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Enzyme Complex Added to Broiler Diets: Effects on Performance, Metabolizable Energy Content, and Nitrogen and Phosphorus Balance[1]

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ABSTRACT

Two trials were performed to evaluate the effects of an enzyme complex (EC) on broiler performance, metabolizable energy, and phosphorus and nitrogen balance. In the first trial, 960 one-day-old male Cobb broilers were randomly distributed in a 3 x 2 factorial arrangement (three nutritional levels and the inclusion or not of EC), totaling six treatments with eight replicates of 20 birds per pen, and reared until 42 days of age. The control diet was formulated to meet the broilers' nutritional requirements. The other diets contained reduced available phosphorus (P), metabolizable energy, and amino acid levels, considering 100% and 150% of the EC nutritional matrix. The inclusion of the EC (200 g/ton) resulted in better broiler performance, improving weight gain in 4% and feed conversion ratio in 3% ($p<0.05$). In the second trial, 240 male broilers were randomly distributed into the same treatments with eight replicates of five birds per cage. The method of total excreta collection was used. There was an interaction ($p<0.05$) between diets and EC for P intake, nitrogen (N) intake, and N retention. The EC supplementation improved nitrogen-corrected apparent metabolizable energy values in 2.02% ($p<0.05$). Significant effects were observed on phosphorus and nitrogen balance ($p<0.05$). Phosphorus retention improved in 10.26%, nitrogen retention increased in 5.3%, while nitrogen excretion decreased in 3.3%. Based on the results of the present study, we recommend the addition of 200 g/t of the enzyme complex to broiler diets, considering nutrient reduction based on 100% of the EC nutritional matrix.

INTRODUCTION

Poultry diets are commonly based on corn and soybean meal, and their energy can be fully utilized when exogenous enzymes are added to the feed (Campestrini *et al.*, 2005). Exogenous enzymes improve the nutrient and energy digestibility of feeds commonly offered to broilers (Yang *et al.,* 2010; Hahn-Didde and Purdum, 2014). In general, enzymes breakdown plant cell walls, reduce feed viscosity, and breakdown proteins and antinutritional factors (Cowieson, 2010, Perazzo Costa *et al.,* 2015). However, these benefits depend on the type of enzymes, and each performs a specific function (e.g., phytase, carbohydrases, and proteases).

In order to improve the availability of nutrients and to minimize the adverse effects of antinutritional factors, diets containing either phytase or carbohydrase are commonly used under practical conditions of commercial broiler nutrition (Francesch & Geraert, 2009; Avila *et al.*, 2012). Previous studies reported improvements in weight gain, feed-to-gain ratio, and carcass traits of broilers supplemented with exogenous enzyme complexes (García *et al.,* 2008; Boguhn and Rodehutscord, 2010; Farran *et al.,* 2010; Campasino *et al.,* 2015).

Different strategies for enzyme utilization are applied (decreasing nutrient specifications or assigning nutrient values to the enzymes) when combining two enzyme products (Avila *et al.*, 2012). Commonly, the use of the nutritional matrix of the enzyme products is recommended for feed formulation, allowing the reduction of dietary energy, phosphorus, protein, and amino acid contents (Campasino *et al.,* 2015), which provides benefits such as reducing nutrient environmental excretion and diet costs (Dersjant-Li *et al.,* 2015). However, studies evaluating the recommendations of reduction of dietary nutrients according to the efficiency of enzyme complexes are needed in order to prevent broiler performance losses.

Thus, this study aimed at evaluating the addition of an enzyme complex on the performance, dietary metabolizable energy content, and nitrogen and phosphorus balance of broilers.

MATERIALS AND METHODS

Two experiments were conducted at the Experimental Poultry Farm of the Animal Science Department of of the Federal University of Viçosa, state of Minas Gerais, Brazil. Animal care procedures throughout the study complied with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the UFV (protocol n. 52/2013).

The same starter and grower corn- and soybean meal-based basal diets (Tables 1 and 2) were fed in both trials.

The enzyme complex (EC) consisted of the commercial product ALLZYME SSF (Alltech Agroindustrial Ltda., PR, Brazil), which contains phytase, protease, xylanase, ß-glucanase, cellulase, amylase, and pectinase.

The experimental treatments (diets) applied in both trials were: positive control (PC); PC + 200 g of Enzyme Complex (EC)/t of feed; negative Control 1 (NC1, 100% of the EC nutritional matrix); NC1 + 200 g of EC/t of feed; negative control 2 (NC2, 150% of the EC nutritional matrix); NC 2 + 200 g of EC/t of feed.

The positive control diet (PC) was formulated according to Rostagno *et al.* (2005) to meet the nutritional requirements of the broilers. The EC was added “on top” (200g/t of feed) in the experimental diets. Based on the positive control diet (PC), the available phosphorus, metabolizable energy and amino acid levels of negative control diets 1 (NC1) and 2 (NC2) were reduced, considering the nutritional matrix of the enzyme complex. Considering 100% of the nutritional matrix of the enzyme complex, the NC1 diet was formulated reducing available phosphorus level in 0.10%, metabolizable energy content in 75.05 kcal/kg, and digestible amino acid content in 2% relative to the PC diet. Considering 150% of the nutritional matrix of the enzyme complex, the NC2 diet was formulated reducing available phosphorus level in 0.15%, metabolizable energy content in 112.6 kcal/kg, and digestible amino acid content in 3% relative to the PC diet.

Table 1 – Ingredients and nutritional composition (%) of the experimental diets to broilers from 1 to 21 days of age.

Feedstuffs	Positive Control (PC)	Negative Control 1 (NC1)	Negative Control 2 (NC2)
Corn	54.895	58.596	60.391
Soybean meal (46%)	38.000	36.400	35.609
Soybean oil	3.000	1.140	0.231
Dicalcium phosphate	1.830	1.283	1.014
Limestone	0.900	1.210	1.385
Salt	0.490	0.490	0.490
DL-Methionine, 99%	0.240	0.226	0.219
L-Lysine HCl, 79%	0.100	0.110	0.115
L-Threonine, 98%	0.010	0.010	0.009
Choline chloride (60%)	0.100	0.100	0.100
[1] Vitamin Supplement	0.120	0.120	0.120
[2] Mineral Supplement	0.050	0.050	0.050
Anticoccidial agent (salinomicyn 12%)	0.055	0.055	0.055
[3] BHT	0.010	0.010	0.010
Starch	0.200	0.200	0.200
Calculated Values			
Crude Protein (%)	21.984	21.565	21.355
Metabolizable Energy (kcal/kg)	3000.0	2925.0	2888.0
Calcium, %	0.902	0.884	0.884
Available Phosphorus, %	0.451	0.350	0.300
Na, %	0.213	0.213	0.214
K, %	0.849	0.830	0.821
Cl, %	0.377	0.380	0.382
Total Lysine, %	1.263	1.236	1.222
Digestible Lysine, %	1.163	1.137	1.125
Digestible Methionine, %	0.544	0.527	0.519
Digestible Met + Cys, %	0.839	0.819	0.810
Digestible Threonine, %	0.755	0.739	0.731
Digestible Tryptophan, %	0.246	0.239	0.236
Digestible Arginine, %	1.414	1.376	1.357
Digestible Valine, %	0.925	0.908	0.899
Total Glycine + Valine, %	2.000	1.960	1.940

[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 – 4.0 g; Vit. B12 – 0.015 g; Pantothenic acid – 12.0 g; Biotin – 0.1 g; Vit. K3 – 3.0 g; Folic acid – 1.0 g; Nicotinic acid – 50.0 g; Selenium – 250.0 mg; Excipient - 1000 g; 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; Excipient - 500 g;

[3] Butylhydroxytoluene 99%.

Table 2 – Ingredients and nutritional composition (%) of the experimental diets to broilers from 21 to 42 days of age.

Feedstuffs	Positive Control (PC)	Negative Control 1 (NC1)	Negative Control 2 (NC2)
Corn	60.287	63.746	65.411
Soybean meal (46%)	31.570	30.098	29.330
Soybean oil	4.250	2.260	1.553
Dicalcium phosphate	1.650	1.113	0.843
Limestone	0.850	1.198	1.374
Salt	0.470	0.469	0.469
DL-Methionine, 99%	0.240	0.214	0.209
L-Lysine HCl, 79%	0.100	0.163	0.171
L-Threonine, 98%	0.010	0.025	0.026
Choline chloride, (60%)	0.100	0.100	0.100
[1] Vitamin Supplement	0.100	0.100	0.100
[2] Mineral Supplement	0.050	0.050	0.050
Anticoccidial agent	0.055	0.055	0.055
[3] BHT	0.010	0.010	0.010
Starch	0.200	0.200	0.200
Calculated Values			
Crude Protein (%)	19.415	19.033	18.820
Metabolizable Energy (kcal/kg)	3150.0	3075.0	3038.0
Calcium, %	0.824	0.824	0.824
Available Phosphorus, %	0.410	0.311	0.261
Na, %	0.205	0.205	0.205
K, %	0.746	0.729	0.720
Cl, %	0.356	0.359	0.361
Total Lysine, %	1.139	1.115	1.104
Digestible Lysine, %	1.050	1.029	1.019
Digestible Methionine, %	0.505	0.487	0.480
Digestible Met + Cys, %	0.775	0.755	0.747
Digestible Threonine, %	0.684	0.669	0.663
Digestible Tryptophan, %	0.213	0.207	0.203
Digestible Arginine, %	1.227	1.193	1.174
Digestible Valine, %	0.820	0.804	0.795
Total Glycine + Serine, %	1.768	1.732	1.712

[1] Vitamin supplement containing (per kg of product): Vit. A – 10,000,000 I.; Vit. D3 – 2,000,000 IU; Vit. E – 30,000 IU; Vit. B1 - 2.0 g; Vit. B2 - 6.0 g; Vit. B6 - 4.0 g; Vit. B12 - 0.015 g; Pantothenic acid - 12.0 g; Biotin - 0.1 g; Vit. K3 - 3.0 g; Folic acid - 1.0 g; Nicotinic acid - 50.0 g; Selenium - 250.0 mg; Excipient - 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; Excipient - 500 g;

[3] Butylhydroxytoluene 99%.

In the first trial, 960 one-d-old male Cobb 500 broiler chicks (average initial weight of 42.5g) were distributed according to a completely randomized design with a 3 x 2 factorial arrangement (three nutritional levels and the inclusion or not of the enzyme complex), totaling six treatments with eight replicates of 20 broilers each. Birds were housed in a conventional masonry broiler house in 1.0 m x 1.5 m pens equipped with tube feeders and nipple drinkers. Birds were offered water and feed *ad libitum* during the entire experimental period.

A minimum-maximum thermometer was used to record daily temperature inside the house. From 1 to 21 days, maximum and minimum temperatures were 31.55 ± 2.99 °C and 22.84 ± 2.24 °C, whereas from 21 to 42 days the maximum and minimum temperatures were 29.47 ± 2.89 °C and 21.76 ± 1.52 °C, respectively. During the trial, maximum and minimum house temperatures recorded were 30.95 ± 2.32 °C and 23.5 ± 2.84 °C, respectively.

Birds were weighed at the beginning of the experiment and at 42 days of age, as well as the feed supplied in order to determine feed intake and weight gain. Subsequently, the feed conversion ratio (FCR) was calculated.

In the second trial, 240 15-day-old male Cobb 500 broiler chicks were distributed according to a completely randomized experimental design into six treatments (identical to first study), with eight replicates of five birds each.

One-d-old chicks were housed in a typical broiler house divided in brooding circles, with the floor covered with wood-shavings litter. Starter feed and water were offered *ad libitum*. On day 14, birds were individually weighed and distributed in battery cages.

The study was carried out for 10 days, including five days for bird adaptation to the cages, diets, and followed by five days of total excreta collection. Excreta were collected twice daily, at 08:00 and 16:00, from the plastic-lined trays under the cages, placed in duly identified plastic bags, and stored in a freezer until the experiment was completed. At the end of the experimental period, excreta were thawed, weighed, and homogenized for the collection of samples (300 g), which were pre-dried in a forced-ventilation oven at 55 °C for 72h.

Excreta samples were analyzed for dry matter, crude protein, gross energy, and phosphorus contents at the Animal Nutrition Laboratory of the Department of Animal Science of UFV.

Nitrogen (N) content in the diets and excreta was determined by the method of Kjeldahl, as described by Silva & Queiroz (2006). Based on the nitrogen balance and gross energy results of the diets and the excreta determined in a bomb calorimeter (model Parr 1271), apparent metabolizable energy corrected for nitrogen balance (AMEn) was calculated using the equation described by Sakomura & Rostagno (2007).

All data collected from both trials were analyzed using the GLM procedure of SAS (SAS Institute, 2010, Version 9.1.3). Data were submitted to analysis of variance (ANOVA) and means were compared by the SNK test at 5% of probability level.

RESULTS AND DISCUSSION

In both trials, the average environmental temperature was below the recommended range for the starter period; however, this does not seem to have affected broiler performance.

In the first trial, there was no significant interaction (p>0.05) between dietary nutritional levels and enzyme complex (EC) for all performance traits (Table 3).

Table 3 – Performance of broilers fed diets supplemented or not with an enzyme complex from 1 to 42 days of age.

Item	Weight Gain (kg)	Feed Intake (kg)	FCR (kg/kg)
Diets[1]			
Positive Control (PC)	2.503^{A}	4.193^{A}	1.676^{A}
Negative Control 1 (NC1)	2.397^{B}	4.086^{B}	1.706^{AB}
Negative Control 2 (NC2)	2.360^{B}	4.069^{B}	1.726^{B}
Enzyme complex			
0	2.373^{x}	4.093	1.725^{y}
200 g/ton	2.466^{y}	4.139	1.679^{x}
CV (%)	2.39	2.38	2.78
p-Value			
Diets	<0.001	<0.001	<0.001
Enzyme complex	<0.001	ns	0.017
Diets x Enzyme complex	ns*	ns	ns

[1]NC1 = PC -75.05 kcal/kg ME, -0.10% available P and -2.00% Digestible AA; NC2 = PC -112.6 kcal/kg ME, -0.15% available P and -3.00% Digestible AA.

A,B Means followed by different superscripts in the same column are different by the SNK test (p<0.05).

x,y Means followed by different superscripts in the same column are different by the F test (p<0.05).

*not significant.

The broilers fed the PC diet showed higher weight gain (5.35 %) and feed intake (2.83%) than those fed the NC1 and NC2 diets, respectively (p<0.05). Despite the higher feed intake observed in birds fed PC diet, their feed-to-gain ratio was equivalent to that of birds fed NC1, and better than that ratio observed in the birds fed the NC2 diet, which may be explained by their higher weight gain.

The broilers fed diets supplemented with the EC gained 3.92% more weight than those fed the diets without EC. These results are consistent with other studies that reported higher weight gain in 42-d-old broilers fed diets with multiple enzyme complexes (Figueiredo *et al.*, 1998; West *et al.*, 2007; García *et al.*, 2008; Farran *et al.*, 2010; Tiwari *et al.*, 2010; Iwahashi *et al*, 2011).

In the present study, feed intake was not affected by EC (p>0.05), which is in agreement with the results reported by several authors (Costa, 1996; Figueiredo *et al.*, 1998). However, the birds fed the diets containing EC presented an improvement by 2.67% in FCR (p<0.05), which indicates that EC dietary supplementation enhanced mainly dietary nutrient absorption efficiency.

Table 4 – Apparent metabolizable energy (AME) and nitrogen-corrected apparent metabolizable energy (AMEn) contents of the diets with the inclusion or not of the enzyme complex.

Item	AME (kcal/kg)	AMEn (kcal/kg)
Diet[1]		
Positive Control (PC)	3426.9^{A}	3334.4^{A}
Negative Control 1 (NC1)	3356.6^{B}	3266.9^{B}
Negative Control 2 (NC2)	3355.9^{B}	3261.2^{B}
Enzyme complex		
0	3345.6^{y}	3254.5^{y}
200 g/ton	3414.2^{x}	3320.5^{x}
CV (%)	2.60	2.54
p-Value		
Diets	0.041	0.03
Enzyme complex	0.009	0.009
Diets x Enzyme complex	ns*	ns

[1]NC1 = PC -75.05 kcal/kg ME, -0.10% available P and -2.00% Digestible AA; NC2 = PC -112.6 kcal/kg ME, -0.15% available P and -3.00% Digestible AA.

A,B Means followed by different superscripts in the same column are different by the SNK test (p<0.05).

x,y Means followed by different superscripts in the same column are different by the F test (p<0.05).

*not significant.

In the second trial, there was no significant interaction between nutritional levels and enzyme complex (p>0.05) on metabolizable energy values (Table 4). AME values were higher than AMEn. According to Leeson & Summers (2001), estimated energy values need to be corrected for nitrogen balance because it is not possible to ensure that all birds will have the same growth rate in a metabolism assay. When determined in growing broilers, AME values are commonly higher than AMEn values because there is high retention of nitrogen for protein deposition, particularly when endogenous and metabolic losses are considered (Nery *et al.*, 2007).

As expected, the diets with reduced nutritional levels negatively influenced apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) values (p<0.05), whereas the PC diet promoted the highest AME and AMEn values. These results suggest that PC diet efficiently met the metabolizable energy requirements of the broilers.

The dietary inclusion of the enzyme complex significantly increased ($p<0.05$) AME and AMEn values on dry matter basis by 68.59 kcal/kg (2.06%) and 65.96 kcal/kg (2.03%), respectively. This suggests that the EC partially increased the availability of dietary non-starch polysaccharides, particularly those present in soybeans, which is rich in oligosaccharides. Several studies reported the capacity of enzymes to improve the nutrient digestibility of corn- and soybean meal-based diets (Brito *et al.*, 2006; Rutherfurd *et al.*, 2007; West *et al.*, 2007; Viana *et al.*, 2009), reducing the influence of antinutritional factors found in these feedstuffs.

Pugh and Charlton (1995) included a multi-enzyme complex, consisting of cellulose, protease, and xylanase (1.0 kg/ton), in broiler diets based on soybean meal and they obtained an increasing by 7.2% in metabolizable energy values. Brito (2003), when supplementing a multi-enzyme complex to broiler diets containing different extruded soybeans, found higher AME (2.69%) and AMEn (2.80%) values compared with non-supplemented diets.

Aiming at reducing layer feed costs, Ny *et al.* (1998) tested two diets: one containing 2872.0 kcal/kg and 17.5% of Crude Protein (CP) level, and the other with 3.5% energy reduction and the same CP level. The reduced-energy diet contained wheat middlings and was supplemented with an enzyme. Layer performance was not influenced by the diets, but a cost reduction of 5.2% was obtained with the reduced-energy diet.

This improvement in energy utilization was also found by other authors. Garcia *et al.* (2000) fed one- to 42-d-old broilers with diets based on soybean meal and extruded soybeans supplemented with α-galactosidade, pectinases, celullase. The authors obtained an improvement by 9% in metabolizable energy utilization. This benefit was also observed by Dänicke *et al.* (2000), Hew *et al.* (1998), and Charlton (1996). Iwahashi (2009), working with growing quails. They also concluded that the supplementation of an enzyme complex (xylanase and β-glucanase) to diets based on corn and soybean meal allowed the reduction of dietary energy up to 119.9 kcal of ME/kg of feed without any negative effects on performance. The differences among studies are possibly due to differences in the type and combination of enzymes applied, in feedstuffs, bird age, and dietary energy content. In fact, exogenous enzymes are commonly supplemented to increase dietary energy levels or to improve energy utilization.

The interaction between nutritional levels and enzyme complex significantly ($p<0.05$) affected phosphorus (P) intake (g/bird), as shown in Table 5. Dietary nutritional levels significantly influenced ($p<0.05$) phosphorus intake, which decreased when nutritional level was reduced. Within nutritional level, the inclusion of the enzyme complex increased phosphorus intake in 3.6% in broilers fed the PC diet, but not when birds were fed the negative control diets.

Table 5 – Phosphorus (P) intake (g/bird) of broilers fed diets containing or not the enzyme complex.

Item	Enzyme complex	
	0	200 g/ton
Diet[1]		
Positive Control (PC)	3.88[Ab]	4.02[Aa]
Negative Control 1 (NC1)	3.20[Ba]	3.14[Ba]
Negative Control 2 (NC2)	2.92[Ca]	2.96[Ca]
p-value		
Diets	<0.001	
Enzyme complex	ns*	
Diets x Enzyme complex	0.015	
CV (%)	2.84	

[1]NC1 = PC -75.05 kcal/kg ME, -0.10% available P and -2.00% Digestible AA; NC2 = PC -112.6 kcal/kg ME, -0.15% available P and -3.00% Digestible AA.

[A,B,C]Means followed by different capital letters in the same column are different by the SNK test ($p<0.05$).

[a,b]Means followed by different small letters in the same row are different by the F test ($p<0.05$).

*not significant.

There was no interaction between nutritional levels and enzyme complex ($p>0.05$) for P excretion (g/bird) and P retention (g/bird) as shown in Table 6. Phosphorus excretion was reduced ($p<0.05$) by 6.82% when the EC was included in the diet, demonstrating that the phytase present in the complex increased the availability of phosphorus present as phytate, increasing its utilization and reducing its excretion. Conversely, P retention increased by 10.3% with EC dietary addition. Higher P retention allows reducing inorganic P dietary inclusion levels as well as its excretion, contributing to reduce environmental pollution. Phosphorus retention was 4.4% higher ($p<0.05$) in the broilers fed the EC, again demonstrating the effect of phytase. Preliminary studies have shown that exogenous phytase may improve phosphorus digestibility, reducing P excretion and increasing P retention in the body (Lelis *et al.*, 2007; Graña, 2008) by breaking down the orthophosphate bound to the phytate molecule.

Table 6 – Phosphorus (P) balance of broilers fed diets supplemented or not with the enzyme complex.

Item	P excretion (g/bird)	P retention (g/bird)
Diet[1]		
Positive Control (PC)	2.05[C]	1.88[A]
Negative Control 1 (NC1)	1.60[B]	1.56[B]
Negative Control 2 (NC2)	1.46[A]	1.48[C]
Enzyme complex		
0	1.76[x]	1.56[y]
200 g/t	1.64[y]	1.72[x]
CV (%)	5.23	7.91
p-Value		
Diets	<0.001	<0.001
Enzyme complex	<0.001	<0.001
Diets x Enzyme complex	ns	ns

[1]NC1 = PC -75.05 kcal/kg ME, -0.10% available P and -2.00% Digestible AA; NC2 = PC -112.6 kcal/kg ME, -0.15% available P and -3.00% Digestible AA.

[A,B,C]Means followed by different superscripts in the same row are different by the SNK test ($p<0.05$).

[x,y]Means followed by different superscripts in the same row are different by the F test ($p<0.05$).

*not significant.

The N excretion (g/bird), intake (g/bird), and retention (g/bird) results are shown on Tables 7 and 8. There was a significant interaction ($p<0.05$) between nutritional levels and EC for N intake and N retention (both in g/bird and as a %).

Table 7 – Nitrogen (N) excretion of broilers fed diets supplemented or not with the enzyme complex.

Item	N excretion (g/bird)
Diet[1]	
Positive Control (PC)	6.86
Negative Control 1 (NC1)	6.80
Negative Control 2 (NC2)	6.90
Enzyme complex	
0	6.97[x]
200 g/t	6.74[y]
CV (%)	3.72
p-Value	
Diets	ns*
Enzyme complex	0.003
Diets x Enzyme complex	ns

[1]NC1 = PC -75.05 kcal/kg ME, -0.10% available P and -2.00% Digestible AA; NC2 = PC -112.6 kcal/kg ME, -0.15% available P and -3.00% Digestible AA.

[x,y]Means followed by different superscripts in the same column are different by the F test ($p<0.05$).

*not significant.

Table 8 – Nitrogen balance of broilers fed diets supplemented or not with the enzyme complex.

Item	N Intake (g/bird)		N Retention (g/bird)	
	Enzyme complex		Enzyme complex	
	0	200 g/ton	0	200 g/ton
Diet[1]				
Positive Control (PC)	19.35[Aa]	18.48[Bb]	12.33[Aa]	11.77[Bb]
Negative Control 1 (NC1)	17.60[Cb]	18.63[Ba]	10.65[Cb]	11.99[Ba]
Negative Control 2 (NC2)	18.52[Bb]	19.51[Aa]	11.59[Bb]	12.64[Aa]
p-value				
Diets	<0.001		<0.001	
Enzyme complex	0.002		<0.001	
Diets x Enzyme complex	<0.001		<0.001	
CV (%)	2.20		3.19	

[1]NC1 = PC -75.05 kcal/kg ME, -0.10% available P and -2.00% Digestible AA; NC2 = PC -112.6 kcal/kg ME, -0.15% available P and -3.00% Digestible AA.

[A,B,C]Means followed by different capital letters in the same column are different by the SNK test ($p<0.05$).

[a,b]Means followed by different small letters in the same row are different by the F test ($p<0.05$).

Dietary nutritional level did not influence ($p>0.05$) N excretion, but it was affected ($p<0.05$) by the addition of the EC in the diets. The supplementation of EC reduced N excretion by 3.3%. The effect of the interaction between dietary nutritional level and EC inclusion was observed only on N intake and N retention. When the EC was not added in the diets, the PC diet presented the highest nitrogen intake and the NC1 diet, the lowest ($p<0.05$). However, when the EC was supplied, the birds fed the NC2 diet showed higher ($p<0.05$) N intake than those fed the PC and NC1 diets, which were not different. Similar results were found for N retention ($p<0.05$). As expected, the NC1 and NC2 diets effectively reduced N intake and N retention, when compared with the PC diet, demonstrating the beneficial effects of the protease present in the EC added to the diets. Previous studies reported that phytate may complex with the protein molecule as well as with amino acids (AA) and small peptides in the gastrointestinal tract (Selle *et al.*, 2000). In addition, it significantly increases AA flow through the small intestine, resulting in higher endogenous AA excretion in broilers (Cowieson *et al.*, 2004).

The results of the present study suggest that the EC supplementation may relieve the adverse effects caused by the protein-phytate complex, reducing the waste of dietary nutrients, such as phosphorus, protein, and carbohydrates that compose the energy fraction of broiler diets.

Therefore, the addition of the enzyme complex to broiler diets resulted in many benefits. It improved broiler performance, with the strongest impact on weight gain (3.92% higher) and feed conversion ratio (2.74% better) between 1 and 42 days of age. AME and AMEn utilization improved by 2.05% and 2.02%, respectively. On average, phosphorus excretion was reduced by 6.82% and P retention increased by 10.26%, while nitrogen excretion was reduced by 3.30% and N retention increased by 5.30%.

CONCLUSION

Based on the results of the present study, we recommend the addition of 200 g of the evaluated enzyme complex per ton of broiler feed, considering the nutrient reduction obtained when 100% of the nutritional matrix of the enzyme complex was considered in the formulation of feeds.

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