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Effects of forage phosphorous content on faecal phosphorous excretion and possible markers of low phosphorous intake in foals fed forage-only diets

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Abstract

Knowledge of endogenous nutrient losses is important when estimating the nutrient requirements of animals. It has been suggested that faecal endogenous phosphorus (P) losses differ between growing and adult horses, but studies on foals are scarce. In addition, studies on foals on forage-only diets with different P contents are lacking. Thus this study: (1) assessed faecal endogenous P losses in foals fed a grass haylage-only diet close to or below estimated P requirements; (2) evaluated use of serum cross-linked carboxyterminal telopeptides of type-I collagen (CTx) as a marker of bone resorption secondary to low-P intake; and (3) examined whether analysis of faecal P concentration on a dry matter (DM) basis could be used as an indicator of P intake. Six foals were fed three grass haylages (fertilised to contain different amounts of P: 1.9, 2.1, 3.0 g/kg DM) for 17-day periods in a Latin square design. Total collection of feaces was performed by the end of each period. Faecal endogenous P losses were estimated using linear regression analysis. There was no difference in the concentration of CTx in plasma between diets in samples collected on the last day of each period. A correlation was found ($y = 0.64x - 1.51$; $r^2 = 0.75$, $p < 0.0001$) between P intake and faecal P content, but regression analysis indicated that underestimation as well as overestimation of intake is likely if faecal P content is used to assess intake. It was concluded that faecal endogenous P losses in foals are low, probably no higher than in adult horses. It was also concluded that plasma CTx cannot be used to assess short-term low-P intake in foals and that faecal P content cannot be used to assess differences in P intake, at least not when P intake is close to or below estimated P requirements.

KEYWORDS

CTx, faeces, forage, horse, phosphorus endogenous, plasma phosphorus

1 | INTRODUCTION

Knowledge of endogenous nutrient losses is important for estimating the nutrient requirements of animals. In adult horses, NRC (2007) has suggested that faecal endogenous phosphorus (P) losses are 8.5–10 mg/kg body weight (BW) and in growing horses 18 mg/kg BW. However, most studies indicate that the losses in growing horses are also ≤10 mg/kg BW (Furtado et al., 2000; Kichura et al., 1983; Ögren et al., 2013; Oliveira et al., 2008; Schryver et al., 1971). Overestimation of endogenous losses results in overestimation of the nutrient requirements of the animal. In the case of P this might not be a problem for the horse as long as calcium (Ca) intake is balanced, but it is well known that P excretion from farm animals contributes to eutrophication (Sharpley et al., 2003). In horses, P is mainly excreted in the faeces (Hintz & Schryver, 1973; Ögren et al., 2013; Schryver et al., 1971) and since horses are commonly kept outdoors and will defecate on any surface, there is a risk of high run-off P losses (Parvage et al., 2013, 2015). In addition, global reserves of phosphate rock are dwindling (Cordell et al., 2009) and feeding excess P to animals must therefore be avoided.

Information on factors that might influence endogenous mineral losses in horses is scarce, but faecal endogenous P losses have been shown not to be related to P intake (Schryver et al., 1971). NRC (2007) suggests that growing horses have higher faecal endogenous P and Ca losses than adults but to our knowledge, there is no published research on endogenous losses of P in foals fed forage-only diets. In cows, it has been suggested that faecal endogenous P losses are dependent to some extent on diet, with e.g. cows on a high-forage diet having greater endogenous losses than cows on a high-concentrate diet (Spiekers et al., 1993). In horses, most studies performed have used diets including cereals (Fowler et al., 2015; Oliveira et al., 2008) and there are only a few using forage-only diets (Ögren et al., 2013; Saastamoinen et al., 2020).

For the purpose of monitoring possible bone resorption, type I collagen (CTx) degradation molecules are suggested to be useful plasma markers (Lepage et al., 2001) and therefore this study evaluated the use of serum cross-linked carboxyterminal telopeptides of CTx as a marker of bone resorption secondary to low-P intake.

The main aims of the present study were to assess faecal endogenous P losses in foals on a grass forage-only diet and also study some potential markers (faecal P content and a marker of bone resorption) of low-P intake in foals. In addition, we examined whether analysis of faecal P content on a dry matter (DM) basis could be used as a marker of P intake in foals on a forage-only diet. Our hypotheses were that P endogenous losses in foals are in the same range as previously reported for growing horses and that P in a faecal spot sample reflect P intake and that CTX in plasma can be used to detect low-P intakes.

2 | MATERIALS AND METHODS

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received by the National Animal Research Committee of Iceland. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. The experiment took place at Hólar University in Iceland.

2.1 | Forage production

Regression analysis was used to estimate the faecal endogenous losses and with this method it is important to have a variation in P intake and also observations as close to zero P intake as possible. To achieve this without using synthetic diets, forages with a variation in P content was produced. Mixed grass (mainly *Poa pratensis and Elytrigia Repens*) haylage was harvested at a vegetative stage after heading—from three neighbouring 0.8-hectare (ha) fields at Hólar (65°44′N, 19°06′W, 160 m above sea level) and wrapped in plastic. The fields were fertilised with the same amount of nitrogen (90 kg N/ha) and potassium (50 kg K/ha) and differed only in P amount, with 0, 29 and 57 kg P/ha being applied to achieve three levels of P content in the harvested forage.

2.2 | Animals, experimental design and forage content

Six foals (colts) of the Icelandic breed were fed the three haylages for 17 days/forage in a Latin square design (Figure 1). At the beginning of the study, the foals were aged 8 ± 1 months and BW was 200 ± 20 kg (mean ± *SD*, electronic scale: Tru-Test model 702, Datamars, New Zealand, accuracy 1%). Foals had been kept on pasture during the summer and had been fed haylage at the side of their dams for several weeks before the study. They were weaned 2 weeks before the study began. The foals were divided into two groups. Within each group, foals were then randomly allocated to one of three haylages in period 1. Haylage samples collected during the study showed that the haylage used contained 1.9 g (low), 2.1 (medium) and 3.0 g (high) P/kg DM. Haylage DM, energy and nutrient content are shown in Table 1. Energy intake was estimated to correspond to a daily weight gain of 370 g (NRC, 2007) where DE/ME ratio of 0.85 was used for energy conversion (Vermorel et al., 1997). The average crude protein (CP) content of the 3 haylages (588 g/CP/d) met or exceeded recommendations (NRC, 2007) assuming adult weight of 370 kg.

2.3 | Management and feeding

The foals were housed individually in boxes (1.5 × 3 m) in which the floor was covered with rubber mats. During the first 11 days

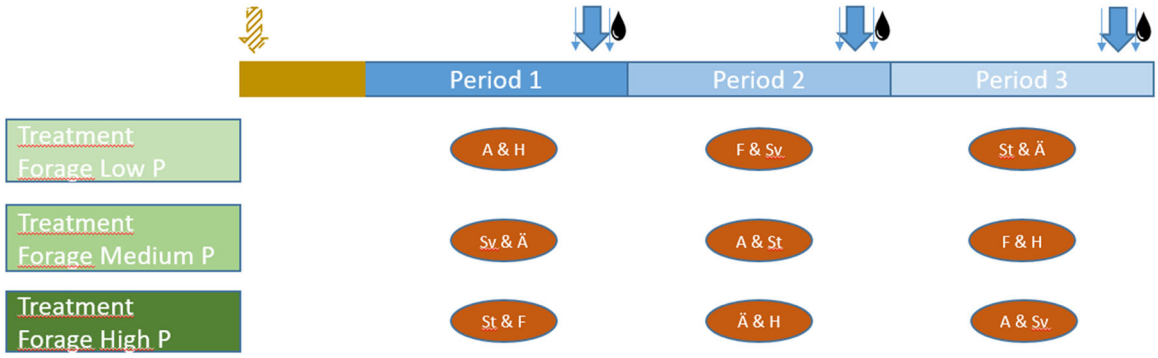

FIGURE 1 Six foals (A, H, Sv, St, F and Ä) were fed three forages with different phosphorous (P) contents (low, medium and high) for 17 days in an unbalanced Latin Square design. During the last 4 days of each period total faecal collection was made (thick arrows) and faecal grab samples were collected on the first and last collection day (thin arrows). A blood sample was collected on the last day (drop). Foals had been kept on pasture during the summer and had been fed haylage at the side of their dams for several weeks before the study. They were weaned approximately 2 weeks prior to the study (hatched arrow). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Dry matter, chemical composition and energy content of three haylages with different phosphorus (P) contents (low-P, medium-P and high-P) fed to six foals in a latin square design.

Item	Diets		
	Low-P	Medium-P	High-P
Haylage			
DM, %	81	82	80
NDF, % of DM	58	57	56
CP, % of DM	13.6	11.3	14.3
ME, MJ/kg[a]	9.6	9.9	10.1
Ash, % of DM	6.5	6.4	6.9
P, % of DM	0.19	0.21	0.30
Ca, % of DM	0.43	0.46	0.49
Mg, % of DM	0.31	0.29	0.34

Abbreviations: DM, dry matter; NDF, neutral detergent fibre.
[a]Calculated value according to Jansson et al. (2011).

of each period the foals spent 4 h/d in a large paddock (12 × 60 m) where they could move freely and during the last 6 days they spent 20 min/d in the paddock under supervision to prevent them from eating dirt from the paddock and making sure no feaces where lost. Daily offered amounts of haylage was 6.2 kg divided into three meals, at 08.00, 14.00 and 20.00 h. The front of each box had an opening and the foals ate haylage from the concrete floor outside the box, this system allowed precise supervision of the feed intake and collection of left overs. Water was provided ad libitum from automatic water bowls. Once per day, 10 mL of a vitamin E-selenium mixture (containing 15% Tranol, 3200 IE/g vitamin A, 320 IE/g vitamin D_3, 11,200 mg/kg vitamin E, 20 mg/kg selenium [Rautt Tranol 16% Lifland]) were added to the water and 15 g of a salt/trace element/vitamin mix (containing per kg: 98.5% salt, magnesium oxide [0.11% Mg], 400 mg copper, 280 mg zinc, 210 mg iron, 200 mg manganese, 15 mg iodine, 15 mg cobalt, 5 mg selenium, 150 mg vitamin E, 20 mg Biotin [HM Hestasteinn Lífland]) were provided in feeding troughs. Weekly, during the whole experimental period the foals live weight was registered. The foals were dewormed (Ivomec®, ivermectin 1%) and kept at the research facility for a minimum of 1 week before the study began and were then fed the medium haylage and the supplements. Before starting the experiment, the foals winter coats where clipped on the belly for hygiene reasons and on the neck to simplify blood sampling. During the study, signs of growth abnormalities were visually monitored by a veterinarian.

2.4 | Sampling of feaces, feed and blood

Total collection of faeces was conducted in all individuals the last 4 days in each period (Figure 1). Faeces were collected from the box floor immediately after defecation. Daily output was weighed and mixed and 5% frozen for further analysis. To compare mineral excretion patterns using faecal spot sampling and total collection of faeces a 250 g spot sample of feaces was also taken on the first and last day of each collection period (Figure 1) and frozen until analysed.

Haylage samples were excavated by drilling and subsamples of 100 g were collected. Haylage samples were taken every third day during the first 11 days, while during the last 6 days' samples of feed and left-overs were taken daily. Refusals or left-overs were removed daily at 10.00 h and weighed (Marel M-serie 1100) on a scale (±2 g sensitivity up to 6 kg). Feed samples and refusals from the collection period were frozen directly after collection and stored until analysis.

Blood samples were taken by venepuncture of the jugular vein on the last day of the faecal collection period (Figure 1) and collected in heparinised tubes (9 mL, Vacuette°; Greine-Bio-One Blood) samples were kept chilled before being centrifuged for 5 min at 520*g* and 20°C (Hettich EBA 12). The plasma fraction was separated and frozen (−20°C) until analysis.

2.5 | Analyses

Before starting the study, haylage samples were analysed for energy, CP, neutral detergent fibre (NDF) and P using NIRS (FOSS). Those data were only used for confirming the differences in P content of the haylages and for deciding the feeding level in the experiment.

During the whole study, daily DM intake was determined as the difference between offered and refused amount of DM. For feed, refusals and faeces, the samples were mixed, dried for 48 h at 60°C and milled to pass through a 1-mm sieve. DM content was determined by overnight drying at 105°C. Ash content was determined by incinerating a 2 g sample at 550°C for 3 h. Feed samples were analysed in duplicates on pooled samples from each period. The concentration of NDF was determined according to Chai and Udén (1998). Analysis of CP was performed according to Kjeldahl (1883), where ammonia nitrogen concentration was determined by direct distillation with a Kjeltec 2460 analyser (FOSS) and N content was multiplied by 6.25 to give the CP content. For estimating the haylages energy content, digestibility coefficient of organic matter (VOS = Ruminal fluid Digestible Organic Matter) and metabolisable energy (ME) content were determined in vitro according to Lindgren (1979) using the formula: MJ ME/kg OM = 0.160 VOS − 1.91 that is then converted to MJ/kg DM. The ME content is, however, based on the ME for ruminants, so it was adjusted for horses using the following equation: $ME_{horse} = 1.12\ (ME_{ruminant}) - 1.1$ (Jansson et al., 2011).

Minerals were analysed at a commercial lab (Agrilan). Phosphorus, Ca and magnesium (Mg) content were analysed by inductively coupled plasma-atomic emission spectroscopy (Spectro Flame, SPECTRO Analytical Instruments) after digestion with nitric acid (Balsberg-Påhlsson, 1990). Acid-insoluble ash (AIA) was analysed by a method described by Van Keulen and Young (1977). The inorganic P in plasma was analysed by a spectrophotometric method (PH 1016; Randox; Henry, 1974; Tietz, 1990) with molybdate as reagent and wavelength 340 nm (Kinetics Spectrophotometer, Ultrospec K 4053; LKB Biochron). Enzyme-linked immunosorbent assay (AC-02F1 lot: 18233, Immunodiagnostic Systems Nordic a/s) was used for analysing CTx in plasma (intra-assay CV 4.2%).

2.6 | Calculations and statistical analyses

In addition to analysis of P data, data on Ca and Mg intake and excretion were analysed to clarify the macro-mineral status. Mineral retention was determined by subtracting mineral faecal output from mineral intake. Faecal endogenous losses were estimated using simple linear regression analysis (P intake vs. faecal excretion of P). For faecal spot samples, AIA was used as an indigestible marker and the apparent total tract digestibility was calculated using the equations:

1. Faecal excretion of X (g/d) = (intake AIAg/kg DM ÷ output AIAg/kg faecal DM) × Xg /kg faecal DM.

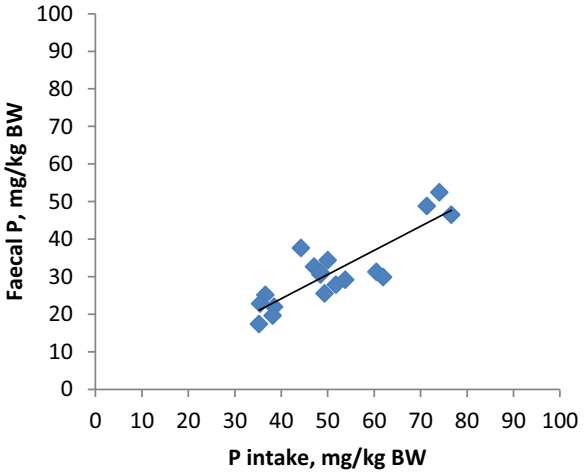

FIGURE 2 Relationship between intake [mg/(kg body weight (BW) and day)] and faecal excretion [mg/(kg BW and day)] of phosphorus (P) ($y = 0.64x - 1.51$; $r^2 = 0.75$, $p < 0.0001$) in six foals fed a forage-only diet with three levels of P. [Color figure can be viewed at wileyonlinelibrary.com]

2. X digestibility (%) = (1 − (faecal excretion of X (g/d) ÷ intake X(g/d)) × 100

A mean value of the two spot samples per treatment for each individual was used for statistical analysis. In one individual, one faecal spot sample was missed and P content analysis in the other sample was an outlier (>2 SD from the mean), so data from this foal were excluded in this analysis.

All data were subjected to analysis of variance (GLM procedure in the Statistical Analysis Systems package 9.2, SAS Inst.) using the model: $Y_{i\,j\,k} = \mu + \alpha_i + \beta_j + \gamma_k + e_{i\,j\,k}$, where $Y_{i\,j\,k}$ is the observation, μ the mean value, α_i the effect of animal, β_j the effect of treatment, γ_k the effect of period and $e_{i\,j\,k}$ the residuals; $e_{i\,j\,k}$ - IND $(0, \delta^2)$. Data were assessed for normal distribution using residual plots. The probability (p) value for significance between treatments was <0.05. Post hoc analysis was made by a Tukey test (significance $p < 0.05$). Values presented are least square means ± standard error of the mean. Regression tests in SAS were used for p-values of correlations. R^2 was calculated in Microsoft Office Excel.

3 | RESULTS

All the foals remained healthy during the study and their daily weight gain was 343 ± 148 g/d (mean ± *SD*).

3.1 | Endogenous losses and faecal P content

Total faecal excretion of P was correlated (P: $y = 0.64x - 1.51$; $r^2 = 0.75$, $p < 0.0001$) to intake and faecal endogenous losses were close to zero (Figure 2). There were correlations between intake and

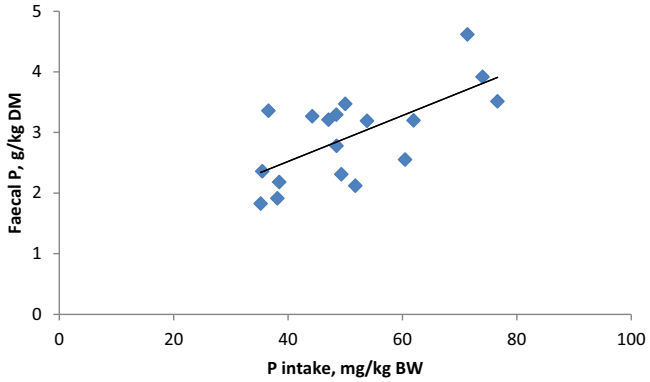

FIGURE 3 Relationship between phosphorus (P) intake and faecal concentration of P on a dry matter (DM) basis ($Y = 0.0379x + 1.006$, $R^2 = 0.44$, $p = 0.0025$). [Color figure can be viewed at wileyonlinelibrary.com]

faecal excretion also for Ca and Mg (Ca: $y = 0.21x + 13.79$, $r^2 = 0.39$, $p < 0.01$; Mg: $y = 0.55x - 0.52$, $r^2 = 0.85$, $p < 0.0001$). There was a correlation between P intake and faecal P concentration on a DM basis, but R^2 was <0.5 (Figure 3).

3.2 | Dietary response

DM intake did not differ between the three diets ($p > 0.05$, mean: 4.50 ± 0.07 kg DM/d) and weight gain was also not different (6.7 ± 3.5 kg, 5.5 ± 2.6 kg and 4.7 ± 4.7 kg for the low-P, medium-P and high-P diet, respectively; $p > 0.05$). Phosphorus intake and faecal excretion were greater with the high-P diet than the low-P and medium-P diets (Table 2). Retention of P was greater with the high-P diet than the low-P diet (Table 2). Calcium intake did not differ between diets, but Mg intake was greater with the high-P diet than the medium-P diet. Faecal excretion and retention of Ca and Mg did not differ between the diets. The apparent total tract digestibility of P, DM, Ca and Mg was unaffected by the three forages (Table 3) and there was no difference between the collection methods used (Table 4). The concentration of CTx in plasma and the plasma P concentration were not affected by diet (Table 5).

4 | DISCUSSION

In this study, faecal endogenous losses of P were estimated to be zero using linear regression analysis (P: $y = 0.64x - 1.51$; $r^2 = 0.75$, $p < 0.0001$). This strengthens the observations made by others, i.e. that faecal endogenous P losses in growing horses are low, possibly 3–10 mg/kg BW as suggested by Schryver et al. (1971), Kichura et al. (1983), Furtado et al. (2000), Oliveira et al. (2008) and Ögren et al. (2013) rather than 18 mg/kg BW as suggested by Cymbaluk et al. (1989) and NRC (2007). The lack of any measurable endogenous losses in the present study and the low losses (2.5 mg/kg BW)

TABLE 2 Intake and faecal excretion of phosphorus (P), calcium (Ca) and magnesium (Mg) in six foals fed haylages with three levels of P (low-P, medium-P and high-P) in a latin square design.

Item	Diet			SEM	*p*-value
	Low-P	Medium-P	High-P		
P					
Intake, g/d	8.8[a]	9.6[a]	13.3[b]	0.3	<0.0001
Faecal excretion, g/d	5.7[a]	5.5[a]	8.3[b]	0.5	<0.01
Retained, g/d	3.1[a]	4.0[a,b]	5.0[b]	0.3	<0.01
Ca					
Intake, g/d	19.7	20.9	21.3	0.7	n.s.
Faecal excretion, g/d	7.0	6.5	7.9	0.4	n.s.
Retained, g/d	12.6	14.4	13.3	0.6	n.s.
Mg					
Intake, g/d	14.3[a,b]	13.3[a]	15.3[b]	0.3	<0.01
Faecal excretion, g/d	7.8	7.2	8.2	0.3	n.s.
Retained, g/d	6.5	6.2	7.1	0.3	n.s.

Note: [a,b]Different subscripts within rows indicate statistically significant ($p < 0.05$) difference between diets.

TABLE 3 Apparent digestibility of dry matter (DM), phosphorus (P), calcium (Ca) and magnesium (Mg) in six foals fed three forages with different P contents (low-P, medium-P and high-P).

Item	Diet			SEM	*p*-value
	Low-P	Medium-P	High-P		
Total collection					
DM, %	49.3	52.0	50.7	1.0	n.s.
P, %	36.6	41.9	38.1	2.8	n.s.
Ca, %	63.6	69.1	62.6	2.1	n.s.
Mg, %	45.7	46.2	46.5	1.4	n.s.

TABLE 4 Apparent digestibility of dry matter (DM), phosphorus (P), calcium (Ca) and magnesium (Mg) in five forage-fed foals, estimated by the total collection method and by the acid-insoluble ash (AIA) method on grab samples.

Item	Method		SEM	*p*-value
	Total collection	Grab samples		
DM, %	49.8	50.1	2.1	n.s.
P, %	37.6	34.3	3.4	n.s.
Ca, %	66.5	66.0	0.9	n.s.
Mg, %	47.1	45.7	2.3	n.s.

TABLE 5 Plasma concentrations of inorganic phosphorus (P_i) and type-I collagen (CTx) in six foals fed three forages with different P contents (low-P, medium-P and high-P).

Item	Diet			SEM	*p*-value
	Low-P	Medium-P	High-P		
Pi, mmol/L	1.47	1.37	1.42	0.17	n.s.
CTx, nm/L	0.450	0.438	0.506	0.025	n.s.

observed by Ögren et al. (2013) in forage-fed yearlings also indicate that losses are not higher on forage diets than on the mixed diets used in previous studies. Moreover, no support was found for the claim that low age increases endogenous losses, since the losses in foals appeared similar to those in the yearlings studied by Ögren et al. (2013). If endogenous losses increase with size of the gastrointestinal tract (also relative to BW), it seems logical that they would be *lower* in foals than in more mature horses.

Assuming 8 g P deposition per kg BW gain, a feed absorption efficiency of 40%–45% (NRC, 2007) and endogenous losses of 10 mg/kg BW, the foals in the present study would have needed 11.5–13 g P/day which is lower than the recommendation of 17 g/day suggested by NRC (2007) for foals with expected adult weight 370 kg, but closer to the German recommendation of 15 g P/day (Flachowsky et al., 2014). This expected amount was not supplied by the low-P or medium-P diet, a finding supported by the increased retention observed as P intake increased. The absorption of 3–5 g P/day probably closely reflects P retention, since only small amounts of P are lost through urinary excretion at low and adequate P contents in the diet (Buchholz-Bryant et al., 2001; Caple et al., 1982; Ögren et al., 2013; Schryver et al., 1971). However, the low level of P intake did not cause any significant treatment effects on CTx and plasma P concentration. Nevertheless, individual plasma P concentration ranged between 0.59 and 1.95 mmol/L (corresponding to 1.8–6.0 mg/dL) and in two individuals on the low-P diet hypophosphataemia (<0.7 mmol/L; Vervuert & Kienzle, 2013) was observed. Despite this and the low Ca intake in the present study (around 75% of NRC (2007) recommendations), no visual growth abnormalities were noted. This was probably due to the short duration of low-P and Ca intake and the fact that the Ca/P ratio still remained balanced in all diets (1.6–2.2:1). To our knowledge, this study is the first to investigate a relationship between P intake and plasma CTx in foals. The lack of response to the low-P diet is in agreement with observations for dairy cows fed low-P (0.32% of DM) and high-P (0.43% of DM) diets (Ekelund et al., 2006).

Although there was a correlation between P intake and faecal P concentration on a DM basis, the faecal concentration could not be used as an indicator of P intake under the experimental conditions applied here. In the three most biased samples (representing 17% of samples), P intake was overestimated or underestimated by 33%–69%. Thus for assessment of P intake in foals, analysis of feed P content is still the best method.

In order to assess faecal mineral endogenous losses properly by simple regression analysis, it is important to have a variation in intake and also to have observations as close to zero intake as possible. This limits the error range when the line is extrapolated to zero intake. However, in equine diets extremely low mineral intakes can only possible be achieved on artificial diets, which might negatively influence gut function and health. Nevertheless, data from Schryver et al. (1971) show that results from linear regression analysis may be comparable to results from radioisotope-labelled P analysis (Ögren et al., 2013), although the authors did not compare the two methods directly. The results also showed that total daily mineral excretion and apparent total tract digestibility can be successfully assessed using spot samples and the AIA method. This confirms conclusions made in a meta-analysis of independent experiments by Sales (2012), where digestibility coefficients of forage-only diets estimated by AIA and total collection were similar.

In conclusion, this study shows that faecal endogenous P losses are low in foals fed forage-only diets and probably similar to those in adult horses. It also shows that CTx cannot be used to assess short-term low-P intake in foals and that faecal P content cannot be used to assess differences in P intake, at least not when P intake is close to or below requirements.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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