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Enzyme Supplementation of Broiler Feeds with Reduced Mineral and Energy Levels

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ABSTRACT

An experiment was conducted with the purpose of evaluating enzyme blends on the performance, carcass traits, and bone mineralization of broilers. In total, 928 one-day-old Cobb 500 male chicks of were used. A completely randomized design with four treatments with eight replicates of 29 birds each was adopted. The evaluated treatments were: 1- Positive Control (PC), feed containing the nutritional recommendations of the genetic company's manual; 2- Negative Control (NC), feed with reductions of 75 kcal/kg AME and 0.10 and 0.12 percent points of phosphorus and calcium, respectively; 3 - NC + enzyme blend (amylase + β-glucanase, xylanase, and phytase; 250 g/t of feed) and 4 - NC + enzyme complex (phytase, amylase, xylanase, glucanase, pectinase, cellulase, and protease; 200 g/t of feed). Birds fed the diet with reduced nutrient levels (NC) presented the worst performance ($p<0.05$). The supplementation of the enzyme blend promoted similar ($p<0.05$) weight gain and feed conversion ratio as those obtained with the PC diet during the period of 1-21 days of age. During the phase of 22-42 days and the entire experimental period, weight gain and feed conversion ratio improved with the use of the enzyme combinations compared with the NC group, but remained lower than the PC group. Enzymes combinations did not affect ($p>0.05$) carcass or parts yields. The broilers fed the reduced-nutrient and energy diet presented lower ($p<0.05$) tibial ash, calcium, and phosphorus contents that the other treatments. The use of enzyme combinations improved the performance of broilers fed diets with reduced nutrient and energy levels.

INTRODUCTION

In Brazil, poultry diets are based on corn and soybean meal which, in spite of presenting high digestibility, can be better utilized. Studies with broilers fed those ingredients have confirmed that the use of exogenous enzymes individually or in combination improves dietary nutrient utilization, resulting in more uniform animal performance (Cowieson & Adeola, 2005; Francesh & Geraert, 2009; Lu *et al*. 2013).

According to Lima (2005), the purpose of adding exogenous enzymes to non-ruminant feeds is to reduce the effects of the antinutritional factors of ingredients that are present in greater or lesser amounts in the diet. Enzymes increase, therefore, the availability of nutrients by breaking down specific chemical structures which endogenous digestive enzymes are not capable of breaking down or break down only partially.

According to Choct (2006), use of commercial enzymes in poultry feeds has evolved greatly in the last few years, based on the efficacy of the new products and a better understanding of the relationship between enzyme activity and substrates available.

Enzyme products containing single enzymes or enzyme blends are currently available in the market. According to Freitas *et al*. (2011), most of the available enzyme products include more than one enzyme activity. However, there are still some single-component products which possess only one enzyme activity, produced by a specific microorganism. Enzyme blends, a term used for products containing enzyme combinations, are obtained from the production of enzymes through the fermentation of several microorganisms individually, which enzymes are only subsequently blended. On the other hand, enzyme complex is derived from the fermentation with a single microorganism that synthesizes several enzymes.

Barbosa *et al*. (2012), studying the efficacy of enzymes in broiler diets, found that the addition of phytase, amylase, xylanase, and protease in diets with reduced metabolizable energy, calcium, and phosphorus content, promoted similar feed intake and weight gain than a diet with adequate nutrient levels. According to the authors, enzyme blends can be added to diets with reduced nutrient levels aiming to maintain broiler growth performance.

The objective of the present experiment was evaluate the use of two different enzyme combinations (a blend and a complex) in diets with reduced nutrient and energy levels on the performance, carcass characteristics, and bone mineralization of broilers.

MATERIAL AND METHODS

The experiment was conducted in the Poultry Production Sector of the Centro de Ciências Agrárias, Ambientais e Biológicas of UFRB, in Cruz das Almas, Bahia, Brazil. A total number of 928 one-d-old male Cobb-500® chicks were acquired from a commercial hatchery. The birds were housed in a conventional poultry experimental facility. Each experimental unit (pen measuring 1.65 x 1.55 m), was equipped with wood-shavings litter, a bell drinker, and a tube feeder.

A completely randomized design with four treatments and eight replicates, totaling 32 experimental units with 29 birds each, was adopted.

The following treatments were evaluated: 1 - positive control (PC) diet, formulated according to the nutrient recommendations of the manual of the Cobb-500® line (Cobb, 2012); 2 - negative control (NC) diet, also supplying the recommendations of Cobb-500® manual, but with reductions of 75 kcal of AMEn, and 0.10 and 0.12 percentage points of available phosphorus and calcium, respectively; 3 - NC + enzyme blend (250g/t); 4 - NC +enzyme complex (200g/t). The dietary energy reduction was obtained by reducing soybean oil levels and making quantitative adjustments.

The enzyme blend included amylase (160 kNU/g) + β-glucanase (280 FGB/g), xylanase (160 FXU/g), and phytase (2000 U/g). These enzymes (single-component) derived from the following microorganisms: *Bacillus amyloliquefaciens* (amylase+β-glucanase), *Thermomyces lanuginosus* (xylanase), and *Citrobacter braakii* (phytase). The enzyme complex consisted of the combination of the enzymes phytase (300 U/g), protease (700 U/g), xylanase (100 U/g), β-glucanase (200 U/g), cellulase (40 U/g), amylase (30 U/g), and pectinase (4000 U/g), produced by solid-state fermentation of the microorganism *Aspergillus niger*.

The nutrient composition of corn, soybean meal, dicalcium phosphate, and limestone was determined and the results were used for the formulation of the experimental diets. The nutrient composition of the other ingredients, corrections for corn and soybean meal amino acids, as well as the metabolizable energy values used for diet formulation followed the recommendations of Rostagno *et al*. (2011). A feeding program (Table 1) with starter (1-10 days), grower (11-22 days) and finisher (23-41 days) diets was adopted.

Both temperature (°C) and relative humidity (%) were daily monitored and recorded by means of a thermo-hygrometer. Based on the data collected daily, weekly maximum and relative humidity averages were calculated as 32.5°C and 58.9%; 34.3°C and 50.6%; 31.3°C and 66.1%; 33.3°C and 56.4%; 35.1°C and 54.3%; 33.2°C and 58.3%, respectively.

Birds and feed offer and residues were weighed on days 22 and 41 days to determine the performance variables feed intake, weight gain, and feed conversion ratio. Pen mortality was recorded daily to calculate livability and to correct feed intake.

On days 22 and 42 days old, one bird per unit, with an average weight of the experimental unit, was sacrificed and their tibiae collected for the determination of bone ash, calcium, and phosphorus contents. The birds slaughtered on day 42, which were selected on the previous day (day 41; until then submitted to the same management and feeding practices) during weighing, were also used for the determination of carcass yield, breast yield, leg (thigh and drumstick) yield, and liver relative weight. Birds were fasted for four hours, individually weighed, and sacrificed. Carcass yield was calculated as the ratio between live weight and carcass weight (carcass with no viscera, feet, head, and neck). Breast and leg yields were calculated as the ratio between carcass weight

Table 1 – Ingredient composition and calculated nutrient levels of the experimental diets.

Ingredients (%)	Phase					
	Starter		Grower		Finisher	
	PC[9]	NC[10]	PC[9]	NC[10]	PC[9]	NC[10]
Corn	54.214	57.087	59.829	62.702	65.440	68.203
Soybean meal	38.932	38.302	33.050	32.420	27.089	26.550
Soybean oil	3.040	1.240	3.551	1.751	4.090	2.310
Dicalcium phosphate	1.547	1.054	1.435	0.942	1.330	0.840
Limestone	0.731	0.733	0.709	0.711	0.717	0.719
Salt	0.464	0.463	0.380	0.379	0.320	0.320
Sodium bicarbonate 27%	0.100	0.100	0.150	0.150	0.200	0.200
Vitamin Premix [1]	0.100	0.100	0.100	0.100	0.100	0.100
Mineral Premix[2]	0.100	0.100	0.100	0.100	0.100	0.100
MHA 84%[3]	0.355	0.352	0.311	0.308	0.270	0.267
L-Lysine HCl 78%	0.177	0.188	0.175	0.186	0.145	0.156
L-Threonine 98%	0.044	0.045	0.033	0.034	0.035	0.036
Salinomicyn 12%[4]	0.050	0.050	0.050	0.050	0.050	0.050
Choline Chloride 60%	0.096	0.096	0.077	0.077	0.067	0.067
Antioxidant[5]	0.010	0.010	0.010	0.010	0.010	0.010
Enramicyn 8%[6]	0.010	0.010	0.010	0.010	0.007	0.007
Bacillus subtilis[7]	0.030	0.030	0.030	0.030	0.030	0.030
Inert material[8]	-	0.040	-	0.040	-	0.035
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutritional composition						
Crude protein (%)	21.50	21.50	19.50	19.50	18.25	18.25
AMEn (kcal/kg)	3,000	2,925	3,100	3,025	3,200	3,125
Calcium (%)	0.900	0.780	0.840	0.720	0.760	0.640
Available phosphorus (%)	0.450	0.350	0.420	0.320	0.380	0.280
Sodium (%)	0.230	0.230	0.210	0.210	0.200	0.200
Digestible lysine (%)	1.180	1.180	1.050	1.050	0.950	0.950
Digestible Met+Cys (%)	0.880	0.880	0.800	0.800	0.740	0.740
Digestible threonine (%)	0.770	0.770	0.690	0.690	0.650	0.650
Digestible tryptophan (%)	0.242	0.240	0.213	0.211	0.196	0.194
Digestible valine (%)	0.901	0.900	0.815	0.813	0.762	0.761

[1] Vitamins supplied per kg feed: vitamin A - 8,000 IU; vitamin D3 - 2,000 IU; vitamin E - 10 IU; vitamin K3 - 1.5 mg; vitamin B1 - 1.0 mg; vitamin B2 - 4.0 mg; vitamin B6 - 1.5 mg; vitamin B12 - 10 µg; pantothenic acid - 8.0 mg; niacin - 25 mg; folic acid - 0.5 mg; biotin - 0,040 mg. [2] Trace minerals supplied per kg of feed: copper - 9.0 mg; iron - 50 mg; Iodine - 1,0 mg; manganese - 70 mg; selenium - 0,3 mg and zinc - 60mg. [3] methionine hydroxy analogue.[4] Salinomycin was withdrawn from the diet during the last week of rearing (36 to 41days); [5] Santoquin - butyl hydroxy toluene and ethoxyquin. [6] The dosage of enramycin was reduced to 4 mg/kg of feed between 36 and 42 days. [7] *Bacillus subtilis* 3 x 10^5 CFU/g feed. [8] The addition of the inert material increased by 0.052 percent points owing to the withdrawal of salinomycin and adjustment of enramycin during the last week of rearing. [9] Positve Control; [10] Negative control.

and their respective weights. Liver relative weight was calculated as the percentage of liver absolute weight relative to carcass weight.

In order to determine bone ash content, the tibiae were dried at 105ºC, defatted in a fat extractor, ground, and samples were burnt at 600ºC. Bone calcium content was determined by atomic absorption spectrophotometry, while bone phosphorus content was determined by UV/visible light spectrophotometry, according to the methodology proposed by the AOAC, method 965-17 (AOAC, 1990).

The results were evaluated for error normality by the Shapiro Wilk test and then submitted to analysis of variance, using SAS statistical package (SAS Institute, 2003). Means were compared by the SNK (Student-Newman-Keuls) test, adopting 5%maximum probability level.

RESULTS AND DISCUSSION

During the period of 1 to 22 days, the best ($p<0.05$) performance responses were obtained in the PC group and in broilers fed the diets supplemented with the enzyme combinations (enzyme blend or enzyme complex) relative to the other treatments. The birds fed the NC diet presented worse feed intake, weight gain, and feed conversion ratio (Table 2) compared with positive control broilers and equal to those fed the diets supplemented with the evaluated enzyme complex.

Table 2 – Performance of 22- and 41-d-old broilers, and carcass yield, cuts yield, and liver relative weight of 42-d-old broilers fed diets supplemented or not with enzyme blends.

Characteristic	Treatments				CV(%)	*p*-value
	PC	NC	NC+EB	NC+EC		
Performance (1 to 22 days old)						
Feed intake (g/bird)	1452a	1372b	1442a	1371b	1.32	0.0001
Weight gain (g/bird)	1023a	902b	1006a	907b	2.41	0.0001
Feed conversion ratio (g/g)	1.419a	1.521b	1.433a	1.511b	2.26	0.0001
Performance (1 to 41 days old)						
Feed intake (g/bird)	4556a	4340b	4495a	4423ab	2.53	0.0044
Weight gain (g/bird)	2701a	2439c	2616b	2534b	3.19	0.0001
Feed conversion ratio (g/g)	1.687a	1.779d	1.718b	1.745c	1.44	0.0001
Livability (%)	98.70	96.15	96.12	96.55	2.97	0.2450
Carcass and cuts yield at 42 days old						
Carcass yield (%)	75.83	73.82	75.17	75.73	2.34	0.1088
Breast yield (%)	36.53	35.84	37.30	36.41	8.67	0.8333
Leg yield (%)	29.90	29.88	30.32	29.73	5.81	0.9155
Liver relative weight (%)	1.79	2.03	1.86	1.68	14.15	0.0801

Means with different letters in the row differ statistically by the SNK test (p<0.05). PC – Positive control, NC – Negative control, NC+EB – Negative control + Enzyme blend , NC+EP – Negative control + Enzyme complex. CV – Coefficient of variation.

The supplementation of the enzyme blend from 1 to 22 days allowed for similar growth performance as the positive-control diet, which mineral and energy levels were not reduced, whereas the birds supplemented with the enzyme complex presented the same performance of those fed the NC diet. These results are consistent with the findings of Barbosa *et al.* (2012), who observed that the supplementation of enzyme blends to diets with reduced mineral and energy levels promoted similar feed intake and weight gain that broilers fed a standard diet during the starter phase. Brito *et al.* (2006), using an enzyme complex containing amylase, protease, and xylanase, observed increased weight gain and improved feed conversion ratio in broilers between 1 to 21 days of age, as opposed to the findings of the present experiment. In addition, Leite *et al.* (2011), who evaluated the same enzyme complex as the current study, found better feed conversion ratio 21-d-old broilers.

The results obtained on day 41 (Table 2) indicate performance differences (p<0.05) among broilers submitted to the different treatments. The birds fed the positive control diet and those fed the NC+EBdiet presented similar (p>0.05) feed intake. The NC group presented lower feed intake, while those fed the NC+EC presented similar feed intake as the PC and NC+EB groups.

Weight gain during the entire rearing period was the highest (p<0.05) in the broilers fed the PC diet, while those fed the NC diet presented the worst result. Regardless of the enzyme combination used, broilers fed enzymes did not achieve the same weight gain as those fed the standard diet.

The best feed conversion ratio (p<0.05) was obtained in birds fed the PC diet, while those fed the NC presented the worst feed conversion ratio. Relative to the broilers supplemented with the enzyme blends, those fed the EB presented worse feed conversion ratio (a difference of 1.55%) than those fed the EC diet (p<0.05). These results are in agreement with the findings of Toledo *et al*. (2007), who evaluated enzyme supplementation to diets with different nutrient densities and observed that the performance of broilers fed a low-density diet supplemented with enzymes was better relative to those fed a low-density diet, but did not achieve the same level as those fed a standard diet. A similar response was observed by Francesh & Geraert (2009), who found that the supplementation of an enzyme blend improved the weight gain and the feed conversion ratio of broilers fed nutrient-reduced diets.

Livability was not significantly different among treatments (Table 2), and the average mortality was below 3.2%.

The nutrient and energy reductions of the NC treatment effectively allowed for the statistical performance differentiation from the PC diet, which was an important initial proposal of this study. On the other hand, Fortes *et al.* (2012), testing the addition of carbohydrases and phytase to a standard diet and to a reduced-nutrient diet, observed that dietary enzyme supplementation promoted higher feed intake and weight gain in 42-d-old broilers, but did not affect feed conversion ratio.

Differently from the results of the present study, Cowieson & Ravindran (2008) found higher weight gain and better feed conversion ratio in broilers fed diets with reduced energy and amino acid levels supplemented with an enzyme complex as compared with a standard diet with no enzyme supplementation. Lu *et al.* (2013) supplementing a blend containing xylanase, β-glucanase, and phytase to diets deficient in energy, protein, calcium, and phosphorus, observed a significant improvement in the performance of broilers supplemented with enzymes.

The high temperatures recorded in weeks 4, 5, and 6 of the rearing period, with maximum temperatures higher than 33ºC, may have influenced broiler performance. According to Bertechini (2012), it is difficult for broilers to exchange heat with the environment, and the main means of heat exchange is by panting. The increased respiratory rate in hot environments leads to a reduction in feed intake, which may affect weight gain. According Lu *et al.* (2007), chicken exposure to high temperatures causes a reduction in feed intake resulting in negative effects on growth rate. In spite of the highest feed intake (absolute) on day 41 obtained by the PC birds (4.556 kg/bird; Table 2), this value is lower than the feed intake estimated in the management guidelines of the broilers' genetic strain (Cobb, 2012), which is 4.766 kg/bird. The high environmental temperatures recorded during the grower phase possibly directly affected the birds' feed intake, resulting in lower weight gain.

Among other reasons, the enzyme supplementation may not have been efficient to allow for similar performance as that obtained with the standard diet due to the higher lipid (oil) level of the positive control diet, which has less impact on the heat increment compared with carbohydrates and proteins. The heat increment represents all energy lost during nutrient digestion, absorption, and metabolism (Sakomura & Rostagno, 2007). Therefore, the broilers fed the PC diet may have been less affected by heat stress and responded better to heat stress during the grower period.

Carcass, breast, and leg yields, and liver relative weight of 42-d-old broilers were not affected (p>0.05) by the mineral differences (0.1 and 0.12 percent points of available phosphorus and calcium) or by the energy difference (75 kcal AME) among the diets, independently of enzyme supplementation. These results are consistent with those of Carvalho *et al*. (2009) and Fortes *et al.* (2012), who supplemented enzyme blends to diets based on corn and soybean meal and found no influence of the treatments on carcass or cuts yield. Santos *et al*. (2008) evaluated the addition of xylanase, amylase, and protease to broiler diets and also did not observe any significant differences in liver relative weight.

Ash, calcium, and phosphorus contents and weights in the tibiae of 22- and 42-d-old broilers are shown in Table 3.

There were no significant differences (p>0.05) in ash, calcium, or phosphorus contents in the tibiaeof 22- or 42-d-old broilers as a function of the diets evaluated.

Ash weights were significantly different (p<0.05) among treatments with PC broilers presenting the highest ash weight, and NC the lowest, whereas

Table 3 – Content (%) and weight (mg) of ashes, calcium and phosphorus in the tibiae of birds 22- and 42-d-old broilers fed diets containing or not enzyme blends.

Characteristic	Treatments				CV(%)	*p*-value
	PC	NC	NC+EB	NC+EC		
			Bone mineralization on day 22			
Ash (%)	53.56	52.60	53.09	53.12	3.15	0.7315
Calcium (%)	16.11	15.85	15.62	16.30	3.61	0.1202
Phosphorus (%)	9.75	9.47	9.70	9.67	4.69	0.6194
Ash (mg)	1,095a	831c	1,011b	968b	8.19	0.0001
Calcium (mg)	329a	250c	297b	297b	7.72	0.0001
Phosphorus (mg)	200a	149c	185b	176b	8.96	0.0001
			Bone mineralization on day 42			
Ash (%)	61.05	59.69	61.22	61.20	2.31	0.1117
Calcium (%)	18.35	18.06	18.66	18.24	5.14	0.6345
Phosphorus (%)	11.34	10.96	11.20	10.94	4.66	0.3509
Ash (mg)	2,834a	2,465b	2,672ab	2,622ab	8.44	0.0230
Calcium (mg)	852	745	817	784	11.10	0.1165
Phosphorus (mg)	527a	452b	490ab	469ab	10.02	0.0290

Means with different letters in the same row differ statistically by the SNK test (p<0.05). PC - Positive control, NC - Negative control, NC+EP - Negative control + Enzyme blend, NC+EP - Negative Control + Enzyme complex. CV - Coefficient of variation.

those supplemented with the enzyme blends (1 or 2) higher ash weight compared with the NC birds when evaluated at 22 days of age. Tibial ash, on a weight basis, showed similar behavior to that observed for weight gain of the birds at the end of the experimental period, as broilers presenting higher weight gain also had higher ash weight.

The broilers fed the NC diet presented the lowest tibial phosphorus (mg) and calcium (mg) weights measured at 22 days of age ($p<0.05$), while the highest values were obtained in the PC broilers. The tibial phosphorus weight obtained with the supplementation of the enzyme blend was similar to that of positive control diet, but was not different from that promoted by enzyme complex. Birds supplemented with enzymes presented similar tibial calcium weight.

Francesch & Geraert (2009) found that reducing dietary nutrient levels resulted in reduced bone ash, calcium, and phosphorus contents in comparison with a standard diet. In addition, those authors observed that supplementing the nutrient-deficient diet with a xylanase, glucanase, and phytase blend, improved the bone mineralization of 21-d-old broilers, promoting similar results as those obtained with a standard diet. However, in the present study, the treatments influenced the weight but not the contents (%) of ashes, calcium and phosphorus.

The tibial ash content obtained in 22-d-old broilers in the present study are different from the responses ash obtained by Shaw *et al*. (2011), who evaluated the addition of different levels of phytase to diets with three different phosphorus levels and found that the highest phytase addition level promoted similar ash content as those found in the broilers fed the highest dietary phosphorus level.

Tibial ash and phosphorus weights determined when broilers were 42 days of age presented similar behavior as those determined when broilers were 22 days old. At 42 days of age, tibial ash and phosphorus weights of the PC-fed birds and those supplemented with both enzyme blends were similar; however, the results obtained with the enzyme treatments were not different from those of the negative control groups. Tibial calcium weight was not affected ($p>0.05$) by the diets evaluated.

The tibial ash and phosphorus content results of the present study are in agreement with those obtained by Oliveira *et al.* (2008), who did not find any effect of the addition of phytase on the tibial ash and phosphorus content of broilers. On the other hand, Onyango *et al*. (2005) reported that phytase supplementation of a low-phosphorus diet restored broiler growth rate, but ash content remained low. In addition, Walk *et al.* (2011), evaluating different of phytase, xylanase and protease combinations in broiler diets with reduced available phosphorus and calcium levels, found effect of enzyme supplementations on the ash content. Possibly, the reduced dietary calcium and phosphorus contents, as well as the reduced energy levels, limited not only muscle growth, but also skeletal growth. Birds may have presented smaller bones, but ash, calcium, and phosphorus contents were similar.

CONCLUSION

The supplementation of enzyme blends to diets with reduced calcium, phosphorus and energy levels allowed for the partial recovery of broiler growth performance and bone characteristics. The single-component enzyme blend (amylase (160 kNU/g) + β-glucanase (280 FGB/g), xylanase (160 FXU/g), and phytase (2000 U/g) provided better performance results than the enzyme complex, (phytase (300 U/g), protease (700 U/g), xylanase (100 U/g), β-glucanase (200 U/g), cellulase (40 U/g), amylase (30 U/g), and pectinase (4000 U/g)) in broilers fed marginal levels of minerals and energy.

Enzyme products did not affect carcass yield and cuts.

Further studies are needed, particularly considering, among other factors, the influence of climate onthe definition of nutrient and energy levels of diets with the inclusion of enzymes.

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