Digestibility of total and phytate phosphorus in young calves

V. Skrivanova[1], M. Marounek[1,2], R. Dvorak[3]

[1]Research Institute of Animal Production, Prague-Uhrineves, Czech Republic
[2]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[3]Clinic of Ruminant Diseases, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: Two experiments were conducted to determine digestibility of total and phytate phosphorus (P) in young calves. In the 1st experiment 14 male calves received a commercial milk replacer (6 l of milk per day) and had free access to a starter concentrate. In the 2nd experiment 21 male calves were divided into 3 groups and fed *ad libitum* a milk replacer (Group I), milk replacer and starter concentrate (Group II), and milk replacer and silaged maize cobs (Group III). Digestibility measurements were carried out at the age of 10 weeks (1st experiment), and 12 and 16 weeks (2nd experiment). In the 1st experiment phytate P accounted for 27.0% of the total P intake. Phytate P was assayed by capillary isotachophoresis. On average, 27.9% of ingested P, but only 3.0% of phytate P were recovered from the faeces. The proportion of phytate P in total faecal P was 5.8%. In the 2nd experiment phytate P accounted for 8.9, 13.8 and 8.6% of total P in diets of calves of Group I, II and III, respectively. On average, 6.6% of ingested P and 3.6% of phytate P were recovered from the faeces. Dry matter of faeces contained total P, phytate P and phosphate P at 9.74, 0.65 and 4.69 mg/g, respectively. Faecal concentrations of total P significantly correlated with those of calcium (r = 0.71). It can be concluded that young calves utilised phytate P very efficiently as no more than a few % of phytate escaped degradation in the digestive tract.

Keywords: digestion; milk nutrition; starter concentrate; faeces

To decrease high feeding costs, expensive milk protein and fat in milk substitutes for young calves are often replaced by ingredients of plant origin. The major portion of phosphorus (P) in these ingredients is present in the form of salts of phytic acid (*myo*-inositolhexaphosphoric acid), which is the primary storage form of P in seeds of plants. Salts of phytic acid, phytates, are also present in various starter concentrates. Contrary to monogastric animals, adult ruminants utilise phytate P very well thank to symbiotic microflora in the rumen (Raun et al., 1956). Several authors concluded that phytates were hydrolysed to inositol and inorganic phosphate in high-producing dairy cows, consuming large quantities of concentrates (Clark et al., 1986; Morse et al., 1992; Marounek et al., 2000). In weaned calves fed a concentrate composed of ground maize, oats and soybean meal, less than 1% of the ingested phytate P was recovered from the faeces (Nelson et al., 1976). Duskova et al. (2001a) found variable concentrations of phytic acid in rectal samples from calves 1, 6 and 13 weeks old, but no data on digestibility of phytate P in milk fed calves are available. Phytate hydrolysis has been attributed to rumen microorganisms, rumen functions, however, are impaired in the milk nutrition period. The aim of our study was to determine the digestibility of phytate P, total P and some other nutrients in young calves fed milk (6 l per day) and starter concentrate *ad libitum* (1st experiment),

Supported by the Ministry of Agriculture of the Czech Republic (Project No. MO2-99-04) and Ministry of Education, Youth and Sports of the Czech Republic (Project No.161700002).

or fed *ad libitum* milk without or with solid feeds (2nd experiment).

MATERIAL AND METHODS

1st experiment

Fourteen Holstein male calves, between 4 and 5 weeks of age, were fed a commercial milk replacer Telasan V (Bodit Ltd., Tachov, Czech Republic). Milk was offered twice a day (6 l in total, 13.5% of solids) and its consumption was measured. A starter concentrate (ČOT-B from Zena Ltd., Mlada Boleslav, Czech Republic) was available *ad libitum*. Ingredients and determined chemical composition of feeds are presented in Table 1. The animals were housed in individual pens that were bedded with wood shavings. Calves were weighed in 10-day intervals. A digestibility trial was carried out at the age of 10 weeks. The preparatory period lasted for one week, then calves received 200 mg of Cr_2O_3 in capsule form twice a day. Feed consumption was recorded. Faeces were collected for 5 days to determine digestibility of phytate and total phosphorus, dry matter, crude protein and ash.

2nd experiment

Twenty-one Holstein male calves, 5 weeks old on average, were divided into three groups. Calves of all groups were fed *ad libitum* a commercial milk replacer Telasan V. In addition, calves of Group II had free access to a commercial starter concentrate ČOT-B. Silaged crushed maize cobs were available *ad libitum* to calves of Group III. Two digestibility trials were carried out as described above, at the age of 12 and 16 weeks.

Analyses and calculations

Phytate P in feeds and faeces was determined by a capillary isotachophoretic method (Duskova et al., 2001b). Faeces were freeze-dried and extracted with chloroform/methanol 2 : 1 in the Soxtec 1043 apparatus (Tecator AB, Sweden) to remove lipids. Phytic acid was then extracted with 0.95 M HCl (50 ml per 5 g sample), precipitated with $FeCl_3$, and ferric phytate dissolved in 1.5 M NaOH. The $Fe(OH)_3$ precipitate was removed by centrifugation, and the supernatant neutralised by adding the catex Dowex 50 WX8 in H^+-cycle. The solution was analysed by means of the column-coupling instrument EA 101 (Villa Labeco Comp., Slovakia). The limit of determination of phytic acid in the extract was ca. 0.3 μmol/ml. The phytate zone was identified on the basis of the step height using a standard solution. In addition, a pooled sample of calf faeces was treated with phytase (Sigma, No. P-1259) as described by Duskova et al. (2001b), and analysed again. Total phosphorus in feeds and faeces was determined by a molybdovanadate reagent, after ashing of dry

Table 1. Composition of feeds of calves

g/kg	Milk replacer[1]	Starter concentrate[2]	Silaged maize cobs
Dry matter	950	889	499
Crude protein	225	190	43.5
Fat	149	32.8	17.1
Fibre	8.8	58.5	82.1
Ash	61.9	57.7	21.4
Phytate P	0.65	2.52	0.01
Total P	7.33	7.31	1.09
Ca	6.68	8.50	1.10

[1]milk replacer Telasan V contained (g/kg): dried whey with palm oil (490), dried buttermilk with palm oil (230), heat-treated soybean meal (70), extracted linseed meal (50), feed casein (50), feed albumin (50), yeast (30), soybean protein (10), citric acid (10), vitamin and mineral supplement (10)

[2]starter ČOT-B contained (g/kg): ground barley (246), wheat (180), extracted soybean meal (172), wheat bran (120), lucerne meal (100), triticale (50), maize (50), rapeseed meal (50), vitamin and mineral supplement (32)

samples at 550°C (AOAC, 1980). Phosphates present in faeces were extracted with 5% (w/v) trichloroacetic acid and determined by the Fiske and SubbaRow method, as modified by Peterson (1978).

Feeds and faeces were air-dried at 105°C to constant weight to estimate the dry matter content. Contents of protein, fat and fibre were determined employing instruments Kjeltec Auto 1030 Analyser, Soxtec 1043 and Fibertec 2010 from Tecator AB Comp. (Sweden), respectively. Calcium and chromium were determined by the atomic absorption spectrometry using a Perkin Elmer 5000 instrument.

Digestibility of nutrients was calculated as described by Kracmar (1981). Data were analysed by one-way analysis of variance, followed by the Bonferroni test where appropriate. Age effect was evaluated by the *t*-test.

RESULTS

The zone of phytates in extracts of feeds and faeces was well separated from zones of lower inositolphosphates and inorganic phosphate, and disappeared in the extract treated with phytase. Samples of feeds and faeces contained a small amount of lower inositolphosphates (Figure 1). Milk replacer, starter concentrate and silaged cobs used in this study contained 0.65, 2.52 and 0.01 g of phytate P per kg, respectively (Table 1). The proportion of phytate P in total P of these feeds was 8.9, 34.5 and 0.9%.

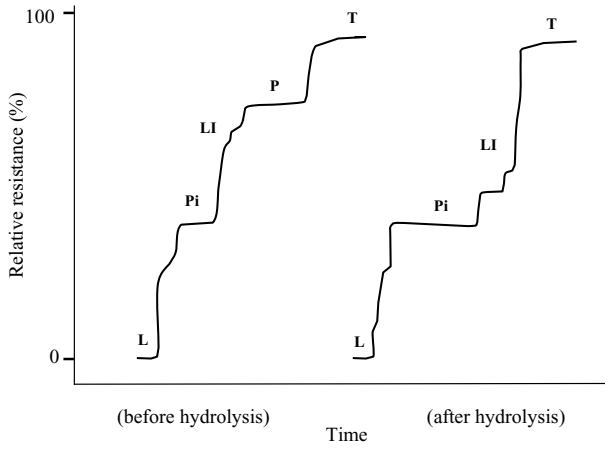

Figure 1. Isotachophoregrams of extracts of faeces of calves before and after hydrolysis of phytic acid by phytase; P = phytate; LI = lower inositolphosphates; Pi = inorganic phosphate; L = leading ion; T = terminating ion

In the 1st experiment, phytate P accounted for 27.0% of the total P intake of calves fed the milk replacer and starter. Digestibility of phytate P was higher than apparent digestibility of total P (Table 2). On average, 3.0% of ingested phytate P and 27.9% of total P were recovered from the faeces. Digestibility of dry matter was higher than that of crude protein (94.8 vs. 72.3%). The concentration of phytate P in faeces ranged from 0 to 1.48 mg/g dry matter. On average, phytate P accounted for 5.8% of total P in faeces.

In the 2nd experiment, phytate P accounted for 8.9, 13.8 and 8.6% of the total P intake in calves of Group I, II and III, respectively. The intake of phytate P was significantly higher ($P < 0.05$) in calves consuming milk replacer and starter

Table 2. Intake of feeds, phytate and total P and calcium, and digestibility of nutrients, and faeces composition in calves fed milk replacer and starter concentrate. Results of the 1st experiment

Age of calves[1]	70 (0.4)
Weight of calves[1]	82.9 (0.8)
Intake (g/day)	
Milk replacer	717 (27)
Starter	1 816 (78)
Total P	18.5 (5)
Phytate P	5.0 (1)
Ca	20.2 (6)
Digestibility (%)	
Dry matter	94.8 (0.3)
Crude protein	72.3 (1.8)
Total P	72.1 (1.4)
Phytate P	97.0 (0.4)
Ca	42.6 (3.3)
Faeces composition	
Dry matter (mg/g)	231 (10)
Crude protein (mg/g DM)	265 (10)
Total P (mg/g DM)	7.09 (0.22)
Phytate P (mg/g DM)	0.41 (0.11)
Ca (mg/g DM)	22.5 (0.1)

Standard errors are given in parentheses
[1]at the start of digestibility trial

(Group II) than in the other calves (Table 3). In one calf the digestibility of phytate P was 88.9%. In other calves it ranged from 92.1 to 99.1%. Apparent digestibility of total P ranged from 74.8 to 97.2%. On average, 3.6% of ingested phytate P and 6.6% of total P were recovered in the faeces. Calves consuming milk replacer and starter concentrate (Group II) digested phytate P, total P, dry matter and crude protein better than calves fed milk replacer with maize cobs (Group III). In 16-week-old calves these differences were statistically significant ($P < 0.05$). At the end of the experiment, calves of both groups consuming solid feeds gained insignificantly more than calves of Group I ($P > 0.05$).

Table 4 presents data on the composition of faeces of calves in the 2nd experiment. Phytate P accounted for on average 6.6% of total P in faeces. The concentration of total P in faeces significantly correlated with that of Ca ($r = 0.71$; $P < 0.001$). Correlation between faecal concentrations of phytate P and Ca was also statistically significant, but less pronounced ($r = 0.36$; $P < 0.02$). Phosphates

Table 3. Intake of feeds, total and phytate P, and nutrient digestibility in calves fed milk replacer (Group I), milk replacer and starter (Group II), and milk replacer and silaged maize cobs (Group III). Results of the 2nd experiment

	Treatment group			Treatment group		
	I	II	III	I	II	III
Age of calves (days)[1]	88 (1.5)	84 (2.3)	88 (1.5)	116 (1.5)	112 (2.3)	116 (1.5)
Weight of calves (kg)[1]	98.5 (4.01)	103.5 (7.75)	101.7 (3.40)	130.7 (9.11)	140.8 (7.26)	138.9 (4.57)
Intake (g/day)						
Milk replacer	2 043 (97.1)	2 157 (61.2)	2 186 (55.2)	2 114 (145.5)	2 343 (137.6)	2 367 (97.5)
Starter	– –	445 (26.5)	– –	– –	633 (35.5)	– –
Silaged cobs	– –	– –	393 (70.3)	– –	– –	817 (59.7)
Total P	15.0^{a} (0.71)	19.1^{b} (0.56)	16.4^{ab} (0.37)	15.5^{x} (1.07)	21.8^{y} (1.09)	18.2^{xy} (0.71)
Phytate P	1.3^{a} (0.06)	2.5^{b} (0.09)	1.4^{ac} (0.06)	1.4^{x} (0.09)	3.1^{y} (0.14)	1.6^{xz} (0.06)
Digestibility (%)						
Dry matter	95.4 (0.68)	95.8 (0.38)	93.1 (1.36)	95.4^{xy} (0.64)	96.7^{x} (0.42)	92.8^{y} (1.44)
Crude protein	93.3 (0.53)	93.4 (0.91)	89.1 (2.34)	93.9^{xy} (0.79)	95.8^{x} (0.42)	90.9^{y} (1.78)
Total P	93.9 (0.94)	94.4 (1.36)	89.7 (3.21)	94.7^{xy} (0.79)	96.2^{x} (0.49)	91.3^{y} (1.97)
Phytate P	95.6 (0.34)	97.2 (0.64)	94.6 (0.98)	97.1^{xy} (0.30)	98.4^{x} (0.19)	95.6^{y} (0.98)

Standard errors are given in parentheses

[1]at the start of digestibility measurement

$^{a,b,c;\ x,y,z}$values in the same row and section with unlike superscript differ significantly at $P < 0.05$

Table 4. Content of phosphorus fractions, calcium and ash in faeces of calves fed milk replacer (Group I), milk replacer and starter (Group II), and milk replacer and silaged maize cobs (Group III). Results of the 2nd experiment

	Treatment group			Treatment group		
	I	II	III	I	II	III
Age of calves (days)[1]	88 (1.5)	84 (2.3)	88 (1.5)	116 (1.5)	112 (2.3)	116 (1.5)
Total P (mg/g DM)	10.4 (0.74)	10.1 (0.86)	9.9 (1.28)	9.2 (0.35)	9.6 (0.62)	9.2 (0.29)
Phytate P (mg/g DM)	0.94 (0.132)	0.76 (0.094)	0.64 (0.102)	0.57 (0.113)	0.57 (0.151)	0.41 (0.110)
Phytate P/total P (%)	9.0 (1.73)	7.5 (0.98)	6.5 (1.88)	6.2 (1.20)	5.9 (1.35)	4.5 (1.24)
Phosphate P (mg/g DM)	3.18 (0.393)	4.92 (0.809)	6.03 (1.364)	4.98* (0.552)	5.48 (0.726)	3.55 (0.416)
Phosphate P/total P (%)	30.6^{a} (5.18)	48.7^{ab} (6.95)	60.9^{b} (9.75)	54.1 (5.10)	57.1 (6.39)	38.6 (3.40)
Ca (mg/g DM)	16.1^{ab} (0.65)	16.4^{a} (0.96)	13.4^{b} (0.70)	12.6* (0.59)	12.9* (0.64)	11.6* (0.37)
Ash (mg/g DM)	127 (4.9)	114 (3.5)	122 (2.4)	126^{x} (5.9)	109^{y} (3.8)	115^{xy}* (1.3)

Standard errors are given in parentheses

[a,b,c; x,y,z]values in the same row and section with unlike superscript differ significantly at $P < 0.05$

*significant effect of age ($P < 0.05$)

extractable with 5% trichloroacetic acid were the principal fraction of P in faeces (48.3% of total P, on average). Concentrations of phytate P, total P and calcium in faeces decreased with age, but only the effect of age on faecal Ca concentration was significant ($P < 0.05$).

DISCUSSION

In the 1st experiment the availability of milk was restricted to 6 l offered per day. In the 2nd experiment milk was available *ad libitum*. Table 3 shows that calves given milk *ad libitum* had limited desire to eat solid feeds. Most nutrients were thus supplied with milk replacer, and, as expected, nutrient digestibility was high. Digestibility of total P and crude protein in calves of 1st experiments was lower. Digestibility of phytate P was high in calves of all groups despite of the fact that rumen functions in young calves are not fully developed. On average, 97.0 and 96.4% of phytate was hydrolysed in the digestive tract of calves in the 1st and the 2nd experiment, respectively. High apparent P digestibility (96.3%) was found by Guilloteau and Toullec (1980) in calves fed skim milk. Corresponding values of apparent P digestibility in older cattle (Witt and Owens, 1983; Klosch et al., 1997) and weaned calves (Challa and Braithwaite, 1988) were lower. Low faecal losses of P (ca. 17 and 10 mg/day per kg of live weight in the 1st and the 2nd experiment, respectively) presumably reflect high P requirement of young, rapidly growing animals.

Calcium hinders the phytate hydrolysis (Maenz et al., 1999; Marounek et al., 2003). Excessive Ca intake increased phytate content in faeces of pigs (Seynaeve et al., 2000), hens (Scheideler and Sell, 1987), and rats (Wise and Gilburt, 1982). In this study faecal concentrations of total and phytate P significantly correlated with concentration of Ca. This can be explained by the formation of insoluble complexes containing both elements. No correlation, however, was found between faecal Ca concentration and total P and phytate P digestibility.

In summary, the present results indicate that only a few % of the ingested phytate P escape hydrolysis in the digestive tract of young calves in the milk nutrition period. Concentrations of phytate P in faeces were well measurable, but much lower than those reported in faeces of pigs and poultry (Duskova et al., 2001b). Our results are in line with the opinion that almost all phytates are degraded in the ruminant digestive tract.

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Received: 03–10–02
Accepted after corrections: 04–02–25

Corresponding Author

Prof. Milan Marounek, Research Institute of Animal Production, Přátelství 815, P.O. Box 1,
104 01 Praha 10-Uhříněves, Czech Republic
Tel. +420 267 009 566, e-mail: marounek@iapg.cas.cz