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Exogenous enzymes in diets for broilers

Enzimas exógenas em dietas para frangos de corte

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SUMMARY

The aimed of the study was to evaluate the effect of the inclusion of different levels of an enzyme complex consisting of phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase on the parameters of performance, carcass yield and meat quality of broilers. Six hundred broiler chicks were used, and the animals were females with one day of age, from the Cobb 500 strain, and distributed in a completely randomized design, with five levels of inclusion of the enzyme complex (0, 100, 200, 300 and 400g/ton), and six repetitions, with twenty animals each. The weight gain, feed conversion ratio, feed intake and production viability were assessed in stages between 1 to 21, 1 to 35 and 1 to 42 days of age. The carcass yield and meat quality were evaluated at 35 and 42 days of age. We evaluated the characteristics of weight loss by cooking, shear force, water holding capacity, pH, lightness and color. The parameters of performance, carcass yield and carcass parts, and meat quality were not affected by the enzyme supplementation of diets fed to broiler chickens (P>0.05), except for the performance characteristics of the breast and the wings at 42 days of age (P<0.05).

Keywords: carcass yield, enzyme complex, meat quality, poultry, performance

RESUMO

O experimento foi realizado com o objetivo de avaliar o efeito da inclusão de diferentes níveis de um complexo enzimático composto por fitase, protease, xilanase, β – glucanase, celulase, amilase e pectinase, em dietas à base de milho e de farelo de soja, sobre os parâmetros de desempenho, de rendimento de carcaça e de qualidade da carne de frangos de corte. Foram utilizados 600 pintos de corte, de um dia de idade, fêmeas, da linhagem Cobb 500, distribuídos em delineamento inteiramente casualizado, com cinco níveis de inclusão do complexo enzimático (0; 100; 200; 300 e 400 g/ton), e seis repetições de 20 aves cada. Foram avaliados, nas fases de 01 a 21, de 01 a 35 e de 01 a 42 dias de idade, o ganho de peso, a conversão alimentar, o consumo de ração e a viabilidade criatória. O rendimento de carcaça e a qualidade da carne foram avaliados aos 35 e aos 42 dias de idade. Avaliou-se as características de perda de peso por cocção, a força de cisalhamento, a capacidade de retenção de água, o pH, a luminosidade e a cor. Os parâmetros de desempenho, de rendimento de carcaça e cortes e de qualidade da carne não foram afetados pela suplementação enzimática das dietas fornecidas aos frangos de corte (P>0,05), exceto para as características de rendimento de peito e de asas aos 42 dias de idade (P<0,05).

Palavras-chave: aves, complexo enzimático, desempenho, qualidade da carne, rendimento de carcaça

INTRODUCTION

Poultry farming is one of the most prominent segments in the global agribusiness. This sector increased significantly after inclusion in the production chain, in the 1980s and mainly due to increasing adoption of technology in the activity. Among the mechanisms of technological, advances in nutrition with the production of food additives allowed greater assimilation of nutrients provided in the diet for broilers resulting in increased performance.

Broiler rations in Brazil are, almost entirely, formulated from two basic ingredients: corn, which is an excellent energy source, and soybean meal, which contributes with high-quality proteins and with great amino acid availability (OPALINSKI et al., 2006). However, it is known that the nutrients originated from these foods are not properly absorbed, mainly because of the presence of anti-nutritional factors, such as NAPs (non-amylaceous polysaccharides) and phytic acid.

Therefore, mechanisms to enhance the performance of foods given to animals were made necessary. Aiming to increase the efficiency of rations, the usage of exogenous enzymes in the feeding of broilers is gaining more space and has become a great alternative, since it enhances food digestibility, minimizing the anti-nutritional effects and promoting the productivity indexes (HOOGE et al., 2010). The use of enzyme complexes is effective, since the wide range of enzymes present in this type of product allows for greater action in different types of substrates and, or, foods utilized in the process of ration fabrication.

The SSF enzyme complex, one of the products available in the market, is showing to be effective. Basically, this type of additive uses fermentation in solid extracts conducted by specific fungi as an enzyme-production mechanism (ROBSON & NIGAN, 2003). This results in the fabrication of a complex with, according to the substrate used in the making, a natural specific combination of enzymes and a greater spectrum of action (MOURA et al., 2012), in addition to the low final cost, which facilitates its addition to broiler rations.

In light of this, this study aimed at evaluating the performance, the carcass yield and the carcass parts, and the quality of the meat for broilers that were submitted to the diets based on corn and soybean meal with different levels of the SSF (solid state fermentation) enzyme complex.

MATERIALS AND METHODS

This experiment was conducted in the facilities of the broiler sector of the Animal Science Department of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM). Six hundred broiler chicks were used, and the animals were females with one day of age, from the Cobb 500 strain. This design was completely randomized with five treatments and six replications with 20 broilers each. The treatments consisted of five inclusion level of enzyme complex (0, 100, 200, 300, 400 g/ton). The enzyme complex SSF is composed of seven distinct enzymes: phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase.

The diets were formulated according to the adaptations by Rostagno et al. (2011). The percent composition and the calculated levels of nutrients for the control diets for the initial stage (1 to 21 days of age), the growing stage (22 to 35 days of age) and the final stage (36 to 42 days of age) are presented on Table 1.

Table 1.Percentage composition and calculated nutrient levels of experimental diets

Ingredients	Initial	Growth	Final
Corn	61.585	63.990	67.136
Soybean mean	33.413	30.255	26.619
Soybean oil	1.169	2.636	3.169
Limestone	0.925	0.816	0.000
Dicalcium phosphate	1.490	1.156	2.040
Common salt	0.456	0.443	0.418
L-Lysine HCl 99%	0.245	0.136	0.104
DL-Methionine 99%	0.289	0.209	0.159
L-Threonine 98%	0.073	0.005	0.000
Mineral supplement[1]	0.050	0.050	0.050
Vitamin supplement[2]	0.100	0.100	0.100
Salinomycin 12%	0.055	0.055	0.055
Antioxidant BHT	0.010	0.010	0.010
Choline chloride 60%	0.100	0.100	0.100
Enzyme complex[3]	0.000	0.000	0.000
Inert[4]	0.040	0.040	0.040
Total	100.0	100.0	100.0
Metabolizable energy, kcal/kg	3000	3100	3150
Crude Protein (%)	20.400	19.000	17.500
Ca (%)	0.809	0.683	0.759
Non-phytate phosphorus (%)	0.386	0.319	0.264
Dig. lysine (%)	1.165	1.005	0.892
Dig. methionine (%)	0.559	0.467	0.403
Dig. methionine + cystine (%)	0.839	0.733	0.651
Na (%)	0.200	0.195	0.185

[1]Safety levels per kg of the product (Min): Folic acid 750mg; Pantothenic acid 12g; B.H.T. 1.000mg; Biotin 25mg; Niacin 35g; Vitamin A 8.000.000 UI; Vitamin B1 1.500mg; Vitamin B12 12.000mg; Vitamin B2 5.000mg; Vitamin B6 2.800mg; Vitamin D3 2.000.000 UI; Vitamin E 15.000 UI; Vitamin K3 1.800mg.
[2]Safety levels per kg of the product (Min): Copper 20g; Iron 96g; Iodine 1.400mg; Manganese 156g; Selenium 360mg; Zync 110g.
[3]Allzyme SSF – Alltech Ind.: minimul levels of enzyme activity: phytase 300 UF/g; protease 700 UI/g; xylanase 100 UI/g; β-glucanase 200 UI/g; cellulase 40 UI/g; α amylase 30 UI/g and pectinase 4000 UI/g.
[4]Washed thin sand.

At 21, 35, and 42 days of age, the animals and the ration leftovers were weighted to allow for the measure the performance variables: RC (ration consumption), WG (weight gain), and FC (feed conversion ratio). Mortality (M) was controlled daily, as well as the measurement of temperature and humidity for the inside of the house, at 9:00a.m. and at 3:00p.m. by using the

thermohygrometer THDL – 400 Instrutherm. Later mortality data were converted in percentage, for production viability (VB) (VB = 100%-M).

At 35 and at 42 days of age, two animals of each experimental group were selected by the average weight of the group (± 5%) for evaluation of the performance for the carcass, breast, leg quarter, wing and abdominal fat. After eight hours of fasting, the animals were packed in boxes and transported to a room lit by artificial blue light. All the slaughtering procedures were approved by the Ethic Committee of UFVJM, process nº 034/12.

After the evisceration, the carcass yield was obtained in relation to the body weight: % CY = (carcass weight x 100/body weight). The yield for the breast, the leg quarter and the wing were calculated in function of the carcass weight: % BY = (weight of the part x 100/carcass weight). The performance for the abdominal fat was calculated in function of the body weight of the animals. For the evaluation of meat quality, cooled, skinless, boneless breast meat. The pH was standardized at room temperature, 25ºC, by means of a pH meter (Tecnopon mPA210) attached to the penetration electrode (Hanna Hl 8314) and introduced directly in the muscle "Pectoralis major". The method described by Hamm (1960) was utilized in order to determine the water retention capacity (WRC).

Weight loss by cooking was achieved with the methodology proposed by Cason et al. (1997). The analysis of the shear force was made by a *StableMicroSystems* TAXT 2 PLUS texturometer attached to a *blade set* V Wanner Bratzler probe. It was considered the force peak of the analysis, therefore determining necessary force for the cuts (OLIVEIRA et al., 2015).Color analysis was conducted with a raw meat sample, with longitudinal cuts in the breast portion made by a Minolta CR 400 colorimeter, with a CIELAB system (L*, a* and b*), where L* = luminosity, a* = red content and b* = yellow content (VAN LAACK et al., 2000).

The dataset were submitted to regression analysis using software SAS (2002). Were tested the linear models and quadratic considering a 5% significance level.

RESULTS AND DISCUSSION

The inclusion of different levels of enzyme complex in the ration of broilers did not significantly influence (P>0.05) the performance of broilers reared during 1 to 21; 1 to 35 and 1 to 42 days of age (Table 2).

Similar results were found by Pinheiro et al. (2008) supplementing broiler diets in the initial stage with carbohydrases and proteases (P>0.05). However, Pucci et al. (2010) observed an improvement (P<0.05) in feed conversion ratio in broilers fed diets based on corn and soybean meal supplemented with EC (amylase, cellulase and protease). Exogenous enzymes added in broiler diets allow supplementation of endogenous enzyme production improves the assimilation of nutrients by the birds (ANGEL et al., 2011); especially in the early stage of life. However, in the supply "on top" as in this study, this effect is reduced and decreases the significant effect detection probability.

Currently in production and marketing of exogenous enzymes for feed supplementation for broiler chickens, using EC have become quite popular. For specific action allows a greater number of substrates. However, in diets with conventional ingredients such as corn and soybean meal, without reducing nutrient

levels, its use may not become attractive. After all, in production diets for broilers, applied for integration companies, often not reduced to its nutritional recovery by increasing the breakdown of anti-nutritional factors of food, caused by the addition of EC. However, it has been observed in field studies that the addition of EC on the level recommended by the manufacturer, provides percentage reduction in feed conversion ratio (FERNANDES et al., 2015). As noted in this study.

Table 2. Average values for weight gain (WG), ration consumption (RC), feed conversion ratio (FC) and viability (VB), of broilers in phases 1 - 21; 1 - 35; 1 - 42 days, submitted to diets containing different levels of enzyme complex (EC)

Variables	Levels of EC Addition (g/ton) – 1 to 21days					CV (%)	P value
	0	100	200	300	400		
WG (g)	857	865	889	875	875	3.17	0.2268 ^{ns}
RC (g)	1203	1209	1209	1217	1209	3.64	0.7414 ^{ns}
FC (g/g)	1.40	1.40	1.36	1.39	1.38	2.15	0.2265 ^{ns}
VB (%)	97.50	95.84	94.50	95.34	97.50	5.32	0.5614 ^{ns}
Variables	Levels of EC Addition (g/ton) – 1 to 35 days					CV (%)	P value
	0	100	200	300	400		
WG (g)	2082	2068	2119	2115	2093	2.47	0.3273 ^{ns}
RC (g)	3176	3161	3191	3185	3165	3.49	0.9845 ^{ns}
FC (g/g)	1.52	1.53	1.50	1.50	1.51	3.09	0.4502 ^{ns}
VB (%)	95.00	91.67	91.78	91.06	95.00	7.20	0.9446 ^{ns}
Variables	Levels of EC Addition (g/ton) – 1 to 42 days					CV (%)	P value
	0	100	200	300	400		
WG (g)	2691	2650	2737	2676	2652	3,53	0,5850 ^{ns}
RC (g)	4324	4332	4476	4367	4386	3,92	0,6952 ^{ns}
FC (g/g)	1,61	1,63	1,63	1,63	1,65	3,53	0,3243 ^{ns}
VB (%)	91,67	90,00	90,20	88,57	94,17	8,43	0,7218 ^{ns}

CV = coefficient of variation (%); P value = significance rate of the regression analysis; ns = no significant (P>0.05).

Pereira et al. (2010) also did not observed significant effect on weight gain and on feed conversion ratio after using EC (protease, amylase and xylanase) in diets for broilers in the stage between days 1 to 35. Nonetheless, Leite et al. (2012) by using the SSF EC in the ration based on millet and soybean meal in comparison with sorghum, observed the beneficial effect (P<0.05) of the inclusion of EC on parameters of performance. Montanhini Neto et al. (2013) observed that the additional a multienzyme complex in broiler diets formulated with non-conventional food, there was increased activity and immunity of the intestinal mucosa intestinal cells. Thus, it is possible to infer that the action of enzyme complexes becomes more effective in diets with unconventional food.

Broilers in the stage between days 1 to 35 are under development and have a complete digestive and absorption systems. However, there is a greater time of contact between the enzyme and the foods, which may lead to an enhancement in performance. De Basílio & Gerig (2011), following this line of reasoning, found a reduction on ration consumption and enhancement in feed conversion ratio (P<0.01) for broilers in the stage between 1 to 35 days, by evaluating the time of

contact between SSF EC and the ration provided. This is because the authors have used diets with reduced nutrient levels, which was not done in this study. Proving this fact, Barbosa et al. (2012) observed a similar effect on weight gain and feed conversion in broilers (1-42 days) fed diets with and without reducing nutrient levels, supplemented with EC (phytase, amylase, xylanase and protease).

The results observed between days 1 to 42 confirm those found by Leite et al. (2011), who did not verify any effects of SSF EC supplementation in diets based on sorghum and soybean meal in the "on top" form (disregarding the advantages of EC in the nutritional matrix for the formulation of diets). After the pre-initial life stage of broilers, specially, the enzyme mechanisms are already established. Thus, it is possible to observe a better nutritional performance after the first week of life. This occurs when broilers are fed with rations that meet their nutritional needs, with enzyme supplementation and provided *ad libitum*, making it difficult to quantify the direct effect of the enzyme on the animal performance (MOURA et al., 2012). Furthermore, according to Olukosi et al. (2007) corn and soybean meal do not have a great contribution to the increase of the viscosity of diets for animals with a gastrointestinal tract in good physiological condition. Hence, there is greater possibility of action of EC in diets formulated with non-conventional foods with higher fiber content and higher presence of anti-nutritional factors, with better proliferative capacity of the crypts and villi height (LORENA-REZENDE et al., 2012; MONTANHINI NETO et al., 2012).

In turn, Bentea et al. (2010), after using SSF EC 200 g/ton + selenium 300 g/ton, observed significant enhancements on weight gain and feed conversion ratio ($P<0.05$), increasing total body weight by up to 19.07% and food conversion by up to 15.35%, considering the total period. Even if this study did not observe any significant effect on the animal performance, percentage increments of 1.71% in weight gain and of 1.23% in feed conversion ratio were observed for the period between 1 to 42 days of age. It is important to notice that the progress on the productive chain as a whole may not be significant and allow for an increase in profitability.

There was no significant effect ($P>0.05$) of the inclusion of different EC SSF on the parameters of carcass yield and meat quality of broilers with 35 days of age. However, this study verified a significant effect ($P<0.05$) for breast yield and wing yield at 42 days for broilers fed with diets supplemented with SSF EC (Table 3).

Figure 1 and 2 schematically represent the significant effects of the inclusion of EC in broiler diets at 42 days, for the breast and wings yields, respectively. Similar results were found by Soto-Salanova et al. (1996) who verified an effect ($P<0.05$) of enzyme supplementation in diets based on corn and soybean meal on the augmentation of the breast muscle in broilers with 42 days of age.

The greatest percentage increase, an average of 2.15% in relation to the other treatments, of the breast, with the enzyme levels recommended by the manufacturer (200g/ton), may have occurred due to the fact that this level provides a better digestibility of the ingredients and, therefore, increases the amount of nutrients available for breast growth. The breast becomes the most important section, it represents about 50% of the total protein of the chicken (ALMEIDA et al., 2002); with increased ability to produce derivatives of chicken feed. These results to poultry industry are very important because

there is a tendency to sell cuts over the whole carcass due to increase in value aggregate. For every other level over the recommended value for EC, this response may have not existed due to the lack of substrate available after the addition of an amount of enzymes greater than the recommended number without considering the nutritional energy matrix and, or, the diet proteins.

Table 3. Average values for carcass yield (CY), breast yield (BY), wing yield (WY), leg quarter yield (LQY) and fat yield (FY), of broilers of 35 and 42 days of age, submitted to diets containing different levels of enzyme complex (EC)

Variables	Levels of EC addition (g/ton) – 35 days					CV (%)	P value
	0	100	200	300	400		
CY	73.54	74.32	74.17	74.04	73.6	1.39	0.9344 ^{ns}
BY	38.61	38.07	38.12	38.71	38.67	3.57	0.6733 ^{ns}
WY	10.47	10.34	10.18	10.32	10.43	4.36	0.8695 ^{ns}
LQY	27.04	26.57	26.60	26.88	26.53	2.45	0.4168 ^{ns}
FY	1.88	1.62	1.41	1.70	1.67	27.36	0.5648 ^{ns}
Variables	Levels of EC addition (g/ton) – 42 days					CV (%)	P value
	0	100	200	300	400		
CY	74.06	73.25	73.21	72.70	75.36	2.61	0.2838 ^{ns}
BY	40.05	40.11	40.41	39.73	38.24	3.65	0.0417 ^{L}
WY	9.73	10.44	10.22	10.84	10.43	5.28	0.0228 ^{L}
LQY	26.46	26.74	26.85	27.21	27.50	6.62	0.3225 ^{ns}
FY	1.76	1.67	1.64	1.98	1.58	26.31	0.8591 ^{ns}

CV = coefficient of variation (%); P value = significance rate of the regression analysis; ns = no significant (P>0.05); L = linear regression (P<0.05).

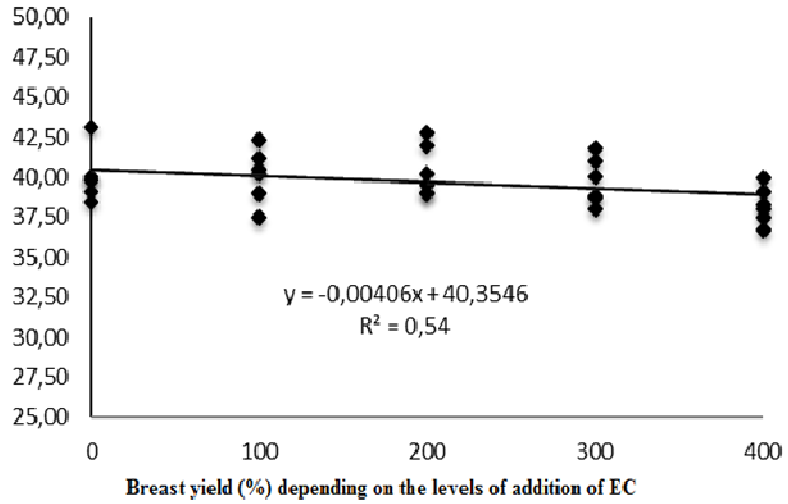

Figure 1. Breast yield (%) of broilers reared at 42 days fed diets supplemented with different levels of EC (0, 100, 200, 300 and 400g/ton)

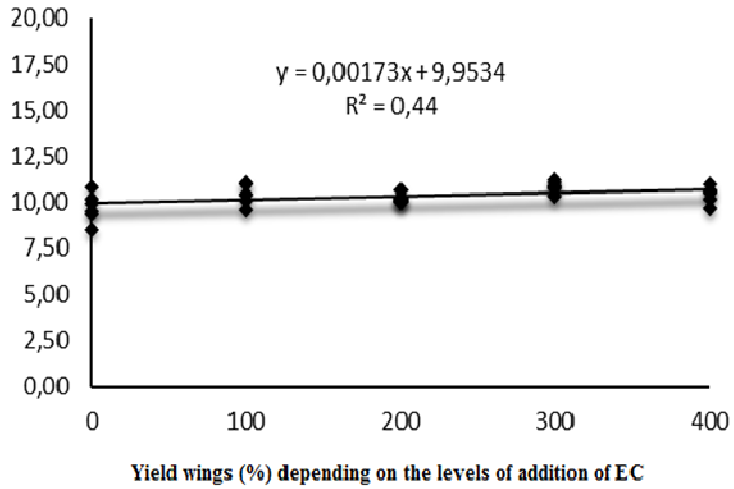

Figure 2. Wings yield (%) of broilers reared at 42 days fed diets supplemented with different levels of EC (0, 100, 200, 300 and 400g/ton)

It may also be due to the low fiber content present in low viscosity diets (SOTO-SALANOVA et al., 1996). Therefore, regarding the rations with EC supplementation in the "on top" form, it is verified that the enzyme does not produce any beneficial effects above the recommended level, which happens due to the quality of the foods used in the formulation and to the meeting of the nutritional demands of the broilers.

Silveira et al. (2010), after studying the use of SSF EC in pelleted diets by analyzing contrasts, were able to verify an effect ($P<0.05$) on the performance of the leg quarter at 21 days of age, with a percentage reduction of 25% in relation to the control diet. However, no significance was observed for the performance of the breast ($P>0.05$). Cardoso et al. (2011) also did not verify any differences ($P>0.05$) on carcass yield for broilers with 42 days of age. Regarding abdominal fat, Souza et al. (2008) observed an increase in the carcass of broilers at 42 days of age. According to Kessler et al. (2000) the most efficient way to avoid fat excess in the carcass is the approximation between energy and protein levels. This fact can be explained by the increase in food digestibility to the level recommended for the addition of the EC, which overestimates the energy values of the ration. Thus, with the energy excess, there is the possibility of greater accumulation of abdominal fat, a fact that was not identified within this study.

Broiler meat, according to Petracci & Baéza (2011), has the following as its main intrinsic attributes: appearance, texture, succulence, flavor, and functionality; coloring is the most important factor that affects the choice of consumers. They also state that the pH is closely related to all the factors that affect meat quality, although this effect is complex. This complexity is due to the many reaction associated with the heme factor, which depends on pH (WERNER et al., 2009). Since all the factors and meat quality parameters will depend directly from the management conditions, lineage, and especially the type of feed provided to the birds (NEVES et al., 2014).

The addition of exogenous enzymes in the feed of broilers, probably, improves nutrient digestibility efficiency and promotes greater carcass yield and meat

deposition (ALLOUCHE et al., 2015). The increase in protein deposition may change the type and shape of fiber to be deposited on the substrate, and may therefore change the meat quality parameters (FATUFE et al., 2004); especially abdominal fat increased efficiency in the use of nutrients. Therefore, the inclusion of the SSF EC did not influence (P>0.05) the parameters of meat quality of broilers with 35 and 42 days of age (Table 4).

Table 4. Average values for weight loss by cooking (WLC), shear force (SF), water retention capacity (WRC), hydrogenionic potential (pH), luminosity (L*), red content (a*) and yellow content (b*) of the breast of broilers with 35 and 42 days of age, submitted to diets containing different levels of enzyme complex (EC)

Variables	Levels of EC addition (g/ton) – 35 days					CV (%)	P value
	0	100	200	300	400		
WLC (%)	29.29	28.76	29.51	29.32	29.23	10.99	0.9137 ^{ns}
SF (kgf.cm^{-2})	2.53	2.65	2.97	2.26	2.77	25.75	0.9200 ^{ns}
WRC (%)	45.61	42.24	46.13	43.35	44.68	10.40	0.9009 ^{ns}
pH	5.92	5.87	5.88	5.83	5.85	1.63	0.1585 ^{ns}
L*	48.11	48.43	50.47	49.3	51.21	5.95	0.0658 ^{ns}
a*	3.84	3.08	3.77	3.16	3.76	20.21	0.9364 ^{ns}
b*	5.03	5.32	5.73	5.32	5.39	22.92	0.6409 ^{ns}
Variables	Levels of EC addition (g/ton) – 42 days					CV (%)	P value
	0	100	200	300	400		
WLC (%)	30.54	26.40	29.54	31.48	26.03	16.79	0.5510 ^{ns}
SF (kgf.cm^{-2})	3.16	3.07	3.33	2.45	3.20	18.61	0.4969 ^{ns}
WRC (%)	44.98	47.13	44.2	48.01	46.71	10.51	0.4850 ^{ns}
pH	5.72	5.67	5.68	5.69	5.71	0.93	0.8317 ^{ns}
L*	49.79	49.37	50.40	49.6	48.22	5.23	0.3751 ^{ns}
a*	3.16	3.07	3.33	2.45	3.20	21.78	0.3226 ^{ns}
b*	7.66	6.72	7.67	7.22	7.82	15.10	0.5782 ^{ns}

CV = coefficient of variation (%); P value = significance rate of the regression analysis; ns = no significant (P>0.05).

According to Werner et al. (2009) the addition of enzymes does not affect quality parameters of the meat; they are interconnected with color and pH, which are mainly hampered by the loss of exudate and temperature pitches. Zakaria et al. (2010) while working with diets based on corn and soybean meal supplemented with the SSF EC, also did not observe any effects (P>0.05) regarding the parameters pH, WLC, WRC, color and luminosity in carcass for broilers at 42 days of age.

The poultry industry is characterized by high production volume, mainly by the broiler industry. In this sense, the productive reality the use of exogenous enzymes can provide obtaining percentage gains in carcass characteristics, making it an extremely attractive alternative from an economic point of view (RAVINDRAN, 2013). However, there greater need for studies that point advantages when it reduces the nutritional value of diets and also those that correlate the cost-benefit of the inclusion of EC in diets for broilers. All these factors combined with the proper management of poultry production will allow consecutive increases in productivity and expansion of Brazilian agribusiness.

Given the above, based on the results

obtained in this study, it can be inferred that the inclusion of the SSF enzyme complex in diets based on corn and soybean meal for broilers, in the levels recommended by the manufacturer, 200g/ton, enhanced the efficiency of the breast and the wing yields at 42 days and did not significantly influence the performance, the carcass yield and the quality of the meat.

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