Effect of microbial phytase on apparent digestibility and retention of phosphorus and nitrogen in growing pigs

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ABSTRACT: Six female pigs of approximately 50 kg BW, fitted with simple T-cannula in the terminal ileum, were used to study the effect of microbial phytase on apparent total tract digestibility and retention of P and N. Three P-adequate diets (digestible P concentration 2.3 g/kg) containing barley (B), soybean meal (S) or their mixture (BS) with or without phytase supplement (1 000 FTU/kg) were fed to pigs using a 6 × 6 Latin square design. The addition of phytase increased ($P < 0.05$) apparent total P digestibility of diets S and BS from 56.5 and 57.2% to 69.0 and 65.2%, respectively, and apparent plant P digestibility of the same diets from 41.3 and 50.0% to 60.5 and 60.0%, respectively. An insignificant improvement in total and plant P digestibilities was found in diet B. Phytase supplementation reduced ($P < 0.05$) P excretion in pigs fed diets S and BS by 25 and 14%, respectively. As compared with diets S and BS, urinary P excretion in pigs fed diet B was much higher, which suggests a lower requirement for available P due to the lower protein deposition and growth rate. Phytase supplementation had no effect on digestibility or retention of N. In all three diets, total tract P digestibility was lower ($P < 0.05$) than ileal digestibility thus indicating a net flux of P into the large intestine.

Keywords: phytase; phosphorus; nitrogen; digestibility; retention; pigs

It is well documented that the supplementation of pig diets with microbial phytase significantly improves the availability of phytate-bound phosphorus, thus reducing its excretion to the environment (Jongbloed et al., 1992; Düngelhoef et al., 1994; Kornegay and Qian, 1996; Omogbenigun et al., 2003). Due to its chelating capacity, phytate may also form complexes with other nutritionally important minerals such as calcium, zinc or copper (Oberleas, 1973) as well as with protein (O'Dell and De Boland, 1976). There is evidence suggesting that the phytate-protein complexes are present not only in plants but they may also be formed *de novo* in the guts, thus compromising the utilization of dietary protein irrespective of its origin (Selle et al., 2000). Other possible consequences of phytate-protein interactions in reducing the utilization of dietary protein have been suggested such as the inhibition of proteolytic enzymes by altering their protein configuration (Singh and Krikorian, 1982) or increase of endogenous nitrogen losses (Ravindran et al., 1999).

However, the results of experiments aimed at the study of the efficacy of microbial phytase for improving protein utilization in pigs were contradictory. Phytase has been reported by several authors to increase protein or amino acid digestibility and retention (Officer and Batterham, 1992; Mroz et al., 1994; Kemme et al., 1999a). Contrary to these observations, there are other studies which failed to demonstrate any significant effect of added phytase on protein utilization in pigs (O'Quinn et al., 1997; Valaja et al., 1998; Näsi et al., 1999; Sands, 2002; Omogbenigun et al., 2003; Walz and Pallauf, 2003).

In most experiments studying the efficacy of microbial phytase, complex diets containing suboptimal levels of total or available phosphorus were used. Less information is available on the response of pigs to phytase supplementation of P-adequate

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diets or diets based on single ingredients. Therefore, the objective of the experiment described herein was to study the effect of microbial phytase on digestibility and retention of N and P in growing pigs fed P-adequate diets based on barley, soybean meal or their mixture.

MATERIAL AND METHODS

Animals and experimental design

Six LW gilts of the Institute herd with an average initial body weight of 34.8 ± 0.7 kg were used in the experiment. The pigs were fitted with simple T-cannulas at the terminal ileum to study ileal digestibility (Nitrayová et al., 2006) and housed in metabolism cages in a thermoneutral environment. After a 14-day adaptation period during which a standard grower diet was offered, the pigs were randomly assigned to six dietary treatments according to a 6 × 6 Latin square design. Within the experiment, there were six consecutive periods, each consisting of a 5-day preliminary period followed by a 2-day collection period. During the collection period, urine was collected quantitatively using bladder catheters draining into carboys containing 50 ml 6M H_2SO_4. Aliquots of the 24-hour collections were obtained and immediately analyzed for total N. Further urine aliquots were stored at 4°C for subsequent analyses of total P. Faeces were collected by grab sampling, freeze-dried, finely ground and analyzed for N, P and Cr_2O_3. All experimental procedures were reviewed and approved by the Ethical Committee of the Research Institute of Animal Production.

Diets and feeding

Three basal diets were formulated to contain barley, soybean meal or their mixture as the sources of N. The content of CP in the soybean meal-based diet was reduced to 196 g/kg using wheat starch. Monocalcium phosphate was added to the diets to keep the digestible P concentration at 2.3 g/kg, which was assumed to meet the P requirement of a 50-kg pig (NRC, 1998). Table values of P digestibility of the ingredients (Šimeček et al., 2000) were used for the calculation. Chromic oxide was added to the diets as a digestibility marker. The composition of basal diets and their analyzed nutrient contents are

Table 1. Composition of basal diets (g/kg, air-dry basis)

	Diet		
	B	S	BS
Ingredient			
Barley	972.0	–	745.0
Soybean meal	–	400.0	230.0
Wheat starch	–	573.0	–
Limestone	12.0	10.5	11.0
Monocalcium phosphate	6.2	6.5	4.5
Salt	4.0	4.0	4.0
Premix[1]	3.0	3.0	3.0
Chromic oxide	3.0	3.0	3.0
Chemical analysis			
Dry matter	894.5	891.4	894.2
Crude protein	137.3	196.1	218.0
Ca	6.16	6.75	6.03
P	5.20	4.37	5.33
Phytase activity (FTU/kg)	332	61	280

[1]supplied per kg of diet: vitamin A 7 200 IU, vitamin D3 1 350 IU, α-tocopherol 18 mg, vitamin B1 0.54 mg, vitamin B2 3.6 mg, vitamin B6 19.5 mg, Ca-pantothenate 10.5 mg, niacin 15 mg, vitamin K3 0.54 mg, biotin 0.06 mg, cyanocobalamine 0.021 mg, choline 102 mg, betaine 51 mg, Fe 60 mg, Zn 90 mg, Mn 42 mg, Cu 21 mg, 0.42 mg, Co 0.54 mg, Se 0.21 mg

given in Table 1. To the basal diets, microbial 6-phytase derived from *Peniophora lycii* (Ronozyme P, DSM Nutritional Products Ltd., Switzerland) was added to provide 1 000 FTU per kg, thus forming six experimental diets. The diets were fed twice daily at 7.00 and 16.00 hours in two equal meals at a daily rate of 75–80 g/kg$^{-0.75}$ in a mash form (water: feed ratio = 2.0 vol/wt). After each meal, water was offered *ad libitum* for 30 min.

Chemical analyses

Analyses of diets, faeces and urine for dry matter, total N, Ca and P were performed in accordance with standard methods of AOAC (1984). Chromic oxide was analyzed by atomic absorption spectrometry as described by Williams et al. (1962). Phytase activity of the basal diets was determined according to Engelen et al. (1994).

Calculations and statistical analysis

Coefficients of apparent digestibility of total P and N were calculated using the following formula:

$$\text{Digestibility (\%)} = 100 \times [1 - (N_f \times C_d)/(N_d \times C_f)]$$

where:

N_d = dietary concentration of the nutrient under study

C_d = dietary concentrations of Cr_2O_3

N_f = concentration of the nutrient in faeces

C_f = concentration of Cr_2O_3 in faeces (all values in g/kg dry matter)

Apparent digestibility of plant P (total P minus monocalcium phosphate P (MCP-P)) was calculated from the mean daily plant P intake (g/d) and its output in faeces (g/d). It was assumed that the apparent digestibility of MCP-P was 85% (Cromwell, 1992). Data were subjected to ANOVA using Unistat 4.53 (1999). When a significant F-value for treatment means ($P < 0.05$) was determined, the differences between means were assessed using Fisher's LSD procedure.

RESULTS

With one exception, all animals remained healthy during the experiment and consumed all the food offered. One pig fed the barley-based diet without phytase suffered from mild diarrhoea for three days in the third period and its ileal digesta were not collected.

Table 2. Effect of microbial phytase (1 000 FTU/kg) on apparent total tract digestibility and retention of P and N in pigs fed barley-, soybean meal- or barley + soybean meal-based diets

	Dietary treatment[1]						Pooled
	B	B + MP	S	S + MP	BS	BS + MP	SEM
Animals per treatment	5	6	6	6	6	6	
Mean body weight (kg)	51.1	49.6	50.5	49.5	51.4	50.6	1.6
P intake (g/d)	7.44^{a}	7.28^{a}	6.10^{a}	6.10^{a}	7.47^{a}	7.47^{a}	0.23
Faecal P (g/d)	3.21^{b}	2.91^{b}	2.64^{b}	1.85^{a}	3.19^{b}	2.62^{b}	0.13
Urinary P (g/d)	0.60^{a}	0.79^{a}	0.04^{b}	0.15^{b}	0.03^{b}	0.19^{b}	0.06
Total P digestibility (%)	57.3^{a}	60.3^{ab}	56.5^{a}	69.0^{c}	57.2^{a}	60.0^{c}	1.1
Plant P digestibility (%)	45.8^{ab}	50.0^{b}	41.3^{a}	60.5^{c}	50.0^{b}	600^{c}	1.6
P retention (g/d)	3.63^{a}	3.59^{a}	3.42^{a}	4.11^{ab}	4.25^{ab}	4.66^{b}	0.14
P excretion (% of P intake)	51.0^{c}	50.5^{c}	44.1^{b}	33.1^{a}	43.2^{b}	37.0^{a}	1.3
N intake (g/d)	31.45^{a}	30.80^{a}	43.81^{b}	43.81^{b}	48.87^{b}	48.87^{b}	1.78
Faecal N (g/d)	5.51^{a}	6.35^{a}	3.02^{b}	3.33^{b}	7.28^{b}	7.43^{b}	0.39
Urinary N (g/d)	15.63^{a}	14.66^{a}	17.48^{a}	17.27^{a}	18.85^{a}	18.88^{a}	0.64
N digestibility (%)	82.3^{ab}	79.5^{a}	93.0^{c}	92.3^{c}	85.1^{b}	85.1^{b}	0.9
N retention (g/d)	10.31^{a}	9.79^{a}	23.30^{b}	23.21^{b}	22.75^{b}	22.56^{b}	1.19
N retention (% of N intake)	33.0^{a}	31.8^{a}	53.9^{b}	52.3^{b}	46.7^{b}	46.2^{b}	1.6

[1]B = barley; S = soyabean; BS = barley + soyabean meal; MP = microbial phytase

The results of the metabolic experiment are summarized in Table 2. The apparent digestibility of total P was similar in all basal diets. The supplementation of microbial phytase improved both total and plant P digestibility in all three diets, the improvement in diets S and BS being significant ($P < 0.05$). Phosphorus retention was similar in all diets and was not significantly affected by phytase supplementation. The excretion of P expressed as a percentage of P intake was reduced ($P < 0.05$) in phytase-supplemented diets S and BS. Pigs fed the barley-based diet excreted significantly higher amounts of P as compared to diets containing soybean meal. The addition of phytase did not show any effect on P excretion in this group.

Due to the higher N intake in pigs fed soybean meal and barley + soybean meal-based diets, both apparent N digestibility and retention were significantly higher as compared with barley-based diet. Microbial phytase supplementation did not improve any parameter of N balance.

DISCUSSION

Despite of the fact that P-adequate diets were used in the present experiment, the supplements of microbial phytase improved apparent total tract P digestibility, thus demonstrating its efficacy. In diets S and BS, the digestibility of total P increased significantly by 22 and 14%, respectively. In diet B, the effect of added phytase was not significant even though the tendency for improvement was also apparent. A more pronounced effect of supplemental phytase was observed when plant P (total P minus MCP-P) digestibility was calculated. In this case, the digestibility of diets S and BS increased by 46 and 20%, respectively. A similar increase was found in pigs consuming all-plant diets without inorganic P supplements (Sands et al., 2001; Fan and Sauer, 2002; Walz and Pallauf, 2003). Due to the improved digestibility, faecal P excretion tended to decrease in pigs fed diets supplemented with phytase. On the other hand, urinary P excretion increased, particularly in diets S and BS. It was shown that the excess of digested P not used for anabolic purposes was excreted via urine and that urinary excretion was the main process responsible for P homeostasis in pigs (Irving, 1964; Rodehutscord et al., 1999). Therefore, it seems that the intake of available P in pigs fed basal diets S and BS was slightly below the optimal requirement while the addition of phytase resulted in its surplus which was excreted. An increase in P retention in phytase-supplemented groups S and BS supports this assumption.

As compared with diets S and BS, urinary P excretion in pigs fed barley-based diets was much higher, which suggests that the requirement for available P was reduced in these animals. Indeed, due to the low dietary level of crude protein in diet B, N retention and consequently the growth rate of pigs fed this diet was considerably lower. Since phosphorus is primarily needed for body growth and bone development, its requirement depends on the level of pig performance. In pigs fed diet B, N intake was clearly the limiting factor that prevented the efficient utilization of available P, thus increasing its urinary excretion.

The apparent digestibility of N was not improved by phytase supplementation. This is in agreement with prior reports by Bruce and Sundstol (1995), O'Quinn et al. (1997), Sands (2002), Walz and Pallauf (2003) and Zobač et al. (2004), who found that phytase had no effect on N digestibility. In the present experiment, phytase also failed to improve N retention, similarly like in experiments by Valaja et al. (1998), Walz and Pallauf (2003) and Johnston et al. (2004). In contrast, there are other reports showing a positive effect of microbial phytase both on N digestibility and N retention (Ketaren et al., 1993; Mroz et al., 1994; Sands et al., 2001). The reasons for these controversial observations are not clear. It is generally accepted that phytate is able to bind proteins and amino acids thus rendering them less available for absorption from the guts. However, as pointed out by Selle et al. (2000), the rationale for the protein responses to microbial phytase remains largely speculative. There are many factors and interactions that may influence the final effect. Thus reducing the Ca and P concentrations in the diet increased the average amino acid digestibility in pigs to the same level as the addition of phytase (Johnston et al., 2004). The addition of Na phytate (which, in theory, forms complexes with proteins) increased ileal N digestibility in pigs to a greater extent than the addition of phytase itself (Kemme et al., 1999a). Even in those studies demonstrating a positive effect of phytase on total N or amino acid digestibility, the improvement in terms of growth rate or protein utilization was seldom observed (Peter and Baker, 2001; Adeola and Sands, 2003). These controversial results call for further research aimed at the identification and quantification of factors affecting phytase efficacy.

Table 3. Comparison of apparent ileal and total tract digestibilities (%) of P and N in pigs fed barley-, soybean meal- or barley + soybean meal-based diets

Diet	Ileal digestibility[1]		Total tract digestibility	
	P	N	P	N
Barley	64.7[a]	68.1[a]	58.9[b]	80.8[c]
Soybean meal	71.3[a]	81.1[b]	6.8[c]	92.6[d]
Barley + soybean meal	68.6[a]	75.2[b]	60.9[c]	85.2[d]
Pooled SEM	1.4	1.1	1.1	0.9

[1]Nitrayová et al. (2005)

[a,b,c,d]means within a row followed by the same superscript are not significantly different ($P < 0.05$)

It is generally recognized that both P and N are absorbed in the hindgut to some extent and, consequently, the total tract digestibility is higher than ileal digestibility. While it is undoubtedly true for N digestibility under normal conditions, the results of experiments estimating the digestibility of P were equivocal. Table 3 gives a comparison of the present data on total tract P and N digestibilities with ileal digestibilities estimated on the same pigs and diets (Nitrayová et al., 2006). For simplicity, the values from both supplemented and unsupplemented diets were combined. In all diets, the total tract digestibility of N was significantly higher ($P < 0.05$) than ileal digestibility while the opposite was true of P digestibility. There are several other reports demonstrating the net movement of total P into the large intestine. Mroz et al. (1994) found that in pigs fed once daily, the ileal P digestibility was by 14% higher than total tract digestibility. However, when the pigs were fed seven times daily, both digestibilities were approximately the same. Jongbloed et al. (1992) observed a higher ileal than total tract P digestibility in pigs fed a maize-soybean meal-based diet but not in pigs fed a complex diet containing maize, tapioca, hominy feed and sunflower meal. In experiments by Partridge (1978), pigs fed a semisynthetic diet had a significant net secretion of P into the large intestine. In other experiments, however, this phenomenon was not observed (Fan et al., 2001; Johnston et al., 2004). To date, it is not clear how feeding frequency or diet composition affects the absorption of P along the alimentary tract and its metabolic fate in the large intestine. As suggested by Jongbloed et al. (1992), there might be regulation mechanisms other than urinary excretion that participate in maintaining P homeostasis.

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