PHYTASE IN RATIONS OF GROWING PIGS: PERFORMANCE, BLOOD PARAMETERS AND BONE MINERAL CONTENT[1]

Fitase em rações para suínos em crescimento: desempenho, parâmetros sanguíneos e teor de minerais nos ossos

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ABSTRACT

It was intended with the present work to verify the effect of phytase on the performance, bone mineralization and content of plasma urea of swine. 80 swine (30 ± 3.1 kg/LW), crossbred (LD x LW) males and females, allocated to a randomized block design (RBD) with four treatments and five replicates. The treatments consisted of a growing swine diet on the basis of corn, soybean meal and defatted rice bran (DRB) supplemented with four levels of phytase (0, 400, 800 and 1200 FTU/kg) The diet was formulated to meet the requirements of growing swine except for available calcium and phosphorus, the levels of which were 0.64 and 0.25%, respectively. The enzyme phytase utilized was Natuphos 5000. Over the experimental period every eight days, blood of two animals per block was collected through a puncture in the sinus orbital, to determine the plasma urea content. At the final of the experimental period, two animals were slaughtered for collection of the metacarpus bone, aiming to verify the mineral content. The variables analyzed were: daily average weight gain (DAWG), daily average ration consumption (DARC), feed conversion (FC), content of plasma urea, content of ashes, calcium, phosphorus, manganese, zinc and magnesium in the bone. The phytase levels provided a linear improvement (P<0.05) on feed conversion and a quadratic effect on swine's plasma urea content. Phytase promoted a linear increase (P<0.05) on the content of calcium and phosphorus in the swine's metacarpus bone. The level of 750 FTU/kg provided the lowest plasma urea content. It is concluded that the use of the enzyme phytase in growing swine diets improved feed conversion, decreased the plasma urea content and increased the contents of calcium and phosphorus in the bone.

Index terms: Enzyme, minerals, plasma, urea.

RESUMO

Objetivou-se, com o presente trabalho, verificar o efeito da fitase sobre o desempenho, mineralização óssea e teor de uréia no plasma de suínos. Foram utilizados 80 animais com 30 ± (3,1 kg), mestiços (LD x LW), machos e fêmeas, distribuídos em um delineamento em blocos casualisados (DBC) com quatro tratamentos e cinco repetições. Os tratamentos consistiram de uma ração à base de milho, farelo de soja e farelo de arroz desengordurado (FAD), suplementada com quatro níveis de fitase (0, 400, 800 e 1200 FTU/kg). A ração foi formulada para atender às exigências de suínos em crescimento, exceto para cálcio e fósforo disponíveis, cujos níveis foram 0,64 e 0,25 %, respectivamente. A enzima fitase utilizada foi a Natuphos 5000. Durante o período experimental, a cada sete dias, foi coletado sangue de dois animais por bloco, por meio de uma punção no *sinus orbital*, para determinar o teor de uréia no plasma. Ao final do período experimental, foram abatidos dois animais por parcela para coleta do osso metacarpo, objetivando verificar o teor de minerais. As variáveis analisadas foram: ganho de peso médio diário (GPMD), consumo de ração médio diário (CRMD), conversão alimentar (CA), teor de uréia no plasma e teor de cinzas, cálcio, fósforo no osso. Os níveis de fitase proporcionaram uma melhoria linear (P<0,05) da conversão alimentar e um efeito quadrático sobre o teor de uréia no plasma dos suínos. A fitase promoveu aumento linear (P<0,05) no teor de cálcio e fósforo no osso metacarpo dos suínos. O nível de 750 FTU/kg proporcionou o menor teor de uréia no plasma. Conclui-se que a utilização da enzima fitase em rações para suínos em crescimento melhorou a conversão alimentar, diminuiu o teor de uréia no plasma e aumentou os teores de cálcio e fósforo no osso.

Termos para indexação: Enzima, minerais, plasma, uréia.

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INTRODUCTION

Pigs and poultry feeding is based on ingredients of vegetable origin, especially corn and soybean meal. About two thirds of the phosphorus contents these ingredients are complexed in the phytase acid molecule and therefore cannot be used by monogastric animals because they do not synthesize the phytase enzyme necessary to hydrolyze this complex. The occurrence of phytase as an anti-nutritional factor for non-ruminants requires phosphorus supplementation with an inorganic source, that is usually expensive, although it is present in the diets in quantities greater than the requirements of the animals. As a consequence, phytase phosphorus, because it is not available, together with the excess of inorganic phosphorus added to the rations, is eliminated in the animal feces.

The phytase enzyme, produced by the microorganism *Aspergillus niger,* has been used successfully in pig and poultry rations to release part of the phosphorus complexed in the form of phytate and improve the digestibility of the gross protein, amino acids and mineral absorption (MCKNIGHT, 1996). Rice is one of the most produced grains in the world, and is used mainly for human consumption, but because its production cost is generally greater than that of corn, only the beneficiation sub-products are used in animal nutrition.

Defatted rice meal is a sub-product that results from the extraction of the rice meal fat by solvent during edible oil production (DOMENE, 1996). Although it contains significant quantities of phosphorus most of it is unavailable, that justifies assessing the effect of phytase in diets containing these feedstuffs to make nitrogen and phosphorus and other minerals more available for the pigs, with decrease in costs and environmental pollution. The objective of the present study was to ascertain the effect of phytase on rations formulated based on corn and soybean meal and defatted rice meal on the performance, better bone mineralization and urea contents in the plasma in growing pigs.

MATERIAL METHODS

The experiment was carried out in the Swine Sector of the Animal Science Department at the Federal University of Lavras (UFLA) from May to June 2002. Eighty mixed breed pigs were used (Large White x Landrace), 40 barrows and 40 gilts, weighing 30.2 ± 3.21 (mean ± SD) kg BW. Two barrows and two gilts were kept in each pens, and each pens was an experimental unit.

The experimental treatments consisted of rations supplemented with 0, 400, 800, 1200 FTU/kg phytase, based on corn, soybean meal and defatted rice meal (FAD) and supplemented with vitamins and minerals. The experimental rations were isoprotein, isocaloric, isocalcium and isolysine, formulated to meet the demands of the pigs according to Rostagno et al. (2000), except for calcium and available phosphorus, whose values were 0.64% and 0.25%, respectively. The chemical and energetic composition of the ingredients and the percentage composition of the experimental rations are shown in Tables 1 and 2. The enzyme Natuphos G 5000 (R), batch 0000301053, was used supplied by the BASF Corp., obtained by the genetic recombination of the *Aspergillus niger* and *Aspergillus ficuun* fungi. The animals were kept in twenty 1.80 x 2 m collective pens, with a floor partially covered with wooden planks, equipped with drinking water and semiautomatic feeder. Pigs had unlimited access to feed and water throughout all experimental period.

TABLE 1 – Chemical Composition of the ingredients used in the experimental diets.

Ingredient[1]	**DM (%)[2]**	**CP (%)[2]**	**DE (Kcal/kg)[3]**	**CF (%)[2]**	**Ca (%)[2]**	**P (%)[2]**
Corn	88.90	8.75	3376	2.34	0.04	0.22
Soybean Meal	89.60	45.86	3476	5.34	0.26	0.43
RiceDefated	89.90	16.31	2531	9.86	0.10	1.99
Bicalcium Phosphate	-	-	-	-	22.04	18.04
Limestone	-	-	-	-	38.54	-

[1]As fed basis.
[2]Analisys from Animal Research Laboratório DZO/UFLA.

[3]**Data from Rostagno et al. (2000).**

TABLE 2 – Composition of experimental diets utilized in the assays.

Ingredient (%)	T1	T2	T3	T4
Corn	65,00	65,00	65,00	65,00
Soybean meal	18,63	18,63	18,63	18,63
Rice , defatted	12,50	12,50	12,50	12,50
Bicalcium Phosphate	0,70	0,70	0,70	0,70
Limestone	1,00	1,00	1,00	1,00
Salt	0,47	0,47	0,47	0,47
Mineral premix[1]	0,10	0,10	0,10	0,10
Vitamine premix[2]	0,10	0,10	0,10	0,10
Kaolim	0,50	0,42	0,35	0,26
Fitase	0,00	0,08	0,16	0,24
Calculated Values				
Digestible energy (Kcal/kg)	3374	3374	3374	3374
Crude protein (%)	16,20	16,20	16,20	16,20
Calcium (%)	0,64	0,64	0,64	0,64
Phosphorus, Total (%)	0,57	0,57	0,57	0,57
Available , Phosphorus (%)	0,25	0,25	0,25	0,25
Lysina (%)	0,82	0,82	0,82	0,82
Methionine+cisthine (%)	0,62	0,62	0,62	0,62
Methionine (%)	0,27	0,27	0,27	0,27
Triptophane (%)	0,16	0,16	0,16	0,16

1 – Minerals, supplied per kilogram of diet: Cu 30.0g; Zn 160.0g; I 1.9g; Fe 100.0g; Mn 70.0g; selenium (0.500g) , Cobalt 0,500g) and Vehicle q.s.p. 1000.0g.

2 – Vitamins, supplied per kilogram of diet: Vit.A 8.000.000 U.I.; Vit.D3, 1.200.000 U.I.; Vit. E, 20.0g; K3, 2.5g; Vit.B1, 1.0g; Vit.B2, 4.0g; Vit.B6, 2.0g; Vit. B12, 20mg; Niacin, 25.0g; Pantothenic Acid 10.0g; Biotin, 50mg; Folic Acid, 600mg; Vit.C, 50.0g; Antioxidant, 125mg; Vehicle q.s.p. 1000.0g.

3 – Based on Rostagno et al. (2000).

The animals were weighed at the start and end of the experiment and the experimental period was 35 days. During the experimental period, blood samples from two animals in each treatment was collected by sinus orbital puncture to analyze the urea contents of the plasma, according to methodology used by Brown and Cline (1974). A total of 56 samples were analyzed. At the end of the experimental period, two animals from each experimental unit were slaughtered, a total of 40 animals. Animals were chosen with weights near to the mean of the experimental plot. After slaughter the metacarpal bone was removed to determine the mineral content in the ashes.

The ingredients and rations were analyzed according to the methods described by AOAC (1990) carried out in the Animal Research Laboratory at the Animal Science Department/UFLA. The mineral solution to determine the bone phosphorus and calcium was obtained from the ashes by the dry path, and phosphorus was determined by the photometric method and calcium was determined by permanganatometry, according to Silva (1998). The plasmatic urea was determined by the calorimetric enzymatic test, using the `LABTEST´ kits. A randomized block design was used, and the criterion to form the plot was the initial weight. The variables analyzed were: mean daily weight gain (DWG), mean daily feed intake (DFI), feed conversion (FC), urea contents in the plasma, calcium and phosphorus ash content in the bones. The results obtained were submitted to analysis of variance and regression by orthogonal polynomial, carried out according to the statistics program SISVAR developed by Ferreira (2000).

RESULTS AND DISCUSSION

Table 3 shows the results for mean daily weight gain (GPMD) the mean daily feed intake (DFI) feed conversion (FC) and urea contents in the pig plasma. No significant effect was observed of the phytase levels on weight gain and feed intake. However, tendencies to greater weight gain resulted in a considerably better feed conversion (P<0.05) with the increase in phytase level in the ration. The better feed conversion presented by the pigs that received phytase shown that the enzyme promoted an increase in the use of the nutrients from the ration, since the weights obtained were similar. Data analysis shown that with the use of phytase, although not significant, weight gain was on averaged 3.12% greater compared to ration without phytase. This resulted in a 3.31% improvement in the feed conversion.

The detailing of the analysis of variance by polynomial regression shown a linear effect (Figure 1) of the phytase levels on feed conversion. These results were also observed by Lei et al. (1993) who assessed supplementation with phytase in rations based on corn and soybean meal and rice meal at the levels of 0, 250, 500, and 750 FTU/kg for growing pigs and also observed a linear effect on feed conversion. Different results were reported by Murray et al. (1997) who also used rations formulated with different ingredients than those used in the present study and obtained increase in weight gain of pigs that consumed rations supplemented with 700 and 1000 FTY/kg with a low level of available phosphorus. A 3.0% linear increase in feed efficiency was reported by Liu et al. (1997) when supplementing rations with levels of 0, 250 and 500 and 750 FTY/kg phytase for growing pigs, results similar to those detected in the present experiment. Although the rations used in the present study were formulated with calcium and phosphorus levels below those required for the growing stage, no damage was detected in the animal performance, because feed conversion improved 3.3%. Libal et al. (1969) reported that the calcium and phosphorus levels can vary considerably in the diets without affecting pigs performance.

Similar results to those obtained in the present experiment were also up obtained by Ketaren et al. (1993), who ascertained improvement in feed conversation when supplementing rations with 1000 FTU/kg phytase for growing pigs. Tables 3 shows that a quadratic effect of the phytase enzyme levels was detected on the urea contents in the plasma. The level of 750 FTU/kg resulted in the lowest urea contents in the plasma (26.14mg/dl) (Figure 2). The quadratic relationship observed among the increasing levels of the phytase enzyme and the urea contents in the plasma suggested a possible action of the enzyme for better use of the protein in the rations.

TABLE 3 – Mean daily weight gain (DWG), daily feed intake (DFI) , feed conversion (FC) and plasma nitrogen ureia (PNU) for growing pigs fed rations with Fitase suplementation.

Fitase Level (FTU/kg)	Analyzed Variables			
	DWG (g) [Ns]	DFI (g) [Ns]	FE*	PNU (mg/dL)**
0	706	1.98	2.82	29.81
400	712	1.97	2.76	27.75
800	728	1.98	2.73	25.06
1200	744	2.04	2..69	27.18
Média	722	1.98	2.75	27.45
CV (%)	4.84	4.94	1.97	11.46

Ns – non significant effect (P>0.05).
***Linear effect (P<0.05).**
****Quadratic effect (P< 0.05).**

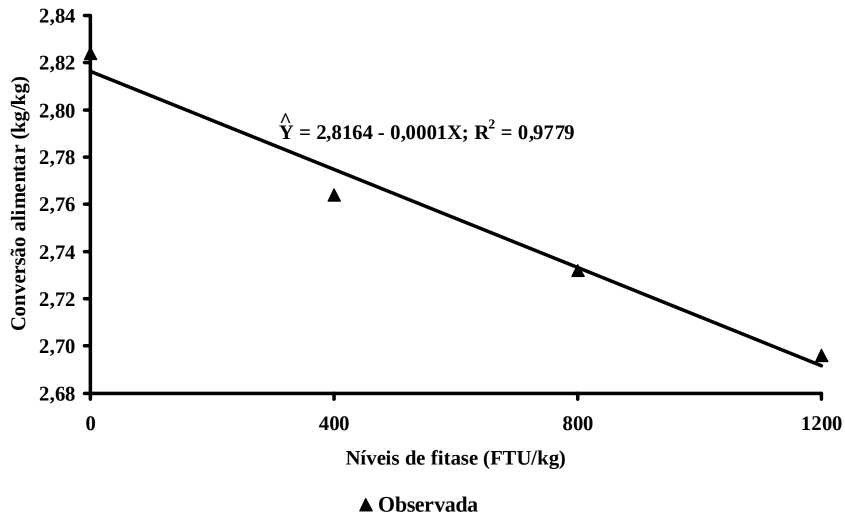

FIGURE 1 – Effect of fitase level in the diets on feed Conversion (FC) of growing pigs.

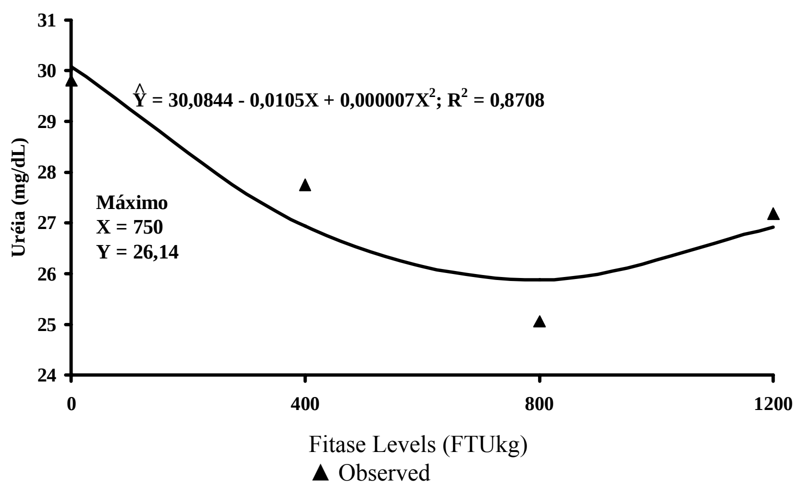

FIGURE 2 – Effect of fitase level in the diets on plasma nitrogen ureia (PNU) of growing pigs.

According to Patterson et al. (1985) urea synthesis rates are influenced by the levels of protein in the diets and by protein catabolism. Eggum (1970) suggested that the low concentration of urea in the bloodstream corresponded to a better use of amino acids. Brown and Cline (1974) reported high urea concentrations in blood serum from pigs fed on amino acid deficient rations. Nevertheless, the same authors noticed that as amino acids were added to the rations, shown reduced urea blood concentrations in the plasma.

Table 4 shows the mean values for ash, calcium and phosphorus in the metacarpal bone of the pigs slaughtered at the end of the experiment.

The ash content in the bone was not affected by phytase levels but the detailing of the analysis of variance by polynomial regression showed a linear increase for calcium and phosphorus in the metacarpal bone in function of the phytase levels (Figures 3 and 4).

TABLE 4 – Average values of ash, calcium and phosphorus in the metacarp bone of growing pigs fed diets supplemented with fitase*.

Fitase levels (FTU/kg)	Analyzed Variables		
	Ash (%)[Ns]	Calcium (%)[1]	Phosphorus (%)[1]
0	51.02	18.95	9.87
400	51.96	19.62	10.20
800	51.77	19.88	10.67
1200	51.65	20.87	11.04
Média geral	51.60	19.83	10.43
CV (%)	2.70	3.69	4.20

***Defatted dry matter.**

[1]Linear effect (P<0.05).

Ns – non significative (P>0.05).

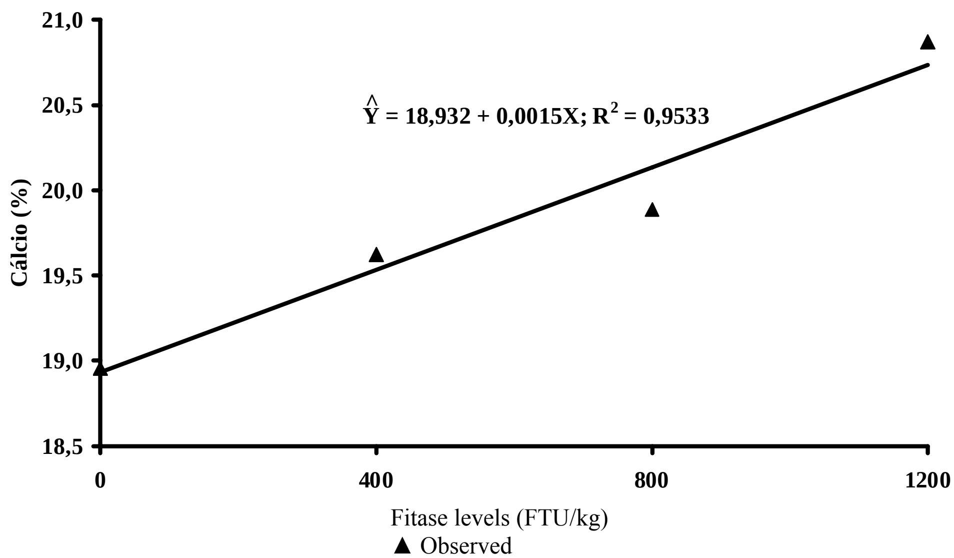

FIGURE 3 – Effect of fitase level in the diets on calcium metacarp bone of growing pigs.

Several authors, including Teichmann et al. (1998) and Zhang (2000) have indicated the percentage of calcium and phosphorus in the bones as the main measure of response to assess the availability of these minerals, because they best reflect variations in the minerals in the rations. The greater phosphorus content in the bones observed in the present experiment, even though the levels of this element were below the animals´ requirements, shown the action of phytase in the release of phosphorus present in the ingredients. This was possible even with the rations containing defatted rice meal, that presented greater total phosphorus concentration, mostly unavailable. Biehl and Baker (1996) worked with rations based on corn and soybean meal with low levels of calcium and available phosphorus supplemented with phytase (0, 300 and 600 FTU/kg) for growing pigs and also reported improvement in the bone characteristics of the animals.

Pointillart (1991) reported the same results when using rations with 20% defatted rice meal for pigs supplemented with phytase at the level of 1200 FTU/kg and observed greater density and also greater mineral content in the pigs bones. These results are similar to those detected in the present study but the author used rations with ideal phosphorus levels. Cromwell et al. (1995) added phytase (500 FTU/kg) for pigs on rations with low total phosphorus (0.3% for the growing and finishing stages) and ascertained that bone resistance was similar to that of the animals that consumed rations with adequate total phosphorus levels. O´Quinn et al. (1997) worked with rations based on sorghum and soybean meal with 0, 300 and 500 FTU/kg for finishing pigs and observed improvement in the bone characteristics and phosphorus concentration in the metatarsal bone of the pigs, that increased linearly with supplementation with phytase, results similar to those reported in the present experiment.

Moreira et al. (2001) used rations based on corn and soybean meal and 17% defatted Rice meal, supplemented with 0, 253,759, and 1265 FTU/kg phytase for growing pigs and observed a quadratic relationship among the phytase levels and phosphorus contents in the bone. These authors did not detect effects on the ash variable in the bones, results also observed in the present study. Lopes et al. (2001) studied phosphorus kinetics in growing pig tissue submitted to rations containing phosphorus of plant origin, supplemented with phytase levels (0, 253,759,1265 and 1748 FTU/kg). The authors observed that the enzyme levels interfered linearly in the inorganic phosphorus concentration in the bone but they did not observe influence of the enzyme on the phosphorus concentration in the liver, kidney, heart or muscles.

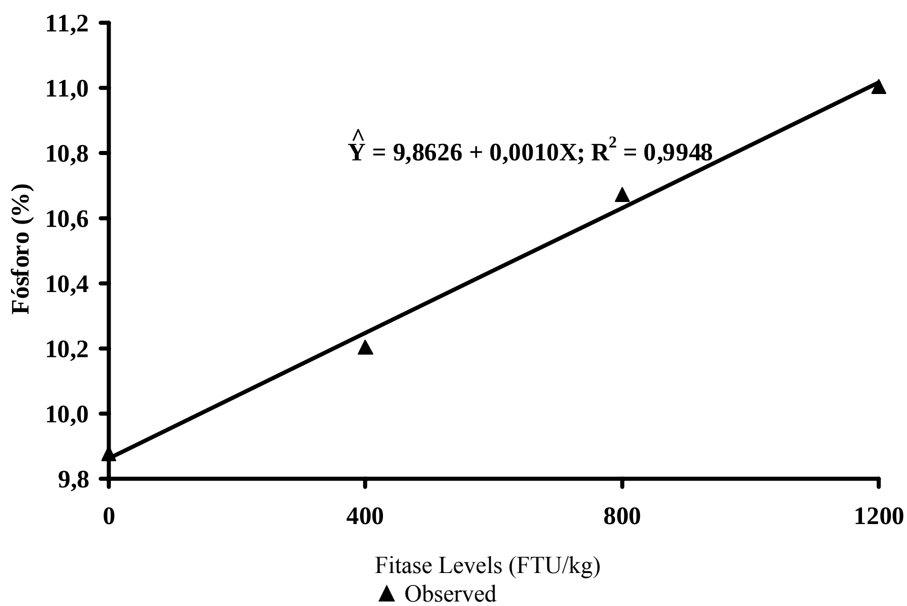

FIGURE 4 – Effect of fitase level in the diets on Phosphorus metacarp bone of growing pigs.

CONCLUSIONS

Phytase enzyme supplementation in rations formulated based on corn soybean meal and defatted rice meal for growing pigs improved linearly the feed conversion and the calcium and phosphorus contents in the bone. The urea content decreased quite drastically in function of the phytase levels. The level of 750 FTU/kg shown the lower urea contents in the plasma.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Agricultural chemicals, contaminants and drugs. In: ______. **Official methods of analysis of the Association of Official Analytical Chemists**. 15. ed. Arlington, 1990. v. 1, 684 p.

BIEHL, R. R.; BAKER, D. H. Effect of supplementation microbial phytase for young pigs fed phosphorus or amino acid-deficient corn-soybean meal diets. **Journal of Animal Science**, Champaign, v. 74, n. 12, p. 2960-2966, Dec. 1996.

BROWN, J. A.; CLINE, T. R. Urea excretion in the pig: an indicator of protein quality and amino acid requirements. **Journal of Nutrition**, Bethesda, v. 104, n. 5, p. 542-545, May 1974.

CROMWELL, G. L. et al. Efficacy of low-activity, microbial phytase in improving the biodisponibility of phosphorus in corn-soybean meal diets for pigs. **Journal of Animal Science**, Champaign, v. 73, n. 2, p. 449-456, 1995.

DOMENE, S. M. A. **Estudo do valor nutritivo mineral do farelo de arroz**: utilização do zinco, ferro cobre, e cálcio pelo rato em crescimento. 1996. 104 f. Tese (Doutorado em Ciência da Nutrição) – Universidade Estadual de Campinas, Campinas, 1996.

EGGUM, B. O. Blood urea measumerents as a technique for assessing protein quality. **British Journal of Nutrition**, Cambridge, v. 24, n. 4, p. 983-988, 1970.

FERREIRA, D. F. **Sistema de análise de variância para dados balanceados**: SISVAR. Lavras: UFLA, 2000.

KETAREN, P. P. et al. Phosphorus studies in pigs: 3. effect of phytase suplentation on the digestibility and availability of phosphorus in soya-bean meal for grower pigs. **British Journal of Nutrition**, Cambridge, v. 70, n. 1, p. 289-311, July 1993.

LEI, X. G. et al. Supplementing corn-soybean meal diets with microbial phytase linearly improves phytase phosphorus utilization by weanling pigs. **Journal of Animal Science**, Champaign, v. 71, n. 12, p. 3359-3367, Feb. 1993.

LIBAL, G. W. et al. Levels of calcium and phosphorus for growing-finishing swine. **Journal of Animal Science**, Champaign, v. 28, n. 2, p. 331-335, Feb. 1969.

LIU, J. et al. Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. **Journal of Animal Science**, Champaign, v. 75, n. 5, p. 1292-1298, Dec. 1997.

LOPES, J. B. et al. Cinética do fósforo em tecidos de suínos alimentados com dietas contendo diferentes níveis da enzima fitase. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 38., 2001, Piracicaba. **Anais**... Piracicaba: SBZ, 2001. p. 717-719.

McKNIGHT, W. F. Technical specifications and properties of phytase. In: COELHO, M. C.; KORNEGAY, E. T. **Phytase in animal nutrition and waste management**: a BASF reference manual 1996. New Jersey: BASF, 1996. p. 1-15.

MOREIRA, J. A. et al. Enzima fitase e farelo de arroz desengordurado para suínos. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 38., 2001, Piracicaba. **Anais**... Piracicaba: SBZ, 2001. p. 703-705.

MURRAY, A. C.; LEWIS, R. D.; AMOS, H. E. The effect of microbial phytase in a pearl millet-soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration and bone mineral density of nursery pigs. **Journal of Animal Science**, Champaign, v. 75, n. 5, p. 1284-1291, 1997.

O'QUINN, P. R.; KNABE, D. A.; GREZ, E. J. Efficacy of Natuphos in sorghum-based diets of finishing swine. **Journal of Animal Science**, Champaign, v. 75, n. 5, p. 1299-1307, May 1997.

PATTERSON, P. H.; CONN, C. N.; HUGHES, I. M. Protein requirements of Mature Working Horses. **Journal of Animal Science**, Champaign, v. 61, n. 1, p. 187-196, July 1985.

POINTILLART, A. Enhancement of phosphorus utilization in growing pigs fed phytase: rich diets by using rye bran. **Journal of Animal Science**, Champaign, v. 69, n. 3, p. 1109-1115, Mar. 1991.

ROSTAGNO, H. S. et al. **Tabelas brasileiras para aves e suínos**: composição de alimentos e exigências nutricionais. 2. ed. Viçosa: UFV, 2000. 141 p.

SILVA, D. J. **Análise de alimentos**: métodos químicos e biológicos. 2. ed. Viçosa: UFV, 1998. 166 p.

TEICHMANN, H. F.; LÓPEZ, J.; LOPEZ, S. E. Efeito da fitase na biodisponibilidade de fósforo em dietas com farelo de arroz integral para frangos de corte. **Revista Brasileira de Zootecnia**, Viçosa, v. 27, n. 2, p. 338-334, mar./abr. 1998.

ZHANG, Z. B. et al. Comparison of phytase from genetically engineered Aspergillus and canola in weanling pig diets. **Journal of Animal Science**, Champaign, v. 78 n. 11, p. 2868-2878, June 2000.