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Endogenous losses and true digestibility of phosphorus in rice bran with or without phytase determined with piglets

Perdas endógenas e digestibilidade verdadeira do fósforo do farelo de arroz integral com ou sem fitase determinado com leitões

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ABSTRACT

The aim of this study was to determine endogenous losses and true total digestibility of phosphorus (P) in piglets fed with diets containing rice bran (RB), and supplemented or not with phytase. The experiment was divided into three periods of 12 days each, seven for adaptation and five for total feces collection, with an interval of three days between periods. Twelve barrows with average body weights of 13.40±1.00kg at 46 days of age were distributed among six treatments consisting of diets formulated with three levels of RB (5%, 10%, and 20%) with or without the addition of 750FTUkg^{-1} of phytase. The amount of total daily feed was divided into four daily meals and water was available ad libitum. The method of total feces collection was used with ferric oxide as the fecal marker. The total consumption of P of RB origin was 0.37, 0.85 and 1.73gd^{-1} for concentrations of 5%, 10% and 20% of RB, respectively. Both RB and phytase levels had an effect on the apparent digestibility of P in RB. Results of the regression analysis of ingested P originating from the RB and absorbed P indicated endogenous losses of 527mg of Pkg^{-1} of dry matter intake, independent of phytase. Additionally, the true digestibility of P in RB was 47%, and it was not influenced by the inclusion of phytase. We concluded that the addition of 750FTU of phytase does not affect endogenous losses or the true digestibility of P in RB.

Key words: *endogenous losses, phytase, regression, total collection.*

RESUMO

O objetivo do estudo foi determinar as perdas endógenas e a digestibilidade total verdadeira do fósforo (P) do farelo de arroz integral (FAI) com ou sem suplementação de fitase. O experimento foi dividido em três períodos de 12 dias cada, sendo, sete de adaptação e cinco de coleta total de fezes, com um intervalo de três dias entre os períodos. Utilizaram-se 12 suínos castrados com peso médio de 13,40±1,00kg e 46 dias de idade, distribuídos em seis tratamentos que consistiram de dietas formuladas com três níveis de FAI (5, 10 e 20%), com adição ou não de 750FTUkg^{-1} de fitase. A quantidade de ração total foi dividida em quatro refeições diárias e a água disponibilizada à vontade. Foi utilizado o método de coleta total de fezes, utilizando o óxido férrico como marcador fecal. O consumo de P com origem no FAI foi de 0,37, 0,85 e 1,73gd^{-1} para os teores de 5, 10 e 20% de FAI, respectivamente. Tanto os níveis FAI como a fitase exerceram efeitos na digestibilidade aparente do P do FAI. Os resultados da análise de regressão entre P ingerido com origem no FAI e P absorvido indicaram perdas endógenas de 527mg de Pkg^{-1} de matéria seca ingerida (DMI), independente do uso da fitase. Além disso, a digestibilidade verdadeira do P no FAI foi de 47% e não sofreu influência da inclusão de fitase. Conclui-se que a adição de 750FTU de fitase não influencia nas perdas endógenas e nem na digestibilidade verdadeira de P no FAI.

Palavras-chave: *coleta total, fitase, perdas endógenas, regressão.*

INTRODUCTION

The amount of total phosphorus (P) in the principal raw materials used for pig diet formulation is variable, as is the proportion of total P that is bioavailable (ROSTAGNO et al., 2011). One of the measures used to express P content in food is digestibility, which indicates the digestive disappearance of P (NRC, 2012). Despite to observed with amino acids, the large intestine seems to have little influence on P homeostasis; consequently, total

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or fecal digestibility is a technically and economically viable alternative to measure P digestion (FAN et al., 2001; BOHLKE et al., 2005). However, it is necessary to make corrections for endogenous losses of P because not all P determined in feces originates from food (ALMEIDA & STEIN, 2012). Therefore, it is possible to calculate the true digestibility of P. True digestibility values are more useful in pig diet formulation, because of high variability in apparent digestibility data, even when considering a single food (FAN et al., 2001). Moreover, the apparent digestibility values of P, estimated for individual foods, are not always additive (ALMEIDA & STEIN, 2012), and this is a fundamental premise for ration formulation using the linear method.

Rice bran (RB) can be included in proportions ranging from 20% to 30% of pigs' diet, in the nursery phase (GOMES et al., 2012).Total phosphorus content of RB is high (1.67%) although about 80% is in the form of phytate, which is largely unavailable for poultry and swine (ROSTAGNO et al., 2011). Since the mid-90s, phytase has been used by animal feed industry to "improve" the bioavailability of P, calcium, amino acids and energy through phytate hydrolysis. Moreover, phytase reduce the excretion of P to the environment (DERSJANT-LI et al., 2014). Because of the possibility of including RB in piglets' diet and its high concentration of phytate, a study was conducted to determine the true total digestibility of P from RB, with or without the addition of phytase for piglets in the nursery phase.

MATERIALS AND METHODS

Twelve castrated pigs were used, in the nursery phase, with an average live weight of 13.40±1.00kg at 46 days old. The animals stayed in metabolic cages maintained in a controlled environment, at an average temperature of 22°C. The experimental period was divided into three blocks lasting 12 days (seven days for adaptation to experimental conditions and five days for fecal collection). Between each 12-day period, a formulated diet was given to meet the animals' nutritional requirements according to ROSTAGNO et al. (2011).

Six semi-purified experimental diets were prepared (Table 1), with two levels of phytase (0.750FTUkg^{-1}) and three levels of RB (5%, 10% and 20%) distributed among six treatments. Due to the use of dried blood plasma (PS; AP920), which contains P as a source of amino acids, a constant inclusion of RB levels was adopted. This strategy aims to ensure similar proportions of dietary P from RB and PS. Limestone and purified cellulose were used to maintain the Ca:P ratio and the same neutral detergent fiber (NDF) between diets, respectively. The animals received mineral and vitamin supplements.

Experimental diets were prepared according to the concept of ideal protein. In the experiment, a phytase was used from the fungus ***Aspergillus Niger***. The total amount of food provided was based on the metabolic weight (kg$^{0.75}$) of the animals and distributed over four meals per day, and water was available for consumption *ad libitum*. Wasted feed was dried in a forced ventilation

Table 1 - Composition of experimental diets.

Ingredients (%)	------------------Diets------------------		
	5% RB	10% RB	20% RB
Rice bran	5.00	10.00	20.00
Starch[1]	69.45	62.25	47.40
Sugar	15.00	15.00	15.00
Blood plasma	3.75	7.50	15.00
Cellulose	3.50	2.30	0.00
Soya oil	0.30	0.10	0.00
Limestone	0.50	1.00	2.00
Salt	0.30	0.30	0.30
Premix[2]	0.10	0.10	0.10
L- lysine	0.90	0.60	0.00
DL- methionine	0.50	0.35	0.20
L- tryptophan	0.20	0.10	0.00
L- threonine	0.50	0.40	0.00
Total	100	100	100
	----------Calculated values----------		
Metabolizable energy. kcalkg^{-1}	3434	3423	3418
Crude protein (%)[(3)]	5.50	7.88	13.57
Lysine (%)	0.98	0.99	1.03
Methionine (%)	0.53	0.43	0.36
Methionine+ Cystine (%)	0.62	0.59	0.69
Threonine (%)	0.66	0.74	0.68
Tryptophan (%)	0.25	0.20	0.20
NDF (%)[(3)]	4.22	4.22	4.30
Calcium (%)[(3)]	0.21	0.41	0.82
Total Phosphorus(%)[(3)]	0.10	0.20	0.40
Ca:P (%)	2.04	2.04	2.04
Lys:ME (%)	2.85	2.91	3.01
P-RB (%)	0.84	1.67	3.34
P-Plasma(%)	0.17	0.34	0.68
P-Plasma/P-RB	0.20	0.20	0.20

[(1)]Inclusion of 7.5g of Natuphos phytase (750FTUkg^{-1}) replacing the starch in treatments with phytase. [(2)]Quantitykg^{-1} of mineral and vitamin mixture Vit A 1.750.000UI; Vit D3 300.000UI; Vit E 3.000mg; Vit K3 400mg; Vit B1 250mg; Vit B2 750mg; Vit B6 250mg; Vit B12 3000mcg; niacin 5000mg. Pantothenic acid 3000mg; choline 3000mg; antioxidant 3750mg; Fe. 80000mg; Cu. 12.000mg; Mn. 70.000mg; Zn. 100.000mg**.** [(3)]Analyzed values.

oven at 60°C for 72h, weighed and deducted from the total amount provided.

The method of total feces collection was used with ferric oxide serving as a fecal marker to study digestibility. Feces were collected twice per day, at 8:00 am and 3:00 pm, packed in plastic bags and stored in a freezer at -18°C. At the end of each block, the material was thawed, homogenized, sampled, weighed and pre-dried in a forced ventilation oven at 60°C, for 72h.

Chemical analyses were performed according to methods outlined in AOAC, 2005. The samples were analyzed for the following components: dry matter (DM), mineral matter (MM), organic matter (OM), NDF, crude protein (CP) and P in ingredients, feed, and excretions. In P analysis, the methodology of wet digestion was used, and both readings were taken using a spectrophotometer. Formulas described by ALMEIDA & STEIN (2011) were used to calculate digestibility coefficients, assuming that P from PS had a digestibility of 92% (ROSTAGNO et al., 2011).

A 2x3, randomized factorial block design was used with two levels of phytase, three levels of RB, and six replications per treatment. The animal was considered an experimental unit. Interactions between main effects were not significant; therefore it was excluded from the statistical model. Results were subjected to analysis of variance and linear regression using the statistical program MINITAB (2013).

RESULTS AND DISCUSSION

The animals remained healthy during the experimental period and feces samples were obtained without any problem. Dry matter intake (DMI) was not affected ($P>0.05$) by RB or phytase and average individual consumption was 378gd^{-1} or 55gkg^{-1}BW$^{0.75}$ (Table 2). Inclusion of RB was associated with a linear increase ($P<0.01$) in the excreted dry matter (DME), and a reduction ($P<0.01$) in digestible dry matter (DMD). Data indicated that for each percentage point of RB increase, there was a decrease ($P<0.01$) of 0.27 percentage points in the DMD of the diet. That effect occurred despite diets had their NDF content equalized with purified cellulose in an attempt to reduce the effect of fiber on DMD. However, it is possible that differences in fiber characteristics between diets, for example, relation between soluble and insoluble fiber, were the cause of the reduction in DMD.

The total apparent digestibility coefficient (ADC) of P coming from RB (P_{RB}) increased with the inclusion of RB (Table 3), and there was a trend of phytase effect in that variable. The negative values of RB digestible P ($PDIG_{RB}$) that were observed when adding 5% RB occurred because the amount of P in feces was larger than the ingested content (YANG et al., 2007).

The addition of phytase did not alter ($P>0.05$) the linear relation intercept between P absorbed coming from RB ($PABS_{RB}$) and P ingested coming from RB ($PING_{RB}$) (mgkg^{-1}DMI) (Figure 1), indicating that phytase had no effect on endogenous losses of phosphorus. These results probably are associated with the fact that the substrate of phytase is phosphorus from phytate which is absent on EBLP (SHEN et al., 2002). Phytase addition did not influence endogenous P losses in diets containing increasing levels of canola or soybean meal (AKINMUSIRE & ADEOLA, 2009).

The average estimate of endogenous P losses was 527mgkg DMI [(663+391)/2)], a value

Table 2 - Body weight, intake, excretion and digestibility of dry matter, and phosphorus balance of diet (gd^{-1}) for piglets fed with diets containing phytase and rice bran (RB) with inclusion levels of 5, 10 and 20%.

Phytase/RB	(0 FTUkg^{-1})			(750 FTUkg^{-1})			Probability		
	5	10	20	5	10	20	SEM	Phytase	RB
ABW (kg)	11.39	13.86	13.33	12.87	14.63	14.21	0.52	0.11	0.01
DMI (gd^{-1})	379.40	394.87	352.19	346.78	387.03	405.30	17.87	0.81	0.32
DME (gd^{-1})	16.12	28.66	36.45	18.15	29.56	39.34	3.77	0.53	0.01
DMD (%)	95.32	93.20	90.76	94.53	93.47	90.89	0.79	0.84	0.00
$PING_D$ (gd^{-1})	0.53	1.28	2.45	0.57	1.27	2.69	0.13	0.40	0.00
$PEXC_D$ (gd^{-1})	0.44	0.75	1.21	0.45	0.59	1.22	0.10	0.63	0.00
$PABS_D$ (gd^{-1})	0.01	0.55	1. 23	0.12	0.68	1.47	0.13	0.24	0.00
$PDIG_D$ (%)	11.71	40.29	49.63	25.58	59.01	53.85	6.25	0.05	0.00

ABW (kg) – Average body weight; DMI (gd^{-1}) – Dry matter intake; DME (gd^{-1}) – Excreted dry matter; DMD(%) – Digestible dry matter; $PING_D$ (gd^{-1}) – Ingested phosphorus from diet; $PEXC_D$ (gd^{-1}) – Excreted phosphorus from diet; $PABS_D$ (gd^{-1}) – Absorbed phosphorus; $PDIG_D$ (%) – Digestible Phosphorus from diet; Linear Regression ($P<0.05$).

Table 3 - Phosphorus balance (gd^{-1}) of rice bran (RB) from piglets fed diets containing phytase and RB with inclusion levels of 5, 10 and 20%.

Phytase	(0FTUkg^{-1})			(750FTUkg^{-1})			Probability		
RB	5	10	20	5	10	20	SEM	Phytase	RB
$PING_{RB}$(gd^{-1})*	0.36	0.86	1.64	0.38	0.85	1.81	0.08	0.39	0.01
$PEXC_{RB}$(gd^{-1})*	0.42	0.68	1.13	0.43	0.55	1.13	0.10	0.60	0.01
$PABS_{RB}$(gd^{-1})*	-0.06	0.51	0.51	-0.04	0.30	0.68	0.11	0.24	0.01
$PDIG_{RB}$(%)*	-18.59	21.44	31.82	-9.75	35.82	35.78	6.72	0.05	0.01

$PING_{RB}$ (g d^{-1}) – Ingested phosphorus from RB; $PEXC_{RB}$ (g d^{-1}) – Excreted phosphorus from RB; $PABS_{RB}$ (g d^{-1}) –Absorbed phosphorus from RB; $PDIG_{RB}$ (%) – Digestible phosphorus from RB; Linear regression* (P<0.05).

different from zero (P<0.01). There was a relatively wide variation in estimates of endogenous P losses determined by regression. Several factors may contribute to the variation in results. It is possible to highlight chemical and physical characteristics of food. This is evident when comparing endogenous losses from pea (160mgkg^{-1}DMI; SHEN et al., 2002) or red rice (725mgkg^{-1}DMI; YANG et al., 2007), for example. However, even when analyzing the results of a single food, such as soybean meal, there are variable endogenous losses, such as 310 (FAN et al., 2001), 450 (AJAKAIYE et al., 2003) and 620mgkg^{-1}DMI (FANG et al., 2007).

True digestibility of P in RB was estimated at 47%. This was the slope of the regression line, and was higher than the value reported in other studies and presented in food composition tables. In ROSTAGNO et al. (2011), for example, the true digestibility of P in RB is 28%, while the standard digestibility is 23% in the NRC (2012). Content of true total digestible P of P in RB usually ranges between 20% and 30%. This is justified because a significant portion of P is in the form of phytate, a molecule that pigs have difficulty digesting because they do not synthesize an enzyme capable of hydrolyzing it (ROJAS & STEIN, 2012).

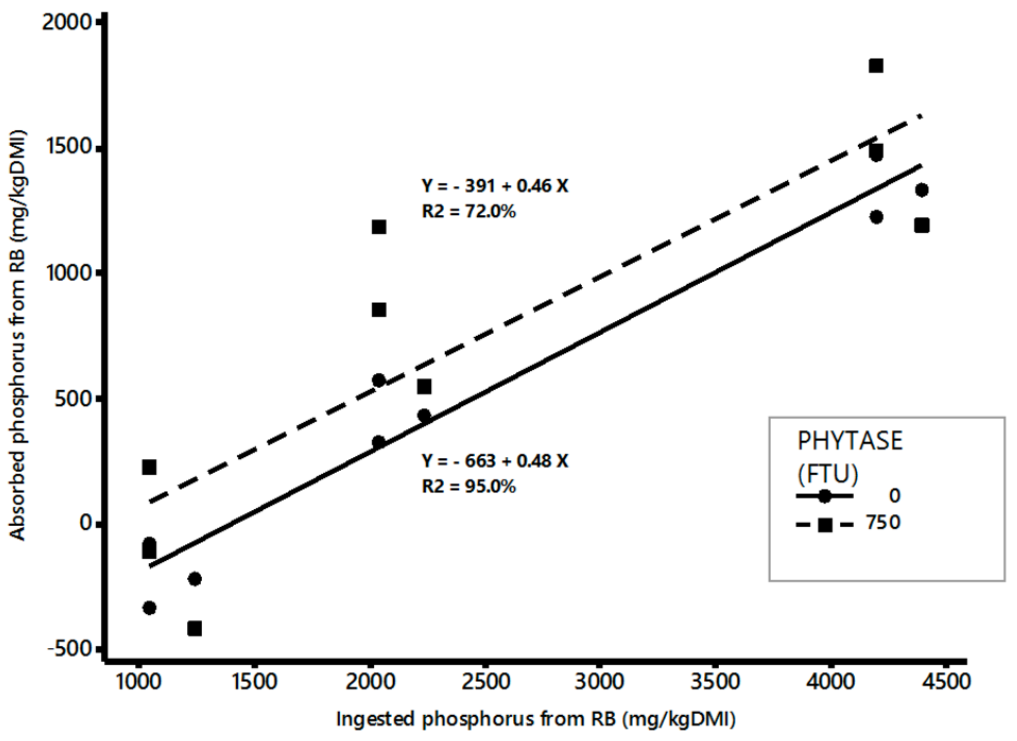

Figure 1 - Endogenous losses (regression line intercept) and true digestibility coefficient (slope of the regression line) of phosphorus in rice bran (RB), with and without addition of phytase, determined with piglets.

Thus, while the value of 47% for P digestibility in RB is higher than that reported in other studies, it is within an expected theoretical amplitude. Whereas the proportion of P in the phytate molecule reported in RB ranges from 64% to 90% (SELLE et al., 2003; GODOY et al., 2005). Assuming that 75% of total P is bound to phytate (ROSTAGNO et al., 2011) and the digestibility coefficient is 80% and 30% for phytic acid and available P, respectively (KIES et al., 2006), it is possible to reach a digestibility coefficient of 43% for total P. Moreover, FIGUEIRÊDO et al. (2000) using the isotope dilution technique reported values near 40% and 68% using the isotope method with and without the presence of 1250FTUkg^{-1} of fungal phytase, respectively, which also suggested that the true total digestibility of P in RB may be higher than the figure usually cited in the literature (ROSTAGNO et al., 2011).

There was no effect of phytase on the true digestibility of P in RB. The reasons for these results are not clear because there are some reports showing that the addition of phytase increases the P digestibility of RB in growing pigs (FIGUEIRÊDO et al., 2000; TRUJILLO et al., 2010). The results cannot exclude live weight as one of the explanations for the lack of effect in this study, since piglets may present a high gastric pH (NARCY et al., 2012), reducing the hydrolytic activity of phytase.

Another possibility to lack of phytase effect could be the phosphorus intake above the requirements of piglets since a condition to observe when using the regression method is that the test nutrient intake must be below requirements (DILGER & ADEOLA, 2006). However, assuming that the total apparent digestibility of total P in the diet is 53.8%, the total consumption of digestible P was approximately 1.45gd^{-1}, a value that represents about 70% of requirements for piglets within the weight range used in this paper (NRC, 2012).

CONCLUSION

We conclude that the addition of 750FTU of phytase does not influenced endogenous losses of phosphorus or the true digestibility of phosphorus in rice bran.

BIOETHICS AND BIOSSECURITY COMMITTE APPROVAL

The experimental protocol was reviewed and approved by the Ethics Committee in Animal Experimentation of the Universidade Federal de Santa Maria (UFSM) (Process n. 021/2013).

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