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Digestibility of the cottonseed meal with or without addition of protease and phytase enzymes in swine diet

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ABSTRACT. This study evaluated the digestibility of cottonseed meal with or without addition of enzymes (phytase and protease) for growing pigs. It was used 18 barrows, housed in metabolism cages, distributed in a completely randomized design, standardizing body weight (bw) with average of 25.8 ± 3.6 kg, with three treatments and six repetitions. The treatments consisted of a reference diet based on corn and soybean meal, the second treatment with replacement of 30% of the reference diet by cottonseed meal without enzymes, and the third with 30% of the reference diet replaced by cottonseed meal with added enzymes. Was determined the digestible protein, digestible energy, digestibility of dry matter, energy and protein. It was also registered the balance of nitrogen and phosphorus. The use of cottonseed meal with the addition of enzymes in diets for growing pigs has no effect on the digestibility of dry matter, gross energy and crude protein, but improved the absorption of phosphorus, consequently reducing its excretion in the feces. There was no improvement in nitrogen balance in the diets containing cottonseed meal with enzymes.

Keywords: alternative food, exogenous enzymes, metabolism, non-ruminants.

Digestibilidade do farelo de algodão com ou sem adição das enzimas protease e fitase na alimentação de suínos

RESUMO. O objetivo do estudo foi avaliar a digestibilidade do farelo de algodão com ou sem adição de enzimas (fitase e protease) para suínos em crescimento. Foram utilizados 18 leitões machos castrados, alojados em gaiolas de metabolismo, distribuídos em delineamento inteiramente casualizado com padronização do peso vivo (PV) com média de 25,8 ± 3,6 kg, com três tratamentos e seis repetições. Os tratamentos consistiram em uma dieta referência à base de milho e farelo de soja, o segundo tratamento com substituição de 30% da dieta referência por farelo de algodão sem enzimas, e o terceiro com substituição de 30% da dieta referência por farelo de algodão com adição de enzimas. Foi determinada a proteína digestível, energia digestível, coeficiente de digestibilidade da matéria seca, da energia e da proteína. Também foi observado o balanço de nitrogênio e fósforo. O uso do farelo de algodão com adição de enzimas em dietas para suínos em crescimento, não afetou os coeficientes de digestibilidade da matéria seca, energia bruta e proteína bruta; porém contribuíram com a redução do fósforo excretado nas fezes e melhorou sua absorção. Não foi observado melhora do balanço de nitrogênio nas dietas contendo farelo de algodão com enzimas.

Palavras-chave: alimento alternativo, enzimas exógenas, metabolismo, não ruminantes.

Introduction

The frequent periods of instability, especially due to the high prices of ingredients that compound the rations, contribute to the need of introducing alternative ingredients that can solve and promote improvement in the meat quality and a reduction in the production cost (TRINDADE NETO et al., 1995).

In this search for alternative ingredients, Brazil stands out by being the fourth largest producer and exporter of cotton, with a cultivated area of 1,390.7 hectares (CONAB, 2011). With the industrialization of cotton, a byproduct is generated, the cottonseed meal, from the decortications of the seed after oil extraction, and this product has been assessed as a food alternative for soybean meal, aiming its inclusion in diets for pigs at different stages of rearing (PAIANO et al., 2006).

The use of cottonseed meal can reduce the costs with rations, but on the other hand, because it is less digestible than soybean and has variable amount of non-starch polysaccharides (NSP), it can cause adverse effect on consumption and physiological changes that alter the use of nutrients (LI et al., 1996). These NSP are resistant to hydrolysis in the digestive tract of non-ruminant animal, representing two problems: reduce the amount of energy for the animal and interfere in the use of other nutrients of the diet. These facts take place because these animals do not synthesize the enzymes necessary for digestion of certain compounds.

The insertion of additives in feed to improve the use of food as, for instance, the use of exogenous enzymes in rations, has the purpose of enhancing the action of endogenous enzymes of the pig, hence increasing the nutrients digestibility of dietary components.

The use of exogenous enzymes (amylase, protease, lipase, phytase, etc) has emerged as an alternative to increase the nutritional value of ingredients with low digestibility and significant fraction of structural non-starch polysaccharides and/or antinutritional factors, which are not hydrolyzed by digestive enzymes of pigs (FURLAN et al., 1997) thus decreasing the viscosity of the diet (PETTERSSON; AMAN, 1989).

The enzymes are globular proteins, of tertiary and quaternary structure, which operate as biological catalysts, speeding up chemical reactions in the organisms, without being changed in this process (CHAMPE; HARVEY, 1989).

Data in literature have shown favorable results regarding the use of enzyme complexes in diets for pigs using different ingredients, which promote improvement in digestibility and carcass quality of these animals, besides reduce environmental impact (HAUSCHILD et al., 2008; NERY et al., 2000; PASCOAL et al., 2008; TEIXEIRA et al., 2005). On the other hand, other studies did not present positive or satisfactory responses when evaluating these same parameters (TENÓRIO FIREMAN et al., 2000; HAUSCHILD et al., 2004; MOREIRA et al., 2009; PASCOAL et al., 2010; RODRIGUES et al., 2002; RUIZ et al., 2008).

Thus, the use of enzymes can be done aiming to complement the synthesis of endogenous enzymes of the animal or provide enzymes not produced by the swine species to degrade specific compound of ingredients of plant origin. In this way, the present study aimed to verify the effect of enzyme supplementation in diets formulated with cottonseed meal on the nutrient digestibility of pigs at growing stages.

Material and methods

The experiment was conducted at the Digestibility Laboratory of the Sector of Swine breeding of the Animal Science Department of the Federal Rural University of Pernambuco (UFRPE). It was used 18 crossbred barrows (Landrace x Large White), with average weight 25.8 ± 3.6 kg. The animals were housed individually in metabolism cages, similar to those described by Pekas (1968).

Treatments were as follows: T1 – reference diet (based on corn and soybean meal); T2 – diet with replacement of 30% of the reference diet by cottonseed meal without enzyme supplementation, and T3 – diet with 30% of the reference diet replaced by cottonseed meal with enzyme supplementation (20 g protease and 15 g phytase per 100 kg of ration).

The reference diet was formulated to meet the nutritional requirements of animals according to the weight range (ROSTAGNO et al., 2005), acording to the Table 1. Treatments T2 and T3 were elaborated through the production of the reference diet, with 30% of this replaced by cottonseed meal.

Table 1. Percentage and calculated composition of the experimental diets for barrows.

Ingredient	T1	T2	T3
		%	
Corn	70.922	49.645	49.645
Soybean meal	24.691	17.284	17.284
Cottonseed meal	---------	30.000	30.000
Soybean oil	0.994	0.696	0.696
Dicalcium phosphate	1.602	1.121	1.121
Limestone	0.579	0.405	0.405
Common salt	0.408	0.286	0.286
DL-Methionine 99%	0.008	0.006	0.006
L-Lysine Hcl	0.252	0.176	0.176
L-Threonine	0.044	0.031	0.031
Vitamin Premix[1]	0.400	0.280	0.280
Mineral Premix[1]	0.100	0.070	0.070
Total	100.00	100.00	100.00
Calculated values			
Crude protein %	17.60	21.26	21.26
Calcium %	0.720	0.573	0.573
Available phosphorus %	0.400	0.367	0.367
Methionine + Cystine %	0.584	0.693	0.693
Lysine %	1.061	1.147	1.147
Methionine %	0.289	0.340	0.340
Analyzed values			
Gross energy (kcal kg^{-1})	3756	3952	3952
Protein (%)	17.45	24.25	24.17
Neutral detergent fiber (%)	18.48	33.78	34.20
Ether extract (%)	5.05	8.21	8.55

[1]Amount per kg/diet: Folic acid 15 mg; Antioxidant- 200 mg; Biotin – 3 mg; Calcium-190 mg; Cobalt-17 mg; Copper-5,000 mg; Iron-2,500 mg; Fluorine-500 mg; Phosphorus-60 g; Iodine-13 mg; Manganese-334 mg; Niacin-479 mg; Calcium Phantothenate -240 mg; Pyridoxine-48 UI; Growth promoter-2.940 mg; Riboflavin-75 mg; Selenium-5 mg; Sodium-60 g; Thiamin-33 mg; Vit. A-150,000 UI; Vit. B12-643 mg; Vit. D3-27,000 UI; Vit. E-450 UI; Vit. K-14 mg; Zinc-2,500 mg.

Maximum and minimum temperatures and relative air humidity were measured daily at 9h00 am and 4h00 pm, respectively, using digital thermometer placed inside the barn, for monitoring the variation of environmental temperature.

The experimental design was completely randomized, with three treatments and six replications. Each repetition was considered an experimental unit represented by a metabolic cage with one animal.

The gossypol content of cottonseed meal was analyzed in the Aggeu Magalhães Research Center (FIOCRUZ/PE) through the methodology described by the American Oil Chemical Society-AOCS (1997). This technique allows measuring the gossypol in the seed, flour and cottonseed cake. The methodology consists of analyzing the gossypol of the sample extracted in the presence of 3-aminopropanol-1, which with a mixture of 2-propanol and hexane allows the determination of free gossypol. Then, using aniline, the gossypol was converted to gossypol-dianiline, whose optical density is measured at 440 nm.

It was used the method of total collection of feces, with the animals housed in metabolism cages for 11 days. The first six days were intended to adapt the animals to the cages and diets, and to determine the individual feed intake. The other five days were intended to collect the feces, twice a day, through collecting boxes below the cages. The calculation of the amount of diet given for each animal was made based on the measured intake, and the metabolic weight of each animal, with the leftovers weighted for determining the diet intake. The feeding was made twice a day, by the morning and afternoon, with the diets previously weighed. The water was given *ad libitum*.

In the diets 1% of ferric oxide powder was used as fecal marker of the beginning and end of collection. The feed intake and the total production of excreta were registered. Daily the excreta were collected, weighed, placed in plastic bags properly labeled and stored at -20ºC.

At the end of the collection period, the samples were defrosted, homogenized and aliquots were taken. The portion of feces were subjected to pre-drying in an oven with forced air ventilation at 55°C for 72 hours, and subsequent milling using a knife mill with sieve 1mm.

The samples of feces, diet and cottonseed meal were taken to the Laboratory of Animal Nutrition of the Animal Science Department (UFRPE) for the analyses of dry matter, crude protein, ether extract, phosphorus and nitrogen according to the methodology described by Silva and Queiroz (2005), and for the determination of the values of neutral detergent fiber and acid detergent fiber of the samples it was adopted the methodologies proposed by Van Soest et al. (1991), the gross energy was determined in Parr calorimeter pump.

The evaluated variables were: apparent digestibility coefficient of dry matter (ADCDM), of crude protein (ADCCP), of gross energy (ADCGE), and the values of digestible protein (DP) and digestible energy (DE). To evaluate the performance variables, at the end of the experimental period, the pigs were weighed to determine the final mean weight, weight gain and feed conversion.

The data were subjected to analysis of variance and the means were compared by the Tukey's test, using the statistical program SISVAR version 4.6 (FERREIRA, 2003).

Results and discussion

Over the experimental period, the temperature inside the installation remained between 23.4 and 34.1°C, and the relative humidity, around 59%.

The free gossypol in the samples of cottonseed meal had a concentration of 32 ppm. This value is below the limit recommended by Gamboa et al. (2001) that affirm that the maximum acceptable value of free gossypol in cottonseed meal used in swine's diet is up to 100 ppm. Thus, the value found for the cottonseed meal produced by the pressing method is below the tolerance value, so it was not necessary to add iron in the diets.

The Table 2 shows the chemical composition of cottonseed meal.

Table 2. Mean values of the contents of dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, and gross energy; content of phosphorus and free gossypol determined for the cottonseed meal.

Chemical and energetic composition[1]	Cottonseed meal[3]
Dry matter, %	93.39
Crude protein, %	24.90
Ether extract, %	9.01
Neutral detergent fiber, %	54.02
Acid detergent fiber, %	32.02
Phosphorus, %	3.80
Free gossypol, ppm	32.00
Gross energy, kcal kg^{-2}	4,484

[1]Analyses performed in the Laboratory of Animal Nutrition of the Zootechny Department from the Federal Rural University of Pernambuco; [2]Analyses performed in the Laboratory of Animal Nutrition of the Federal University of Viçosa, Minas Gerais State; [3]Cottonseed meal – Agropecuária São José.

The value of dry matter (DM) was higher to that observed by Santos et al. (2005) that found values of 89.1%, but their values of crude protein (CP) (32.1%) and acid detergent fiber (ADF) (34.1%) were higher than those of the present study. Moreira et al. (2006) observed lower values for DM, ether extract (EE) and neutral detergent fiber (NDF), with 91.90%, 0.77% and 38.69%, respectively; however the values of CP (36.22%) and ADF

(27.18%) were greater than those found in this study. The values of DM and EE were similar to the values recorded by Carvalho et al. (2010), which were 95.91% of DM and 9.80% of EE. These results point out that the ingredient has great variability that may be due to the different type of soils, climate, and processing methods.

In relation to ADF and NDF, the values are close to those verified by Carvalho et al. (2010), indicating that the cottonseed meal can present similar contents of husk in the seed.

The value determined for gross energy was 4,484 kcal kg^{-1}, close to that determined by Carvalho et al. (2010) and Albino et al. (1994) who found for the cottonseed meal 4,867 and 4,345 kcal, respectively. These values were higher than those observed by Dávila et al. (2007), Santos et al. (2005) and Rostagno et al. (2005).

No significant difference was detected between the diets with and without enzymes on the nutrient digestibility, as shown in Table 3.

The supplementation of enzymes in the diets with cottonseed meal had no effect on the digestibility of dry matter. The results of lower digestibility coefficient of DM for the diets with cottonseed meal can be associated to the level of fiber of the product, since high level of fiber in the diet for swine's can affect negatively the digestibility of dry matter (ANDERSON; LINDBERG, 1997). The fiber may have caused a reduction in the digestibility of dry matter due to the increase in the rate of passage through the intestinal tract (STANOGIAS; PEARCE, 1985).

Regarding the digestibility coefficient of CP the observed results showed a slight increase in the diets with enzymes, but not significant. This can be attributed to the smaller contact surface of the substrate with the enzyme, which makes the substrate unavailable to the action of proteases of the digestive tract of the animal. Another probable explanation is the short exposure time to enzymes, besides the fact of not being used any enzyme that could contribute to the digestion of fibrous portion, because it is possible that lignified tissues encircle protein and carbohydrates, protecting them from the enzymatic action, contributing to the non-digestion of these nutrients (BEDFORD, 2000), once the goal of the study was to evaluate only the effect of the two enzymes.

Importantly emphasizing that, not always the supplementation of digestive enzyme provides positive effects. For the enzyme to act, it is necessary the specific substrate in the diet and the adequate dosage of enzymes with ability to overcome the barriers in the gastrointestinal tract. One way to evaluate the digestibility efficiency would be by adding exogenous enzymes that degrade the fiber of the cottonseed meal, which probably would provide a better absorption of nutrients from this ingredient, which could contribute to the animal performance and to lower the final cost of production.

As for the digestibility coefficient of the gross energy, no significant difference was detected between the diets with and without addition of enzymes. This can be due to the greater amount of fibers in the diets with cottonseed meal, which negatively affect the digestibility coefficient of energy (WENK, 2001).

No significant difference between the treatments with cottonseed meal was observed, concerning the ingested content of nitrogen. There was a higher intake of nitrogen because the diets with cottonseed meal have provided a slight increase of CP in the diet.

The diets with cottonseed meal had higher amount of retained nitrogen. In some studies, the retention was lower in the diets with less protein (KEPHART; SHERRITT, 1990; KERR; EASTER, 1995), which corroborates the results found in this study.

Table 4 can be observed the values of nitrogen ingested, absorbed, retained and excreted in feces and urine of pigs submitted to different treatments.

Table 3. Values of the apparent digestibility coefficient of dry matter, crude protein, gross energy, phosphorus and values of digestible protein and digestible energy of the diets.

Treatment	Parameters					
	ADCDM %	ADCCP %	ADCGE %	CP %	DE (kcal kg^{-1})	ADCP %
Reference diet	90.99^a	74.25^a	89.25^a	12.9^b	3352^a	63.47
30% Cottonseed meal without enzyme	80.21^b	63.13^b	79.61^b	15.31^a	3164^b	62.78
30% Cottonseed meal with enzyme	80.36^b	67.48^b	78.68^b	16.31^a	3105^b	72.95
CV (%)	2.05	6.45	3.06	6.91	5.06	5.50
Significance level	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.01$

Treatments followed by different letters are significantly different by the Tukey's test, at 5% significance level; CV – coefficient of variation; ADCDM – Apparent Digestibility Coefficient of Dry Matter; ADCCP – Apparent Digestibility Coefficient of Crude Protein; ADCGE – Apparent Digestibility Coefficient of Gross Energy; DP – Digestible Protein; DE – Digestible Energy; ADCP – Apparent Digestibility Coefficient of Phosphorus.

Table 4. Mean of nitrogen ingested, excreted in feces, in urine, absorbed, and retained, in the experimental treatments with growing pigs.

Treatment	Parameters				
	Nitrogen intake (g day^{-1})	Fecal nitrogen (g day^{-1})	Nitrogen in the urine (g day^{-1})	Nitrogen retained (g day^{-1})	Absorbed nitrogen (g day^{-1})
Reference diet	46.01^{b}	4.06^{b}	15.73^{ns}	26.56^{b}	41.95^{b}
30% Cottonseed meal without enzyme	64.20^{a}	7.26^{a}	16.31^{ns}	40.63^{a}	56.94^{a}
30% Cottonseed meal with enzyme	63.77^{a}	7.23^{a}	18.26^{ns}	38.27^{a}	56.53^{a}
CV (%)	5.04	17.42	21.44	14.41	5.69
Significance level	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.01$	$p < 0.01$

Treatments followed by different letters are significantly different by the Tukey's test, at 5% significance level; Treatments followed by NS are not significantly different by the Tukey's test; CV - coefficient of variation.

The nitrogen excreted in the feces was greater in the treatments with cottonseed meal, regardless the usage or not enzymes. This can be related to fibrous ingredients and the presence of PNA, since they stimulate the fermentation in large intestine, increasing thus microbial population (WENK, 2001). These mechanisms stimulate the transfer of plasma urea to the large intestine, which is converted into ammonia by bacterial ureatic (MAMLOF; HAKANSSON, 1984). This process also increases the fecal nitrogen (GALASSI et al., 2004). When proteins are not well used, there is a high excretion of nitrogen, causing a waste of a nutrient with high cost and potential pollutant effect (FERKET, 1996). These results are similar to those achieved by Figueroa et al. (2002) and Le Bellego et al. (2001) that also verified an increase in fecal nitrogen with increasing protein in the diet.

In relation to the urinary nitrogen, it was not observed any significant difference between the treatments, however when analyzing the ratio between nitrogen retained and absorbed nitrogen, there was a greater absorption of nitrogen for the diets with cottonseed meal. Oliveira et al. (2007) verified a directly proportional relationship between the nitrogen intake and nitrogen absorbed.

The results of the mean values of intake, excretion, and absorption of phosphorus are listed in Table 5.

By analyzing the amount of phosphorus in the feces, it was observed that the diet with 30% of cottonseed meal with addition of enzymes provided a lower excretion of phosphorus. This can be associated with the action of the enzyme phytase that provides a better use of phytic phosphorus, and may reduce by 20 to 30% the excretion of this mineral (SIMONS; VERSTEEGH, 1990). This same result was registered by Figueirêdo et al. (2000), examining the action of phytase on the biological availability of phosphorus, in diets with brown rice meal, for growing swines, and observed a reduction in fecal phosphorus in the treatments using phytase.

Considering the absorbed phosphorus, the treatment with 30% of cottonseed meal with enzymes had the best result. This result can be related to the high digestibility of phosphorus in the diet due to the addition of phytase. According to Zimmermann et al. (2003) the supplementation with phytase in diets with cottonseed meal increases linearly the absorption of phosphorus, due to the better use of phytic phosphorus.

The addition of enzymes (protease and phytase) to swine diets has not promoted better digestibility of nutrients in the diets, as well as, has not reduced the nitrogen excretion. Nevertheless, it decreased the excretion of phosphorus, which may favor a reduction in environmental impact caused by this mineral.

The results of the nutrients digestibility are probably associated with the difficulties that the enzymes have to act on the cellular content of fibrous food, or because the time period had been minimal for the action of these enzymes. Finally, it would be required the use of exogenous enzymes able to promote the disruption of the cell wall of the food, so that other enzymes could access the other nutrients of the food.

Table 5. Mean of phosphorus ingested, excreted in feces, and absorbed in the experimental treatments with growing pigs.

Treatment	Parameters assessed		
	Intake of Phosphorus (g day^{-1})	Fecal Phosphorus (g day^{-1})	Absorbed Phosphorus (g day^{-1})
Reference diet	54.73^{b}	19.13^{b}	35.60^{c}
30% Cottonseed meal without enzyme	75.45^{a}	25.93^{a}	49.52^{b}
30% Cottonseed meal with enzyme	80.72^{a}	20.76^{b}	58.29^{a}
CV (%)	5.04	15.39	9.35
Significance level	$p < 0.05$	$p < 0.05$	$p < 0.05$

Treatments followed by different letters are significantly different by the Tukey's test, at 5% significance level; CV - coefficient of variation.

Conclusion

The use of the cottonseed meal with addition of enzymes in diets for growing pigs has no effect on the digestibility coefficients of dry matter, gross energy and crude protein.

The use of phytase reduces the content of phosphorus in the feces and improves the absorption of this mineral in diets with cottonseed meal for barrows.

By replacing 30% of the reference diet by the cottonseed meal with or without adding protease and phytase provides better result in the content of digestible protein calculated for diets with cottonseed meal with values of 15.3 and 16.3%, with and without enzymes, respectively.

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