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NONRUMINANTS NUTRITION

Effect of different levels of supplementary alpha-amylase in finishing broilers

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ABSTRACT. The objective of this study was to investigate the effects of alpha-amylase supplementation on performance and carcass and cuts yield in broilers during finishing phase (21 to 42 days). A completely randomized experimental design with six treatments and eight replications was used: Positive Control (PC): no enzyme; Negative control (NC): without enzyme formulated with 6.34% less energy (- 6.34% ME); NC1: with 250 g ton^{-1} of alpha-amylase; NC2: with 500 g ton^{-1} of alpha-amylase; NC3: with 750 g ton^{-1} of alpha-amylase; NC4: with 1000 g ton^{-1} of alpha-amylase. Significant differences in weight gain, feed intake and feed conversion were reported with the inclusion of alpha-amylase. The NC treatment with inclusion of amylase provided improvement values of weight gain, feed conversion and feed intake, but do not was similarly in comparison to those obtained in broilers fed a diet that completely met the requirements during this phase. There was no dietary influence on carcass parameters. The use of alpha-amylase was effective in improving performance in broilers fed a diet of 200 kcal kg^{-1} of reduced metabolizable energy.

Keywords: energy reduction; enzyme; performance.

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Introduction

The current poultry industry has been effective in producing good quality food with high biological value for the consumer. For that reason, alternatives that maximize the use of nutrients in broilers have been sought to meet market demand both in quality and quantity. Energy utilization is largely studied since it directly influences the absorption of the other nutrients and because the main dietary source is corn. This ingredient comprises about 30 to 40% of poultry diets in Brazil. Despite the high nutrient utilization, especially in corn, a great part of these nutrients is not utilized by the birds.

Therefore, it is important to include exogenous enzymes in the diets to allow a better utilization of these nutrients and to maximize the absorption of the remaining nutrients. Based on this, the use of alternatives that maximize energy utilization is a trend that has been diffused among farmers and researchers. Therefore, it is important to include exogenous enzymes in the feeds to allow a better utilization of these nutrients and to maximize the absorption of the remaining nutrients.

The main constituent of the grains used to formulate corn-soybean meal-based diets is starch, which is responsible for the energy content in these diets for monogastric animals, with greater impact on the energy balance. Its digestion is carried out by the action of amylase, which catalyzes the hydrolysis of the starch, resulting in maltoses and dextrins, which are then absorbed. Dietary multienzyme in complete diets can increase nutrient utilization by poultry (Slominski, 2011). Adeola and Ileleji (2009) recently reported that the ME and ME_n contents of corn distillers grains (CDG) with soluble in a practical corn-soybean meal-based diet for broiler chickens was determined by the regression method to be 2,904 and 2,787 kcal kg^{-1} of DM, respectively. We are not aware of data on the energy value of CDG for broiler chickens.

The pancreas of broiler chickens produces amylase, also called pancreatic alpha-amylase, which randomly cleaves the alpha 1-4 glycosidic bonds of amylose to generate maltose and maltotriose or maltose, glucose and dextrin from amylopectin. By including exogenous enzymes in the diets, such as amylase, there is an increase in energy utilization, probably by breaking large amounts of starch during the digestion process, increasing energy digestibility. Woyengo, Patterson, Slominski, Beltranena, and Zijlstra (2016), studying the supplementation of rations with an enzymatic complex containing cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, and protease, verified an increase in the availability of metabolizable energy.

The results of researches on the inclusion of amylases in feeds for broilers are quite variable and depend on some factors such as evaluation, ingredients present in the formulations, concentration of the enzymatic products, age of birds, particle size, growth conditions, amount of resistant starch and the amylose and amylopectin ratio present in the diets (Amerah, Romero, Awati, & Ravindran, 2016; Bedford & Cowieson, 2012; Yuan et al., 2017).

Although there are some studies with negative results and the various contradictions between the results of the surveys that evaluate the inclusion of enzymes in broiler feeds, the use of amylase is well diffused among poultry farmers. Further studies are needed to evaluate the real influence of the inclusion of these enzymes on broiler diets.

The aim of this study was to evaluate the effects of exogenous alpha-amylase supplementation in corn-soybean meal-based diets on performance and carcass and cuts yield in broilers during finishing phase (21 to 42 days).

Material and methods

The methodology used was approved and conducted according to the institutional committee on animal use protocol number of Federal University of Lavras. The trial was conducted in Lavras-Minas Gerais-Brazil, and geographic coordinates (Latitude: 21° 14 '43 "S, Longitude: 44° 59' 59" W, Altitude: 919m).

A total of 960 Cobb-500 male broilers were homogeneously raised up to 18 days of age (handling and nutrition). On the 18th day, the broilers were divided into homogeneous groups according to weight (720 ± 40 g) and were distributed in 48 experimental boxes. From that point, they were fed the experimental diets. The evaluation started three days after adaptation, in the 21st day. Twenty broilers were housed per box (3m^2) with wood shavings litter, in a completely randomized experimental design with six treatments and eight replicates each. The boxes were equipped with a tubular feeder and a bell type drinking fountain. Six treatments were evaluated: Positive Control (PC): no enzyme; Negative control (NC): without enzyme formulated with 6.34% less energy (- 6.34% ME); NC1: with 250 g ton^{-1} of alpha-amylase; NC2: with 500 g ton^{-1} of alpha-amylase; NC3: with 750 g ton^{-1} of alpha-amylase; NC4: with 1000 g ton^{-1} of alpha-amylase. The alpha-amylase presents an activity, according to the manufacturer, of 56,000 KNU g^{-1}.

Experimental diets (Table 1) were formulated based on corn and soybean meal according to the recommendations described in the Cobb-500 Lineage Manual (2015). The birds were weighed at the beginning and at the end of the experimental period, as well as the feed supply and leftovers at the end of the trial (42 days of age), to determine feed intake (FI), weight gain (WG) and feed conversion ratio (FCR), all expressed in grams. During the experimental period, the birds received food and water ad libitum.

At the end of the experimental period, two birds per plot, which were in the average weight (3100 ± 100 g) of the plot, were slaughtered after a 6-hour fasting, following the standards stipulated by the Ethics, Bioethics and Animal Welfare Committee, to evaluate yield and carcass measurements. The carcasses were kept in ice buckets for one hour and then divided into head, feet, neck, chest, leg and thigh, liver and pancreas. All carcass constituents were weighed. Yields of carcass, breast, leg and thigh, liver, pancreas and abdominal fat were calculated in relation to the weight of the eviscerated carcass, without considering the weight of head, neck, feet and edible offal.

The averages of feed intake (FI), weight gain (WG), feed conversion (FC) and carcass measurements were submitted to analysis of variance using the SISVAR (System for analysis of variance) (Ferreira, 2011), and the treatments were compared by the Tukey test at 5% probability.

Results and discussion

The performance of broiler chickens during the 21 to 42-day-old phase are shown in Table 2. All variables of performance: feed intake (FI), weight gain (WG) and feed conversion (FC) were influenced ($p < 0.05$) by the diet. The reduction of 200 kcal ME in negative control diet (NC) decreased ($p < 0.05$) weight gain, feed intake and feed conversion compared to that obtained in positive control diet (PC). This proves the accuracy of the trial to distinguish between experimental diets.

Table 1. Composition and calculated analysis of diets for broiler chickens during finishing phase (21 to 42 days).

Ingredients	NC	NC + 250g ton^{-1} amylase	NC + 500g ton^{-1} amylase	NC + 750g ton^{-1} amylase	NC + 1000g ton^{-1} amylase	PC
Corn	62.131	62.131	62.131	62.131	62.131	61.277
Soybean meal, 45%	31.379	31.379	31.379	31.379	31.379	30.626
Soybean oil	2.925	2.925	2.925	2.925	2.925	4.559
Dicalcium Phosphate, 18/24	1.575	1.575	1.575	1.575	1.575	1.587
Limestone	0.818	0.818	0.818	0.818	0.818	0.808
Salt	0.427	0.427	0.427	0.427	0.427	0.428
Dl-Methionine, 98%	0.227	0.227	0.227	0.227	0.227	0.213
L-Lysine, 79%	0.184	0.184	0.184	0.184	0.184	0.168
Vitamin Premix1	0.100	0.100	0.100	0.100	0.100	0.100
Choline Chloride, 70%	0.020	0.020	0.020	0.020	0.020	0.020
Mineral Premix2	0.100	0.100	0.100	0.100	0.100	0.100
L-Threonine, 99%	0.014	0.014	0.014	0.014	0.014	0.014
Kaolin	0.100	0.075	0.050	0.025	0.000	0.100
Alpha-amylase	0.000	0.025	0.050	0.075	0.100	0.000
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutritional and energetic composition						
Metabolizable energy (kcal kg^{-1})	2950	2950	2950	2950	2950	3150
Crude protein (g kg^{-1})	20.00	20.00	20.00	20.00	20.00	20.00
Methionine (g kg^{-1})	0.51	0.51	0.51	0.51	0.51	0.51
Methionine + Cystine (g kg^{-1})	0.78	0.78	0.78	0.78	0.78	0.78
Lysine (g kg^{-1})	1.09	1.09	1.09	1.09	1.09	1.09
Calcium (g kg^{-1})	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus (g kg^{-1})	0.43	0.43	0.43	0.43	0.43	0.43
Sodium (g kg^{-1})	0.20	0.20	0.20	0.20	0.20	0.20

[1] The premix supplied the following per kilogram of diet: Vit. A – 12.000 IU, Vit. D - 2200 IU, Vit. E - 30 mg, Vit. K - 2.5 mg, Niacin - 53 mg, Folic Acid - 1.0 mg, Pantothenic Acid - 13 mg, Biotin – 110 µg, Vit. B1 - 2.2 mg, Vit. B2 - 6 mg, Vit. B6 - 3.3 mg, Vit. B12 - 16 µg, Se - 0.25 mg.[2] The premix supplied the following per kilogram of diet: Fe - 50 mg, Co 8.5 mg, Zn - 70 mg, Mn - 75 mg, I – 1.5 mg and Co – 0.2 mg.NC - Negative Control with reduced energy (6.34%); PC - Positive Control.

Table 2. Performance (FI – Feed intake; WG – Weight gain; FC – Feed conversion) of broilers from 21 to 42 days old fed diets with different levels of amylase.

Treatments	Feed intake, g bird^{-1}	Weight gain, g bird^{-1}	Feed conversion, g g^{-1}
Positive control (PC) without enzyme	3430 ab	1748 a	1.96 c
Negative control (NC) without enzyme	3485 a	1497 c	2.33 a
NC with 250 g/ton of amylase	3312 ab	1561 b	2.12 b
NC with 500 g/ton of amylase	3237 b	1530 b	2.12 b
NC with 750 g/ton of amylase	3294 ab	1535 b	2.15 b
NC with 1000 g/ton of amylase	3331 ab	1605 b	2.08 b
Probability			
Treatments	0.041	0.003	0.012
CV %	2.78	3.07	2.37

*Means followed by different letters in the same column are statistically different according to the Tukey test ($p < 0.05$).

There was a reduction ($p < 0.05$) in feed intake when 500 g ton^{-1} amylase was used, in relation to the negative control diet without enzyme inclusion. Therefore, the amylase was able to increase energy supply in broilers fed diets with reduction of 200 kcal ME. This proves that the inclusion of amylase can maximize the use of dietary nutrients, especially energy. The higher the nutrient availability in the intestinal lumen, the lower the need to consume in order to meet physiological requirements.

The weight gain (WG) was influenced ($p < 0.05$) by the ME reduction of 6.34% in comparison to the diet that met the energy requirements of these birds (PC). The inclusion of amylase promoted WG improvements in broilers fed diets with less energy, especially for the inclusion of 1000 g ton^{-1} of amylase, which resulted in higher WG compared to broilers fed diets with different enzyme inclusion levels. However, even the level of 1000 g ton^{-1} of alpha amylase was not enough to result in similar weight gain to that of birds fed the control diet (PC), which met the metabolizable energy requirement of broilers.

Regarding the feed conversion (FC), the diet with reduced energy and without amylase resulted in higher FC compared to the other diets. The inclusion of amylase resulted in lower FC when compared to the negative control diet (NC) without enzyme inclusion, which proves that the inclusion of alpha amylase was efficient in improving the feed conversion of broilers. However, even with the inclusion of amylase, it was not enough to result in similar feed conversion to that of broilers fed diets that met all the nutritional requirements.

The inclusion of enzyme provided better results for FI, WG and FC in relation to the diet without enzyme and with reduced energy, but it was not enough to be equivalent to the PC diet, which met all the nutritional requirements without enzyme inclusion. This result indicates that the use of the enzyme could not exceed this difference, even using 1 kg ton^{-1} of amylase, that is, the alpha-amylase was not able to increase energy supply of the negative control diet to be equivalent to the PC diet as regards the performance variables analyzed.

Valadares et al. (2016) in a research to evaluate the nutrient content and to determine the metabolizable energy of corn residual without and with the use of the alpha-amylase enzyme, found that the addition of the enzyme improved the utilization of the energy of the ingredient, providing effect on the energy metabolism coefficient. Yu and Chung (2004) observed differences in feed intake, in which broilers fed the negative control (reduction of 3% ME) had higher feed intake than broilers fed the other treatments. Adeola, Jendza, Southern, Powell, and Owusu-Asiedu (2010), who used carbohydrates (xylanase and amylase) in poultry diets and found a reduction in feed intake due to the better utilization of dietary metabolizable energy. According to these authors, carbohydrases, such as amylase, can hydrolyze structural carbohydrates, reducing the barrier to nutrient digestibility and increasing ingredient utilization. However, there are several possible mechanisms to explain the improvement related to the inclusion of amylase on feed intake. These results were expected due to the better nutrient utilization in diets supplemented with soluble carbohydrases capable of hydrolyzing the carbohydrates that alter the viscosity of the digesta, as occurs with the amylase (Adeola et al., 2010; Bedford & Cowieson, 2012). The results these study indicate the efficiency of increasing enzyme inclusion in poultry diets, confirming the results presented by Zanella, Sakomura, Silversides, Fiqueirdo, and Pack (1999) and Gracia, Aranibar, Lazaro, Medel, and Mateos (2003), who observed a positive effect of exogenous alpha-amylase supplementation in poultry diets. In addition, it favors a better animal performance and starch digestibility, which increases the metabolizable energy in corn-soybean meal-based diets and, consequently, the development of broilers.

Sayehban et al. (2016) included an enzymatic complex in the broiler ration, but found no significant effect on broiler performance. The authors cite that they found this because the experimental designs that include enzymes generally focus on the addition of enzymes while discounting their nutritional value in the diets. The experimental diet, used by the authors, met all nutritional requirements of the evaluated broiler strain, independently of the ENZ inclusion. Therefore, the nutrients provided by enzymes exceeded the requirements of the birds. Similarly, Cardoso et al. (2011) did not observe significant differences in the feed intake of broilers fed diets with the addition of enzyme complex or isolated enzymes, regardless of whether the ration has low levels of metabolizable energy or not. Costa, Clementino, Jácome, Nascimento, and Pereira (2004) reported no difference in WG with the inclusion of amylase but reduced feed intake and better FC were observed the higher the inclusion of alpha-amylase (200, 400 and 600 g ton^{-1}) in the diet.

Kaczmarek et al. (2014) in a study to evaluate the effect of enzymatic supplementation using isolated amylase or amylase plus protease in corn and soybean meal rations on performance of broiler chickens during on growth, found that there was no effect of supplementation enzymatic activity on weight gain and on feed conversion. Sorbara et al. (2009), when using an enzymatic complex containing the enzyme amylase, found that birds fed addition of enzymes showed lower feed intake and weight gain, and there was no difference in feed conversion. Alefzadeh et al. (2016) using an enzyme complex (containing phytase, β glucanase, α-amylase, cellulase, pectinase, xylanase, lipase, protease, amilo-glucosidase, hemicellulase, pentosonase, acid phytase, and acid phosphatase) in rations of broilers, verified a better weight gain for the broilers fed the enzymatic complex.

The results of the carcass evaluation are presented in Table 3. There was no dietary influence ($p > 0.05$) on carcass parameters. Therefore, the experimental diets, even with a reduction of 6.3% of the metabolizable energy, were not sufficient to modify the carcass measurements. Hence, the difference in 6.34% ME is not enough to alter the yields of carcass, breast, leg and thigh and yields of the pancreas, liver and abdominal fat.

Similarly, Zanella et al. (1999), Torres et al. (2003) and Bedford and Cowieson (2012) did not find differences in carcass yield by using diets with enzyme inclusion and energy reduction (ME).

The results obtained in the present study also agree with the obtained by Fortes et al. (2012) and Cardoso et al. (2011) who did not find significant difference carcass yield and chick cuts at the end of the creation, where the enzymatic supplementation had no effect significance of any of the characteristics evaluated.

Table 3. Averages (%) of eviscerated carcass (EV), breast yield (BY), leg and thigh yield (LTY), abdominal fat (AF), liver and pancreas in broiler chickens fed diets with different levels of amylase and reduced energy.

Treatments	EV	BY	LTY	AF	Liver	Pancreas
Positive control (PC) without enzyme	78.5	36.3	31.5	3.55	2.86	0.219
Negative control (NC) without enzyme	77.8	36.4	31.1	3.62	2.82	0.220
NC with 250 g ton^{-1} of amylase	78.5	35.5	31.3	3.48	2.73	0.240
NC with 500 g ton^{-1} of amylase	78.6	35.7	32.9	3.50	2.72	0.205
NC with 750 g ton^{-1} of amylase	78.6	35.8	31.8	3.57	2.81	0.235
NC with 1000 g ton^{-1} of amylase	79.5	35.0	32.7	4.01	2.68	0.225
Probability						
Treatments	0.967	0.567	0.883	0.754	0.682	0.575
CV %	1.48	3.19	3.50	28.90	5.36	11.27

Tukey test ($p > 0.05$).

Mahagna, Nir, Larbier, and Nitsan (1995) found that the secretion of amylase and proteases (trypsin and chymotrypsin) by the pancreas was reduced when broilers were fed diets supplemented with amylase and protease. However, in the present study, the relative weight of the pancreas was not influenced even with the inclusion of 1000 g ton^{-1} of exogenous amylase. Therefore, to reduce the pancreatic weight due to the addition of exogenous enzymes, there may be other factors and relationships other than simply inclusion of carbohydrases, such as alpha-amylase, given that in the experiment performed by Mahagna et al. (1995) two enzymes were used simultaneously and in this study only alpha-amylase was used.

Alefzadeh et al. (2016) using an enzyme complex (containing phytase, β glucanase, α-amylase, cellulase, pectinase, xylanase, lipase, protease, amilo-glucosidase, hemicellulase, pentosonase, acid phytase, and acid phosphatase) in rations of broilers, verified an increase in drumstick weight, but there was no increase on breast weight or empty carcass weight.

Conclusion

The use of alpha-amylase, above 250 g ton^{-1} up to 1000 g ton^{-1}, was effective to reduce poor performance in broilers submitted a diet with a reduction of 200 kcal of metabolizable energy. However, when metabolizable energy is reduced in broiler feeds, it is advisable to include the alpha-amylase enzyme for better performance.

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