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Effect of phytase on true total tract digestibility of phosphorus in full-fat rice bran determined in pigs by regression method

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Abstract: The goal of this study was to determine the effect of phytase on true total tract digestibility of phosphorus (TTDP) and endogenous losses of phosphorus (ELP) of full-fat rice bran (FFRB) by simple linear regression. Twelve castrated pigs in the growing phase with an average live weight of 57.00 ± 2.50 kg were used. The experiment lasted 36 days, divided into three periods of 12 days each. The experimental design consisted of randomized blocks in a 2 × 3 factorial scheme, with two levels of fungal phytase (0 and 750 FTU kg^{-1}) and three levels of phosphorus (P) coming from FFRB (5%, 10%, and 20%), totaling six treatments with six replicates. The P coming from FFRB recovered in feces was influenced by phytase ($P < 0.008$) and by the amount of ingested FFRB ($P < 0.001$). The average value of ELP was 524 mg/kg dry matter intake (DMI). The TTDP of FFRB was increased by 20 percentage points due to phytase addition. In conclusion, the ELP represented 524 mg/kg DMI on average. The addition of 750 FTU of phytase increased the TTDP of FFRB, which was 32.60% and 52.60% without and with phytase, respectively.

Key words: Digestibility, fungal phytase, phosphorus endogenous losses, phytate, regression method

1. Introduction

Total digestibility is used as an indirect measure of the availability of phosphorus (P) in feed for pigs (1–3), due to the high correlation between digestibility and availability. On average, values expressed in the total digestibility correspond to 90% of availability values (4). In addition, total digestibility trials are faster and less expensive, and they can be performed more frequently when compared to comparative slaughter experiments.

The total tract digestibility can be expressed as apparent or true, but the former values are more suitable for use in the formulation of pig diets. This occurs because of high variability in apparent digestibility data, even when considering a single feedstuff (5). Moreover, the apparent digestibility values of P estimated for individual foods are not always additive (6), and this is a fundamental assumption for diet formulations using the linear method.

For the calculation of true total tract digestibility of P, it is necessary to make corrections for endogenous losses of P, which comes from the salivary juice, gastric secretion, biliary secretion, pancreatic secretion, and cell desquamation, and can be determined by a P-free diet or estimated by regression method (5). In the first case, the idea is to quantify the P recovered in feces of pigs fed a diet free of P or with highly digestible P content (7). In the regression method, diets with increasing levels of P are provided to the animals and the data of ingested and absorbed P are adjusted by simple linear regression. In this case, the intercept is an estimative of endogenous losses of P, and the slope of the regression line represents true digestibility of P (8).

Rice bran (RB) is a feedstuff with the potential to be used in diets for growing and finishing pigs. RB has 90% the metabolizable energy, 110% the digestible lysine, and five times more total P than corn. However, a significant portion of the P present in RB is bound to phytate molecules, which have low digestibility in pigs (2).

The nutritional value of RB can be increased by raising the P digestibility using phytases, enzymes that promote phytate hydrolysis releasing P for absorption and reducing their amount in feces. Thus, an experiment was carried out to determine the effect of phytase on true total tract digestibility of P (TTDP) and on endogenous losses of P (ELP) of RB by simple linear regression.

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2. Materials and methods

2.1. Experimental design, animals, and diets

Twelve castrated pigs in the growing phase with an average live weight of 57.00 ± 2.50 kg at 46 days old were used. The animals were housed in metabolic cages maintained in a controlled room at an average temperature of 22 °C. The experimental period was divided into three blocks, each lasting 12 days (7 days for adaptation to experimental conditions and 5 days for fecal collection). Between each 12-day period, a diet formulated to meet the animals' nutritional requirements was given as an attempt to reestablish their body reserves (2).

Six semipurified diets were prepared (Table 1), with two levels of phytase (750 FTU kg^{-1}) and three levels of RB (5%, 10%, and 20%) distributed among six treatments. Spray-dried porcine plasma (SDPP) was used as a source of amino acids. The Ca:P ratio was similar between treatments and the animals received vitamin and mineral supplementation. The experimental diets were prepared according to the concept of ideal protein. A fungal phytase

Table 1. Proximate and chemical composition of experimental diets, as-fed basis.

Ingredients (%)	Diets		
Full-fat rice bran	5.00	10.00	20.00
Starch	69.05	61.85	47.00
Sugar	15.00	15.00	15.00
Spray-dried porcine blood plasma	3.75	7.50	15.00
Purified cellulose	3.50	2.30	-
Soya oil	0.30	0.10	-
Limestone	0.50	1.00	2.00
Salt	0.30	0.30	0.30
Premix*	0.50	0.50	0.50
L-lysine	0.90	0.60	-
DL-methionine	0.50	0.35	0.20
L-tryptophan	0.20	0.10	-
L-threonine	0.50	0.40	-
Total	100	100	100
	Composition		
Metabolizable energy, kcal/kg	3434	3423	3418
Crude protein (%)**	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
Neutral detergent fiber (%)**	4.22	4.22	4.30
Calcium (%)**	0.21	0.41	0.82
Total phosphorus (%)**	0.17	0.32	0.66
Ca:P (%)	1,23	1,28	1,24
Lis:ME (%)	2.85	2.91	3.01

*Amount/kg of vitamin and mineral premix: Vit A 1,750,000 UI; Vit D3 300,000 UI; Vit E 3000 mg; Vit K3 400 mg; Vit B1 250 mg; Vit B2 750 mg; Vit B6 250 mg; Vit B12 3000 µg; niacin 5000 mg, pantothenic acid 3000 mg; choline, 3000 mg; antioxidant 3750 mg; Fe, 80,000 mg; Cu, 12,000 mg; Mn, 70,000 mg; Zn, 100,000 mg. Inclusion of 7.5 g of fungal phytase (750 FTU/kg^{-1}) replacing starch in treatments with phytase.
**Analyzed values.

with a minimum enzymatic activity of 5000 FTU per gram of product was used in the diets. This phytase originates from *Aspergillus niger* and is extensively used in the pig industry. This enzyme is a 3-phytase, which means that the dephosphorylation of phytic acid begins at position three on the inositol ring, with an optimum pH and temperature for action of 2–5.5 and 65 °C, respectively (9).

The animals were fed based on metabolic weight ($kg^{0.75}$) and the total amount was divided into four meals per day (0800, 1130, 1400, and 1730 hours), while water was available ad libitum. Wasted feed was dried in a forced ventilation oven at 60 °C for 72 h, weighed, and deducted from the total amount provided.

2.2. Data and sample: collections and analysis

We used total collection of feces, employing the marker-to-marker approach using ferric oxide (1%) as a fecal marker. Feces were collected twice daily, placed in plastic bags, and kept in a freezer at −18 °C. At the end of each period, the feces were thawed and homogenized, and an aliquot was removed for drying at 65 °C in a forced air oven before being milled for chemical analysis.

Chemical analyses of dry matter (method 930.15), mineral matter (MM, method 942.05), crude protein (method 977.02), and P (method 946.06) in ingredients, feed, and excretions were performed according to methods outlined by the AOAC (10). Neutral detergent fiber and acid detergent fiber were determined according to Van Soest et al. (11). The methodology of wet digestion was used, and both readings were taken with a spectrophotometer for P analysis.

2.3. Calculations and statistical analysis

Equations described by Almeida and Stein (12) were used to calculate digestibility coefficients, P content in RB, and the digestibility of P in RB, assuming that P from SDPP had digestibility of 98% (3).

The experimental design consisted of randomized blocks in a 2 × 3 factorial scheme, with two levels of phytase and three levels of P from RB, totaling six treatments with six replicates in three periods. The experimental unit was a pig, and an alpha level of 0.05 was used to test the statistical significance. The data were subjected to analysis of variance using the general linear model. Interactions were not detected and they were removed from the model. The ELP from full-fat RB (FFRB) was obtained by the regression method and is represented by the intercept of the linear relationship between the absorbed and ingested P, while the TTDP in FFRB was obtained from the slope of this equation (13). The slopes and intercepts from both equations (phytase versus no phytase) were compared using Student's t-test. All statistical analyses were performed using the statistical software MINITAB (14).

3. Results

The MM intake (MMI g/day) and excreted MM (MME g/day) were influenced ($P < 0.000$) by FFRB levels (Table 2). The MM absorption (MMA) was not influenced by phytase ($P > 0.05$), but only for FFRB content, at least in absolute terms. When expressed in proportional terms (MMD, %), no effect ($P > 0.05$) of FFRB was detected on digestibility.

As expected, there was an increase in P intake when higher proportions of FFRB were added in diets. The P from FFRB recovered in feces was influenced by phytase ($P < 0.008$) and by the amount of ingested FFRB ($P < 0.001$). This means an increase in P absorption both in grams per day and as a proportion of ingested P (%).

The apparent digestibility of P (ADP) in FFRB was affected by FFRB levels ($P < 0.001$) and by phytase addition

Table 2. Mineral matter balance of growing pigs fed diets with full-fat rice bran (FFRB) without and with fungal phytase.

Phytase FFRB (%)	(0 FTU/kg⁻¹)			(750 FTU/kg⁻¹)				Probability	
	5	10	20	5	10	20	SEM	Phytase	FFRB
Replications	6	6	6	6	6	6	-	-	-
ALW (kg)	55.85	58.10	59.4	56.38	56.77	59.49	-	-	-
DMI (g/day)	1290.10	1394.44	1454.74	1273.89	1386.87	1421.13	80.78	0.779	0.170
MMI (g/day)*	16.72	34.44	73.56	16.61	34.25	73.44	3.09	0.934	0.000
MME (g/day)*	9.20	19.18	37.75	9.97	17.47	42.95	2.60	0.577	0.000
MMA (g/day)*	7.52	14.65	35.94	6.54	16.78	30.48	1.98	0.391	0.000
MMD (%)	43.17	41.82	45.82	42.91	49.70	41.05	3.85	0.744	0.728

ALW (kg), Average live weight; DMI(g/day), dry matter intake; MMI (g/day), mineral matter ingested; MME (g/day), mineral matter excreted; MMA (g/day), mineral matter absorbed; MMD (%), mineral matter digestibility.
*Linear regression ($P < 0.05$).

($P < 0.001$) (Table 3). The ADP presented as negative values (–31.62 and –18.56 without and with phytase, respectively) when FFRB represented only 5% of diet.

For the determination of ELP and TTDP simple linear regressions between apparent digestible P and ingested P were used, generating the equations $y = -0.511 + 0.326x$ ($r^2 = 0.54$) and $y = -0.537 + 0.526x$ ($r^2 = 0.61$) without and with phytase respectively. There was no difference ($P > 0.05$) when the intercepts were compared, resulting in an average value of ELP of 524 mg/kg dry matter intake (DMI) (Table 4).

The TTDP values, estimated by the slope of the regression line, of FFRB were 32.60% and 52.60% without and with phytase, respectively. There was an increase of 20 percentage points in the P digestion due to phytase addition, which represents an increase of 60% in phytate hydrolysis.

4. Discussion

The MMI (g/day) was influenced by the FFRB level in diets due to the high mineral content of this ingredient. However, the mineral fraction of FFRB has low digestibility because of its high phytate levels (2), which probably caused the increase in MME (g/day). The proportionality between ingested and excreted mineral matter indicates that the increase in MMA was constant in relation to FFRB content and, on average, represented 48.5%. There was no difference on MMD (%) due to phytase or FFRB level. According to our expectations the proportion of MM of endogenous origin in feces increases as the dietary MM concentration decreases, creating an inverse relationship between MM level and MM digestibility (13). However, the results obtained did not confirm this statement, probably because the fiber from FFRB had stimulated "extra" endogenous losses of MM that are not stimulated by the fiber coming from the purified cellulose, used to maintain equal NDF between diets (15).

The difference in ingested P is also expected due to high P content and increasing inclusion levels of FFRB. The P from FFRB recovered in feces was influenced by phytase and by the amount of ingested FFRB, resulting in an increase in P absorption, both in grams per day and as a proportion of ingested P (%). The release of phytic acid, catalyzed by the phytase enzyme, occurs gradually through successive phytate hydrolysis until only myo-inositol and phosphate molecules remain (9). The phytase efficiency in phytic acid hydrolysis has been shown in studies by Almeida and Stein (6), Rojas and Stein (16) and Casas and Stein (17).

Negative values of ADP probably occurred because the fecal P was higher than the ingested P, due to nondietary P in feces. Similar results can be seen in studies that evaluated the P digestibility in corn (13) and brown rice (18).

Considering the phytase effect on P digestibility, two groups were made (with and without phytase) and a linear equation was adjusted for each group individually. The regression equation intercept between the ingested and absorbed P (mg/kg DMI) was an estimate of ELP (5,19). The R^2 value of the equations indicated that 54% and 61% of the variation in the dependent variable was explained by FFRB inclusion levels. In the literature, values ranging from 0.75 to 0.92 can be observed (19,20). This variation may occur by differences in experimental conditions such as animals, basal diets, and tested feedstuffs. In our study, the average value of ELP was 524 mg/kg DMI, which was not influenced by phytase. Considering that the substrate for phytase action is the P bound to phytate, it is not expected that the enzyme acts on ELP since this is phytate-free (13). Phytase addition did not influence endogenous P losses in diets containing increased levels of canola or soybean meal (19).

In the literature, the existence of variation when the ELP values were estimated by regression method is evident for either the same or different feedstuffs, which

Table 3. Phosphorus (P) balance and apparent digestibility of P in full-fat rice bran (FFRB) determined in growing pigs without and with phytase.

Phytase FFRB (%)	(0 FTU/kg^{-1})			(750 FTU/kg^{-1})				Probability	
	5	10	20	5	10	20	SEM	Phytase	FFRB
PI (g/day)*	1.18	2.57	5.33	1.17	2.54	5.32	0.22	0.894	0.000
PF (g/day)*	1.48	2.00	4.53	1.32	1.52	3.29	0.26	0.008	0.000
PA (g/day)*	–0.37	0.69	1.14	–0.22	1.09	2.20	0.25	0.016	0.000
ADP (%)*	–31.62	25.00	18.76	–18.56	42.30	39.72	5.14	0.000	0.000

PI (g/day), Ingested phosphorus; PF (g/day), fecal phosphorus; PA (g/day), absorbed phosphorus; ADP (%), apparent digestibility of phosphorus.
*Linear regression ($P < 0.05$).

Table 4. Endogenous loss and true total tract digestibility of phosphorus in full-fat rice bran (FFRB) determined in growing pigs by regression method without and with phytase.

Phytase	0 FTU/kg^{-1}		750 FTU/kg^{-1}	
Linear equation parameters	Intercept	Slope	Intercept	Slope
	-0.511	0.326	-0.537	0.526
Standard error	158,000	0.077	212,000	0.104
Probability value	0.006	0.001	0.023	0.000
R^2	0.540		0.610	
Endogenous phosphorus (mg/kg DMI/day)	511,000^x		537,000^x	
True total tract digestibility of P in FFRB (%)	32.600^x		52.600^y	

DMI, Dry matter intake; intercept is an estimate of endogenous phosphorus losses, while the slope represents the true total tract digestibility of P in FFRB. Lines with a different superscript are statistically different by Student's t-test (P < 0.05).

may be a result of differences between feedstuffs used in the basal diet, such as in the experimental methodology among studies. Dealing with soybean meal, Akinmusire and Adeola (19) observed basal ELP of 158 mg/kg DMI, whereas Zou et al. (21) and Liu et al. (22) reported losses of 294 and 434 mg/kg DMI for the same feedstuff, respectively.

The TTDP was estimated by the slope of the regression line through the relationship between ingested and absorbed P. Our values are higher than those reported by Leske and Coon (23), who observed an increase of 45% (33.2 × 48.0%) for phytate hydrolysis due to phytase addition. However, Agudelo et al. (24) related values of 110% (15 × 31.5%) in FFRB P digestibility due to phytase activity. According to the presented results, it is clear that exogenous phytase incorporation has been effective in phytate hydrolysis and FFRB organic P release. However, the range of these responses has considerable variation, resulting in different TTDP values. Factors such as optimal pH range, enzyme resistance to endogenous proteases, enzyme/substrate specificity, enzyme:substrate ratio, Ca:P ratio, and inorganic P content in the diet can interfere in the amplitude of the obtained response by phytase addition (15). Additionally, the amount of P bound to phytate molecules can range from 64% to 90% (25,26), which also may influence the response.

In our study the TTDP in FFRB was higher than the standardized values obtained by Casas and Stein (17), which were 26.4% and 41.3% without and with phytase supplementation, respectively. However, Abelilla (27) reported standardized TTDP in FFRB of 50% and 63% without and with phytase, respectively. The explanation for the variation could be related to the amount of phytic acid present in this byproduct, which can vary due to differences in the production process (17).

Stein et al. (28) recommended that basal amino acid flux be measured routinely in studies aiming to evaluate ileal digestibility because discrepancies are detected even with standardized experimental and analytical procedures. Considering the high variability observed among studies, this recommendation can be extended to studies aiming to determine the TTDP and the ELP, with or without addition of enzymes, in feedstuffs.

The correct measure of P content in feedstuffs for pigs is important from environmental and economic perspectives. Additionally, phytase has been shown to be effective in increasing P digestibility and the quantification of these effects is important for feed manufacturers and nutritionists using diets with FFRB.

In conclusion, the ELP represented 524 mg/kg DMI on average. Furthermore, phytase addition can contribute to phytic P release. In our study, 750 FTU of fungal phytase increased the TTDP of FFRB, which was 32.60% and 52.60% without and with phytase, respectively. Nevertheless, there are others phytases used in the pig industry that can be tested in further experiments. Considering the practicality of the regression method to estimate the TTDP, many other ingredients can be evaluated.

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