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Determination of nutrient and energy values of cottonseed meal supplemented or not with phytase and protease for broiler chicks

Alcilene Maria Andrade Tavares-Samay[1], Wilson Moreira Dutra Junior[2], Liliane Olímpio Palhares[2], Cláudia da Costa Lopes[3*], Carlos Bôa-Viagem Rabello[2], Andrew Henrique da Silva Cavalcanti Coelho[2]

[1] Instituto Federal de Educação, Ciência e Tecnologia de Pernambuco, Campus Belo Jardim, Belo Jardim, PE, Brasil.
[2] Universidade Federal Rural de Pernambuco, Departamento de Zootecnia, Recife, PE, Brasil.
[3] Universidade Federal do Amazonas, Instituto de Ciências Sociais, Educação e Zootecnia, Departamento de Zootecnia, Parintins, AM, Brasil.

***Corresponding author:**
cclway@yahoo.com.br

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ABSTRACT - The objective of this study was to determine the nutritional and energy compositions of cottonseed meal (CM), with or without enzyme supplementation, for broilers at different ages. A total of 672 male Cobb 500 chickens were distributed into four metabolism and ileal-digestibility trials. The experimental design was completely randomized, with four treatments and six replicates. The following treatments were tested: a corn- and soybean meal-based control diet without enzymes; control diet with enzyme addition; control diet with 25% replaced by CM; and control diet with enzyme addition and 25% replaced by CM. The following variables were investigated: apparent metabolizable energy (AME); nitrogen-corrected AME (AMEn); apparent metabolizability coefficients of dry matter, gross energy, phosphorus, and calcium; apparent ileal digestibility coefficients of dry matter, crude protein, calcium, and phosphorus; and digestible protein of CM. Enzyme supplementation did not affect the energy values of CM. The average values obtained in the pre-starter, starter, grower, and finisher phases were 2,958; 2,554; 1,676, and 1,963 kcal kg^{-1} for AME and 2,519; 2,282; 1,423, and 1,680 kcal kg^{-1} for AMEn, respectively. Enzyme addition improves the apparent digestibility coefficients of phosphorus and calcium of cottonseed meal in the grower phase. However, enzyme supplementation does not affect the ileal digestibility coefficients of these ingredients in broilers.

Keywords: alternative feedstuffs, amino acids, enzymes, metabolizable energy

Introduction

Nutritionally efficient and economically viable diets for broilers are formulated on the basis of digestibility results, since the energy density of a diet directly interferes with animal performance (Brumano et al., 2006). Soybean meal is the most commonly used protein ingredient in poultry feeding; however, its price fluctuates largely due to climatic variables affecting its productivity and because it is widely consumed by humans.

For this reason, researchers are constantly investigating feedstuffs considered alternatives to poultry. One of such is cottonseed meal (CM), a byproduct of the cotton industry obtained after the oil-extraction step. On a global scale, it is the largest protein source available for animal feeding, after soybean meal

only (USDA, 2016). However, the use of CM in poultry diets is limited because of its chemical variability, as it contains crude protein contents ranging from 29.98 to 39.21%, crude fiber contents from 12.6 to 23.7%, total phosphorus contents from 0.87 to 1.03%, and lysine from 1.21 to 1.62% (Rostagno et al., 2017). The presence of gossypol, a toxic polyphenolic compound found in the cottonseed (Dalle Zotte et al., 2013) that reduces lysine utilization by broilers (Nagalakshmi et al., 2007), is another factor restricting its use.

One of the ways to maximize the utilization of these alternative ingredients by broilers is the addition of exogenous enzymes to their diet, which improve the utilization of nutrients, allowing to reduce levels of metabolizable energy and amino acids of the diets without negatively affecting animal performance. Phytases increase phosphorus availability, whereas proteases improve protein digestion. Furthermore, they allow for lesser environmental pollution due to the decreased excretion of nitrogen and phosphorus (P), since 77% of the total P in CM is in phytate-P form (Selle and Ravindran, 2007).

When combined, these two enzymes can improve the utilization of CM by broilers, allowing for a reduction in the dietary inclusion of soybean meal. Considering the above-described, the present study was undertaken to determine the nutritional, energy, and amino acid compositions of cottonseed meal with or without phytase and protease supplementation. The apparent ileal digestibility coefficients of crude protein, calcium, and phosphorus in broilers at different ages were also evaluated.

Material and Methods

All experimental procedures were conducted in accordance with the recommendations of the local Ethics Committee (case no. 042/2013). The study took place in Recife, PE, Brazil (8°02'10" S and 34°95'39" W, 18 m asl).

Four metabolism and ileal-digestibility trials were carried out consecutively, involving 672 male Cobb 500 chickens. This total corresponded to 240 chickens in the pre-starter phase (one to seven days), 192 in the starter phase (13 to 21 days), 144 in the grower phase (25 to 33 days), and 96 in the finisher phase (34 to 42 days).

Broilers were kept in three-floor batteries with cages (1.00 × 0.50 × 0.50 m) equipped with trough feeders and cup drinkers. A completely randomized design with four treatments and six replicates was adopted, totaling 24 experimental units in all phases.

The whole experiment lasted eight days, which consisted of four days dedicated to adaptation and four days for total excreta collection, except for the pre-starter phase, which was composed of three days of acclimation and four days of data collection. The last day of each phase was used for collection of ileal content. During each experimental period, the following air temperature and air relative humidity values were recorded in the pre-starter, starter, grower, and finisher phases: 31.5, 30.3, 29.1, and 28.5 °C and 58.3, 68.4, 63.8, and 68.4%, respectively.

The following treatments were examined in the study: a corn- and soybean meal-based control diet without enzymes; control diet with enzyme addition; control diet with 25% replaced by CM; and control diet with enzyme addition and 25% replaced by CM. The exogenous enzymes used in the rations were supplemented with no nutritional reductions in the diets, following the manufacturer's instructions: phytase (15 g 100 kg^{-1}), corresponding to 10,000 units of phytase per gram, and protease (20 g 100 kg^{-1}), corresponding to 84,500 units of protease per gram.

The cottonseed meal used in this study was obtained after mechanical pressing. Before it was incorporated into the diets, it was treated with ferrous sulfate at the ratio of 40 g 100 kg^{-1} to prevent the negative effect of gossypol. The chemical and energy composition of CM (Table 1) was analyzed according to methodologies described by AOAC (2000). The free gossypol content in it was determined as described in method Ba 7-58 of the American Oil Chemical Society (AOCS, 2009), whereas its

Table 1 - Chemical, energy, and amino acid compositions of cottonseed meal

Nutrient	Composition (g kg^{-1})	Amino acid	Total (g kg^{-1})	Digestibility[1] (g kg^{-1})
Dry matter	945.8	Arginine	28.4	20.8
Crude protein	242.8	Histidine	6.9	5.0
Ether extract	79.4	Isoleucine	8.2	7.4
Neutral detergent fiber	435.6	Leucine	15.1	11.1
Acid detergent fiber	318.0	Lysine	10.7	7.8
Mineral matter	75.1	Methionine	3.7	2.8
Calcium	8.8	Methionine + cystine	8.1	5.5
Phosphorus	6.3	Phenylalanine	13.9	10.8
Gross energy (kcal kg^{-1})	4,614	Threonine	8.2	6.3
Free gossypol	0.479	Valine	11.4	8.6

[1] Values estimated from the amino acid digestibility coefficients tabulated by Rostagno et al. (2017).

amino acid composition was analyzed by high-performance liquid chromatography (HPLC) through a P 6.1L AZURA® bomb.

The experimental diets (Table 2) were formulated to meet the nutritional requirements of broilers according to each rearing phase, following the recommendations of Rostagno et al. (2005). Water and feed were available *ad libitum* throughout the experimental period.

The total excreta collection method, described by Sibbald and Slinger (1963), was employed to determine the metabolizable energy value. Ferric oxide was used in the diets as a fecal marker, at the rate of 1%. During the collection period, excreta were harvested daily, weighed, identified, and stored in a freezer at –20 °C. At the end of the experimental period, they were thawed, homogenized, pre-dried in a forced-air oven at 55 °C for 72 h, ground, and sent to the laboratory.

Samples of excreta, ingredients, and diets were analyzed to determine the concentrations of dry matter (DM), nitrogen, P, and calcium (Ca) (AOAC, 2000). Gross energy (GE) was determined in a bomb calorimeter. Based on the obtained data, we determined the values of apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn), by employing equations proposed by Matterson et al. (1965), and calculated the apparent metabolizability coefficients of GE, DM, P, and Ca.

To determine apparent ileal digestibility, 1% of the chromic oxide (Cr_2O_3) indicator was added to the experimental diets on the last four days of each experimental period, as described by Sakomura and Rostagno (2016).

On the 7th, 21st, 33rd, and 42nd days, 2 h before slaughter, broilers were stimulated to consume feed to ensure a larger amount of material to be collected. After this time, all broilers were slaughtered by cervical displacement, and the digesta content present in the ileum was harvested, identified, and stored in a freezer at –20 °C. Subsequently, the samples were thawed and lyophilized for 24 h (–50 °C; –80 kPa). Next, they were ground through a ball mill and analyzed in the laboratory for the concentrations of DM, CP, Ca, P, and chromium (Cr) (AOAC, 2000).

Based on the results of the analyses of diets and digesta, we determined the apparent ileal digestibility coefficients of DM, CP, Ca, and P, in addition to digestible protein, using the following formulae:

Indigestibility factor (IF):

$$IF = \text{Diet indicator} / \text{Digesta indicator} \quad (1)$$

Apparent digestibility coefficient of dry matter:

$$ADCDM\ (\%) = 100 - (IF \times 100) \quad (2)$$

Apparent digestibility coefficient of nutrient:

$$ADC\ \text{of nutrient}\ (\%) = \text{Nutrient diet} - (\text{nutrient digesta} \times IF) / \text{nutrient diet} \times 100 \quad (3)$$

Digestible protein (DP):

$$DP = CP\ (diet / feed) \times ADCCP\ (diet / feed) / 100 \quad (4)$$

All the evaluated variables were subjected to analysis of variance and comparison of means by the t test at the 5% probability level, using SAS software (Statistical Analysis System, version 9.4).

Table 2 - Composition and nutritional values of experimental diets

Item	Experimental ration							
	Pre-starter		Starter		Grower		Finisher	
	CD	CDE	CD	CDE	CD	CDE	CD	CDE
Ingredient (g kg^{-1})								
Corn	554.43	554.43	593.87	593.87	622.16	622.16	663.88	663.88
Soybean meal	385.64	385.64	342.63	342.63	306.93	306.93	267.01	267.01
Dicalcium phosphate	19.10	19.10	18.18	18.18	16.75	16.75	15.25	15.25
Limestone	8.55	8.55	8.35	8.35	7.93	7.93	7.59	7.59
Soybean oil	17.15	17.15	24.39	24.39	34.23	34.23	33.77	33.77
Salt	4.56	4.56	4.45	4.45	4.24	4.24	4.03	4.03
DL-methionine	3.25	3.25	2.38	2.38	2.19	2.19	2.17	2.17
L-lysine	3.55	3.55	2.61	2.61	2.54	2.54	3.07	3.07
L-threonine	1.42	1.42	0.79	0.79	0.68	0.68	0.88	0.88
Mineral[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Inert[3]	0.35	0.00	0.35	0.00	0.35	0.00	0.35	0.00
Phytase	0.00	0.15	0.00	0.15	0.00	0.15	0.00	0.15
Protease	0.00	0.20	0.00	0.20	0.00	0.20	0.00	0.20
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Nutritional content (calculated)								
Metabolizable energy (kcal kg^{-1})	2,960		3,050		3,150		3,200	
Crude protein (g kg^{-1})	229.5		211.4		197.3		183.4	
Calcium (g kg^{-1})	9.42		8.99		8.37		7.75	
Available phosphorus (g kg^{-1})	4.71		4.49		4.18		3.86	
Sodium (g kg^{-1})	2.24		2.18		2.08		1.98	
Chlorine (g kg^{-1})	3.03		2.99		2.87		2.76	
Potassium (g kg^{-1})	8.76		8.09		7.51		6.91	
Fat (g kg^{-1})	41.49		49.42		59.65		60.09	
Linoleic acid (g kg^{-1})	22.63		26.98		32.58		32.84	
Crude fiber (g kg^{-1})	33.64		31.86		30.30		28.75	
Digestible amino acids (g kg^{-1})								
Methionine + cystine	9.68		8.44		7.91		7.55	
Methionine	6.35		5.31		4.94		4.75	
Lysine	13.63		11.90		10.99		10.48	
Threonine	8.86		7.73		7.14		6.81	
Tryptophan	2.56		2.33		2.13		1.92	
Arginine	14.31		13.11		12.10		11.00	
Valine	9.25		8.57		7.99		7.37	

CD - control diet without enzymes; CDE - control diet with enzyme addition.
[1] Level/kg of ration: Mn, 75,000 mg; Zn, 70,000 mg; Fe, 60,000 mg; Cu, 85,000 mg; I, 1500 mg; Co, 200 mg.
[2] Level/kg of ration: vitamin A, 1,000,000 UI; vitamin B3, 2,000,000 UI; vitamin E, 20,000 mg; vitamin K3, 4000 mg; vitamin B1, 1880 mg; vitamin B2, 5000 mg; vitamin B6, 2000 mg; vitamin B12, 1000 mg; niacin, 30,000 mg; pantothenic acid, 13,500 mg; folic acid, 500 mg; selenium, 250 mg; antioxidant, 100,000 mg.
[3] Washed and sieved sand.

Results

Phytase and protease addition in the pre-starter, starter, and finisher phases did not affect (P>0.05) the energy values or metabolizability coefficients of DM, GE, Ca, and P of cottonseed meal (Table 3). However, in the grower phase, the apparent metabolizability coefficients of P and Ca rose with the addition of enzymes, validating the efficiency of supplementation.

There were differences between the AME and AMEn values in the pre-starter, starter, grower, and finisher phases (439, 272, 253, and 283 kcal kg^{-1}, respectively). The AME values found in CM (Table 3) were higher than the AMEn values measured in the ingredient, suggesting that the nitrogen balance exhibited by the broilers was positive. In other words, there was retention of nitrogen derived from the ingredient for protein tissue deposition.

In the evaluation of CM, with or without enzyme addition, on the apparent ileal digestibility coefficients of DM, CP, Ca, and P, or on digestible protein during all phases (Table 4), no effect (P>0.05) of enzyme supplementation was found on the utilization of the ingredient.

Table 3 - Apparent metabolizable energy (AME) values determined, nitrogen-corrected AME (AMEn), and apparent metabolizability coefficients of dry matter (AMCDM), gross energy (AMCGE), phosphorus (AMCP), and calcium (AMCCa) of cottonseed meal with or without enzymes in broiler diets

	Cottonseed meal			P	CV (%)
	Without enzymes	With enzymes	Average		
		Pre-starter (one to seven days)			
AME (kcal kg^{-1})	3,001	2,915	2,958	NS	2.88
AMEn (kcal kg^{-1})	2,558	2,480	2,519	NS	3.30
AMCDM (%)	52.72	52.49	52.16	NS	7.81
AMCGE (%)	68.53	66.69	67.61	NS	3.76
AMCP (%)	15.06	15.56	15.31	NS	4.53
AMCCa (%)	15.06	15.26	15.16	NS	5.50
		Starter (13 to 21 days)			
AME (kcal kg^{-1})	2,722	2,386	2,554	NS	12.87
AMEn (kcal kg^{-1})	2,356	2,208	2,282	NS	9.43
AMCDM (%)	47.15	45.68	46.42	NS	8.19
AMCGE (%)	64.90	60.21	62.55	NS	8.86
AMCP (%)	12.59	13.00	12.80	NS	3.81
AMCCa (%)	16.53	17.02	16.78	NS	3.32
		Grower (25 to 33 days)			
AME (kcal kg^{-1})	1,580	1,771	1,676	NS	12.06
AMEn (kcal kg^{-1})	1,384	1,461	1,423	NS	13.20
AMCDM (%)	25.57	28.56	27.07	NS	12.09
AMCGE (%)	37.65	39.60	39.62	NS	6.77
AMCP (%)	12.18b	13.81a	13.00	0.002	5.51
AMCCa (%)	14.27b	16.11a	15.19	0.002	4.96
		Finisher (34 to 42 days)			
AME (kcal kg^{-1})	2,101	1,825	1,963	NS	12.44
AMEn (kcal kg^{-1})	1,798	1,562	1,680	NS	12.97
AMCDM (%)	37.25	38.51	37.88	NS	12.57
AMCGE (%)	49.66	42.44	46.05	NS	12.84
AMCP (%)	9.58	10.25	9.92	NS	8.31
AMCCa (%)	7.71	8.556	8.13	NS	9.67

P - probability; CV; coefficient of variation; NS - not significant.
Average values followed by the same letter in the row do not differ significantly by the t test at the 5% probability.

Table 4 - Apparent ileal digestibility coefficients of dry matter (ADCDM), crude protein (ADCCP), calcium (ADCCa), and phosphorus (ADCP) and digestible protein (DP) values of cottonseed meal with or without enzymes

	Cottonseed meal			P	CV (%)
	Without enzymes	With enzymes	Average		
	Pre-starter (one to seven days)				
ADCDM (%)	38.91	40.98	39.95	NS	4.21
ADCCP (%)	77.69	76.51	77.10	NS	3.25
DP (%)	16.68	16.47	16.58	NS	1.07
ADCCa (%)	17.14	17.25	17.20	NS	3.32
ADCP (%)	18.77	19.19	18.98	NS	4.05
	Starter (13 to 21 days)				
ADCDM (%)	27.53	27.61	27.57	NS	3.01
ADCCP (%)	76.11	77.89	77.00	NS	4.02
DP (%)	16.85	16.34	16.60	NS	1.00
ADCCa (%)	13.81	14.16	13.99	NS	6.60
ADCP (%)	20.39	20.81	20.60	NS	5.36
	Grower (25 to 33 days)				
ADCDM (%)	30.09	30.82	30.46	NS	5.10
ADCCP (%)	67.97	67.90	67.94	NS	1.01
DP (%)	27.85	28.69	28.27	NS	2.03
ADCCa (%)	14.39	14.86	14.63	NS	4.62
ADCP (%)	14.96	15.21	15.09	NS	5.21
	Finisher (34 to 42 days)				
ADCDM (%)	34.02	33.62	33.82	NS	4.02
ADCCP (%)	87.48	86.16	86.82	NS	4.73
DP (%)	26.81	24.93	25.87	NS	2.27
ADCCa (%)	12.86	13.14	13.00	NS	5.98
ADCP (%)	15.80	16.35	16.08	NS	4.71

P - probability; CV; coefficient of variation; NS - not significant.

Discussion

The statistical insignificance of the metabolizable energy values in CM (Table 3) in the pre-starter, starter, and finisher phases showed that enzyme supplementation without nutritional reductions in the diet was not efficient with respect to availability of some nutrients and energy of the feedstuffs.

Sheehan (2011) stated that enzymatic reactions follow the principle that the product of an enzymatic reaction is the result of the interaction between enzyme and substrate. This fact could be explained by the inadequate conditions for the activity of enzymes in the gastrointestinal tract of broilers, which is possibly assumed due to the insufficient enzyme-substrate bond on the surface of the intestinal mucosa. This is caused by the immaturity of that compartment in terms of morphological and physiological development, especially during the initial life stages, due to the short residence time of the digesta in the digestive tract of these animals. Svihus (2014) explains that the passage rate of the digesta may influence the utilization of the diet, alter the intake capacity of a feedstuff, and determine the time during which nutrients will be exposed to the action of digestive enzymes and intestinal absorptive surface.

Additionally, there are physiological limits imposed by the digestive tract conditions in response to enzyme activities. Enzymes may not be able to overcome the physiological conditions of poultry. These barriers are related to pH and the residence time of the feed in the digestive tract, which may even antagonize digestive enzymes in those animals (Cowieson, 2010).

For this reason, McCleary (2001) and Ravindran (2013) declared that the enzymatic activity in poultry diets should be sufficiently high to ensure its effect, given the very physiology of broilers. Cardoso et al. (2011) also highlighted the need for further research aiming to clarify uncertainties regarding enzymatic activity and its mechanism of action.

The AME values were higher than the AMEn values determined in CM (Table 3), indicating that the nitrogen balance shown by the broilers was positive, i.e., there was retention of the nitrogen derived from the ingredient for protein tissue deposition. According to Generoso et al. (2008), this is more perceptible when correction is made for endogenous and metabolic losses. Those authors also stressed that the retained-nitrogen value in broilers fed *ad libitum* is greater than zero, making the AME higher than the AMEn values.

In the first two phases, the average AMEn values (2,519 and 2,282 kcal kg^{-1}) were above the 1,666 kcal kg^{-1} (as-is basis) tabulated by Rostagno et al. (2017). However, they are within the range of 1,901 to 2,811 kcal kg^{-1} recommended by Nagalakshmi et al. (2007). For the grower and finisher phases, in turn, Generoso et al. (2008) reported variations in the energy values of CM from 1,625 to 1,786 kcal kg^{-1} for AME and 1,605 to 1,734 kcal kg^{-1} for AMEn, which are similar to those obtained in the present study.

Overall, the energy values of CM, mainly in the initial phases, were higher than those obtained in the grower and finisher phases even without enzyme addition. This fact emphasizes the importance of analyzing the development of organs in young broilers, since little research has been done on the absorptive capacity of the small intestine of broilers during the post-hatching period. Longo et al. (2005) stated that variations found in energy values indicate that the metabolic characteristics of each phase of broiler development can affect the energy value of feedstuffs and, consequently, alter the metabolizable energy value provided in the diet.

In the grower phase (25 to 33 days of age), enzyme supplementation provided better utilization of P and Ca in CM, as can be observed by the 13.3 and 12.9% increase in the digestibility coefficients of P and Ca, respectively, when enzyme supplementation was provided. This increased digestibility may be associated with supplementation with the phytase enzyme. Phytase enables the degradation of the phytic acid present in the plants, acting on the release of part of the fixed phosphorus and other minerals found available from the formation of insoluble complexes in the form of chelates with magnesium, zinc, and copper cations. As a result, the solubility and digestibility of these nutrients is increased (Selle and Ravindran, 2007; Singh and Satyanarayana, 2011; Gupta et al., 2013), contributing to a reduction in their excretion into the environment.

No significant differences were observed in the ileal digestibility coefficients during the pre-starter, starter, and finisher phases (Table 4). In this regard, it can be observed that the effect of enzymes, especially phytase, on the ileal digestibility and utilization of protein has been inconsistent, with highly conflicting information (Kong and Adeola, 2011). However, there are positive reports about the use of phytases on the digestibility of protein, amino acids, and phosphorus in broiler diets, such as those found by Cowieson et al. (2017), He et al. (2017), and Zouaoui et al. (2018).

The mechanism of action of protease on the ileal digestibility of protein may be considered dependent on the quality of dietary protein, since protein digestibility is benefited when protease is included in corn- and soybean meal-based diets (He et al., 2017). In the current experimental conditions, imbalanced diets were used to evaluate one ingredient with antinutritional factors. According to Murugesan et al. (2014), higher concentrations of protease or its association with carbohydrates might provide an improvement in protein digestibility.

Studies involving protease alone; enzyme complexes containing phytase, xylanase, amylase, and proteases (Barbosa et al., 2014); or even enzyme complexes containing proteases and phytases (Murugesan et al., 2014) showed improvements in the ileal digestibility of DM, CP, and minerals. However, Kong and Adeola (2011) found no additional effect of enzymes on the ileal digestibility of nutrients in broilers.

According to Barbosa et al. (2014), enzyme supplementation in diets with adequate nutritional levels does not lead to increased nutrient digestibility. Nevertheless, when this supplementation is performed along with a reduction of dietary nutritional levels, digestibility coefficients improve. The supplementation strategy used in this study (without reductions in the nutritional levels of the diet) was possibly responsible for the lack of effects on the digestibility of the evaluated nutrients.

In view of the results obtained in this study, further research should be undertaken to better examine the utilization of CM in broiler diets involving enzyme supplementation with reductions of dietary nutritional levels and considering the physiological development of the digestive tract of broilers at different ages.

Conclusions

Enzyme supplementation does not affect the energy values or the ileal digestibility coefficients of cottonseed meal. However, the enzyme rather increases the apparent digestibility coefficients of phosphorus and calcium during the grower phase of broilers, being able to provide less excretion of these nutrients in the environment.

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