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EFFECT OF MICROBIAL PHYTASE ON APPARENT ILEAL AMINO ACID DIGESTIBILITY OF BARLEY AND SOYABEAN MEAL DIETS IN GROWING PIGS

Patráš P., Nitrayová S., Heger J., Sommer A., Dolešová P., Chrenková M.
Slovak Agricultural Research Centre, Research Institute of Animal Production, Nitra, Slovakia

ABSTRACT. Six ileally cannulated pigs (mean initial body weight 34.8 kg) were used to study the effect of microbial phytase on apparent ileal digestibility of P, total N and amino acids. Two P-adequate diets (digestible P concentration 2.3 g/kg) containing barley (B), and soyabean meal (S) with or without phytase supplement (1000 FTU/kg) were fed to pigs using a 4 x 6 Latin square design. To the basal diets, microbial 6-phytase derived from *Peniophora lycii* (Ronozyme P, DSM Nutritional Products Ltd, Switzerland) was added. 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/kg0.75.

Within the experiment, there were four consecutive periods, each consisting of a 7-day preliminary period followed by a 24-hour collection period. During the collection period, samples of ideal digesta were collected. Chromic oxide was added to the diets as an indigestible marker.

In general, nutrient digestibility of soyabean meal was significantly higher than that of barley. The addition of phytase increased apparent ileal P digestibility of diets B and S by 16.5 and 19.2 %. There was no effect of phytase on the ileal digestibility of total N. Apparent ileal digestibility of amino acids tended to increase in the both diet supplemented with phytase, but no significant difference was found for any amino acid as compared with the unsupplemented diet.

Supplementation of P-adequate diets based on barley and soyabean meal with microbial phytase significantly improved the apparent ileal digestibility of P, but had no effect on the digestibility of N. The response of amino acid digestibility to phytase addition was small and inconsistent. The present results do not support the idea of using "amino acid equivalency value" of phytase in least-cost formulation of compound feeds for pigs.

Key words: phytase, ileal digestibility, phosphorus, nitrogen, amino acids, pigs

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It is well documented that the supplementation of pig diets with microbial phytase significantly improves the availability of phytate-bound phosphorus (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 gut, 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).

The results of experiments studying the effect of exogenous phytase on amino acid digestibility in pigs are conflicting. 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. 1999). 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 effect of microbial phytase on protein utilization, complex diets containing suboptimal levels of total phosphorus were used. Less is known on the protein responses to phytase supplementation of P-adequate diets or diets based on single ingredients. Therefore, the objective of the present experiment was to study the effect of microbial phytase on apparent ileal digestibility of amino acids, N and P in growing pigs fed on P-adequate diets based on barley and soyabean meal.

Six pigs with an average initial body weight of 34.8±0.7 kg were used throughout the experiment. The pigs were fitted with simple T-cannulas at terminal ileum and housed in metabolism cages in a thermoneutral environment. After a 14-day recovery period, during which a standard grower diet was offered, the pigs were randomly assigned to four dietary treatments according to a 4 x 6 Latin square design. Within the experiment, there were four consecutive periods, each consisting of a 7-d preliminary period followed by a 24-h collection period. During the collection period, samples of ileal digesta were collected in polyethylene bags attached to the cannula barrel in one-hour intervals. Digesta samples were acidified with 6M H2SO4 to pH 3.5, frozen at -20^{o}C and stored for subsequent analysis of amino acids, N, P and Cr2O3. All experimental procedures were reviewed and approved by the Ethical Committee of the Research Institute of Animal Production.

Two basal diets were formulated to contain barley and soyabean meal as the sources of N. The content of CP in the soyabean meal-based diet was reduced to 168 g/kg using wheat starch. Dicalcium 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 an indigestible marker. The composition of basal diets and calculated nutrient contents are given in

Table I and the analyzed nutrient composition is presented in Table II. To the basal diets, microbial 6-phytase derived from Peniophora lycii (Ronozyme P, DSM Nutritional Products Ltd, Switzerland) was added to provide 1000 FTU per kg, thus forming four 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 .

Analyses of diet and digesta samples for dry matter, total N, Ca and P were performed in accordance with standard methods of AOAC (1984). Chromic oxide was analysed by atomic absorption spectrometry as described by Williams et al. (1962). The amino acid composition of diets and digesta was analysed by ion-exchange chromatography (AAA400 automatic analyzer, Ingos, Prague) using the manufacturer's recommendations.

Coefficients of apparent ileal digestibility of amino acids, N and P were calculated using the following formula:

Digestibility (%) = 100 x [1-(Ni x Cd)/(Nd x Ci)]

where Nd = dietary concentration of the nutrient under study, Cd = dietary concentration of Cr2O3, Ni = concentration of the nutrient in ileal digesta and Ci = concentration of Cr2O3 in ileal digesta (all values in g/kg dry matter). Data were subjected to ANOVA using Unistat 4.53 package (1999). When a significant F-value for treatment means ($P<0.05$) was observed, the differences between means were assessed using Duncan's multiple range test (Duncan 1955).

Table I. ***Composition of basal diets (g/kg)***

	Diets	
	B	S
Barley	972.0	-
Soyabean meal	-	400.0
Wheat starch	-	573.0
Limestone	12.0	10.5
Monocalcium phosphate	6.2	6.5
Salt	4.0	4.0
Premix1	3.0	3.0
Chromic oxide	3.0	3.0
Calculated nutrient content		
Crude protein	119	168
Ca	6.0	6.0
Total P	5.1	4.2
Digestible P	2.3	2.3

[1] Supplied per kg of diet: vit. A 2.400.000 IU, vit. D3 450. 000 IU, α– tocopherol 6.000 mg, vit. B1 180 mg, vit. B2 1.200 mg, vit. B6 500 mg, Ca-panthothen 3.5000 mg, niacin 5.000 mg, vit. K3 180 mg, biotin 20 mg, cyanocobalamin 7 mg, choline 34.000 mg,betain 17.000 mg, Fe 20 000 mg, Zn 30. 000 mg, Mn 14. 000 mg, Cu 7. 000 mg, I 140 mg, Co 180 mg, Se 70 mg.

Table II. ***Analyzed nutrient contents of basal diets (g/kg, on air-dry basis)***

	Diets	
	B	S
Dry matter	894.50	891.40
Crude protein	137.30	196.00
Ca	6.16	6.75
Total P	5.20	4.37
Alanine	4.50	8.12
Arginine	6.74	15.80
Aspartic acid	8.15	24.23
Cystine	2.34	2.25
Glutamic acid	30.56	37.61
Glycine	5.01	8.67
Histidine	3.26	6.07
Isoleucine	4.16	8.60
Leucine	8.85	15.93
Lysine	4.61	12.86
Methionine	2.30	2.56
Phenylalanine	6.30	9.69
Proline	15.71	11.08
Serine	5.86	10.76
Threonine	4.67	8.99
Tyrosine	4.62	8.36
Valine	6.03	9.59

Pigs recovered quickly from surgery and their mean body weight gain was 579±12 g/day during the experiment. With one exception, all animals remained healthy and consumed all the food offered. One pig fed the barley-based diet without phytase suf-

fered from mild diarrhoea for three days in the third period and its ileal digesta was not collected. Mean body weight of pigs during the experiment was 49.4±1.4 kg.

Mean estimates of apparent ileal digestibilities of dry matter, N and P are given in Table III. In general, nutrient digestibility of soyabean meal was significantly higher than that of barley. The addition of phytase increased apparent ileal P digestibility of diets B and S by 16.5 and 19.2 %. There was no effect of phytase on the ileal digestibility of total N.

Table III. ***Effect of microbial phytase on apparent ileal digestibility of dry matter, nitrogen and phosphorus in pigs fed barley- and soyabean meal-based diets***

	n	Phytase	Digestibility (%)					
		FTU/kg	DM		N		P	
Barley	5	0	63.2	bc	68.0	a	59.4	a
	6	1000	65.7	c	68.0	a	69.2	bc
Soyaben meal	6	0	76.5	d	80.6	cd	65.0	ab
	6	1000	78.8	d	81.5	d	77.5	d
Pooled SEM			1.5		1.1		1.4	

a,b Means within a column followed by the same superscript are not significantly different (P<0.05)

The data on the apparent ileal digestibilities of amino acids are summarized in Table IV. Similarly to the digestibility of N, the amino acid digestibilities of soyabean meal were higher when compared to barley. In both ingredients, the lowest values were found for lysine and threonine while the highest digestibilities were observed in methionine and arginine. The estimated data agreed fairly well with those published in the literature (Amipig 2000, Pedersen and Boisen 2002), the only exception being the digestibility of methionine that exceeded the upper limit of a range given by Pedersen and Boisen (2002). Phytase supplementation had no significant effect on apparent ileal digestibilities of amino acids.

The significant improvement of apparent ileal digestibility of total P in all diets due to the addition of microbial phytase demonstrated its efficacy. However, the observed response was lower than that found in most other experiments (Jongbloed et al. 1992, Mroz et al. 1994, Kemme et al. 1999b, Omogbenigun et al. 2003). This was expected, since about one third of total dietary P was provided in the form of highly digestible monocalcium phosphate, thus decreasing the relative proportion of phytate P in experimental diets. There is a paucity of data in the literature regarding the effect of added phytase on P digestibility in P-adequate diets. Johnston et al. (2004) studied the digestibility of nutrients in 50 kg pigs that were fed maize and soyabean meal-based diets containing 1.9 or 0.9 g/kg of available P. The addition of phytase to low-P diet increased apparent ileal P digestibility by 44 % whereas the improvement in the diet containing higher P level was only 21 %, even though the concentration of phytate P in both diets was approximately the same. An improvement in apparent total tract P digestibility of similar order in pigs fed a P-adequate diet was reported by Xavier et al. (2004).

In the present experiment, the apparent ileal N digestibility was not affected by the addition of microbial phytase. This is in agreement with prior reports by Bruce and Sundstol (1995), O'Quinn et al. (1997), Walz and Pallauf (2003) and Omogbenigun et al. (2003) who found that phytase had no effect on N digestibility in pigs. Also in experiments carried out by Kemme et al. (1999), phytase failed to improve ileal digestibility of N in spite of the fact that the digestibility of most amino acids increased. The protein response of pigs to phytase does not seem to be related to dietary protein concentration since phytase supplementation had no effect on faecal N digestibility, both in low-protein and high-protein diets (Sands, 2002). On the other hand, Mroz et al. (1994) found a significant positive effect of added phytase on faecal but not on ileal N digestibility.

Table IV. ***Effect of microbial phytase on apparent ileal digestibility of amino acids (%) in pigs fed barley and soyabean meal based diets***

Amino acid	Dietary treatment1				Pooled
	B	B + MP	S	S + MP	SEM
n	5	6	6	6	
Arginine	76.2	75.6	91.6	92.3	1.2
Histidine	74.7	74.1	86.4	86.8	1.0
Isoleucine	77.0	76.5	89.3	89.9	1.0
Leucine	77.0	76.5	87.8	88.5	0.9
Lysine	70.5	69.0	88.5	89.1	1.5
Methionine	80.9	81.9	91.4	91.1	0.9
Phenylalanine	73.3	71.3	86.5	85.7	1.1
Threonine	64.1	62.9	79.1	79.9	1.4
Valine	75.6	75.4	86.5	87.2	0.9
Alanine	63.7	63.3	83.5	83.7	1.5
Aspartic acid	69.7	69.0	86.2	87.1	1.4
Cystine	76.5	76.6	76.7	77.1	1.0
Glutamic acid	81.6	81.9	90.0	90.4	0.8
Glycine	55.9	58.3	78.2	78.4	1.6
Proline	77.3	75.9	80.6	79.9	0.9
Serine	73.0	73.0	86.2	86.5	1.0
Tyrosine	72.0	67.3	83.2	84.3	1.4
Essential AA	74.4	73.7	87.5	87.8	0.4
Non-essential AA	73.4	72.9	83.1	83.4	0.6

B = barley; S = soyabean meal; MP = microbial phytase

The data summarized in Table III showed that the addition of microbial phytase had no significant effect on apparent ileal digestibility of amino acids. The published reports as to the efficacy of microbial phytase for improving amino acid digestibility are conflicting and inconsistent. While there are experiments in which an improvement in ileal digestibility was demonstrated for at least some amino acids (Officer and Batterham 1992; Mroz et al., 1994; Kemme et al., 1999; Johnston et al., 2004), no favourable effect of phytase supplementation was found in other studies (Valaja et al., 1998; Traylor et al., 2001, Copado et al., 2003, Cervantes et al. 2004). Recently, Liao et al. (2005) conducted a series of experiments on weanling pigs in which the efficacy of phytase supplements to various P-adequate diets was studied. Out of four experiments, only one resulted in an improvement or a tendency toward improvement in the apparent amino acid digestibilities. The authors suggested that the amino acid response to phytase supplementation might depend on diet composition. Discrepancies also exist in the phytase-mediated response of individual amino acids. Thus the addition of phytase resulted in a marked increase in the ileal digestibility of threonine in the experiment by Kemme et al. (1999), while Mroz et al. (1994) and Cervantes et al. (2004) found a slight decrease in the diges-

tibility of this amino acid in phytase-supplemented pigs. Even in those studies demonstrating a positive effect of phytase on total N or amino acid digestibility, the improvement in terms of growth performance or protein utilization has seldom been observed (Peter and Baker, 2001; Adeola and Sands, 2003).

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 gut. However, as pointed out by Selle et al. (2000), the rationale for the protein responses to microbial phytase remains largely speculative. It seems that the efficacy of phytase may be affected by many factors such as the dietary concentration and source of phytin, level of available P, protein quality, presence of bivalent cations, feed processing, animal species and genotype, or the experimental technique used (Adeola and Sands, 2003). Even though the inclusion of phytase into compound feeds for pigs is primarily motivated by its effect on phytate P availability, the improvement of protein and amino acid utilization would be of great practical importance and the cost effectiveness of the enzyme would be enhanced substantially. However, further research is needed to clarify the effects of intrinsic and extrinsic factors on phytase efficacy and their interactions. Considering the uncertainties surrounding this area and a lack of unambiguous quantitative data, the least-cost formulation of pig diets based on an assumption that phytase improves amino acid digestibility does not seem to be fully justified.

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