Apparent Digestibility of Phosphorus in Experimental Feeds and the Effect of Commercial Phytase

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ABSTRACT : The digestibility (apparent) of P and other nutrients from the RSM, SBM and 2 batches of maize, wheat and barley was investigated in two experiments with 24 castrated male growing pigs. The effect of supplemental microbial phytase (1,000 U/kg) was also evaluated. The diets contained 25% RSM (Exp. 1) or 40% SBM (Exp. 2) and had no inorganic P. In each period of digestive trial, after 9 days of adaptation, faeces were collected for 5 days. The digestibility of P contained in the RSM and SBM was calculated by difference method. The P digestibility in maize, wheat, barley was on average 20, 34 and 36%, respectively. The digestibility of P in the RSM and SBM estimated from maize-based diets were 19 and 24 %, respectively. Kind of cereal grain had significant ($P < 0.05$) influence on the digestibility of P which was lower in the diets based on maize than wheat or barley. The digestibility of P significantly increased with the supplemental microbial phytase (on an average of 17%). Moreover, inclusion of enzyme into the diets positively affected digestibility of other nutrients, namely the protein and organic matter.
(**Key Words:** Pigs, RSM, SBM, Phytase, Digestibility)

INTRODUCTION

Knowledge of the availability of P in feed ingredients is a valuable information that can be used in the formulation diets for nonruminants.

The phosphorus in cereals and oilseeds is poorly available for pigs (Cromwell, 1992). This is mainly due to the proportion of phytate P in feedstuffs and the lack of intrinsic phytase (Jongbloed and Kemme, 1990; Düngelhoef et al., 1994).

A large proportion (over 55%) of the phosphorus found in cereals and oilseeds is bound in phytate (Jongbloed et al., 1991), the inositol hexaphosphate from which it must be liberated in the gastrointestinal tract to be available for absorption. The degradation of phytate is possible due to activity of the enzyme phytase, which can be found in some seeds. However, the level of the native enzyme in plant feeds is usually too low for sufficient break down of P-phytate. Many authors recently reported that the availability of P was increased by the inclusion of microbial phytase in plant-based diets (Näsi, 1990; Düngelhoef et al., 1994; Näsi et al., 1995).

The diets for pigs based on maize, barley, wheat and soybean meal (SBM) considerably differ in P availability due to the intrinsic phytase activity. It could be shown, that maize and SBM contain no or extremely low phytase activities (Lantzsch, 1990) and in both P availability is lower than in wheat and barley.

In some countries (eg. in Poland) the oil meal from rape of low-glucosinolate variations (RSM) is widely used as a protein source which is successfully replacing the SBM in pig nutrition. Besides having a well-balanced amino acid composition, the RSM also contains considerable amount of P in comparison to the other plant feedstuffs. However, its availability seems to be even lower than from SBM and most cereals (Cromwell, 1992), but little attention has so far been given to this problem.

The aim of the study was to determine the influence of different cereal grains (maize, wheat and barley) and addition of exogenous phytase to diets with the SBM or RSM on digestibility of P in pigs.

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Received January 25, 1997; Accepted November 4, 1997

MATERIALS AND METHODS

The digestibility (apparent) of P in maize, wheat, barley, rapeseed meal and soybean meal was studied. The chemical composition of the ingredients is shown in table 1.

Two digestive trials (Exp. 1 and 2) were carried out following the procedures described by Jongbloed and Kemme (1990).

The maize, wheat and barley as single feeds (served as the basal diets) were tested in each trial. RSM was investigated in Exp. 1, and SBM was investigated in Exp. 2. Digestibility of P in RSM and SBM was determined according to difference method. The procedure was designed to ensure that at least 50% of total P came from the test material. The tested diets contained 25% of RSM and 40% of SBM (table 2 and 3).

Table 1. Chemical composition of the feed ingredients used in the Exp. 1 and 2 (g/kg dry matter)

Nutrients	Maize		Wheat		Barley		RSM	SBM
	1	2	1	2	1	2	1	2
Protein	109.8	95.2	130.1	142.8	135.9	109.3	372.2	479.9
Ether Extract	45.9	52.3	23.0	23.9	22.9	20.5	39.5	13.3
Crude fibre	16.2	21.4	23.9	23.9	50.0	47.5	137.9	68.1
Ash	30.2	25.5	35.5	30.1	43.9	37.1	77.7	69.1
Ca	0.3	0.3	0.4	0.4	0.7	0.4	7.9	5.0
P - total	3.9	0.4	4.2	3.7	4.8	4.8	14.1	6.6
P - phytate (%)	71	76	64	59	62	56	73	62
Phytase (U/kg)	56	224	936	2,089	750	829	17	184
Glucosinolates, μM/g DMFF		–		–		–	11.1	–

Table 2. Composition of the basal and tested diets (Exp. 1)[1]

Diet	Basal			RSM + Basal		
	Maize	Wheat	Barley	Maize	Wheat	Barley
Ingredients (g/kg)						
Cereal grain	978	977	976	725	725	724
RSM	–	–	–	250	250	250
Premix*	5	5	5	5	5	5
Limestone	11	11	12	13	13	14
NaCl	3	3	3	3	3	3
Cr_2O_3	4	4	4	4	4	4
Nutrients (g/kg DM)						
Protein	105.6	129.3	133.6	169.9	192.4	197.3
EE	45.9	23.0	22.9	41.8	23.1	25.5
CF	16.1	23.9	44.8	51.8	58.8	68.4
NFE	802.1	778.3	756.6	689.7	674.1	659.5
Ash	30.2	25.6	42.2	46.8	51.6	55.9
P	3.8	4.2	4.8	5.0	6.3	6.9

[1] Abbrevations in this and next tables mean: DM - dry matter, EE - ether extract, CF - crude fibre, NFE - Nitrogen - free extractives, P - phosphorus.

* Amounts of vitamins and microelements supplied per kg of diet: vitamin A - 15,000 IU, vitamin D_3 - 2,000 IU, vitamin E - 15 mg, vitamin K_3 - 1.5 mg, vitamin B_1 - 1.0 mg, vitamin B_2 - 5 mg, vitamin B_6 - 1.5 mg, vitamin B_{12} - 0.015 mg, biotin - 0.03 mg, folic acid - 0.5 mg, nicotinic acid - 15 mg, calcium pantothenate - 8 mg, choline chloride - 150 mg, Mn - 30 mg, Zn - 40 mg, Cu - 15 mg, Fe - 60 mg, I - 0.5 mg, Se - 0.15 mg, Co - 0.6 mg, virginiamycin (StafacR) - 5 mg.

Table 3. Composition of the basal and tested diets (Exp. 2)

Diet	Basal			RSM + Basal		
	Maize	Wheat	Barley	Maize	Wheat	Barley
Ingredients (g/kg)						
Cereal grain	982	981	972	582	582	580
SBM	–	–	–	400	400	400
Premix*	5	5	5	5	5	5
Limestone	8	9	11	8	8	10
NaCl	3	3	3	3	3	3
Cr_2O_3	2	2	2	2	2	2
Nutrients (g/kg DM)						
Protein	95.4	142.8	109.3	251.0	276.7	249.5
EE	52.3	23.9	20.5	33.6	19.2	17.6
CF	21.4	23.9	47.5	52.1	46.8	64.3
NFE	805.6	779.7	785.8	614.9	606.9	610.6
Ash	25.5	30.1	37.1	48.4	50.4	58.0
P	3.2	4.0	4.1	4.4	4.8	5.6

* Amounts of vitamins and microelements supplied per kg of diet: vitamin A - 15,000 IU, vitamin D_3 - 2,000 IU, vitamin E - 15 mg, vitamin K_3 - 1.5 mg vitamin B_1 - 1.0 mg, vitamin B_2 - 5 mg, vitamin B_6 - 1.5 mg, vitamin B_{12} - 0.015 mg, biotin - 0.03 mg, folic acid - 0.5 mg, nicotinic acid - 15 mg, calcium pantothenate - 8 mg, choline chloride - 150 mg, Mn - 30 mg, Zn - 40 mg, Cu - 15 mg, Fe - 60 mg, I - 0.5 mg, Se - 0.15 mg, Co - 0.6 mg, virginiamycin (StafacR) - 5 mg.

Vitamins and essential trace elements were added to the diets, but no inorganic P was supplemented. The Ca/P-total ratio in the diets was maintained at 1.3 : 1 by addition of limestone. The composition of all experimental diets is shown in table 2 and 3.

To investigate the effects of supplemental microbial phytase on P digestibility, RSM and SBM diets were either unsupplemented or supplemented with microbial phytase{1,000 U/kg, ALLZYME (Alltech, Inc. Biotechnology Center, 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356 USA) in Exp. 1 and NATUPHOS (BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany) in Exp. 2}. The diets were not pelleted.

In each trial 12 castrate male pigs (Polish Landrace), initially weighing approximately 35 kg, were kept in metabolic cages and fed twice daily, at 08 : 00 and 14 : 00. The feed was offered in wet form (1 : 1). Daily rations were calculated to the 60% of voluntary feed intake, as reported by the ARC (1981). Each experimental period comprised 9 days of adjustment and 5 days of faeces collection. In each experiments (1 and 2) four pigs per 3 treatments and 3 cereals were used. A total of 72 individual collections (2 × 3 × 3 × 4) of faeces have been made. Digestibility of nutrients was measured by indirect method with Cr_2O_3 as indicator.

Analyses of feeds and faeces were done according to the AOAC (1990) standard methods. Cr was analyzed according to the method of Hinsberg et al. (1953). P was determined photometrically using the vanado-molybdate method. Ca was determined from ash solutions by atomic absorption spectrophotometry. Phytase activity of feeds was measured according to Eeckhout and Paepe (1994), one unit (U) of phytase activity being defined as the activity liberating 1 μmol of inorganic phosphate per minute from 0.0015 mol of sodium phytate at 37℃ and pH 5.5. The activity of intrinsic phytase was not assayed in the feeds used in Exp. 2, and will be determined later. Determination of phytate was done according to Tangkongchitr et al. (1981) modified by Antoniewicz et al. (1992).

Calculation and statistics

Digestibility of P and other nutrients in single feeds was measured directly. Digestibility of P (Pdig), in RSM and SBM was calculated by difference from the test diets and respective basal diets by using the following formula:

$$Pdig = \{dt - (db*a)\}/(1 - a)$$

where

dt – digestibility of P in the test diet
db – digestibility of P in the basal diet
a – proportion of P from the basal diet to the total P

Differences in nutrients digestibility of individual ingredients as well as in unsupplemented and phytase supplemented mixtures were evaluated by analysis of variance according ANOVA (Statgrafics, 1993).

RESULTS AND DISCUSSION

The activity of phytase in both maize and RSM was low (60 U/kg, table 1). In contrast, the phytase activity in barley and wheat was considerably higher (750 and 936 U/kg, respectively).

The digestibility of P in the single cereal grains ranged from 18.2 to 35.8%. (table 4). As in other investigations (Calvert et al., 1978; Jongbloed, 1987; Jongbloed and Kemme, 1990; Düngelhoef et al., 1994; Wecke et al., 1994), maize was consistently low in P digestibility (18-22%). This figure is considerably less than the traditionally accepted value of 30-35% (i.e., one-third of the total P). The above value was estimated by the proportion of non-phytate P in total P (Cromwell, 1989), not on pig digestibility. The digestibility of P in wheat and barley was significantly higher (32-36%), and it was attributed to the presence of naturally-occurring phytase in the seed coat. Our estimates for cereal grains are similar to those obtained by other workers (Calvert et al., 1978; Jongbloed, 1987; Barrier-Guillot et al., 1996).

There were some differences in the apparent digestibility of other nutrients as compared with values usually found (INRA, 1984), eg., protein digestibility in the wheat was higher than in barley and maize.

Table 4. Nutrient digestibility (%) of cereal grains (Exp. 1 and 2)

Trials	Exp.	Maize	Wheat	Barley	SE
Protein	1	77.7	79.1	74.6	1.27
	2	74.7^{a}	81.3^{b}	71.1^{b}	1.35
EE	1	57.1^{A}	22.1^{B}	19.7^{B}	1.70
	2	76.1^{A}	44.4^{B}	35.6^{C}	0.71
CF	1	40.3^{a}	18.4^{B}	25.1^{ab}	2.41
	2	47.9^{A}	31.8^{B}	31.9^{B}	1.24
NFE	1	95.1^{A}	93.6^{B}	91.5^{C}	0.13
	2	93.5^{Aa}	95.5^{A}	92.8^{Bb}	0.13
Ash	1	27.6	30.4	27.6	0.77
	2	37.3^{ab}	41.0^{a}	35.5^{b}	0.70
P	1	18.2^{A}	31.8^{B}	35.7^{B}	1.04
	2	21.6^{A}	35.8^{B}	35.8^{B}	1.02

^{a,b} Means within the same row with different superscript differ at $P < 0.05$.

^{A,B,C} Means within the same row with different superscript differ at $P < 0.01$.

Table 5. Nutrient digestibility (%) of diets without (−) and with (+) supplementation of microbial phytase (Exp. 1)

Phytase	RSM + maize		RSM + wheat		RSM + barley		SE	Effect of	
	−	+	−	+	−	+		cereal	enzyme
Protein	77.8	83.8	84.6	87.5	80.0	86.3	0.50	**	**
EE	50.8	53.5	20.2	26.7	22.3	38.6	1.37	**	*
CF	52.1	70.0	45.9	59.9	42.7	54.0	1.44	**	**
NFE	92.1	93.2	93.2	93.0	89.6	92.9	0.21	**	**
Ash	30.1	35.8	39.7	48.6	38.4	48.6	0.46	**	**
P	18.5	27.7	35.0	46.0	35.4	50.1	0.71	**	**

* $P < 0.05$.
** $P < 0.01$.

Table 6. Nutrient digestibility (%) of diets without (−) and with (+) supplementation of microbial phytase (Exp. 2)

Phytase	SBM + maize		SBM + wheat		SBM + barley		SE	Effect of	
	−	+	−	+	−	+		cereal	enzyme
Protein	84.5	87.5	87.9	89.9	84.1	87.5	0.40	**	**
EE	61.6	73.1	29.8	31.3	33.1	50.3	1.35	**	**
CF	56.4	67.1	49.1	63.0	38.1	61.0	1.22	**	**
NFE	91.2	92.9	92.9	93.7	90.9	93.9	0.21	−	**
Ash	47.1	61.0	48.6	59.0	46.1	48.9	0.55	−	**
P	22.4	56.1	36.6	54.5	32.1	46.9	0.82	*	**

* $P < 0.05$.
** $P < 0.01$.

In tables 5 and 6 the digestibilities of nutrients from RSM and SBM/cereal diets, unsupplemented or supplemented with microbial phytase are presented. The enzyme supplementation significantly increased P digestibility on average by 13.6% in RSM groups (from 27.6 to 41.2%) and by 22.1% in SBM groups (from 30.4 to 52.1%). However, there was found a rather weak response in RSM/maize group (table 5). It is unexpected in comparison with results of other researchers (Nair, 1990; Näsi et al., 1995) and needs further investigations.

The average effect of enzyme supplementation amounted to 17% increase in P digestibility (table 7). It also shows the effect of individual cereals on P digestibility. As expected, the digestibility of P in maize-based diets was the lowest (31.2%) and in wheat-based diets the highest (43.1%). The digestibility of P in barley-based diets was somewhat lower than in wheat-diets. Therefore, it could be said, that kind of cereal grains has significant influence on the P digestibility of the whole diet, but the supplementation with commercial phytase is necessary to further improve P availability.

Table 7. Effect of microbial phytase suplementation on the digestibility of phosphorus (Exp. 1 and 2)

Diets	Digestibility of Phosphorus, %		Mean
Microbial phytase (U/kg)	0	1,000	
RSM + maize	18.5	27.7	23.1
SBM + maize	22.4	56.1	39.3
Mean	20.5	41.9	31.2
RSM + wheat	35.0	46.0	40.5
SBM + wheat	36.6	54.5	45.6
Mean	35.8	50.3	43.1
RSM + barley	35.4	50.1	42.8
SBM + barley	32.1	46.9	39.6
Mean	33.8	48.5	41.2
Total	30.0	46.9	

Digestibility of P in high-protein feedstuffs depends on the type of basal diet. Digestibility of P in RSM and SBM estimated from maize-based diet was 19 and 24% (table 8). These figures are similar to those obtained by Liu et al. (1995) and Jongbloed (1987). In contrast, if wheat and barley were used in the basal diet, the estimates were significantly higher: 38 and 35% for RSM and 37 and 28% for SBM, respectively (table 8). It is probably due to the presence of intrinsic phytase in these cereals which might affect the P digestibility.

Table 8. Digestibility of P (%) in RSM and SBM determined by difference method from diets based on three cereals

Basal diet	Digestibility of P	
	RSM	SBM
Maize	19	24
Wheat	38	37
Barley	35	28

It is also important to point out that in this study microbial phytase supplementation significantly increased the digestibility of other nutrients besides P. It suggests that addition of commercial phytase could insert to the diets some amounts of amylolytic and proteolytic enzymes besides of phytase. The results of our study indicate that phytase supplementation can improve the energy value of plant feeds.

From the result of this experiment, it could be concluded as follows:

1. Digestibility of P in maize is lower (18-22%) than in wheat or barley (32-36%).
2. The P digestibility in RSM and SBM estimated from the maize-based diets is below 25%. It is somewhat lower in RSM than SBM.
3. Digestibility of P of protein components estimated from the mixture of plant feedstuffs depends on the kind of cereal ingredients. In the diets with maize it is always lower than with wheat or barley.
4. Diet supplementation by commercial phytase (1,000 U/kg) increased P-digestibility about 17%.
5. It seems that diet supplementation by microbial phytase is necessary even in the case of diets based on the cereals with high activity of intrinsic phytase.
6. Microbial phytase addition can improve the digestibilities of other nutrients beside of P.

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