Phosphorus balance and fecal losses in growing Standardbred horses in training fed forage-only diets

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ABSTRACT: This study examined the P balance and fecal P losses in growing Standardbred horses in training fed a forage-only diet with or without P supplementation and assessed the magnitude and proportion of the soluble, inorganic P (Pi) fraction in feces. Fourteen Standardbred horses (aged 20.0 ± 0.3 mo) adapted to ad libitum intake of grass forage containing 0.25% P were used in a crossover experiment investigating 2 dietary treatments with (high-P) and without (low-P) mineral supplementation for 6 d. Daily feed intake and refusals were weighed. Spot samples of feces were collected twice daily on d 4 to 6 and analyzed for total P and Pi. Acid-insoluble ash was used as a marker for total fecal output. Spot samples of urine were collected once on d 4 to 6 and analyzed for P and creatinine. Daily P intake was greater \( (P < 0.001) \) for the high-P diet (32.0 ± 0.6 g) than the low-P diet (17.5 ± 0.6 g), and the individual intake ranged from 13.3 to 38.4 g/d. Total fecal excretion of P was also greater \( (P < 0.001) \) for the high-P diet (30.3 ± 0.8 g/d) than the low-P diet (17.0 ± 0.8 g/d) whereas excretion in urine was less than 0.2 g/d on both diets. Using simple regression analysis, fecal endogenous P losses were estimated to be less than 10 mg/kg BW. Phosphorus retention was 1.6 ± 0.6 and 0.3 ± 0.6 g/d on the high- and low-P diets, respectively, but only that for the high-P diet was greater \( (P < 0.05) \) than 0 g/d. The proportion of Pi of total fecal P excretion was 100 ± 3% for the high-P diet and 87 ± 3% for the low-P diet \( (P = 0.005) \) and Pi increased linearly with P intake \( (y = 1.10x - 4.44; r^2 = 0.91; CV = 9.9%; P < 0.001) \).

In conclusion, on this forage-only diet significant retention of P occurred at a daily P intake of 7.1 g/100 kg BW. Phosphorus was mainly excreted in feces and both total fecal P and Pi excretion had a strong relationship to P intake. More than 80% of total P appeared to be soluble. Fecal endogenous P losses were similar to those described previously in growing horses.

Key words: balance, forage, growth, horses, phosphorus, soluble

INTRODUCTION

Knowledge of endogenous nutrient losses is important in estimating the nutrient requirements of animals (Scott et al., 1995). In NRC (2007), the endogenous fecal P losses in growing horses are estimated at 18 mg/kg BW, which is 80% greater than in the previous version of NRC (1989). This increase seems to be based mainly on results of 1 study (Cymbaluk et al., 1989) whereas other studies indicate that losses are approximately 10 mg/kg BW (Schryver et al., 1971b; Kichura et al., 1983; Furtado et al., 2000; Oliveira et al., 2008). The new estimated value of losses may increase the risk of overfeeding P to growing horses if it is used as the basis for recommendations on P requirements. This may not be a problem for the horses, but it is well known that excretion of P from farm animals contributes to surface water pollution and eutrophication (Sharpley et al., 2003). In addition, global reserve of phosphate rock is dwindling, and within 20 to 50 yr, the demand for P will exceed supply (Cordell et al., 2009). Therefore, feeding excess P to animals must be avoided. The inorganic soluble P (Pi) fraction in feces is assumed to be the most vulnerable regarding potential runoff losses. In horses, P is mainly absorbed in the large intestine (Schryver et al., 1972; Meyer et al., 1982).
whereas P is mainly excreted in the feces (Schryver et al., 1971b; Hintz and Schryver, 1973). Our pilot study indicated that the proportion of P_i of total fecal P in foals, on both forage-only and forage-oats diets, may be greater than 90% (Löf, 2009).

Previous studies of endogenous P losses in growing horses have used a diet that included cereals and, as far as we know, there are no observations on forage-only diets. In addition, there are no observations on endogenous P losses in young horses in training, a group of horses likely to be supplemented with minerals in practice. Therefore, the objective of the study was to investigate P balance in growing horses in training fed a forage-only diet with or without mineral supplementation and to estimate endogenous fecal P losses. A secondary objective was to assess the relationship between fecal P excretion and P intake and the proportion of soluble P of total P in feaces. The hypotheses were that endogenous P losses are lower than 18 mg/kg BW and that the proportion of P_i is high and positively related to P intake.

MATERIAL AND METHODS

The study was performed at the Swedish National Trotting School, Wången, Sweden, and the protocol was approved by the Umeå Local Ethics Committee.

Horses and Management

Fourteen healthy Standardbred trotters [13 geldings and 1 stallion; aged 20.0 ± 0.3 mo and BW of 450 ± 54 kg (weighed without feed and water restriction)] in training were used. The average weight gain during 2 mo prior and after the study was 0.2 kg/d. All horses were subjected to the same training program both before and during the study (training, 4 d/wk; trotting at speed of 2 to 3 min/km for 4 to 7 km on a race track). On days without training, all horses were still walked in a circular walker machine for 30 to 60 min once or twice per day. Horses were kept individually in wooden box stalls (9 m²) on sawdust litter and had free access to water from buckets. Before the study, horses spent 6 to 8 h/d together in a paddock but during the experimental days, horses were kept indoors, except during exercise. The study was conducted during wintertime, with an outdoor temperature of −2 to −17°C. The paddocks were completely covered by snow and the horses had no access to soil.

Experimental Design and Diets

The horses were randomly allocated to 1 of 2 dietary treatments with (high-P) and without (low-P) P supplementation for 6 experimental d in a crossover design with 12 d in between. Collection of feces and urine was performed during the last 3 d in each experimental period. Before the experimental days, all horses were fed the same mineral supplement as in the high-P diet. Withholding the mineral supplement for 3 d before the collection period was considered a sufficient washout period, based on other studies of excretion patterns after abrupt changes on forage-only diets (Connysson et al., 2006; Muhonen et al., 2008), to determine treatment effects on pooled data. Capple et al. (1982) have also reported that urinary excretions had changed markedly 3 d after a change in mineral intake, indicating a rapid response to changes in such intakes.

All horses had been fed a forage-only diet (meadow fescue and timothy haylage wrapped in 350-kg bales) ad libitum and the mineral supplement (Miner Vit, Lantmänn Krafft AB, Falkenberg, Sweden) used in the high-P diet for at least 2 mo before the study, and during the study horses were still fed forage ad libitum. Forage allowances (provided 3 times daily in a trough) were weighed, and ad libitum was defined as the feed refusal of >10%. With the high-P diet, 150 g/d of the mineral supplement (6.44% monocalcium phosphate, 6.76% calcium carbonate, 5.93% magnesium phosphate and magnesium oxide, 0.5% vitamin E, and 0.0015% sodium) was offered whereas with the low-P diet, only Se and vitamin E were offered in amount corresponding to that in the high-P diet [50 g/d; water solution with 1% vitamin E and 0.002% sodium selenite (preserver 1,2-propandiol and emulsifier Bredol); Protect E-Selen; Lantmänn Lantbruk, Malmö, Sweden]. Along with the supplements, which were fed once per day, all horses were also fed 250 g pelleted alfalfa (Lantmänn Krafft AB, Falkenberg, Sweden) to ensure complete supplement intake. The CP, ME, Ca, P, and Mg contents of feedstuffs are shown in Table 1.

Feed Sample Collection and Analysis

All feed and refusals were weighed daily. A forage sample was collected from the bale used at every feed allowance preparation and refusals were collected individually. Forage and refusals samples were frozen at −20°C pending further analysis. Daily DMI was determined as the difference between offered and refused DM. Before analysis, feed and individual refusals from each period was mixed, dried for 16 h at 60°C, and ground in a 1-mm hammer mill (Slagy 200 B; Kamas, Malmö, Sweden). Dry matter content was determined by overnight drying at 105°C. Both lignin and AIA were analyzed. Acid insoluble ash was analyzed using the method described by Van Keulen and Young (1977). Acid detergent fiber and permanganate lignin were determined according to the method of Van Soest et al. (1991). Crude protein and energy content were analyzed by near-infrared spectroscopy (FOSS, Göteborg, Sweden). Calibration for determination of CP content was performed by Dumas combustion.
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(Wiles et al., 1998) and calibration for determination of rumen digestible OM and calculations of ME for horses was performed according to Lindgren (1979). Phosphorus, Ca, and Mg content were analyzed by inductively coupled plasma–atomic emission spectroscopy (Spectro Flame; Spectro Analytical Instruments, Kleve, Germany) after digestion with nitric acid (Balsberg-Påhlsson, 1990). In addition to P, Ca and Mg contents were analyzed because Ca uptake, at least, can be affected by P intake levels in horses (Schryver et al., 1971a).

Urine Sample Collection and Analysis

One spot sample of urine per horse was collected once during the last 3 d in each treatment period. Urine samples were analyzed for creatinine using a colorimetric method with picric acid in an autoanalyzer (Technicon 1974b; Technicon, Dublin, Ireland). Minerals were analyzed using a spectrophotometer (Spectro Flame; Spectro Analytical Instruments) after extraction in nitric acid and hydrogen peroxide (Swedish Standard 028311; Naturvårdsverket, Stockholm, Sweden).

Fecal Sample Collection and Analysis

Spot samples of fresh feces were taken from the floor adjacent to defecation twice daily in the last 3 d of each treatment period. The minimum period between samplings was 8 h. Fecal samples were immediately frozen at −20°C. Before analysis, the samples were thawed and pooled per horse and treatment period and mixed. Samples for analysis of AIA, ADF, lignin, total P, Ca, and Mg contents were dried for 48 h at 65°C and ground using a 1-mm screen in a hammer drill (Kamas, Slagy 200 B, Malmö, Sweden) and then analyzed by the same methods as those described for feed. Fecal P, was analyzed using a modified version of the method described by Dou et al. (2007). Frozen feces were pooled per horse and treatment period, ground, and mixed and a 5-g sample of the wet (thawed) feces was mixed with 0.1% HCl solution to total mass of 100 g. The samples were shaken for 1 h, centrifuged at 1,800 × g for 5 min at 19°C, and gravity filtered through Whatman 42 paper. The P in feces was analyzed by a spectrophotometric method (PH 1016; Randox, Antrim, UK; Henry, 1974; Tietz, 1990) with molybdate as reagent and wavelength 340 nm (Kinetics Spectrophotometer, Ultrospec K 4053; LKB Biochron, Cambridge, UK). Intra-assay CV was 4.2%, and the minimum detectable concentration was 1 g/kg DM.

Calculations and Statistical Analyses

Apparent digestibility was calculated using AIA and lignin as indigestible markers according to these equations:

\[
\text{Fecal excretion of } X (\text{g/d}) = \frac{(\text{AIA or lignin intake, g/kg DM/ AIA or lignin output, g/kg fecal DM})}{X \text{ g/kg fecal DM}}
\]

and

\[
X \text{ digestibility (%) = } 1 - \frac{\text{fecal excretion of } X (\text{g/d})/\text{intake } X (\text{g/d})}{100}
\]

Mineral retention was determined by subtracting mineral fecal and urine output from mineral intake. Creatinine was used for calculating the amount of urine excreted daily, assuming 1.15 mg/(kg BW·h) of creatinine excretion in urine (Meyer and Staderman, 1990). Fecal endogenous losses were estimated using simple linear regression analysis (regression of fecal nutrient excretion on intake) and then fecal excretion was extrapolated to 0 intakes.

All data were subjected to ANOVA (GLM procedure; SAS Inst. Inc., Cary, NC) using the model

\[Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk},\]

in which \(Y_{ijk}\) is the observation, \(\mu\) is the mean value, \(\alpha_i\) is the effect of animal, \(\beta_j\) is the effect of treatment, \(\gamma_k\) is the effect of period, and \(e_{ijk}\) is the residuals \([e_{ijk} \sim \text{IND}(0,\sigma^2)]\). Retention data was tested for the hypothesis that it differed from 0. Values are presented as least square mean ± SEM. The PROC REG of SAS was used to obtain \(r^2\), CV, and \(P\)-values. The level of significance between treatment least square means, for difference from 0, and relationships was set at \(P < 0.05\).

<table>
<thead>
<tr>
<th>Item</th>
<th>Forage (range)</th>
<th>Alfalfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>62 (58 to 69)</td>
<td>89</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>14.3 (13.8 to 14.9)</td>
<td>15.0</td>
</tr>
<tr>
<td>P, % of DM</td>
<td>0.25 (0.24 to 0.27)</td>
<td>0.25</td>
</tr>
<tr>
<td>Ca, % of DM</td>
<td>0.56 (0.52 to 0.60)</td>
<td>1.76</td>
</tr>
<tr>
<td>Mg, % of DM</td>
<td>0.20 (0.19 to 0.22)</td>
<td>0.20</td>
</tr>
<tr>
<td>ME, MJ/kg</td>
<td>10.6 (10.0 to 11.3)</td>
<td>8.5</td>
</tr>
</tbody>
</table>

1 Fed ad libitum.
2 250 g/d pelleted alfalfa (Lantmännen Krafft AB, Falkenberg, Sweden).
3 Values from the manufacturer (Lantmännen Krafft AB, Falkenberg, Sweden).
RESULTS

Dry Matter Intake and Marker Evaluation

Dry matter intake was greater \( (P < 0.001) \) for the high-P diet than for the low-P diet \( (9.4 \pm 0.2 \text{ and } 7.2 \pm 0.2 \text{ kg/d, respectively}) \), but the range overlapped between treatments \( (7.6 \text{ to } 11.4 \text{ kg/d for the high-P diet and } 5.4 \text{ to } 10.0 \text{ kg/d for the low-P diet}) \). In addition, AIA and lignin intake were greater for the high-P diet than the low-P diet \[ 211 \pm 7 \text{ g/d compared with } 154 \pm 7 \text{ g/d (} P < 0.01) \] and \[ 452 \pm 22 \text{ g/d compared with } 313 \pm 22 \text{ g/d (} P = 0.01) \], respectively. Linear regression between P intake and P fecal excretion using lignin as a marker resulted in estimated fecal excretion of more than 100% of the P intake, and this method was, therefore, concluded to be unreliable (data not shown). Hereafter, only data obtained using AIA as a marker are used unless otherwise stated.

Phosphorus, Calcium, and Magnesium Intake and Estimated Excretion and Retention

As expected, Ca, P, and Mg intake was greater for the high-P diet than for the low-P diet (Table 2). The Ca:P ratio was approximately 1.9:1 and 2.2:1 on the high- and low-P diet, respectively. There were no differences in estimated urinary excretion between treatments for P, Ca, or Mg (Table 2). The main excretion route of P, Ca, and Mg was feces, and fecal excretion of all 3 elements was greater for the high-P diet than the low-P diet (Table 2). There were also strong positive relationships across diets between P, Ca, and Mg intake and estimated fecal excretion (Fig. 1).

There were no differences in Ca and P estimated retention between the high- and low-P diets, respectively, but in the low-P diet, the estimated retentions of Ca and P were not statistically different from 0 (Table 2). Estimated retention of Mg was more positive on the high-P diet than the low-P diet, but it was not statistically different from 0 on the low-P diet. According to simple linear regression analysis, the estimated fecal endogenous losses were 2.5 mg/kg BW for P and 11.5 mg/kg BW for Ca, but there were no fecal endogenous losses of Mg (Fig. 1).

<table>
<thead>
<tr>
<th>Item</th>
<th>High-P</th>
<th>Low-P</th>
<th>SEM</th>
<th>Treatment</th>
<th>High-P</th>
<th>Low-P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ca</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake, g/d</td>
<td>60.5</td>
<td>39.0</td>
<td>0.6</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal excretion, g/d</td>
<td>25.5</td>
<td>18.0</td>
<td>0.8</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine excretion, g/d</td>
<td>22.1</td>
<td>13.4</td>
<td>4.4</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained, g/d</td>
<td>13.0</td>
<td>7.6</td>
<td>4.8</td>
<td>0.44</td>
<td>&lt;0.05</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake, g/d</td>
<td>32.0</td>
<td>17.5</td>
<td>0.6</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal excretion, g/d</td>
<td>30.3</td>
<td>17.0</td>
<td>0.8</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine excretion, g/d</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained, g/d</td>
<td>1.6</td>
<td>0.3</td>
<td>0.6</td>
<td>0.16</td>
<td>&lt;0.05</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Mg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake, g/d</td>
<td>27.4</td>
<td>14.6</td>
<td>0.5</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal excretion, g/d</td>
<td>16.6</td>
<td>7.8</td>
<td>0.8</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine excretion, g/d</td>
<td>5.4</td>
<td>5.1</td>
<td>0.4</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retained, g/d</td>
<td>5.4</td>
<td>1.7</td>
<td>1.2</td>
<td>0.04</td>
<td>&lt;0.05</td>
<td>0.18</td>
</tr>
</tbody>
</table>

1 Treatment = \( P \)-values for differences between treatments; High- and Low-P = \( P \)-value on the difference from 0 (H ≠ 0) for retention.
Phosphorus balance and fecal losses in horses

Table 3. Estimated apparent digestibility by using AIA and lignin as internal markers in young horses fed a forage-only diet with (high-P) or without (low-P) a mineral supplement

<table>
<thead>
<tr>
<th>Item</th>
<th>High-P</th>
<th>Low-P</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>57.6</td>
<td>57.6</td>
<td>0.9</td>
<td>1.00</td>
</tr>
<tr>
<td>P, %</td>
<td>5.4</td>
<td>2.0</td>
<td>2.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Ca, %</td>
<td>58.0</td>
<td>53.6</td>
<td>1.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Mg, %</td>
<td>39.1</td>
<td>45.3</td>
<td>4.0</td>
<td>0.29</td>
</tr>
<tr>
<td>Lignin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>47.6</td>
<td>43.8</td>
<td>2.3</td>
<td>0.31</td>
</tr>
<tr>
<td>P, %</td>
<td>–18.2</td>
<td>–32.6</td>
<td>6.6</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Fecal Soluble Phosphorus

The P fraction in feces was 7.7 ± 0.1 g/kg DM for the high-P diet and 4.8 ± 0.1 g/kg DM for the low-P diet (P < 0.001). The P fraction of total P was greater (P < 0.001) for the high-P diet (100 ± 3%) than the low-P diet (87 ± 3%). Across diets, there were strong positive relationships between P intake and total P (y = 0.93x + 0.59, r² = 0.93, CV = 8.5, P < 0.001) and P (y = 1.10x – 4.44, r² = 0.91, CV = 9.9, P < 0.001) fractions, respectively, but between P intake and the insoluble P fraction the relationship was weak (y = –0.16x + 5.04, r² = 0.21, CV = 29.3, P < 0.05).

Apparent Digestibility

There were no differences between treatments in apparent digestibility of DM (Table 3). The apparent digestibility of P was less than 6% on both diets and apparent digestibility for Ca and Mg was between 50 and 60% and 39 to 46% for both diets, respectively.

DISCUSSION

In the present study, P intake corresponded to about 130 (32 g) and 70% (17 g) of the suggested requirement on the high- and low-P diets, respectively (NRC, 2007). The P balance data confirm that the requirement in these horses was somewhere in the range of 17 to 32 g/d [3.8 to 7.1 g/(100 kg BW·d)] because retention was observed with the high-P diet but not with the low-P diet. This is in agreement with Nielsen et al. (1998) who studied 12 Quarter Horse geldings of similar age (24 mo) and BW during the first 4 mo of training and found that a daily intake of 20 to 24 g P resulted in P retention of 2.5 to 6 g/d whereas increasing intake to 25 to 27.3 g/day resulted in no further effect on retention. However, an estimated P retention of 1.6 g/d, as observed with the high-P diet in the present study, might be an underestimation based on the comparatively high estimated Ca retention on this diet and the high estimated fecal P excretion, which resulted in very low apparent digestibility. Nielsen et al. (1998) reported that during the first 3 to 4 mo of training in young horses, every gram of retained P corresponded to Ca retention of 3.9 to 4.4 g. Based on their results and the Ca retention observed in the present study, the expected P retention would be 2.8 to 3.3 g/d.

Data from the present study also indicated that estimated fecal endogenous losses of P are less than 18 mg/kg BW suggested by NRC (2007). Our estimated value (2.5 mg/kg BW) is even less than earlier observations by Schryver et al. (1971b), Kichura et al. (1983), Furtado et al. (2000), and Oliveira et al. (2008), all using radioisotope-labeled P analysis and sedentary horses. The reason for the numerical difference is unclear, but it might be due to the methods used or to the fact that these horses were exercised although we do not see any good physiological evidence for the latter. Data from Schryver et al. (1971b) also show that the 2 methods (radioisotope-labeled P analysis and linear regression analysis) could be comparable because applying linear regression analysis to their data results in a similar outcome to using radioisotope-labeled P although the authors did not compare the 2 methods. On the other hand, it is questionable whether it is appropriate to extrapolate the regression line close to 0 P intakes because it is not known if P excretion is truly linear.

Cymbaluk et al. (1989) estimated endogenous P losses in growing horses to be 18 mg/kg BW using an equation that indicated an interrelationship of fecal Ca with fecal P excretion whereas if only simple linear regression analysis had been used, the estimated fecal endogenous P losses would have been much greater (42 mg/kg BW). Earlier studies have shown that Ca intakes of about 300 mg/kg BW decreases P absorption and greater intakes result in no further decrease (van Doorn et al., 2004). However, the present study does not provide any support for the assumption that fecal P excretion has to be corrected for Ca excretion, at least not for Ca intakes in the range 65 to 170 mg/kg BW. In the study by Cymbaluk et al. (1989), the relationship between P intake and fecal output was also much weaker than that in the present study. Therefore, we suggest that fecal endogenous losses in young horses are approximately 10 mg/kg BW, which agrees with several others (Schryver et al., 1971b; Kichura et al., 1983; Furtado et al., 2000; Oliveira et al., 2008), and the present NRC (2007) recommendations overestimates the endogenous losses and thus the P requirements.

The estimated fecal endogenous losses of Ca and Mg were within a reasonable range of values reported earlier in studies using radioisotope-labeled analysis [Ca: 12 vs. 20 mg/kg BW (Schryver et al., 1970) and Mg: 0 vs. 2 mg/kg BW (Hintz and Schryver, 1973)]. However, the dietary fraction of Ca and Mg excreted in feces is smaller and more variable than P (Hintz and Schryver, 1973), and therefore, our balance data and endogenous losses, which
are based on spot samples, for Ca and Mg should be interpreted with caution.

The present study indicates that analysis of fecal spot samples can be a useful tool to assess P overfeeding and to estimate the amounts of P that could easily contribute to eutrophication on surface waters. As expected, a high proportion of the P intake was excreted in feces, as reported previously by Schryver et al. (1971b), Cymbaluk et al. (1989), Nielsen et al. (1998), and van Doorn et al. (2004). More than 80% of the P excreted in feces was soluble and the proportion was greater than that observed in feces from dairy cows analyzed by the same method (Dou et al., 2007; Nordqvist, 2012) and also greater than the water soluble P proportion observed in feces from horses (Hainze et al., 2004). However, the use of a diluted acid solution (as in the present study) for extraction of soluble P has been suggested to be more accurate because the fraction is sensitive to variations in fecal pH and Ca content and that can be overcome by the use of a diluted acid solution (Chapuis-Lardy et al., 2004; Dou et al., 2007). A proportion of P\textsubscript{i} > 100% (as in the high-P diet) is, of course, not possible and is most likely due to variations in methodological accuracies and not exactly the same material were used for the total P and P\textsubscript{i} analyses.

The total and soluble P concentrations and the proportion of soluble P\textsubscript{i} in feces were also greater with the high-P diet than the low-P diet. Those findings indicate that the P sources used in the high-P diet might have very high solubility in the digestive tract of the horse. This is in accordance with findings in other species (i.e., cattle, swine, chickens, and turkeys; Peeher, 1972). Chapuis-Lardy et al. (2004) suggested that increased dietary P, both organic and inorganic, increases the amount of P excreted and the proportion of water-soluble P in feces from dairy cows, and the present study indicates the same response in horses. We also observed a positive linear relationship between daily P intake and fecal excretion of soluble P\textsubscript{i}, and similar observations have been made in dairy cows by Dou et al. (2007) and Nordqvist (2012). With respect to runoff losses, the high fraction of P\textsubscript{i} in feces from horses might be serious, especially in the horse management systems where diets are produced with or including P from the global reserve and horses are kept on land with no or limited vegetation. On the other hand, horse manure can also be recommended as a fertilizer when P is needed.

Calcium intake was above the level recommended by NRC (2007) in both treatments (180% with the high-P diet and 115% with the low-P diet), but no retention was observed with the low-P diet. This might indicate that these horses needed more than 39 g/d or that Ca retention was limited by low-P intake. The Mg intake corresponded to 220 and 114% of the NRC (2007) recommendation for the high- and low-P diet, respectively, but with the low-P diet, no retention was observed. Similarly, Nielsen et al. (1998) observed Mg retention only in young horses in training receiving more than 15 g Mg/d. However, as mentioned earlier, the balance data for both Ca and Mg in the present study should be interpreted with caution because spot sampling and not total collection were used to assess urinary losses. Although feces are the main excretion route for both Ca and Mg, urinary excretion plays a major role in regulating the balance (Schryver et al., 1970; Hintz and Schryver, 1973; Meyer and Staderman, 1990).

In addition to being relatively young and inexperienced, all horses used were privately owned and intended for a future racing career. Therefore, there were limited possibilities to interfere with the management, and instead of total collection, spot sampling was used. Lignin was rejected as a reliable marker to calculate digestibility because the actual value was greatly underestimated, probably because of the lack of recovery in feces (Miriaglia et al., 1999). This is also consistent with recent findings by Sales (2012). In that study, a meta-analysis of independent experiments showed that digestibility coefficients of forage-only diets were similarly estimated by AIA and total collection whereas acid detergent lignin resulted in lower apparent digestibility coefficients with both forage-only and forage and concentrate diets. We therefore suggest that lignin cannot be used as a reliable indigestible marker in horses. De Marco et al. (2012) have also shown that using AIA as an internal marker led to average values similar to those obtained with total collection. Fecal recovery is reported to be more reliable when the AIA level in feed is increased (McCarthy et al., 1974). In the present study, the diet contained, on average, 24 g AIA/kg DM, far beyond the estimated threshold of 7.5 g AIA/kg DM suggested by Thonney et al. (1985) for reliability of using AIA as a marker in digestibility studies in dairy cows.

The reason for the difference in voluntary DM intake between the high- and low-P diets in the present study is unclear. However, Ott and Asquith (1989) also found greater voluntary DM intake in a diet supplemented with trace minerals, Ca, and P compared with a basal diet. These findings are interesting for future research.

In conclusion, on the forage-only diets tested in the present study, considerable P retention was observed with the high-P diet (7.1 g/100 kg BW) and estimated fecal endogenous P losses were similar to observations in previous studies on growing horses but not as high as those suggested by NRC (2007). In addition, it is concluded that the soluble P fraction corresponded to more than 80% of the total P and that both total and soluble P\textsubscript{i} had a strong positive relationship to P intake.
Phosphorus balance and fecal losses in horses

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