Phosphorus to horses and cows

by

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LICENTIATE THESIS

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Abstract

It is important from a nutritional perspective to meet the phosphorus (P) requirements of animals. From an environmental perspective however, it is important not to feed more P than necessary, because excessive P will mainly be excreted with feces, and the soluble P fraction is vulnerable to runoff losses and may contribute to eutrophication. The recommended dietary P levels to dairy cows have successively decreased over the years but recently, an increase in the requirements to growing horses was suggested because fecal endogenous P losses might be higher than previously estimated. The main aim of this thesis was to assess fecal P losses in growing horses, and in brood mares and dairy cows kept on pasture in Sweden.

In paper I, P balance and fecal P losses in growing horses in training fed a forage-only diet *ad lib* with (HP) or without (LP) P supplementation was studied. In addition, the proportion of the extractable (in dilute HCl solution) inorganic P fraction (P_i) of total P (TP) in feces was assessed. Fourteen Standardbred horses (aged 20 months) were used in a cross-over experiment. Feed intake was measured and spot samples of urine and feces were collected. Acid insoluble ash was used as indigestible marker to assess daily fecal output. Estimated P retention was low (<1.7 g/day) and only that for diet HP was significantly higher than 0 g/d. The fecal endogenous P losses were estimated to be less than 10 mg/kg body weight, which do not support the suggested increase in P requirements. The proportion of P_i of TP was > 85% and P_i increased linearly with P intake.

In paper II, TP and P_i in feces from lactating and non-lactating dairy cows (n=167) and brood mares (n=74) kept on pasture was investigated and also the use of P supplements. Samples of feces, forages and pastures biomass were collected. The proportion of P_i of TP was greater for lactating horses than lactating cows (63±4 *vs* 49±5 %). Supplementation of lactating animals with P generally matched their estimated requirements and was therefore justified, while supplementation of non-lactating animals could not be justified.

In conclusion, an increase in P requirement to growing horses seems not to be justified, and the high proportion of P_i in feces from horses indicates that P overfeeding of horses might be potentially more harmful to the environment than P overfeeding of dairy cows.
Keywords: Ad libitum, dairy cows, feces, forage-only, horses, pasture, phosphorus

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ögren, G., K. Holtenius, and A. Jansson. 2013. Phosphorus Balance and Fecal Loss in Growing Standardbred Horses in Training Fed Forage-only Diets. *Journal of Animal Science*. 91. (in press)

II Ögren, G., A. Jansson, and K. Holtenius. 2013. Comparison of fecal phosphorus excretion in grazing horses and cows. (manuscript)

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Licentiate degree

The Licentiate degree, which requires two years of full-time postgraduate study, is intended to guarantee, by means of course work and the completion of a dissertation, that the recipient

- ✓ has demonstrated an ability to investigate and to solve problems scientifically
- ✓ is conversant with general scientific methodology and is familiar with the more important research methods within his or her subject area
- ✓ is knowledgeable within his or her area of expertise and has contributed to the development of this area through his or her own research
- ✓ is able to utilize the scientific literature within the subject area and relate it to his or her research results
- ✓ has in the planning and execution of research, as well as in the analysis of results, worked both independently and in cooperation with others
- ✓ has experience in presenting and discussing research results, both orally and in writing

Abbreviations

ADG	Average Daily Gain
AIA	Acid Insoluble Ash
BW	Body Weight
Ca	Calcium
CP	Crude Protein
DM	Dry Matter
ME	Metabolizable Energy
Mg	Magnesium
P	Phosphorus
P_i	Inorganic P
SP	Acid extractable (Soluble) P_i
TP	Total Phosphorus
VDMI	Voluntary Dry Matter Intake

1 Introduction

Science has never demonstrated any benefit of feeding phosphorus (P) in excess to animals, and optimizing P intake in animals is crucial since P is a non-renewable resource. It has been predicted that P demand will increase and lead to a global peak in P production within 30 years, after which phosphate rock reserve will decline rapidly (Buckingham & Jasinski, 2006). Inorganic phosphate is mined mainly for use in industry and as a P fertilizer in agriculture. In animal farming, inorganic P is also used for mineral supplementation and in processed concentrates and P is the most expensive macro mineral in dairy cow diets (NRC, 2001).

Recommended dietary P levels to meet dairy cow requirements have successively decreased over the past 50 years (ARC 1965, 1980; NRC 2001). However, NRC (2007) recently increased the recommended level for growing horses, mainly based on one study reported that endogenous losses are much higher for growing horses than for adult horses. Most growing horses in Sweden will require mineral supplementation to meet the estimated P requirements according to these revised recommendations.

Diseases associated with P deficiency in horses and cows are very unusual in Sweden, and in fact studies in the USA, Canada and Sweden indicate that dairy cows are more likely to be fed P in excess (Dou et al., 2002; Kebreab et al., 2008; Nordqvist, 2012). Excess dietary P is mainly excreted in feces in both horses and cows and such P excretion may have consequences for the environment. A large amount of the fecal P excreted is in the form of soluble, inorganic P and this fraction is potentially vulnerable to runoff losses to surface water and can thus contribute to eutrophication (Sharpley et al. 2003).

In summary, it is important from a nutritional perspective to meet the P requirements of animals, while from environmental perspective it is important not to feed more P than necessary. Measuring P excretion in feces from animals, especially animals kept on pasture such as horses and cows, is important to minimize the risk for eutrophication and it may have a potential to be an easy, cheap and useful tool to assess P status in horses and cows.

2 Background

2.1 Phosphorus in nature

Phosphorus is essential for all living organisms. It is highly reactive and never occurs as free P in nature, but instead found as phosphate, i.e bound to oxygen (Vance et al., 2003). Phosphorus has many functions that are rather similar for plants and animals; in energy metabolism (ATP), as part of RNA and DNA, as phospholipids in membranes, in acid-base balance and in enzymatic activities (Smith, 2002; Sjaastad et al. 2003). Phosphates are either found as organic compounds, which are defined as ester-bound phosphates, where P is bound to an amino acid, lipid or carbohydrate, or as inorganic compounds, which are highly water-soluble.

Phosphorus in plants is essential for early root development and for maturation of the plant (Smith, 2002). Plants contain both inorganic and organic P and the cytoplasm concentration of inorganic P is 5-10 mmol/L. Plants absorb P from the soil and the availability depends mainly on the concentration of P and the pH in the soil. When plant materials are returned to the soil the organic P they contain is mineralized by soil microbes and becomes available to plants as inorganic phosphates (Sanyal & DeDatta, 1991).

2.2 Horses and cows

Approximately 1% of the body weight in horses and cows is phosphorus, with 85 % of this occurring in the skeleton and teeth, 15 % in blood and soft tissues and 0.1 % in the extracellular fluid (Toribio, 2011). The form of ionizied phosphate is pH dependent and under physiological pH only HPO_4^{2-} and

$H_2PO_4^-$ exists in measurable concentrations. Phosphate is the second most dominant anion in the intracellular body fluid. Normal serum P_i concentrations range is 3-8 mg/dL (1.0-2.6 mmol/L) for horses and 4-8 mg/dL (1.3-2.6 mmol/L) for ruminants, with the higher values for growing animals (Ternouth, 1990; Frape, 2010; Toribio, 2011). Phosphorus is mainly excreted in feces and only small amounts are excreted through urine, sweat and tissue losses (Meyer & Staderman, 1990; van Doorn et al., 2011).

The anatomy and metabolism of the digestive tract of cows and horses differ, the most obvious difference being that cows are foregut fermenters and horses are hindgut fermenters. Some of the anatomical and physiological differences between cows and horses are important for P utilisation. In cows most feed fermentation occurs in the rumen where the microbial activity is high. Dietary P is partly utilized by the rumen microorganisms and small amounts of P are absorbed in the rumen (Breves & Schröder, 1991). Most of the P absorption in ruminants occurs in the small intestines (Sjaastad et al, 2003). A significant amount of the absorbed P is recycled with saliva, and during rumination large volumes of saliva are secreted to the rumen (Pfeffer et al., 1970) (Figure 1).

In horses, available dietary P is absorbed in the small intestines (Figure 2). However, feed fermentation in horses occurs in the hindgut and dietary P needs to reach the large intestines to be available to microorganisms (Dyer et al., 2002). An *in vitro* study by Cehak et al. (2012) indicates a significant P secretion in the small intestines and a net absorption in the small intestines and dorsal colon of horses. This agrees with previous findings by Schryver et al., (1972), but the mechanism of P secretion in the small intestines remains to be investigated (Cehak et al. 2012).

Phosphorus is absorbed by two separate mechanisms: the paracellular pathway which involves passive diffusion down an electrochemical gradient, and the transcellular pathway, which is Na^+-dependent, carrier-mediated and regulated by 1.25-dihydroxyvitamin D_3. The paracellular pathway is more important when the intraluminal P concentration is high. The systemic homeostatic regulation of P is a complex multiorgan process, which involves the parathyroid gland, intestines, bone and kidney (Berndt & Kumar, 2008). The hormones involved are 1.25-dihydroxyvitamin D_3 (also called calcitriol), parathyroid hormone (PTH), calcitonin, and fibroblast growth factor 23 (FGF23). However, the role of vitamin D in regulating serum P levels in horses is unproven (Breidenbach et al, 1998).

Intestinal P absorption is reduced in horses if the diet contain large amounts of phytate P (organic P), since the microbes in the hind-gut in horses only produce small amounts of phytates. In contrast, ruminants are able to utilize phytate P. High Ca intake (300 mg/kg BW) reduce the P absorption (van

Doorn et al., 2004). Phosphorus absorption is approximately 65-80 % of P intake in cows and 35-45% in horses (NRC, 2001; NRC 2007). Fecal P losses represent about 95-98% of total P excretion, while about 1% is excreted via urine and 1% via sweat and cell debris. Excretion via urine is important for P homeostasis in plasma in horses when P intake is high, above 4 g/kg DM (Caple et al., 1982), and when plasma P_i concentration increased from 2 mmol/L to 4 mmol/L significant P was lost via urine in calves (Challa & Braithwaite, 1988). The urinary P concentration is usually low in dairy cows; however, it may vary with a few individual animals occasionally having high concentrations (Wu et al., 2000).

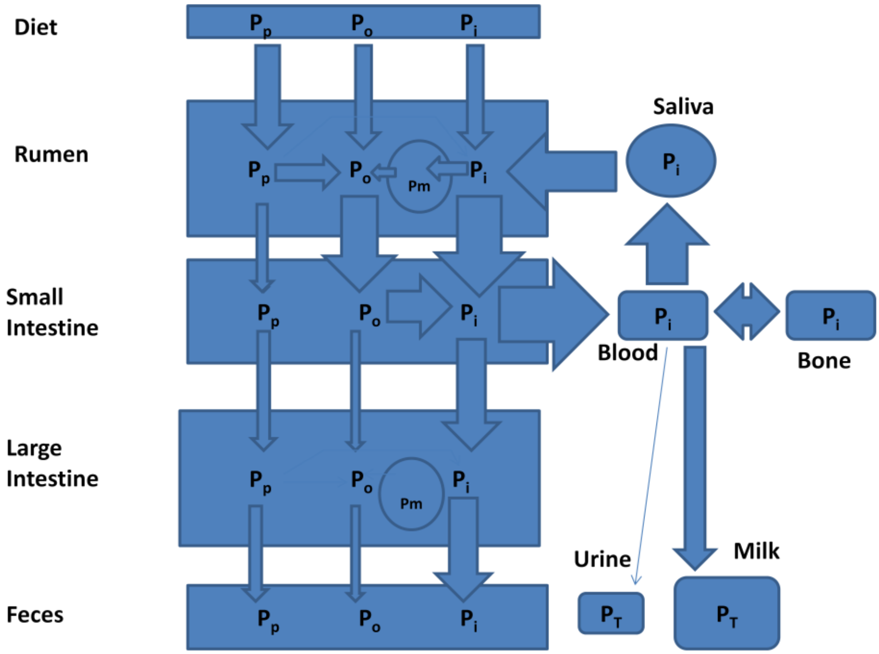

Figure 1. Schematic model of phosphorus (P) absorption and metabolism in a lactating cow fed a complete feed diet. Size of arrows indicates magnitude of flux between compartments. P_p = phytate P; P_o = organic P excluding phytate; P_i = inorganic P; P_m = microbal P, P_T = total P (modified from Hills et al., 2008).

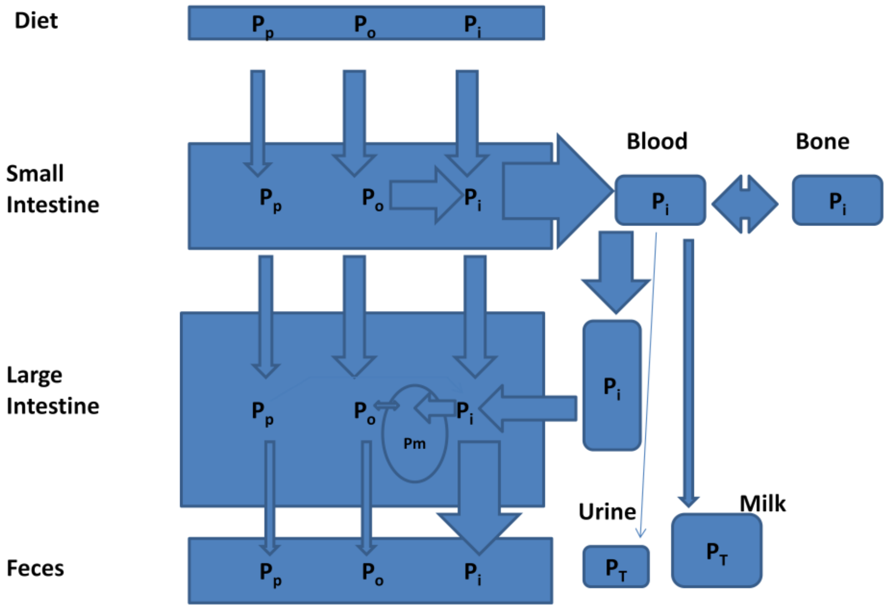

Figure 2. Schematic model of phosphorus (P) absorption and metabolism in a lactating horse fed a forage-only diet. Size of arrows indicates magnitude of flux between compartments. P_p = phytate P; P_o = organic P excluding phytate; P_i = inorganic P P_m = microbal P, P_T = total P.

2.3 Feces

Phosphorus losses in feces are suggested to originate from three different sources: 1) dietary unavailable P, which cannot be absorbed, 2) P inevitable losses from the body and microbes (endogenous losses) and 3) dietary P that is available but exceeds the P requirements (Spiekers et al., 1993). The latter fraction is suggested to be largely, if not completely, present in soluble inorganic P form in cows (Dou et al, 2002).

2.4 Phosphorus requirements

Nutrients are always lost through bodily excretes and cell debris (endogenous losses), and this occurs even if there is no additional intake of the nutrient. The maintenance requirement of a nutrient is the estimated intake that will balance these losses. The recently increased P recommendations for growing horses are mainly based on one study by Cymbaluk et al. (1989), which suggested that fecal endogenous losses are 80 % higher than the previously estimated value (18 mg/kg BW compared with 10 mg/kg BW). Previous studies have mainly used isotope-labeled P for estimating endogenous losses (Schryver et al. 1971, Kichura et al., 1983, Furtado et al., 2000; Oliveira et al., 2008), while Cymbaluk et al. (1989) extrapolated a simple regression line of P intake against fecal P excretion to zero intake. However, data from Schryver et al. (1971) show that the two methods may be comparable, because in that study linear regression analysis resulted in a similar outcome to using radioisotope-labeled P. In adult cows similar values (10-13 mg/kg BW) have been estimated (ARC, 1980; Cymbaluk, 1990). However, in the current nutrient recommendation for cows (NRC, 2001), fecal P endogenous losses are based on fecal dry matter excretion, which is related to dry matter intake (DMI).

2.5 Environmental considerations

Unfortunately, the P excreted by animals may be risk causing environmental damage by running off to surface water and contributing to eutrophication. High P concentrations in surface waters stimulate algae growth and when these algae die dissolved O_2 is depleted and the aquatic environment is negatively affected (Sharpley et al., 1994). Animal excreta spread on growing pastures are utilized by growing plants and tend not to be a problem. However, if the pastures have little vegetation and if the soil is compacted by animal tramping then P runoff is more likely to occur.

3 Aims of the thesis

- To investigate P balance and estimate endogenous fecal P losses in growing horses on forage-only diets
- To describe the variability in total P and acid-extracted inorganic P in feces from lactating and non-lactating horses and cows grazing Swedish pastures., and to evaluate if P in feces is affected by inorganic P supplementation
- To evaluate whether acid-extractable inorganic P as a proportion of total P in feces from horses and cows is influenced by animal species and lactation stage.

4 Materials and Methods

4.1 Experimental designs

In the experiment described in Paper I, 14 horses were fed a forage only diet supplemented or not supplemented with P (monocalcium phosphate) in a cross-over design for six days. The average age of the horses was 20 months and they followed their normal training schedule during the experiment. The horses were fed forage *ad libitum* resulting in continuous intake of P. Feed intake was measured and feed and left-overs were analyzed for dry matter (DM) and mineral content. Spot-samples of feces were collected during days 4-6 in each period and acid insoluble ash (AIA) was used as an internal marker for calculating fecal output. A complementary study was also performed, in which six Icelandic stallions (aged 8 months) were fed forage-only diets with different P content (1.9, 2.1 and 3.0 g P/kg DM, representing low, medium and high P diets) in a Latin Square design for 54 days. Horses in the complementary study were kept in metabolism stalls and total collection of feces was made during the last four days of each period. Feed allowances, feed refusals and feces were weighed and analyzed using the same methods as in the main experiment (Paper I).

In Paper II samples of feces, forage, and grass were collected from pastured animals on dairy cow (n=9) and horse stud farms (n=5). In addition, information on feed and mineral allowances and nutrient content was collected from the farmers. Feces samples from four groups of 2-5 animals from each farm were mixed before analysis. In total, 241 feces samples were collected from horses (n=74) and cows (n=167). Mineral content and metabolizable energy (ME) in forage and grass were analyzed separately for each farm. In dairy cows, energy intake (and consequently dry matter intake) was estimated according to NRC (2001). The ME requirement for lactating cows was based on mean annual production of energy corrected milk (ECM). Estimates of daily P intake were based on forage and pasture analyses and the amounts of concentrates and supplements offered. For non-lactating cows the voluntary daily DM intake on pasture was estimated to be 88 g DM/ kg $BW^{0.75}$according to Menard et al. (2002). Voluntary DM intake of pasture or roughage in lactating mares and mares in late pregnancy was estimated to be 113 g DM/kg $BW^{0.75}$ according to Grace et al. (2002) and in non lactating adult horses 99 g DM /kg $BW^{0.75}$according to Chenost & Martin-Rosset (1985).

4.2 Laboratory analyses

The soluble, inorganic P (SP) in feces samples was analyzed by a modified version of the method described by Dou et al. (2007), for which 5 g of wet feces were mixed with 0,1% HCl-solution to a total mass of 100 g. The samples were shaken, centrifuged and gravity-filtered. Soluble P in feces was then analyzed using a commercially available colorimeric kit (PH 1016, Randox) with molybdate as reagent and P concentrations measured by spectrophotometry at wave length 340 nm.

Total phosphorus (TP) in feed and feces was analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) after digestion with nitric acid (Balsberg- Påhlsson, 1990). Dry matter in feces and feed were determined by drying at 105 C and ash content by incineration at 550 C. Total mineral content in feed and feces was analyzed by the same method as total P. Feed was also analyzed for metabolizable energy (ME) and crude protein by near infrared spectroscopy (NIRS). More details of the laboratory procedures can be found in papers I and II.

4.3 Statistical analyses

All data were subjected to one-way ANOVA using the GLM procedure in SAS. PROC CORR test in SAS was used to determine *P*-values for correlations, correlation coefficient (r^2) and coefficient of variation (CV). The models in GLM and the calculations are described in detail in Papers I and II.

4.4 Calculations

In Paper I, dry matter intake was determined by subtracting dry matter of leftovers from dietary dry matter allowance. Fecal output was calculated as:

Fecal excretion of P (g/d) = (Intake AIA or lignin g/kg DM /Output AIA or lignin g/ kg fecal DM) x fecal P g/kg fecal DM.

Creatinine was used for calculating the amount of urinary daily excretion, assuming constant daily creatinine excretion in urine of 28 mg/kg BW (Meyer & Staderman, 1990). Mineral retention was determined by subtracting mineral fecal and urine output from mineral intake. Fecal P endogenous losses were estimated by extrapolating the regression line for P fecal excretions (mg/kg BW) as a function of P intake to zero P intake.

In Paper II, feed intake in the animals was estimated using equations suggested in previous studies on similar type of animals (Chenost & Martin-Rosset, 1985; Grace et al., 2002; Menard et al., 2002). The body weights values used in calculations was 450 kg for heifers, 620 kg for dry cows and 500 kg for adult horses. Phosphorus intake was calculated by the equation:

Total P intake (g/d) = (pasture or forage concentration of P (g/kg DM) * pasture or forage DM intake (kg/d)) + (P g/kg DM concentrate * concentrate DM intake kg/d) + (P g/kg DM supplement * supplement DM intake kg/d)

5 Results

5.1 Phosphorus balances

The P intakes ranged between 30 and 85 mg/kg BW in Paper I and between 35 and 77 mg/kg BW in the complementary study. In the main experiment (Paper I), there were no difference in P retentions between the diet supplemented with P (high P diet) and the non-supplemented diet (low P diet, Table 1). In the complementary study, the P retention was greater than in the main study and also greater on the high P diet than on the other diets (Table 1). The P intake and retention values were similar for the low and medium P diet.

The apparent P digestibility was low in horses aged 20 months (Paper I) and appeared to be much higher in younger growing horses (8 months; Table 1). The apparent DM digestibility was 58 % on both diets in Paper I, and 49%, 52% and 51% for the low, medium and high P diets, respectively, in the complementary study.

Table 1. *Phosphorus balance and apparent digestibility in 14 Standardbred horses (age 20 months, BW 400 - 496 kg) fed a forage only diet supplemented or not supplemented with P and six Icelandic horses (age 8 months, BW 176 - 234 kg) fed forage-only diet with different P contents. Values shown are least mean square ± standard error of the mean.*

	Horses 8 months			Horses 20 months	
Item	Diet Low P	Diet Medium P	Diet High P	Diet Low P	Diet High P
Balance dat[1]					
P intake g/d	8.8 ± 0.3	9.6 ± 0.3	13.3 ± 0.3	17.5 ± 0.6	32.0 ± 0.6
Fecal P excretion g/d	5.7 ± 0.5	5.5 ± 0.5	8.3 ± 0.5	17.0 ± 0.8	30.3 ± 0.8
P retention g/d	3.1 ± 0.3	4.0 ± 0.3	5.0 ± 0.3	0.2 ± 0.6	1.5 ± 0.6
Apparent digestibility					
P %	37 ± 3	42 ± 3	38 ± 3	2 ± 2	5 ± 2

5.2 Fecal endogenous losses

Using simple linear regression analysis, the estimated endogenous P losses in feces amounted to 2.5 mg/kg BW in the older growing horses (Paper I) and when data for the younger growing horses were added to the data-set the estimated losses became negative (Figure 3).

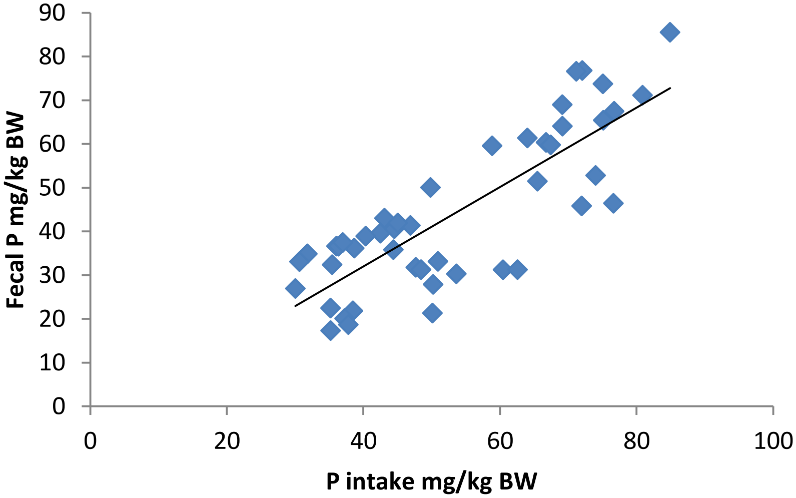

Figure 3. Relationship between P intake and fecal P losses in 14 Standardbred horses (age 20 months, Paper I) and six Icelandic horses (age 8 months) fed forage only diets (two and three diets, respectively) (y=0.91x-4.35, r^2=0.67).

5.3 Phosphorus fractions excreted in feces from horses

The estimated daily fecal DM excretion ranged from 2.3 to 5.2 kg in Paper I, while fecal DM ranged from1.7 to 2.8 kg in the complementary study. Total P in feces was in range 5.1-9.4 g/kg DM in the main study in (Paper I), 1.8-4.6 g/kg DM in the complementary study, and 2.8-10.2 g/kg DM in Paper II. The P_i fraction of TP in feces was greater for growing horses fed a forage-only diet (Paper I) than for mares on pasture (Paper II) (Table 2).

Table 2. *Dietary P(g/kg DM), total P (g/kg DM) and the proportion of P_i of total P (%) in feces from 6 Icelandic horses, aged 8 months; 14 Standardbred horses, aged 20 months, fed forage only diets (Paper I), and 74 brood mares on pasture (Paper II) presented as LSMeans.*

	Horses 8 months, forage-only diet			Horses 20 months, forage-only diet		Mares on pasture	
	Low P diet	Medium P diet	High P diet	Low P diet	High P diet	Lact [1]	Non-lact[2]
DietaryP,g/kg DM	1.9	2.1	3.0	2.4	3.4	u	u
SEM[3]	0.1	0.1	0.1	0.02	0.02		
TP, g/kg DM	2.7	2.5	3.6	5.6	7.7	5.6	6.5
SEM	0.1	0.1	0.1	0.1	0.1	0.6	0.9
P_i of TP, %	n	n	n	87	100	63	61
SEM				3	3	4	6

[1] lactating
[2]non-lactating
[3]Standard Error of Mean
[u] unknown
[n] not analyzed

5.4 Phosphorus fractions excreted in feces from cows

The total P concentration in feces from pastured cows was in the range 4.4-14.9 g/kg DM. Total P, extractable $P_{i,}$ and extractable P_i of TP fractions were greater in feces from non-lactating cows than from lactating cows (Table 3).

Table 3. *Fecal content (g/kg DM) and proportion of P_i of total P (TP) in lactating and non-lactating cows kept on pasture only, or on pasture complemented with forage, concentrates, and mineral supplements.*

	TP, g/kg DM	P_i of TP, %
COWS		
Lactating cows	$7.0^a \pm 0.5$	$49^a \pm 5$
Non lactating cows	$7.6^a \pm 0.5$	$57^b \pm 5$

[ab] different subscripts within columns indicates a statistically difference between effect of animal species*lactation (*P*<0.05).

5.5 Dietary P and the correlation with fecal P

In the main study in Paper I, there were positive correlations between P intake and total P in feces (g/d) (r^2 = 0.93) and between P intake and P_i in feces (r^2 = 0.91), but the correlation between P intake and the insoluble P fraction in feces was weak (r^2 = 0.21). The correlation coefficient between P intake and total P in feces (g/d) in the complementary study was 0.77. However, no correlation was found between dietary P intake (g/kg DM) and TP in feces (g/kg DM).

In Paper II, the calculated P intake and P in feces for horses and non-lactating cows showed no correlation. In dairy cows, estimated P intake and P in feces (g/d) were correlated, but no correlation was found between estimated P concentrations in the diet and the analyzed P concentrations in feces (g/kg DM).

6 Discussion

The P requirement for horses in Paper I was estimated to be about 53 mg/kg BW to meet P requirement for moderate to heavy exercise (NRC 2007).This value is midway between that provided by low and high P diets. The recommended requirement for growing horses is 18 g P/d for each kg gain (NRC, 2007). The younger growing horses (in the complementary study) gained about 600 g/d and P requirement according to NRC (2007) was estimated to be 52 mg/kg BW, which was not met by the low and medium P diets. However, P retention was close to zero for horses aged 20 months in the main study (Paper I), while it was 3-5 g/d for the 8-month old horses in the complementary study, which indicates that P requirements differs between horses in Paper I and the complementary study. The result of Paper I agrees with Nielsen et al. (1998), who reported that variations in P intake did not influence P retention in 2 year-old Quarter horses in training, when they were fed 18-27 g/d, and the low P retention is also in agreement with previous P balance studies investigating horses aged 18-24 months (Cymbaluk et al., 1989; Buchholtz-Bryant et al., 2004; van Doorn et al., 2004).

The value for endogenous P losses in feces of 18 mg/kg BW for growing horses, as reported by Cymbaluk et al. (1989), has been accepted in P recommendations for horses, e.g. NRC (2007) and Frape (2010). Unfortunately, there are also some irregularities in the calculations made by Cymbaluk et al. (1989), because their original result of 42 mg/kg BW was corrected for high Ca intake. The P and Ca intakes in their study was approximately 96 and 143 mg/kg BW, respectively, while P intake in the main study in Paper I and the complementary study ranged between 30 and 85 mg /kg BW, and Ca intake between 66 and 132 mg/kg BW. Obviously, the high value of fecal endogenous P losses reported by Cymbaluk et al. (1989) was not reproducible in our studies. One explanation may be that they used a rather high P intake in their study, while horses in the main study in Paper I and the complementary study were fed low P diets. The minimum amount of P excreted in feces was 17 mg/kg BW, indicating that endogenous P loss in feces is probably less than this value.

In Paper II, cows were generally fed higher dietary P than horses. Dairy farms had pastures with P content adapted to lactating animals and this resulted in high dietary P content for the non-lactating cows. Nevertheless, 33% of the dairy farms supplemented their non-lactating cows with P, which was not justified. Most of the horse stud farms had P content in pasture that was close to meeting the P requirement, and supplemented horses with P when required. However, non-lactating horses require lower P content in pastures than lactating horses, and for these horses the estimated dietary P exceeded their requirements.

Cows had a higher concentration of TP and extractable P_i in feces than horses, but the proportion of extractable P_i of TP was higher in horses. According to Dou et al. (2002), this proportion is related to P status (adequate or excess) in dairy cows, and thus lactating cows in the present study appeared to be fed adequate P. On the other hand, non-lactating cows had higher P concentrations in feces than lactating cows, which indicate that dietary P was more likely fed in excess than deficiency. Horses in Paper I had a much higher proportion of extractable P_i of TP than pastured horses in Paper II. In Paper I the mean value for the proportion of extractable P_i of TP was 93%, which is in agreement with results from Löf (2013) in a study with horses aged 8 to 12 months. However, the result of 100% extractable P_i of TP (with HP diet in Paper I) is not reliable, thus indicating that some analytical error has occurred. The high proportion of P_i in TP found in feces from the horses in Paper I indicates that horses may be able to secrete significant amounts of P via the intestines, due to an increased P concentration in the interstitial P pool with increased P intake as a corresponding mechanism to secretion of P rich saliva in ruminants. Another explanation of the high proportion of P_i in TP may be that microbes in the hindgut in horses are very effective in mineralizing organic P. However, more research is needed before conclusions of P status in horses (adequate or excess) can be drawn by using data from extractable P_i in feces samples from horses.

7 Conclusions

- Phosphorus retention and apparent digestibility is high in young horses and decline with age.

- The recently reported endogenous loss value of 18 mg P/kg BW appears to be less relevant in estimations of P requirements for growing horses than the previously suggested value of 10 mg/kg BW.

- Feeding mineral supplements to non-lactating horses and cows (apart from animals in late pregnancy) grazing Swedish pastures appear often not to be justified.

- Horses appear to have a greater proportion of extractable P_i of total P excreted in feces than cows.

8 Populärvetenskaplig sammanfattning

8.1 Bakgrund

Fosfor är ett livsnödvändigt ämne för alla levande celler och det måste ingå i kosten eftersom det inte kan tillverkas av kroppen. Den största mängden hittas i skelett och tänder, där ca 80 % av kroppens fosfor finns bundet i hårda kristaller. Den fosfor som finns i kroppens mjuka vävnader befinner sig framför allt inuti celler.

Hos gräsätande djur har tarmmikrober en stor betydelse för ämnesomsättningen. Mikroberna finner man i grovtarmen på hästar, där de förjäser fodret som sedan kan utnyttjas som energi. Hos nöt sker denna process i den första förmagen, våmmen. Mikroberna kräver fosfor för sin överlevnad och sina funktioner, men naturligtvis behöver hästar och kor behöver också fosfor till sig själva, små mängder för att överleva, men betydligt större mängder för att producera mjölk. När djuren får i sig mer fosfor än de behöver utsöndras överskottet i träcken. Överskott utsöndras främst i en lättlöslig, oorganisk form. Andelen löslig fosfor i träcken från mjölkkor har i tidigare studier visats vara kopplat till behovet av fosfor, utsöndring i träcken ökar när intaget överskrider behovet. I träck som utsöndras på bete är lösligt fosfor lättillgänglig för växterna, men lättlöslig fosfor kan också rinna med ytvatten till närliggande vattendrag. Alger tar upp då upp fosfor, varpå pH-värdet i vattnet sänks och det skapas gynnsamma förhållanden för giftiga blå-gröna alger att tillväxa och blomma. En ond cirkel kan uppstå som leder till en ändrad miljö för alla levande organismer i vattnet.

Fosfor i form av fosfatmalm från gruvor är en ändlig naturresurs som kan vara på väg att sina. När jordens befolkning växer behövs mera mat och föra att få stora skördar så gödslas åkrar med handelsgödsel som är tillverkat av fosfatmalm. Det är därför viktigt att inte slösa med fosfor. Att utfodra djur med bara den mängd fosfor som de behöver är ett sätt att spara på jordens fosfor reserv.

För att uppskatta behovet av fosfor är det viktigt att känna till hur stora de s.k. endogena förlusterna är, det är den mängd som alltid utsöndras från kroppen via kroppsvätskor och döda celler. De svenska rekommendationerna till häst har hittills baserats på de amerikanska. I dess senaste utgåva (NRC, 2007) har man hos växande hästar höjt de uppskattade endogena fosforförlusterna till 18 mg/kg kroppsvikt, en höjning med 80 %.

Syftet med våra försök var att:

- Undersöka om den av NRC rekommenderade höjningen av de uppskattade endogena förlusterna av P i träcken hos växande hästar är motiverad
- Undersöka om andelen löslig oorganisk P av totala P innehållet i träck från häst är högre än i träck från kor
- Utvärdera om andelen löslig oorganisk P av totala P innehållet i träck påverkas av om djuret mjölkar eller inte.

8.2 Resultat av försöken

Två fosforbalansstudier är utförda på växande hästar. Upptaget av fosfor var betydligt högre hos de yngre hästarna, 7-9 månader gamla, än i hos hästar i åldern 20 månader. Detta tyder på att fosforbehovet var högre hos de växande unghästarna än hos de äldre hästarna, men kan också bero på att unghästarna bättre utnyttjande fosfor som fanns i fodret. I våra studier fann vi inget belägg för att uppvärdera de endogena förlusterna, snarare tvärtom, då våra uppskattade endogena förluster som högst blev 2,5 mg/ kg kroppsvikt.

I den andra studien togs träckprov från betande hästar och kor. Resultatet av studien visar stor variationen i träckens fosforkoncentration, både mellan gårdar men även i olika hagar på samma gård. Andelen löslig, oorganisk fosfor i träck var i storleksordningen 40 - 100 % av totala fosforutsöndringen och andelen var högre hos mjölkande hästar än hos mjölkkor. Vi samlade också uppgifter om hur de betande djuren tillskottutfodrades. Samtliga mjölkkor fick ensilage, kraftfoder och mineraler som komplement till betet. Ickemjölkade kor betade liksom de mjölkande korna på fosforrika beten. Många av gårdarna gav ändå extra mineraler till de kor som inte producerade mjölk, vilket ledde till att de flesta av dessa kor fick i sig, och utsöndrade, mer fosfor än nödvändigt. De mjölkande (digivande) hästarna var däremot inte alltid utfodrade med tillräckligt mycket fosfor. Flera av hästgårdarna hade fosfornivåer i betet som gjorde att tillskott av fosfor via kraftfoder eller mineraler behövdes.

8.3 Praktisk tillämpning av resultaten

Vid beräkning av fosforbehovet till växande hästar bör det tidigare föreslagna värdet, 10 mg/kg kroppsvikt, användas för de oundvikliga fosforförluster som utsöndras i träcken. Svenska utfodringsrekommendationer följer dessa värden för växande hästar och våra forskningsresultat visar att de inte behöver höjas enligt den amerikanska rekommendationen. En höjning som skulle innebära att svenska växande hästar behövde utfodras med ca 50 ton extra fosfor.

När vi jämförde fosforinnehållet i foderstater till hästar och kor visade det sig att korna åt betydligt mera fosfor än hästarna. Detta är helt naturligt när det gäller mjölkande djur eftersom kor producerar betydligt mera mjölk än hästar gör. Men även kor som inte producerade mjölk fick mycket fosfor i sitt foder. Våra resultat tyder på att de flesta kvigor och sinkor inte behöver utfodras med extra fosfor när det går på bete. Däremot var fosforinnehållet lågt i beten till hästar och de flesta högdräktiga och digivande stona behövde extra tillskott av fosfor. Slutsatsen är att man bör ta prover från sina beteshagar för att se över näringsinnehållet i bete, för att varken under- eller överutfodra med fosfor.

Trots att de hästarna i fältstudien hade ett lägre uppskattat intag av fosfor så utsöndrade de generellt högre andel lättlöslig oorganisk P i träcken än korna. Den lättlösliga oorganiska fosforfraktionen i träcken är den fraktion som är mest skadlig för miljö eftersom den lätt kan rinna till närliggande vattendrag. I och omkring hagar och rastfållor för hästar är det klokt att införa åtgärder för att förhindra fosforläckage. Till exempel bör mindre hagar mockas ofta och vid större hagar bör man ha vegetation i och kring hagen som både kan utnyttja fosfor och bromsa avrinningen till närliggande vattendrag. Ett annat alternativ är att anlägga dammar i anslutning till hagen för att samla upp avrinningsvatten.

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