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ISOTACHOPHORETIC DETERMINATION OF PHYTIC ACID IN THE FEED AND FAECES OF RABBITS

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Abstract **- Phytic acid concentration was measured in the feed and faeces of five rabbits, fed a commercial concentrate, by means of the capillary isotachophoresis. Prior to analysis, phytic acid was separated from interferring compounds by acid extraction and iron precipitation. The feed contained 17.2 g of phytic acid per kg of dry matter (42.5% of total P). Faeces of rabbits contained 2.62 - 8.21 g of phytic acid and 18.4 - 21.5 g of total P per kg of dry matter. Phytic acid represented 4.0 - 11.0% of total P in faeces of rabbits.**

INTRODUCTION

Phytic acid (*myo*-inositol hexaphosphoric acid) is present in substantial quantities in cereals, oil-seeds and seeds of leguminous plants. Phytic acid has often been considered as an antinutrient due to its tremendous potential for forming complexes with positively charged cations and proteins. Cations of concern in this respect include zinc, iron, calcium and copper (ZHOU and ERDMAN, 1995). Phytic acid is not absorbed from digesta into the blood. The hydrolysis of phytic acid to inositol and phosphate in the digestive tract is carried out by action of plant phytases, phytases of microorganisms and intestinal mucosal phytases. Phytic acid is dephosphorylated incompletely in monogastric animals such as pigs and poultry (TAYLOR, 1965), but the release of phosphate appears to be much better in ruminants such as cattle (NELSON *et al.,* 1976). Therefore, inorganic phosphate has to be added to pig and poultry diets to satisfy the phosphorus requirement. This consequently contributes to phosphorus pollution problems in areas of intensive livestock production. In these areas the quantity of phosphorus excreted in animal faeces should be diminished. To our knowledge, there are no data on efficiency of phytic acid hydrolysis in the digestive tract of rabbits, and on concentration of phytic acid in rabbit faeces. According to MATEOS and DE BLAS (1998), an almost complete utilization of phytate phosphorus should be expected. Phytic acid determination in feeds is relatively easy. Most methods are based on the insolubility of ferric phytate and colorimetric determination of iron or phosphate content of the precipitate, or on the decrease of iron in the supernatant. Determination of phytic acid in faeces is difficult, due to the fact that faeces contain phosphates which co-precipitate with iron, thus disturbing colorimetric measurements, and colloid substances which complicate other analytical techniques. Several authors measured phytic acid content in samples of feeds, digesta and faeces of pigs and poultry by the HPLC method (e.g. SANDBERG

et al., 1993; KIISKINEN *et al.,* 1994). Reliable quantiative determination of phytic acid in this way, however, involves several steps : extraction by HCl, separation of phytic acid from crude extract by a ion-exchange chromatography, evaporation of the eluate under vacuum and HPLC analysis. The aim of our study was to measure the phytic acid concentration in the feed and faeces of rabbits using an instrument for capillary isotachophoresis. A suitable technique for this purpose seemed to be that used by BLATNY *et al.* (1995) for isotachophoretic assay of phytic acid in cereals and feeds. Precipitation of ferric phytate, as described by KIKUNAGA *et al.* (1985) was used to separate phytic acid from interferring compounds, and to increase the phytic acid concentration in analyzed solutions (DUŠKOVÁ *et al.,* 2000).

MATERIALS AND METHODS

The volume-coupling instrument Ionosep 900.1 (Recman Ostrava, Czech Republic) was used. The instrument was equipped with Teflon capillaries: preseparation capillary 50x1 mm, separation capillary 150x0.45 mm, detection capillary 70x0.3 mm. Detection was carried out with a contactless high-frequency conductivity cell. The following reagents were necessary :
phytic acid (as dodecasodium salt), Sigma (No. P-3168)
BTP: 1.3-bis[tris(hydroxymethyl)methylamino]propane, Sigma (No. B-6755)
HPMC: hydroxypropylmethylcellulose, Sigma (No. H-7509)
MES: 2-(N-morpholino)ethanesulphonic acid monohydrate, Sigma (No. M-5287)
tiron: 4.5-dihydroxy-1,3-benzenedisulphonic acid disodium salt, Fluka (No. 89460)
creatinine, Fluka (No. 27910)
wheat phytase (EC 3.1.3.26), Sigma (No. P-1259): 0.04 unit per 1 mg of solid.

Feed and faeces of five eleven-week-old Hyla 2000 rabbits were sampled. The composition of the feed is shown in Table 1.

Table 1: Composition of rabbit diet

Components	(%)	Nutrients	(g/kg)
Alfalfa meal	35	Dry matter	881
Wheat bran	22	Crude protein	169
Extracted sunflower meal	20	Crude fibre	172
Barley	10	Starch	163
Oats	10	N-free extract	439
Vitamin-mineral supplement	1.5	Fat	27
Rapeseed oil	1.5	Ash	73

Fifty ml of 0.95 M HCl were added to 5 g of the sample of a feed. After shaking at the room temperature for 1 h, the mixture was diluted to 100 ml and centrifuged at 15 000 rpm for 20 min, at 4 °C. The supernatant was separated from pellets and phytic acid present in 10 ml of the extract was precipitated by 0.22 M $FeCl_3$. Seven times as much $FeCl_3$ as phytic acid on the molar basis needed to be added. The mixture was heated in a boiling water bath for 45 minutes, cooled, and centrifuged at 6 000 rpm for 10 min. The supernatant was carefully separated from the precipitate by means of a syringe and transferred into a 25 ml volumetric flask. The precipitate was washed with 5 ml of 0.15 M HCl, centrifuged at 6 000 rpm for 10

minutes, resuspended in deionized water and centrifuged again. The washing with deionized water was repeated once more and the combined supernatants in a final volume of 25 ml were used for Fe^{3+} determination.

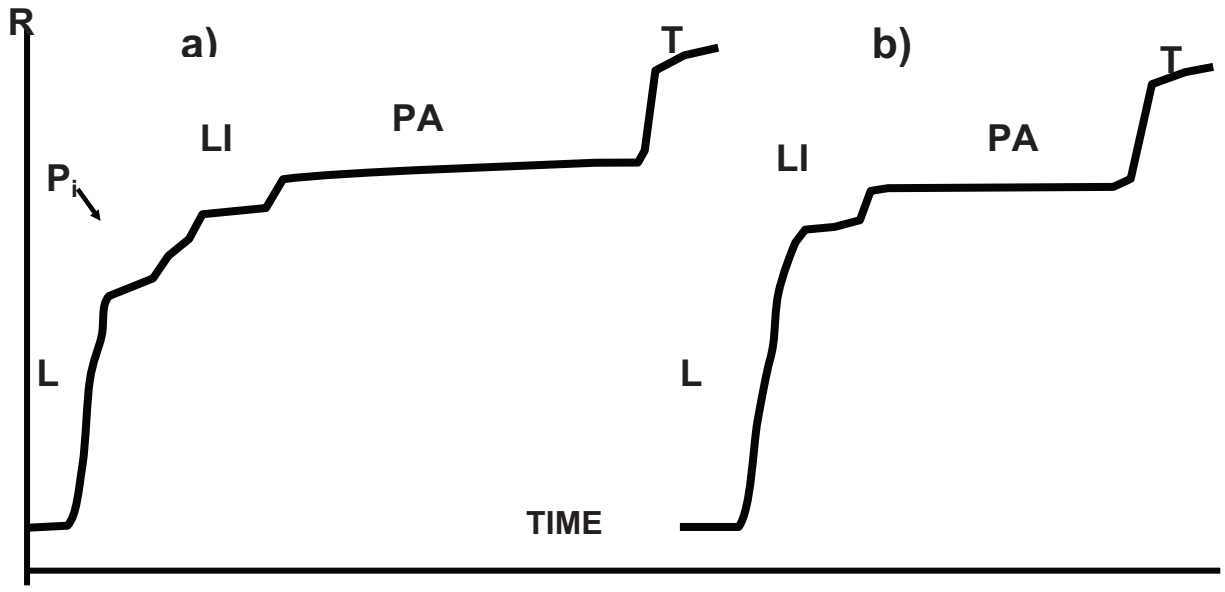
To determine Fe^{3+}, 2.5 ml of a tiron solution (40 g l^{-1}), 10 ml of 0.1 M sodium acetate buffer (pH 4.7), appropriate amount of the combined supernatant (0.3 – 1 ml, depending on Fe^{3+} content) and water were mixed to obtain a final volume of 25 ml. After 5 min, the absorbance was measured at 565 nm against a blank. The readings were converted to iron concentrations by means of a calibration graph prepared with standard Fe^{3+} solutions (0 – 8 mM). Iron concentration in the supernatant was used for calculation of Fe present in the precipitate. To dissolve the precipitate, 1.5 M NaOH was added at 9 mol per 1 mol of Fe precipitated. The mixture was vigorously shaken for 15 min, then water was added (10 times as much water as 1.5 M NaOH), and ferric hydroxide separated by centrifugation at 6 000 rpm for 10 min. The supernatant was transferred into a small flask using a syringe. Ferric hydroxide precipitate was washed twice with deionized water, centrifuged at 6 000 rpm for 10 min, and all supernatants were pooled. The pH of the pooled supernatant was adjusted to 6.0 – 6.4 by hydrochloric acid and the volume was brought to 100 ml with deionized water. This solution was analyzed by isotachophoresis. The operation conditions were as recommended by BLATNY *et al.* (1995) : leading electrolyte (pH 6.2) containing HCl (10 mM), BTP (5.5 mM) and HPMC (1 g l^{-1}); terminating electrolyte (pH 6.2) containing 5 mM MES. Time of separation varied from 25 to 35 min. An external calibration method was used with solutions of dodecasodium salt of phytic acid as standards. Seven calibration points were measured in the concentration range from 0.01 to 0.12 mM.

A slightly different extraction procedure was used to extract phytic acid from faecal samples. Eighty ml of 0.65 M HCl were added to 10 g of faeces in 100 ml volumetric flasks. The mixture was first kept in an ultrasonic water bath for 6 min, then shaken at the room temperature for 1 h and diluted to 100 ml. The other procedure was the same as described above. Phytic acid zone was identified on the basis of the step height, using a standard solution. Phytic acid zone in precipitated samples was well separated and of sufficient length. In a separate experiment, extracts were diluted 1 : 1 with a buffer prepared by titration of 0.04 M HCl with a creatinine solution to pH 5.15. Phytase was added to this solution at 4.9 mg/5 ml and incubated at 53°C for 80 min. Total phosphorus was determined after digestion of feeds and faeces with concentrated H_2SO_4 and Se at 420°C in the block digester DS 20 (Tecator, Sweden). Phosphorus in the mineralizate was assayed using the Fiske and SubbaRow method.

RESULTS AND DISCUSSION

Isotachophoregrams of extracts of the feed and faeces of rabbits are shown in Figure 1. No phytic acid zone appeared in samples treated with phytase (data not shown). In a separate experiment, we examined the limit of determination of phytic acid by the iron precipiation method, using standard solutions of phytic acid. The limit of determination of phytic acid was *ca* 3 μmol per 10 ml. This corresponds to 2 g kg^{-1} of phytic acid in a sample of faeces or 4 g kg^{-1} of phytic acid in a sample of a feed. The recovery of phytic acid precipitated from ten 10 ml samples of 1 mM phytic acid was 93.74 ± 7.40%.

Figure 1 – Isotachopherograms of extracts of the feed (a) and faeces (b) of rabbits. PA, phytic acid; LI, lower inositolphosphates; P_i, inorganic phosphate; L, leading ion (chloride); T, terminating ion (MES).



Results of analyses are summarized in Table 2. The feed contained 17.2 g of phytic acid per kg of dry matter (42.5% of total P). Faeces of rabbits contained 2.62 - 8.21 g of phytic acid and 18.4 - 21.5 g of the total P per kg of dry matter. Phytic acid represented 4.0 - 11.0% of the total P in rabbit faeces.

Table 2: Concentration of phytic acid in the feed and faeces of rabbits

Samples	DM	Total P	Phytic acid		
	(%)	(g/kg DM)	(g/kg)	(g/kg DM)	(% of total P)*
Feed	93.13	11.40	16.04	17.22	42.52
Faeces					
1	51.72	19.82	1.57	3.04	4.32
2	34.78	21.55	2.06	5.92	7.74
3	37.50	20.49	2.99	7.99	10.98
4	47.12	21.01	3.87	8.21	11.01
5	53.12	18.37	1.39	2.62	4.02

DM, Dry matter
* Phytic acid contains 28.15% P

Using the same isotachophoretic method, we found that faeces of pigs contained 8.8 - 14.2 g of phytic acid, and 16.0 - 23.3 g of total P per kg of dry matter. Corresponding findings in laying hens were 23.7 g of phytic acid and 18.4 g of total P per kg of dry matter (DUŠKOVÁ *et al.,* 2000). EDWARDS (1993) found by means of a HPLC method 13.0 ± 1.8 g of phytic acid per kg of dry matter of droppings of chickens fed a diet without a phytase supplement. MORSE *et al*. (1992) reported 1.94 ± 1.39 g of phytic acid per kg of dehydrated faeces of dairy cows. It follows from these data that phytic acid concentration in faeces of rabbits was higher than that in cattle, but lower than in faeces of pigs and poultry. Phytic acid concentration in the feed of rabbit was higher than that in feeds of pigs and hens (DUŠKOVÁ *et al.,*

2000), probably because of high bran content. Available literature indicates that typical poultry diets contain 0.87 - 1.40% of phytic acid (RAVINDRAN *et al.,*1995). Similar data were published for feed mixtures for pigs (JONGBLOED *et al.,* 1992). No attempt to measure phosphorus and / or phytic acid digestibility was done in this study. Phosphorus content in rabbit faeces was rather high, similar to that in faeces of pigs fed diets without phytase (21.0 and 16.3 g P per kg of dry matter, as reported by SIMONS *et al*., 1990), but higher than in excreta of broilers (8.0 – 16.4 g P per kg of dry matter, as reported by ZOBAČ *et al*., 1995). In our opinion, relatively low content of phytic acid in rabbit faeces can be explained by microbial activity in the rabbit hindgut.

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