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Nutritional and energetic value of rice by-products, with or without phytase, for growing pigs

Valor nutricional e energético de sub-produtos do arroz, com ou sem fitase, para suínos em crescimento

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ABSTRACT

The objective of this study was to evaluate nutritional and energetic value of rice by-products, with or without phytase, using growing pigs. Thirty-six male pigs were housed in individual metabolic cages. Total collection of feces and urine was carried out in two periods of ten days: five days for adaptation and five days for collection. A randomized blocks design was used, considering the sampling period as a block, with five treatments and seven replicates. Two control diets (with and without phytase - Phy) were used in the digestibility calculations, the latter in order to evaluate the enzyme influence on energy digestibility of the tested ingredients. The control diet was replaced by 30% of one of the ingredients: defatted rice bran (DRB) with and without Phy and dephytinised defatted rice bran (DDRB). The use of Phy in the control diet did not influence DRB+Phy energy digestibility. Relative to DRB+Phy, dephytinised defatted rice bran had higher contents of ME and digestible protein but lower digestible P and Ca. Phy supplementation increased Ca and P utilization of DRB and improved energy and protein digestibility. The DRB without Phy showed the lowest digestibility coefficients for all responses. Metabolizable energy, digestible protein, phosphorus and calcium of DRB, DRB+Phy and DDRB were respectively, 2140, 2288 and 2519kcal kg-1; 79.25, 92.41 and 107.10g kg^{-1}; 1.62, 3.41, and 2.11g kg^{-1} and 2.80, 3.79 and 2.90g kg^{-1}.

Key words: *alternative ingredients, dephytinization, enzyme, nutrients.*

RESUMO

O objetivo do presente trabalho foi avaliar o valor nutricional e energético de subprodutos do arroz, na presença ou não de fitase, para suínos em crescimento. Foram utilizados 36 suínos machos, castrados, alojados em gaiolas metabólicas individuais. Realizou-se a coleta total de fezes e urina em dois períodos de dez dias: cinco de adaptação e cinco de coleta. Utilizou-se o delineamento de blocos ao acaso, tendo-se considerado o período de coleta como bloco, com cinco tratamentos e sete repetições. Duas dietas controle (com e sem fitase - Fit) foram utilizadas nos cálculos de digestibilidade, a última para avaliar a influência da enzima na digestibilidade da energia dos ingredientes teste. A dieta controle foi substituída em 30% pelos subprodutos testados: farelo de arroz desengordurado (FAD) com ou sem Fit e farelo de arroz desengordurado desfitinizado (FADD). O uso de fitase na dieta controle não influenciou na digestibilidade da energia do FAD+Fit. Comparado ao FAD+Fit, o farelo de arroz desengordurado desfitinizado apresentou mais EM e proteína digestível, mas menos P e Ca digestíveis. A suplementação de fitase melhorou a utilização do P e Ca do FAD e aumentou a digestibilidade da energia e da proteína. O FAD sem fitase apresentou os menores coeficientes de digestibilidade para todas as respostas. Energia metabolizável, proteína, fósforo e cálcio digestíveis do FAD, FAD+Fit e FADD foram, respectivamente, 2.140; 2.288 e 2.519kcal kg^{-1}; 79,25; 92,41 e 107,10g kg^{-1}; 1,62; 3,41 e 2,11g kg^{-1} e 2,80; 3,79 e 2,90g kg^{-1}.

Palavras-chave: *desfitinização, enzima, ingredientes alternativos, nutrientes.*

INTRODUCTION

Grain-based diets contain enough total P to meet the requirements of pigs. However, 60 to 80% of P is in the form of phytate (SELLE et al., 2011); hence, the bioavailability of this P is low in pigs. Defatted rice bran contains high levels of crude protein (15.3%) and P (1.89%) (ROSTAGNO

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et al., 2011). However, about 85% of the total P is unavailable to non-ruminant animals as it is bound up in phytate (ROSTAGNO et al., 2011). This impairs P absorption by pigs and facilitates chelation with other minerals. Previous research by HASTAD et al. (2005) determined that some varieties of low-phytate corn had a greater energy content compared with conventional varieties.

The use of phytase (Phy) to increase the use of phytic acid is already known in the monogastric feed industry. Phytase catalyzes the hydrolytic degradation of phytic acid (myo-inositol hexaphosphate) and its salts (phytate), resulting in inositol, inositol monophosphate and inorganic phosphate (MULLANEY et al., 2000). According to LIAO et al. (2002), phytate may decrease starch digestibility by several mechanisms. Supplementation of phytase may release starch and α-amylase, thereby increasing the digestible energy content of the diets.

Besides the use of phytase, phytic acid removal from vegetable ingredients through industrial processes and/or laboratory methods have been employed successfully by SERVI et al. (2008) and CANAN et al. (2011). The removal of phytic acid from rice bran through these techniques aims to produce, as the main product, phytate, and generate as a by-product dephytinised defatted rice bran. Phytate is used in cosmetics and in the pharmaceutical industry, to help prevent certain human diseases such as diabetes (LEE et al., 2006) and kidney stones (SAW et al., 2007), and in the food industry to inhibit oxidative peroxidation (LEE & HENDRICKS et al., 1995). Nevertheless, dephytinised rice bran can be used in the feed industry, and may have higher nutrient availability because of its low content of phytate phosphorus. The aim of this study was to evaluate nutritional and energetic value of rice-by products, with or without phytase, using growing pigs.

MATERIAL AND METHODS

Thirty-six crossbred barrows were used (PIC sire and female DB-DanBred). The experiment was conducted in metabolic cages and divided into two periods of 10 consecutive days, with 5 days of adaptation and 5 days of total feces and urine collection. Pigs weighed 29±0.73kg and 37±0.55kg at the beginning of the first and second period, respectively.

The dephytinisation process consisted of maceration of the defatted rice bran (DRB), solubilizing the bran in an aqueous solution followed by acidification of the solution. The methodology was developed by the Federal University of Santa Maria and a private company (INGAL®). The same source of DRB used to feed the animals was used for the dephytinisation process.

A randomized blocks design was used, considering the sampling period as a block, with five treatments and seven replicates. The experimental unit was the pig. The treatments were: (CONT) Control diet (ground corn and soybean meal); (CONT+Phy) Control diet with 100mg kg^{-1} phytase; (CONT+DRB) 700g kg^{-1} Control diet and 300g kg^{-1} DRB (defatted rice bran); (CONT+DRB+Phy) 700g kg^{-1} Control diet, 300g kg^{-1} DRB and 200mg kg^{-1} phytase; (CONT+DDRB) 700g kg^{-1} Control diet, 300g kg^{-1} dephytinised defatted rice bran. The control diet with phytase was introduced because phytase could affect the reference diet, delivering nutrients that could be attributed erroneously to DRB.

The enzyme used was Quantum Blue®, an enhanced 6-phytase from ***Escherichia coli*** and with an activity of 6,800 FTU g^{-1}. The digestibility of the DRB, DRB+Phy and DDRD were calculated by the difference (indirect) approach (ADEOLA, 2001).

Pigs received water *ad libitum* and the amount of feed, distributed in two daily meals 9h and 17h, was adjusted to give an energy consumption of 2.6 times maintenance, estimated as 250kcal kg^{-1} EM×$LW^{0.60}$ (NOBLET & SHI, 1993). The Control diet was formulated according to the nutritional recommendations proposed by ROSTAGNO et al. (2011) (Table 1) for growing pigs. Rice bran was included in the diet by a difference (indirect) approach, wherein 300g kg^{-1} of the control diet on a dry matter basis was replaced with the test ingredients.

Total feces and urine were collected, and the beginning and end of each collection determined by the appearance of marked feces with the addition of 0.25% of ferric oxide (Fe_2O_3) in the diets. Feces were collected twice a day (10h and 18h), weighed, packed in plastic bags and stored in a freezer at -10°C. At the end of the experiment, the feces were homogenized and sampled (300g kg^{-1}), dried in a forced air oven at 60°C for 72 hours, and ground for later analysis. Urine was collected in plastic buckets together with 5ml H_2SO_4. The volume was weighed daily, and a 10% aliquot was removed and refrigerated at -10°C.

Diet, defatted and dephytinised rice bran and feces samples were ground in a knife mill with a one millimeter sieve, and analyzed for dry matter, crude protein, phosphorus and calcium. Gross energy was analyzed by bomb calorimetry (model C2000 - IKA Werke GmbH and Co. KG, Staufen,

Table 1 - Diet formulation and nutrient content of the control diet (CONT), defatted rice bran (DRB) and dephytinised defatted rice bran (DDRB).

Ingredients (%)	CONT		
Corn	70.10		
Soybean meal 44 CP	24.20		
Soybean oil	2.00		
Limestone	1.40		
Dicalcium phosphate	1.20		
Salt	0.50		
L-Lysine HCl (78%)	0.16		
DL-Methionine	0.23		
Ethoxiquin	0.03		
Choline Cl (60%)	0.07		
Vitamin premix*	0.04		
Mineral premix**	0.07		
Calculated and analyzed composition	CONT	DRB	DDRB
Dry matter (%)[1]	88.10	87.70	87.70
Crude protein (%)[1]	17.00	15.30	16.30
Digestible lysine (%)[1]	0.89	-	-
Digestible methionine (%)[1]	0.28	-	-
Met+cys (%)[1]	0.53	-	-
Total calcium (%)[2]	0.84	0.07	0.04
Total phosphorus (%)[2]	0.49	2.68	1.17
Phytate (%)[3]	0.24	2.16	0.94
Sodium (%)[1]	0.20	-	-
Ash (%)[2]	0.50	-	-
Ether extract (%)[2]	4.80	-	-
Starch (%)[2]	-	47.8	50.00
Gross energy (kcal kg^{-1})[2]	3,953	3,494	3,771

*Vitamin premix provided the following per kilogram of diet: Vitamin A, 11,200IU; Vitamin D3, 2,100IU; Vitamin E, 25.2mg; Vitamin K, 2.8mg; Vitamin B1, 2.24mg; Vitamin B2, 7.14mg; Vitamin B6, 2.17mg; Vitamin B12, 26µg; pantothenic acid, 18.2mg; niacin, 36.4mg; folic acid, 0.63mg; Biotin, 126mcg.
**Mineral premix provided the following per kilogram of diet: Se, 0.39mg; I, 0.46mg; Fe, 52mg; Cu, 10.4mg; Zn, 104mg; Mn, 39mg.
[1]Calculated data.
[2]Analyzed data.
[3]Date were calculated according to NRC (2012).

Germany). Urine samples from each animal were dried in a forced air oven at 60°C for 72 hours, and also analyzed for gross energy.

Determination of the coefficients of digestibility (CD) of dry matter (CDDM), gross energy (CDGE), crude protein (CDCP), calcium and phosphorus for the diets and ingredients were calculated. The values of digestible protein (DP) in the defatted and dephytinised rice bran were determined according to SAKOMURA & ROSTAGNO (2007). To calculate DE and ME of the ingredients, CAMPBELL et al. (1983) formula was used. From this calculation, the energy values of the two control diets, CONT and CONT+Phy were compared.

Data were analyzed in a completely randomized design, considering the period as a block, by ANOVA and LS Means using the GLM procedure (Statistical Analysis System, version 9.2). Pooled SEM was calculated by averaging the SEM calculated by the GLM procedure of SAS for the variable of interest. A level of $P<0.05$ was defined as indicating statistical significance.

RESULTS AND DISCUSSION

In the comparison of the two control diets (with and without Phy), there was no difference in the energy values obtained, independently of the use of phytase. Thus, all other calculations were developed using the control diet without phytase.

Dry matter intake was similar for all treatments, as expected since feed was provided in accordance with metabolic weight and the pigs had similar body weights (Table 2). The coefficients of digestibility of dry matter, gross energy, crude protein, Ca, P, as well as DE and ME of the two control diets were high compared to the other diets. This result is consistent, since the control diets had adequate nutritional balance in their formulations and less fiber due to the absence of rice bran (Table 1). However, CONT+Phy showed a higher P digestibility. Dietary starch and fiber are important nutrients for pigs but are underappreciated in their complexity (REGMI et al., 2011). Increasing rice bran levels in growing and finishing pig diets had a linear effect on nutrients digestibility (KUNRATH et al., 2010), related to the increase in fiber content, since increasing dietary fiber increases the rate of digesta passage (PARTANEN et al., 2007) and negatively affects the digestibility of many nutrients, even fiber itself (GOMES et al., 2007).

The addition of phytase to the diet with rice bran not only improved the use of Ca and P, an effect that is already well known in the literature (OMOGBENIGNUN et al., 2003), but also improved digestibility of dry matter, energy and protein, similar to the findings of ZANELLA et al. (1999). The diet with dephytinised DRB had lower dry matter digestibility than the diet with DRB+Phy, but similar gross energy and crude protein digestibility. The DE and ME of the diet with dephytinised DRB were higher compared to rice bran-containing diets. For the DDRB diet, Ca and P digestibility were lower than those for the

Table 2 - Dry matter intake, coefficients of digestibility of the control diet (CONT), CONT + phytase (CONT+Phy), CONT + defatted rice bran (CONT+DRB), CONT+DRB+Phy and CONT+dephytinised defatted rice bran (CONT+DDRB) in growing pigs.

	---Diets---					P	SEM**
Dry matter intake (g day^{-1})	CONT*	CONT+ Phy	CONT+ DRB	CONT+ DRB+ Phy	CONT+ DDRB		
	1.55	1.55	1.58	1.54	1.54	0.5	0.001
---Apparent digestibility---							
Dry matter (%)	87.3^a	87.4^a	77.5^d	80.1^b	78.8^c	<0.001	0.004
Gross energy (%)	88.4^a	88.1^a	81.3^c	82.6^b	81.8^{bc}	<0.001	0.003
Crude protein (%)	85.2^a	84.9^a	74.4^c	77.7^b	78.3^b	<0.001	0.007
Calcium (%)	65.1^a	68.2^a	41.1^c	55.7^b	44.4^c	<0.001	0.014
Phosphorus (%)	47.1^b	56.0^a	15.1^e	34.4^c	28.9^d	<0.001	0.019
DE (kcal kg^{-1})	3,493^a	3,470^a	3,121^d	3,183^c	3,229^b	<0.001	0.172
ME (kcal kg^{-1})	3,407^a	3,386^a	3,041^d	3,095^c	3,181^b	<0.024	0.188

Means sharing the same letters in rows did not differ for the LS-Means (P>0.05).
*Data represents the average of seven replicates, except for CONT+DRB+Phy, which had eight replicates.
**Standard error of mean

DRB without Phy. The DRB diet generally had the lowest nutrient digestibilities.

Regarding the digestibility of the test ingredients (Table 3), control diet of dry matter, gross energy and crude protein were higher for DRB+Phy and Dephytinised DRB than DRB. These values agree with those obtained by NAMKUNG & LEESON (1999), in which the use of phytase increased energy, minerals, and amino acid availability in the diet of broilers. In the present study, the dephytinisation process had similar effect to the use of phytase enzyme in the animal.

The digestible protein (DP) of rice bran in this study was lower than that found by ROSTAGNO et al. (2011) (79.3 vs 114.1g kg^{-1}), although similar values for crude protein content were noted (15.3 and 15.5%, respectively). A low crude protein digestibility (56.6%) was also observed by KUNRATH et al. (2010), and is close to the value seen in this experiment (51.8%), and lower than the values suggested by ROSTAGNO et al. (2011; 74.6%). These variations may be associated with intrinsic and/or extrinsic ingredient characteristics, such as source and processing. In the two cases cited as having low digestibility, the rice bran was from the south of Brazil. The type of rice processing can also influence the chemical composition of the bran (DENARDIN et al., 2004).

Table 3 - Coefficients of digestibility of the ingredients defatted rice bran (DRB), defatted rice bran + phytase (DRB+Phy) and dephytinised defatted rice bran (DDRB) in growing pigs.

	---Ingrediets---			P	SEM**
	DRB*	DRB+ Phy	DDRB		
---Apparent digestibility---					
Dry matter (%)	54.7^b	63.3^a	60.6^a	<0.003	0.016
Gross energy (%)	62.4^b	67.1^a	69.0^a	<0.020	0.020
Crude protein (%)	51.8^b	60.4^a	65.7^a	<0.003	0.023
Calcium (%)	-0.5^b	-0.2^a	-0.4^b	<0.001	0.000
Phosphorus (%)	-0.1^c	0.5^a	0.05^b	<0.001	0.001
Digestible Ca (g kg^{-1})	2.80^b	3.79^a	2.90^b	<0.001	0.000
Digestible P (g kg^{-1})	1.62^b	3.41^a	2.11^b	<0.001	0.000
Digestible protein (g kg^{-1})	79.25^c	92.41^b	107.10^a	<0.001	0.004
Digestible energy (kcal kg^{-1})	2,181^c	2,343^b	2,545^a	<0.001	0.046
Metabolizable energy (kcal kg^{-1})	2,140^c	2,288^b	2,519^a	<0.001	0.049

Means followed by same letters in rows did not differ for the LS-Means (P>0.05).
*Data represents the average of seven replicates, except for DRB+Phy, which had eight replicates.
**Standard error of mean.

The Ca and P digestibility was greater for DRB+Phy than for DRB. The phytase enzyme has proven to be successful for degrading phytic acid in the gastrointestinal tract of pigs, with increases in the amounts of calcium and phosphorus available, a response that confirms the results in table 2 and is in agreement with TRUJILLO & LINDEMANN (2010). The negative values seen in this experiment for Ca and P digestibility may be associated with the high level of complexed P in rice bran and endogenous Ca and P. The antinutritive properties of phytate include forming complexes with amino acids, inhibiting porcine proteolytic enzymes, and increasing endogenous amino acid secretions, thereby reducing apparent amino acids digestibility and decreasing availability of P, Ca, and other divalent minerals (SELLE & RAVINDRAN, 2008).

The reduced coefficients of digestibility of Ca and P in Dephytinised rice bran compared to DRB+Phy may be associated with high amounts of phytic acid still remaining in the former after the dephytinisation process. The rice bran and Dephytinised rice bran had 2.68 and 1.17% of total phosphorus, respectively (Table 1), showing that 44% of the total phosphorus remained in the dephytinised bran. According to SERVI et al. (2008), reduction of phytic acid by more than 90% could significantly improve bioavailability and absorption of minerals and proteins. Since dephytinisation did not remove all P, one can assume that some of remaining phosphorus was in the form of phytate, and supplementation with phytase could be positive. The dephytinised rice bran showed the highest values of digestible protein, DE and ME ($P<0.001$), compared to rice bran independently of the use of phytase. The higher crude protein and gross energy found in Dephytinised, compared to rice bran (16.3% and 3,771kcal kg^{-1} vs. 15.3% and 3,494kcal kg^{-1}, respectively, Table 1), and the slightly higher amount of starch (50.0 vs 47.8%) explain the higher DE values of DDRB. According to HURRELL (2004), removing phytic acid facilitates ingredient digestion because it prevents the chelation of myo-inositol hexaphosphate molecules with multivalent metal ions, especially zinc and calcium. Phytate also binds with proteins, amino acids, starch and enzymes such pepsin, trypsin and alpha amylase, compromising digestion and absorption (CASEY & WALSH, 2004). The low nutrient digestibility observed with DRB compared to DRB+Phy was also observed by SILVA et al. (2005), when phytase supplementation improved the apparent ileal digestibility of amino acids from defatted rice bran and soybean meal in growing pigs.

CONCLUSION

The dephytinization process and phytase addition improved the nutrient digestibility of defatted rice bran. Metabolizable energy, digestible protein, phosphorus and calcium of DRB, DRB+Phy and DDRB were respectively, 2,140; 2,288 and 2,519kcal kg^{-1}; 79.25; 92.41 and 107.10g kg^{-1}; 1.62; 3.41, and 2.11g kg^{-1} and 2.80; 3.79 and 2.90g kg^{-1}.

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ETHICS AND ANIMAL EXPERIMENTATION COMMITTEE

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