamento experimental foi inteiramente casualizado, incluindo três tratamentos com cinco repetições (n=15) cada: a) controle positivo (PC), dieta calculada para machos com desempenho médio; b) controle negativo + enzimas exógenas (CN+EE), dieta mínima de nutrientes de acordo com as exigências da fase de produção, e foi adicionada a mistura enzimática; c) controle negativo (CN), teores mínimos de nutrientes para cada fase. Aos 21 dias, o grupo PC apresentou maior ganho de peso e menor conversão alimentar que o NC (P<0,05). Aos 42 dias, PC apresentou menor consumo de ração que NC (P=0,040), enquanto foi observada menor conversão alimentar nos grupos PC e NC+EE que NC (P=0,001). O índice de eficiência de produção foi maior no tratamento PC, mas o tratamento NC+EE foi maior que o NC (P=0,001). Considerando os custos de produção e peso corporal, verificamos que as aves NC+EE tiveram maior rentabilidade. Portanto, concluímos que a mistura de enzimas exógenas adicionada a uma dieta com níveis nutricionais mínimos tem aplicação prática no sistema de produção de frangos de corte.
Palavras-chave: Carboidrases, polissacarídeos não amiláceos, proteases.

INTRODUCTION

Brazilian broiler diets are mainly based on corn and soybeans, which are ingredients that have some antinutritional factors that lead to low digestibility (REDA et al., 2020; İNCILI, et al., 2020; ALFAIA et al., 2021). In addition, corn and soybeans vary in price due to production and market factors (FARAHAT et al., 2021., YEGANI & KORVER, 2013). Among the antinutritional factors associated with these ingredients, we highlight the presence of insoluble and soluble non-starch polysaccharides (NSP); corn contains 6.83% of total NSP, while soybean meal with 45% of protein contains 16.46% of total NSP (ROSTAGNO et al., 2017). NSPs occur in the form of pectins, hemicelluloses, xylanases, and mannoses, which for birds are not digestible. They also increase intestinal viscosity, increasing food passage time and mucosal renewal rate; this culminates in endogenous excretion of amino acids (SINGH et al., 2019). To minimize these problems, additives such as prebiotics, probiotics, antibiotics, and exogenous enzymes are administrated to broiler diets, aiming to improve digestibility, contributing to improve animal performance and the economy of the rearing system (FROEBEL et al., 2019).
Regarding enzymes, xylanases and proteases improve the zootechnical performance of broiler chickens since they enhance weight gain by increasing the availability of absorbable substrates, complementing the secretion of amylase that helps degrade starch and other enzymes that are present in digestive processes (LLAMAS et al., 2021). The

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primary function of α-galactosidase and β-glucanase is to break beta 1,4-bonds, thereby improving the digestibility and absorption of nutrients, consequently reducing production costs (Karunaratne et al., 2021). Therefore, this study aimed to determine whether a blend of exogenous enzymes (alpha-amylase, alpha-galactosidase, beta-glucanase, cellulase, mannanase, pectinase, protease, and xylanase) in a feed formulated to meet the minimum nutritional requirements can maintain zootechnical performance, reduce production costs, and maintain meat metabolism and quality.

MATERIALS AND METHODS

2.1 Enzyme product

The product (FRA® Octazyme C Dry) was purchased from Feedis (São Paulo, Brazil). This product is marketed as a zootechnical additive formulated with a mixture of exogenous enzymes with a minimum activity of 160,000 BXU/g of xylanase, 1,000 IU/g of mannanase, 10,000 IU/g of α-amylase, 20,000 BU/g 1.3(4)-β-glucanase, 1,500 U/g of protease, 3,200 IU/g of cellulase, 2100 IU/g pectinase and 80 GALU/g α-galactosidase. The commercial indication of this product is to improve feed digestibility and nutrient absorption by the animal. The recommended concentration of the product is 50 mg of blend/kg of feed, which provides 8,000 BXU of xylanase, 50 IU mannanase, 500 IU amylase, 1,000 BU glucanase, 75 U protease, 160 IU cellulase, 105 IU pectinase, and 4 GALU galactosidase.

2.2 Animals and experimental design

Was purchased 225 one-day-old male Cobb500 chicks from a commercial company in Chapecó, Brazil. The animals were housed in pens (1.2 x 2 m), with fresh wood (first batch) in order to use as a poultry litter. During the entire production cycle (42 days) feed and water was given ad libitum. Heat was provided using an electric lamp/box and gas hoods in the central region of the shed, aiming to maintain an environmental temperature of 28 to 32 ºC in the first 21 days of the experimente; after this period the temperature of the environment was natural (variation between 11ºC at night and 30ºC during the day). Light management followed the recommendations of the lineage manual (COBB, 2012). Feed was provided in tubular feeders for most of the production cycle, except for the first seven days of life when infant feeders were left on the ground on brown paper. Drinking water was provided using nipple-type drinkers, weekly regulated according to the age of the birds.

The experiment lasted 42 days. Two distinct diets were formulated; both were formulated based on corn and soy, according to the recommendations of the Brazilian Table of Poultry and Swine (ROSTAGNO et al., 2017) for medium performance chickens (Ration A) and minimum performance (Ration B). Three formulations were generated considering the production cycle; starter phase (1 to 21 days), growth phase (22 to 35 days), and finisher phase (36 to 42 days). The conventional growth enhancer used in Feeds A and B was enramycin 0.10 g/kg of feed and diclazuril 0.3 g/kg of feed in the starter and grower phases (Table 1).

The experimental design was completely randomized, with three treatments of five replications each and 15 chickens by replication: 1) positive control (PC), calculated diet (Ration A) based on the 2017 Brazilian poultry table for males with average performance (Rostagno et al.,

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2017); 2) negative control + exogenous enzymes (NC+EE): birds that received a minimum nutrient diet (Ration B) according to the minimun requirements of the production phase and the enzymatic blend was added (50 mg/kg); and 3) negative control (NC): birds that received the diet in order to meet the minimum levels of nutrients for each phase (Ration B).

Table 1. Feed and nutritional composition of the experimental diets were identified as "Ration A" (for medium performance of chickens) and "Ration B" (for minimum performance of chickens).

Ingredient	Starter Ration A	Starter Ration B	Grower Ration A	Grower Ration B	Finisher Ration A	Finisher Ration B
Corn, g/kg	479.57	505.46	509.73	535.91	614.49	640.68
Soy flour (46%), g/kg	433.08	428.35	391.84	387.05	304.46	299.68
Soybean oil. g/kg	44.95	24.47	61.35	40.77	49.18	28.61
Dicalcium phosphate, g/kg	18.94	18.87	14.53	14.46	10.92	1.85
Limestone, g/kg	8.32	8.38	8.36	8.42	7.14	7.21
Salt, g/kg	4.60	4.60	4.25	4.24	4.07	4.07
Premix vit/min	4.00	4.00	4.00	4.00	4.00	4.00
DL-Methionine (99%), g/kg	3.34	2.98	3.16	2.80	2.54	2.19
L-Lysine HCl, g/kg	2.21	1.81	1.81	1.42	2.22	1.83
L-Threonine	0.82	0.58	0.66	0.42	0.64	0.39
L-Valine	0.17	-	0.31	-	0.31	-
Calculated values						
Metabolizable energy, kcal/kg	3050	2945	3200	3095	3250	3145
Crude protein, g/kg	243.00	242.34	226.20	225.54	195.40	194.74
Digestible lysine, g/kg	13.63	13.26	12.35	11.98	10.67	10.29
Digestible met. + cys., g/kg	9.68	9.36	9.14	8.83	7.90	7.58
Digestible threonine, g/kg	8.86	8.67	8.15	7.93	7.04	6.82
Digestible tryptophan, g/kg	2.80	2.79	2.58	2.56	2.11	2.10
Digestible valine, g/kg	10.06	9.89	9.51	9.21	8.22	7.92
Calcium, g/kg	9.42	9.42	8.22	8.22	6.61	6.61
Available phosphate, g/kg	4.71	4.71	3200	3.84	3.09	3.09
Sodium, g/kg	2.27	2.27	226.20	2.11	2.01	2.01

[1] Vitamin supplement containing per kg of product: Vit. A - 10,000,000 IU; Vit. D3 - 2,000,000 IU; Vit. E - 30,000 IU; Vit. B1 - 2.0 g; Vit. B2 - 6.0 g; Vit. B6 - 4.0g; Vit. B12 - 0.015g; Pantothenic Acid - 12.0 g; Biotin - 0.1 g; Vit. K3 - 3.0g; Folic Acid - 1.0 g; Nicotinic Acid - 50.0 g; Selenium - 250.0 mg; and Excipient q.s.p – 1000 g.
[2] Mineral supplement containing per kg of product: Iron - 100.0 g; Cobalt - 2.0 g; Copper - 20.0 g; Manganese - 160.0 g; Zinc - 100.0 g; Iodine - 2.0 g; and Excipient q.s.p – 1000 g .

2.3 Performance

On days 1, 21, 35, and 42, body weight was determined by using a digital scale. On days 21 (period 1 to 21), 35 (period 22 to 35), and 42 (period 36 to 42) the amount of feed intake (supplied – leftovers) was measured. Based on feed inatke and weight gain, was the feed conversion were calculated. Feed

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conversion was obtained through the feed consumed divided by the average weight of birds. All variables were corrected for dead broilers during the experimental period. At the end of the production cycle, the productive efficiency index (PEI) was calculated, according to the formula described below (STRINGHINI et al. 2006): PEI= (Body weight × Viability)/(Age at slaughter × Feed conversion).

2.4 Collection of blood and muscle samples

On day 42, blood from the ulnar vein was collected from eight birds of each treatment group using insulin syringes. Blood was placed in microtubes for subsequent centrifugation at 3,500 rpm for 10 minutes to obtain serum for biochemical analysis. Then, the blood was frozen at - 20 °C.

One chicken per repetition (five per treatment) was sacrificed by cervical dislocation and the pectoral muscle was removed. The muscle was placed in the refrigerator (8 °C) for five hours until the physicochemical composition of the meat was analyzed. Then, the abdominal cavity was opened, a fragment of the intestine (jejunum) was removed, and the sample was preserved in 10% formalin for histopathological analysis.

2.5 Histopathology

The samples were processed, and slides with histological sections were generated and stained with hematoxylin and eosin. The height of the villi and the depth of the crypts were determined according to Caruso & Demonte (2005), a methodology described in detail by Galli et al. (2020). This information allowed us to calculate the villi/crypt ratio.

2.6 Serum biochemistry

The biochemical variables analyzed were total protein, albumin, triglycerides, cholesterol, uric acid, glucose, alanine aminotransferase, and aspartate aminotransferase, using commercial analytical kits (Analisa®) and a BioPlus semi-automatic biochemical analyzer (Bio-2000®). Globulin values were calculated as total protein – albumin.

2.7 Meat quality analysis

The pectoralis muscle was refrigerated for five hours after which the pH was measured using an electrode. In muscle fragments, luminosity (L*), red intensity (a*), and yellow intensity (b*) were measured using a Minolta Chome meter. Cooking water losses (CWL) and water holding capacity (WHC) were measured as described by Hamm (1960) and Honikel (1987), where: a) WHC was measured in a meat fragment held under pressure to check for water loss; b) the method for CWL consisted of using a fragment of meat and placing it in a heater for cooking. The samples used to determine the CWL were used to measure the shear force (SF), using cuts with muscle fibers with orientation perpendicular to the instrument blade (Texture Analyzer TA -XT2i), coupled to a device (Warner-Bratzler) that provided the force required to cut the sample. SF was expressed as kgf/cm.

2.8. *Cost-effectiveness*

All ingredients used to produce the feeds were purchased from agroindustries in Chapecó, Brazil; in this way, was obtained the commercial cost of each ingredient per kilogram to calculate the cost of production of each of the feeds produced ("A" and "B") used for groups PC, NC+EE, and NC (Table 7). The experiment allowed us to obtain data on body weight and feed intake for each of the treatments and to calculate the feed production costs of each treatment (PC, NC+EE, and NC) and the production costs of the 75 chickens of each treatment in this experiment (five

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replicates, with 15 replicates per experiment).

2.8 Statistical analysis

Data was subjected to normality testing (Shapiro–Wilk). Data that was not normally distributed was transformed to logarithms. Was applied the one-way analysis of variance test for normally distributed data and, to compare between groups, was used the Tukey test using Assistat software. The results were expressed as mean and standard deviation, and a significant difference was considered when P-value ≤ 0.05.

RESULTS

3.1 Performance

The performance results are shown in Table 2. At 21 days, the PC group showed greater weight gain and lower feed conversion than the NC ($P < 0.05$). On day 35 of the experiment, there was no difference between groups for performance ($P > 0.05$). At 42 days, PC had lower feed intake than NC ($P = 0.040$), and a lower feed conversion was found in PC and NC+EE than NC ($P = 0.001$). On day 42, we observed no difference in body weight ($P > 0.05$). The production efficiency index results are shown in Table 3. The production efficiency index was higher in the PC treatment than the NC+EE, which was also higher than the NC treatment ($P = 0.001$).

Table 2. Performance (mean and standard deviation) of broilers of the 3 treatment groups.

Day 21			
Treatment	Weight gain (g)	Feed intake (g)	FC
PC: positive control	940 (35)[a]	1263 (52)	1.34 (0.01)[b]
NC + EE: negative control + exogenous enzyme	909 (35)[ab]	1248 (81)	1.37 (0.03)[ab]
NC: negative control	864 (49)[b]	1250 (107)	1.44 (0.11)[a]
P-value	**0.034**	0.796	**0.050**
Day 35			
Treatment	Weight gain (g)	Feed intake (g)	FC
CP: positive control	2186 (122)	3244 (156)	1.49 (0.17)
NC + EE: negative control + exogenous enzyme	2134 (180)	3244 (247)	1.53 (0.18)
NC: negative control	2115 (133)	3290 (105)	1.55 (0.15)
P-value	0.527	0.847	0.101
Day 42			
Treatment	Weight gain (g)	Feed intake (g)	FC
CP: positive control	2836 (104)	4495 (255)[b]	1.58 (0.04) [b]
NC + EE: negative control + exogenous enzyme	2819 (128)	4495 (507)[ab]	1.59 (0.03) [b]
NC: negative control	2783 (102)	4674 (257)[a]	1.68 (0.07) [a]
P-value	0.076	**0.040**	**0.001**

[a,b] Different letters in the same column indicate significant differences between groups using the Tukey test.

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Table 3. Production efficiency index values of broilers of the 3 treatment groups.

Treatment	Productive Efficiency Index (PEI)
PC: positive control	423.7^a
NC + EE: negative control + exogenous enzyme	402.2^b
NC: negative control	375.9^c
P-value	**0.001**

$^{a-c}$ Different letters in the same column indicate significant differences between groups using the Tukey test.

3.2 Histopathology

The histopathological analysis is shown in Table 4. Intestinal villi were higher in the NC group than in the other groups (P < 000.1). The crypts were deeper in the NC than the NC+EE group, and both were deeper than the PC (P < 0.001). The crypt-villus ratio was higher in the PC group, followed by NC+EE, and the smallest villus/crypt ratio was found in the intestine of birds in the NC group (P < 0.001).

Table 4. Villus, crypt, and villus/crypt ratio of the jejunum of broilers of the 3 treatment groups.

Treatment	Villus	Crypt	Villus/crypt
PC: positive control	1159 ± 96^b	186 ± 34^c	6.22 ± 0.43^a
NC + EE: negative control + exogenous enzyme	1310 ± 124^b	226 ± 31^b	5.79 ± 0.18^b
NC: negative control	1737 ± 214^a	330 ± 54^a	5.26 ± 0.27^c
P-value	**P < 000.1**	**P < 000.1**	**P < 000.1**

$^{a-c}$ Different letters in the same column indicate significant differences between groups using the Tukey test.

3.3 Serum biochemistry

Metabolism results are shown in Table 5. Total protein levels were higher in serum from the PC group than NC (P = 0.05). Globulin levels were higher in the PC group than in the NC (P = 0.026). Higher cholesterol levels were observed in the serum of birds from the NC+EE group than PC and NC (P < 0.001). Uric acid levels were higher in the NC+EE group than the others (P < 0.05). Albumin, triglycerides, and glucose levels did not differ between treatments (P > 0.05).

Table 5. Serum biochemistry of broilers of the 3 treatment groups.

Treatment	TP	ALB	GLO	TRI	CHO	GLU	UA
CP: positive control	4.30^a (0.22)	1.63 (0.21)	2.66^a (0.21)	58.0 (12.1)	119.8^b (24.8)	276 (28.7)	5.83^{ab} (0.74)
NC + EE: negative control + exogenous enzyme	4.11^{ab} (0.21)	1.80 (0.18)	2.31^{ab} (0.36)	59.0 (8.32)	167.5^a (14.6)	290 (20.9)	7.25^a (0.99)
NC: negative control	3.91^b (0.17)	1.78 (0.31)	2.13^b (0.23)	62.1 (10.9)	111.5^b (20.5)	269 (17.6)	5.15^b (0.46)
P-value	**0.050***	**0.367**	**0.026***	**0.825**	**< 0.001***	**0.742**	**0.012***

^{a,b} Different letters on the same line indicate significant differences between groups using the Tukey test.

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Note: TP – Total protein; ALB – Albumin; GLO – globulin; TRI – triglyceride; CHO – cholesterol; UA – uric acid; GLU – glucose; ALT – alanine aminotransferase; AST – aspartate aminotransferase.

3.4 Physicochemical composition in meat

The results are shown in Table 6. The intensity of red (color a) was higher in poultry meat from the NC group, followed by higher rates in the meat from the NC+EE group than the PC (P = 0.039). The water retention capacity was higher in the PC group than the NC (P = 0.045). The loss of water by cooking was lower in the PC group than in the NC (P = 0.001). The pH, yellow intensity (color b), and shear strength did not differ between treatments (P > 0.05).

Table 6. Physicochemical composition in the meat of broilers of the 3 treatment groups.

Treatment	Cor L	Cor a	Cor b	pH	WRC	WLC	SF
CP: positive control	52.8 (1.74)	-1.016[b] (0.08)	11.6 (1.45)	5.70 (0.10)	80.7[a] (4.35)	22.9[b] (3.82)	8016 (2525)
NC + EE: negative control + exogenous enzyme	54.1 (2.07)	0.094[ab] (0.05)	11.7 (0.85)	5.66 (0.18)	77.0[ab] (3.20)	25.7[ab] (1.96)	7336 (2472)
NC: negative control	54.2 (2.39)	0.548[a] (0.09)	10.5 (0.68)	5.66 (0.12)	74.2[b] (4.96)	28.4[a] (4.29)	7143 (1446)
P-value	**0.257**	**0.039***	**0.10**	**0.974**	**0.045***	**0.001**	**0.562**

[a,b] Different letters in the same column indicate significant differences between groups using the Tukey test.
Note: WRC - Water Retention Capacity (%), WLC - water loss by cooking (%), SF - Shear force (kgf/cm)

3.5 Cost-effective production

Table 7 displays the results of body weight and feed consumption during the 42 days of the experiment. The calculations showed that the diet made available to the PC chickens had a higher cost than the other treatments. In this study, the profitability of using the exogenous enzyme in a diet for the minimum performance of broilers was 8.59% higher than a diet for the average performance of broilers (Table 7).

Table 7. Profit margin return analysis for positive control treatments (PC - diet calculated based on the 2017 Brazilian table of poultry for males with medium performance), negative control + exogenous enzymes (NC+EE - birds that received a diet minimum nutrient according to the requirements of the production phase and the enzymatic blend was added) and negative control (NC - birds that received the diet in order to meet the minimum levels of nutrients for each phase).

Zootechnical Indices - 42-day cycle	PC	NC+EE	NC
Weight (kg/bird)	2.836	2.819	2.783
Consumption (kg/bird)	4.495	4.495	4.674
Average feed cost (R$/ton)	1.496.55	1.398.95	1.388.81
Cost per 75 birds (R$)	504.52	471.62	486.84
Billing for 75 birds (R$)	829.53	824.55	814.02
Profit margin (R$)	325.00	352.93	327.17

Note: The profitability of the NC+EE group was 8.59% higher than the PC group.

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DISCUSSION

The been looking for alternatives to reduce the cost of diets while reducing the antinutritional effects and increasing the amount of substrate at the intestinal level is research focus (WALK et al., 2019; SINGH et al., 2019). In this study, was introduced exogenous enzymes in a diet formulated to meet the minimum needs of broilers, and we found that these birds had a higher productivity index than control birds that received the same diet except for exogenous enzymes. This is because birds do not possess endogenous enzymes capable of digesting NSP, such as arabinoxylans, beta-glucans, lecithins, and pectins (MUSIGWA et al., 2021). In addition, the viscosity of the intestinal chyme reduces the rate of passage of feed, which interferes with endogenous enzymatic action and nutrient absorption (NGUYEN et al., 2021). Gallardo et al. (2020) reported that adding exogenous enzymes and fiber degrading additives improved the digestibility and nutritional value of feeds because it supported the degradation of carbohydrates and proteins, favoring the animal digestibility process.

In the present study, was observed that the birds that received the diet to obtain medium performance had good productivity in terms of body weight, with a tendency to be higher than the birds that consumed the diet with minimal nutrient requirements. This experiment did not include a major microbiological challenge as the bedding was not reused, thereby avoiding energy expenditure to mount an immune response. This condition allowed the birds to use the nutrients for muscle tissue deposition. The inclusion of the enzymatic blend, improved digestibility, and indirect effect and excellent on villous/crypt ratio resulted in a good productivity index and muscle growth. The results were promising and allow the realization of new research that simulates field conditions, i.e., sanitary challenge conditions where birds are kept on used bedding.

Non-starch polysaccharides reduce serum lipid particles; thus, when exogenous enzymes are used, this production increases lipid levels (GALLHER et al., 2000), explaining the increase in cholesterol concentration in the present study. In the 1990s, researchers reported a hypercholesteremic effect related to the presence of NSPs (RAZDAN et al., 1997). Was believe that the increase in cholesterol is a consequence of the production of micelles, the production of which is impaired in diets that contain high levels of NSP, especially soluble NSP, which create a gelatinous mass and hinder enzyme action. The uric acid concentration displayed behavior similar to cholesterol, which we believe is related to the action of exogenous enzymes, which may have favored the absorption of amino acids; nevertheless, we cannot rule out a predisposition to a metabolic disorder. It is important to remember that uric acid levels indicate the efficiency of the use of amino acids in broiler chickens (ZHAI et al., 2016), and its increase is related to increased protein catabolism (SAHEBI-ALA et al., 2021), in addition to being related to an increase in protein turnover and, with it, endogenous losses of nitrogen and ammonia.

In the present study, the consumption of a diet covering minimal requirements increased the height of the villi and crypt

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depth, a change that was minimized by supplementing the diet with a blend of exogenous enzymes. The consumption of feeds with high levels of NSP impairs intestinal morphology (KERMANSHAHI et al., 2018); these authors found that consumption of pectin (soluble polysaccharides) resulted in an increase in intestinal viscosity, which decreased digestibility by interfering with the process of digestion; however, this did not occur when they included cellulose (insoluble polysaccharides) in the chicken diet.

In the present study, there was an increase in villi in group NC birds, which is probably related to a compensatory effect on the organism of the birds that were receiving a diet with lower metabolizable energy and needed to absorb as many nutrients as possible; the greater crypt depth may also be related to the compensatory effect; however, was believe it is a consequence of the adverse effects of NSPs and lower humoral immune response in birds. In addition, birds in the NC group had lower serum total protein concentrations due to a lower concentration of globulins. Globulins are immunological proteins related to the acute response to invasion by pathogens and others of an antibody nature, such as immunoglobulins. The results suggest that the diet with minimum requirements (NC group) compromised the immune response of birds, which may be related to the reduction of immunoglobulins such as IgA that are important for protecting the intestinal mucosa. This mechanism may explain the greater depth of the crypt that usually occurs when the intestinal mucosa is damaged, accompanied by an increase in the rate of cell renewal.

The greater water holding capacity in the PC group than the NC means that there was an increase in the water content present in the muscle, which increases the juiciness of the meat (GUARAGNI et al., 2020), and this is also related to less protein denaturation (SÁNCHEZ-ZAPATA et al., 2010). Less water loss during cooking was also observed in the PC group than NC, which can be attributed to less moisture and fat loss during cooking, a fact related to the protein matrix's ability to retain water and prevent lipid migration. Increasing the capacity to retain water and reducing water loss by cooking is desirable for industry and consumers. They represent economic losses by causing changes in the composition (vitamins and amino acids) and nutritional value of the product (SÁNCHEZ-ZAPATA et al., 2010). Was found that the nutritional level of the diet directly affects the quality of the meat, and the blend of exogenous enzymes cannot reverse this problem, only minimize the degree of changes.

CONCLUSION

The addition of a blend of exogenous enzymes (xylanase, mannanase, amylase, glucanase, cellulase, pectinase, protease and galactosidase) in a broiler diet with minimum bird performance requirement minimized the adverse effects on productivity rates (which are consequences of a lower metabolic energy diet) and favored the absorption of carbohydrates and amino acids by chickens. The inclusion of the enzymatic blend also favored intestinal health, in addition to increasing levels of globulins which are immunological proteins with several biological functions. The meat of chickens that consumed the minimum diet was redder and had a lower water retention capacity and greater loss of

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water by cooking; these changes were not corrected with the enzyme blend in the birds' diet; the addition only minimized the negative changes. Adding a blend of exogenous enzymes in the lower energy diet is an interesting and practical option, especially when feed costs are high, as has been the case in Brazil in recent years; the additives minimized the adverse effects on productivity, leaving the feed with a shorter course. Was found that the profitability of the supplemented diet was higher than the diet provided to the chickens for average performance.

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ETHICS COMMITTEE

All procedures for this project were approved by the *Comitê de Ética do Uso de Animais na Pesquisa* (CEUA) of the *Universidade do Estado de Santa Catarina*, under the protocol number 7175220620, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

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