Effect of excessive inorganic phosphorus supplied by abomasal infusion on inorganic phosphorus metabolism in dairy cows

by

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Dedication

To my parents for their support and encouragement in my entire life
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Abstract

The effects of inorganic phosphorus (P\textsubscript{i}) supplied by abomasal infusion on phosphorus metabolism were tested in three cows of the Swedish red and white breed in a study with a 3×3 Latin square design. Monosodium dihydrogen orthophosphate dihydrate (NaH\textsubscript{2}PO\textsubscript{4}.2H\textsubscript{2}O) solution with three different levels of P (0, 14.4 and 28.8 g/d) was infused in the abomasum for four days in each experimental period. Blood, rumen liquid, feces and urine samples were collected. Co-EDTA was used as a marker for the estimation of rumen liquid phase volume and rumen fluid outflow rate. P\textsubscript{i} absorption tended to increase as P supply increased. This was accompanied by a tendency for the reduction of P\textsubscript{i} absorption efficiency suggesting mechanisms in the small intestine regulating P\textsubscript{i} absorption. It appeared that the fractional absorption rate of P\textsubscript{i} was negatively related to the increasing amount of P supply. In addition, P infusion resulted in increased salivary P\textsubscript{i} concentration. It was suggested that salivary glands could have increased P\textsubscript{i} secretion in response to increased P\textsubscript{i} flux in plasma. This was evidenced from the unchanged plasma P\textsubscript{i} concentration in relation to increased P\textsubscript{i} absorption. Urinary P\textsubscript{i} excretion played a negligible role in P balance. It was concluded that P homeostasis was controlled within gut through regulation of P\textsubscript{i} uptake, salivary P\textsubscript{i} secretion and fecal P\textsubscript{i} excretion.

Introduction

Phosphorous (P) is an essential mineral that is involved in various biological functions such as energy metabolism pathways, DNA and RNA synthesis, bone formation and cell signaling (Hill et al., 2008). P is fundamental for animal health and production performance (Valk et al., 2000; Dou et al., 2007; Ferris et al., 2010). P deficiency results in the reduction of feed intake (Ternouth, 1990) and growth (NRC, 2001). Milk production and fertility are also impaired if P supply does not meet the animal’s requirement (NRC, 2001). In the body, about 80-85 per cent of total P is found in bones and teeth (McDonald et al., 2002). The rest of P is found in soft tissues and body fluids (McDonald et al., 2002). Rumen microbes have a great need for P (Van Soest, 1994). It was reported that the P requirement of rumen microbes could be greater than the total P requirement of the host animal (Preston and Pfander, 1964; Kincaid and Rodehutscord, 2005). Feed intake, organic matter fermentation and microbial protein synthesis are suppressed if the P requirement of rumen microbes is not met (Kincaid and Rodehutscord, 2005). Salivary P secretion and dietary P intake are two sources of P supply for rumen microbes (Kincaid and Rodehutscord, 2005).

In a modern animal farming, P accumulation on the farm level is a common challenging issue. This is caused when P input into the farm exceeds the P output in the forms of animal products (Valk and Šebek, 1999; Dou et al., 2007). P is considered a major pollutant to freshwater because it increases the growth of green algae in lakes and rivers (Bravo et al., 2003), causing eutrophication (Kebreab et al., 2009). In ruminants, the major
route of P excretion is through feces (Bravo et al., 2003). Fecal P loss consists of unabsorbed dietary P and of endogenous P originating from saliva, intestinal cells, digestive tract secretions and rumen microbes (Bravo et al., 2003). Urinary P excretion in ruminants is normally insignificant (Dias et al., 2006a; Dias et al., 2006b) but when the diet does not sufficiently stimulate saliva secretion (e.g. feedstuff with low fibre quality like hydrolyzed sugarcane bagasse), the urinary P loss increases (Dias et al., 2006a). The P excretion from urine also increases in late lactation in response to the reduction of P requirement for milk synthesis (Wu et al., 2001). Beside environmental concerns, optimizing P intake and utilization is also important from economical aspects because the sources of inorganic P are limited (Kebreab et al., 2009). Therefore, to achieve both environmental and economical benefits, it is a necessity to improve our knowledge about P metabolism in ruminants.

In ruminants, different mechanisms contribute to maintain P homeostasis; P absorption from digestive tract, endogenous P secretion into saliva, deposition and mobilization of P in bones and P reabsorption in kidneys (Seheted, 2004). However, P balance is mainly achieved within digestive tract (Scott et al., 1985; Valk et al., 2000) because salivary P secretion and endogenous fecal excretion of P are the predominant regulatory mechanisms of P homeostasis (NRC, 2001).

In the digestive tract of ruminants, P of endogenous origin comes mainly from salivary P secretion (Breves and Schröder, 1991). Salivary P which is inorganic (Kebreab and Vitti, 2005; Hill et al., 2008) acts as a pH buffer in the rumen (McDonald et al., 2002). Daily salivary P i secretion in sheep and cows could range from 5 to 10 and from 30 to 60 g/d, respectively (Breves et al., 1987; Reinhardt et al., 1988; Scott, 1988 cited by Breves and Schröder, 1991). In dairy cows, salivary P i concentration could be 4 to 5 times higher than plasma P i concentration (NRC, 2001). This indicates that salivary glands are able to concentrate the plasma P i (Breves and Schröder, 1991). The influence of the salivary glands on the regulation of the P i level in plasma is yet to be investigated. There is a controversy about the relation between plasma and saliva P i concentrations. It was pointed out that in ruminants, P i concentration in parotid saliva is mainly related to the plasma P i concentration (Breves and Schröder, 1991). A linear relation between plasma and salivary P i concentrations was also reported in small ruminants by Mañas-Almendros et al. (1982). On the other hand, Riad et al. (1987) showed that salivary P i concentration and secretion in cattle was regulated by plasma 1,25-dihydroxycholecalciferol (1,25-(OH)2D3) concentration rather than the plasma P i concentration. 1,25-(OH)2D3 is a product of vitamin D metabolism in the kidneys (McDonald et al., 2002). Increased plasma concentration of 1,25-(OH)2D3 resulted in the reduction of salivary P i concentration and secretion and vice versa (Riad et al., 1987). Bovine species could have receptors for 1,25-(OH)2D3 in parotid glands (Riad et al., 1987). There are also different opinions about the relation between dietary P supply and salivary P i secretion. Scott et al. (1985) and Challa et al. (1989) reported a positive relation between P intake and salivary P i secretion in sheep and growing calves respectively. On the other hand, it was mentioned that as the dietary P intake increased, the salivary P i secretion declined in dairy cows (Khorasani et al., 1997).
In ruminants, absorption of P mainly occurs in the intestine (Pfeffer et al., 2005). The region between proximal duodenum and terminal ileum is considered as main site of intestinal P absorption (Pfeffer et al., 2005). P is absorbed in an inorganic form (Hill et al., 2008). P in the intestinal digesta is mainly monovalent dihydrogen phosphate (H$_2$PO$_4^-$) (Pfeffer et al., 2005). The major proportion of intestinal H$_2$PO$_4^-$ originates from salivary P$_i$ secretion (Shirazi-Beechey et al., 1991). In blood plasma, P$_i$ is mainly found as the bivalent monohydrogen phosphate (HPO$_2^-$) (Pfeffer et al., 2005).

In ruminants, dietary P above the requirement is mostly excreted in feces (Wu et al., 2000; Pffefer et al., 2005). However, the mechanisms involved in fecal P excretion are not fully understood. A direct positive relation between P intake and endogenous fecal losses of P was reported in several studies (Challa and Braithwaite, 1988a; Challa and Braithwaite, 1988b; Scott et al., 1985; Challa et al., 1989). Challa et al. (1989) pointed out that the increased endogenous fecal P in response to increased P supply was due to the rise of salivary P$_i$ secretion. It has been suggested that endogenous fecal P accounts for approximately 75% of total fecal losses of P in sheep and cattle (Ternouth, 1989; Coates and Ternouth, 1992; Bortolussi et al., 1996). By reviewing studies from 1951 to 1996, Pfeffer et al. (2005) concluded that endogenous fecal losses of P accounted for about two-thirds to three-quarters of total fecal P in ruminants. The main origin of this fraction of fecal P is believed to be salivary P$_i$ (Challa et al., 1989; Ternouth, 1990; Coates and Ternouth, 1992; Dias et al., 2006a; Dias et al., 2006b; Dias et al., 2006c). Reinhardt et al. (1988) concluded that salivary P$_i$ secretion could be the main route of P excretion in ruminants. However, salivary P$_i$ is highly available for absorption. This raises the question how salivary P$_i$ could have such a large contribution on fecal P excretion. Challa and Braithwaite (1988b) claimed that salivary P$_i$ had higher availability than dietary P but lower availability than P in mineral supplements in growing calves. It was also reported that absorption efficiency of salivary P$_i$ would remain nearly constant at different rates of salivary P$_i$ secretion (Challa et al., 1989). However, salivary P$_i$ and P$_i$ originated from dietary P are identical and they are completely mixed in the rumen (Pffefer et al., 2005). As a result, P$_i$ is absorbed irrespective to its origin in small intestine (Pffefer et al., 2005). It thus appears a differentiation between absorption efficiency of exogenous and endogenous P hardly could be justified (Pffefer et al., 2005). Coates and Ternouth (1992) and Bortolussi et al. (1996) found that as the P supply increased in cattle, the proportion of endogenous fecal P to total fecal P was reduced. However, even in a high P diet, endogenous fecal P accounted for more than 66% of total fecal P (Coates and Ternouth, 1992; Bortolussi et al., 1996). Since salivary P makes a larger contribution to the P content within the digestive tract than the dietary P does (McDonald et al., 2002), it appears that it is the ratio between endogenous (mainly from saliva) and exogenous P in gut which determines the proportion of different fractions of fecal P (Pffefer et al., 2005).

It is generally believed that P$_i$ absorption in dairy cows is under regulation of two distinct mechanisms; active transport and passive transport (NRC, 2001). Active transport of P is vitamin D dependent (Horst, 1986; NRC, 2001). Production of 1,25-(OH)$_2$D$_3$ is stimulated when dietary P is insufficient resulting in more efficient P absorption (Horst, 1986). In small ruminants, H$^+$ (Shirazi-Beechey et al., 1991; Shirazi-Beechey et al., 1996;
Huber et al., 2002) and Na\(^+\) (Schröder et al., 1995; Schröder and Breves, 1996; Huber et al., 2002) dependent transport systems have been described for P\(_i\) absorption in duodenum and jejunum respectively. The H\(^+\) dependent absorption mechanism of P\(_i\) in duodenum could be due to the acidic pH value of digesta in duodenum resulting in pH gradient dependency of P\(_i\) absorption (Shirazi-Beechey et al., 1989; Shirazi-Beechey et al., 1991). On the other hand, when P supply exceeds the requirement, passive absorption is the predominant mechanism of P\(_i\) uptake (Horst, 1986; NRC, 2001). P\(_i\) absorption, therefore, is assumed to be directly related to the amount of P\(_i\) entering into the small intestine (NRC, 2001). A positive relation between P supply and P\(_i\) absorption was reported in several studies (Scott et al., 1985; Challa et al., 1989; Khorasani et al., 1997). However, it was found that the increased P\(_i\) absorption in relation to increased P supply was accompanied by reduced P\(_i\) absorption efficiency in sheep (Scott et al., 1985) and growing calves (Challa et al., 1989). It was suggested that the saturation of a carrier facilitated transport mechanism could be the reason for the reduction of P\(_i\) absorption efficiency (Care et al., 1980 cited by Scott, 1988). However, such a study has not been previously performed in adult dairy cows. Therefore, the main aim of this experiment was to investigate the relation between excessive P supply and P\(_i\) uptake in adult dairy cows. It was hypothesized that the fractional absorption of P\(_i\) would be negatively related to the amount of P\(_i\) entering into the small intestine. The impacts of excessive P supply on P\(_i\) kinetics were also investigated.

Materials and Methods

Animals, diets and experimental design

Three non-lactating non-pregnant rumen fistulated cows of the Swedish red and white breed were used. The body weight ranged from 629 to 743 kg. The diet consisted of 4 kg of hay and 1.8 kg of concentrate fed every day at 8:00 h and 16:00 h. Total dry matter (DM) intake was 5.3 kg/d. The feed intake provided 17.8 g of P/d. Cows were kept in a tie stall barn with free access to water and salt lick. The experiment had a 3×3 Latin square design with four days interval between treatments.

Experimental procedure and treatments

Monosodium dihydrogen orthophosphate dihydrate (NaH\(_2\)PO\(_4\).2H\(_2\)O) dissolved in water was used as the phosphate solution. Three solutions were prepared provided 0, 14.4 and 28.8 g of P/d. Solutions were continuously infused into the abomasum for four days. The infusion protocol is given in Table 1. At the end of each experimental period, the tubes used for the infusion were removed from the abomasum.
### Table 1. Concentration of phosphate solution and the rate of P infusion.

<table>
<thead>
<tr>
<th>P infusion (g/d)</th>
<th>NaH$_2$PO$_4$.2H$_2$O (g/l)</th>
<th>P concentration (g/l)</th>
<th>Volume of the phosphate solution (ml/d)</th>
<th>Infusion rate of P (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>4800</td>
<td>Water only</td>
</tr>
<tr>
<td>14.4</td>
<td>15.11</td>
<td>3</td>
<td>4800</td>
<td>14.4</td>
</tr>
<tr>
<td>28.8</td>
<td>30.22</td>
<td>6</td>
<td>4800</td>
<td>28.8</td>
</tr>
</tbody>
</table>

Picture 1. Tubes with anchors placed into the abomasum.

Picture 2. Infusion Tubes after placing into the abomasum.
Sample collection, preparation and chemical analysis

One grab sample of hay and one grab sample of concentrate were collected per each experimental period. Rumen fluid, blood, feces and urine samples were collected daily throughout of each experimental period. Rumen fluid and blood samples were collected at 12:00 h and 9:00 h, respectively. Blood plasma was harvested after centrifugation of the blood samples for 15 minutes at 1800 x g. Two grab samples of feces, one in the morning and one in the afternoon, were collected. Urine samples were collected once a day. All samples obtained from the animals were frozen on a daily basis. After completion of experiment, the collected samples were subjected to the sample preparation for the chemical analysis.

The collected hay and concentrate samples were pooled into one sample of each. The pooled hay sample was chopped by the chopper machine to the cutting length of 5 cm. Hay and concentrate samples were then dried in the oven over night at 60°C. DM content of feed samples at 60°C was calculated by dividing the net weight of dried samples to the net weight of samples before drying. It was followed by grinding the samples in a Cyclone mill (Tecator Cyclotec 1093, Höganäs, Sweden). Following that, 1-2 g of feed samples were dried at 103°C over night and the DM content of samples at 103°C was calculated. The original DM content of feed samples were calculated by multiplying the DM content at 60°C to the DM content at 103°C. The same procedure was applied for the DM determination of thawed fecal samples. However, after drying the fecal samples at 60°C, they were grinded by using a Hammer mill (Kamas Slagy 200B, Malmö, Sweden). The rumen fluid samples were thawed and centrifuged for 5 minutes at 1800 x g and subsamples of rumen fluid were taken afterwards. The urine and plasma samples were thawed and no further sample preparation were applied to them. The Pi concentration of fecal samples was measured on the fresh thawed fecal samples. The corresponding thawed fecal samples collected in the morning and in the afternoon were pooled into one sample. They were then subjected to the sample preparation according to Nordqvist et al. (2009). However, distilled water was used instead of the hydrochloric acid.

The Pi concentration in rumen fluid, plasma, fecal and urine samples were measured by a colorimetric method using Randox inorganic phosphorus colorimetric kit (Phos, Antrim, United Kingdom) on a spectrophotometer (LKB-Biochrom, Ultrospec K, Cambridge, England). Atomic absorption spectrophotometry (ANALYST 100, Perking Elmer, Norwalk Connecticut, USA) was applied for the measurement of Cobalt (Co) concentration in the rumen fluid samples. The P concentration in feed samples was measured by inductively coupled plasma optical emission spectrometry known as ICP-technique (Spectroflame, Spectro Analytical Instruments GmbH, Kleve, Germany).

Calculation

It was assumed that Pi was evenly distributed in the fluid phase of the rumen. In addition, it was assumed that the fluid turnover in rumen followed first order kinetics. Cobalt-Lithium EDTA was used as fluid marker in order to estimate the rumen fluid volume and its outflow rate. Eight g of Cobalt-Lithium EDTA dissolved in 350 ml of water was poured into the rumen during the last infusion day. It was followed by eight hours rumen fluid collection.
on an hourly basis. By plotting the Co concentration against time and using the exponential function, the rumen fluid volume (L/d) and its outflow rate (L/h) was estimated. P outflow rate from rumen (g/h) was calculated by multiplying the P concentration of the rumen fluid (g/L) to the rumen fluid outflow rate (L/h). The amount of P entered into the small intestine in each treatment (g/d) was calculated by adding the amount of infused P (g/d) to the corresponding P outflow from rumen (g/d). DM digestibility was estimated by means of an internal marker. Acid insoluble ash (AIA) was used as the marker. AIA concentrations in feed and fecal samples were determined by a modified method of 2N HCl procedure described by Keulen and Young (1977).

**Statistical analysis**

Statistical analysis of data was carried out using the Mixed procedure of SAS (SAS system for windows, release 9.1; SAS institute Inc., Cary, NC, USA). The mixed model included the main effects of treatment and period as fixed factors and cow within treatment as a random factor. Polynomial contrasts were constructed to determine the nature of changes in response variables to increasing P infusion. The effect was considered significant when $P < 0.05$ and trend when $0.05 \leq P \leq 0.10$. Data were presented as least square means ± pooled standard error of a mean.

**Results**

Digestibility of dry matter (DM) was not affected with P infusion (Table 2). It ranged from 718 to 729 (g/kg). However, fecal DM tended to decline as the level of infused P increased (Table 2).

<table>
<thead>
<tr>
<th>Variables</th>
<th>P infusion (g/d)</th>
<th>Statistical significance, $P&gt;F^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14.4</td>
</tr>
<tr>
<td>Digestibility of DM (g/kg)</td>
<td>718</td>
<td>719</td>
</tr>
<tr>
<td>Fecal DM content (g/kg)</td>
<td>184</td>
<td>184</td>
</tr>
</tbody>
</table>

1Data are least square means and standard errors of means (SEM) (n = 3).

2 Linear = linear effect of P infusion level.

The rumen fluid volume and its outflow rate ranged from 76 to 100 (L) and from 5 to 5.7 (L/h), respectively. P infusion had no effect on these parameters ($P>0.1$). There was also no effect of P infusion on P outflow from rumen and P content of the rumen fluid. But the P concentration of rumen fluid tended to increase as P supply increased (Table 3).
Table 3. Effect of different amounts of P infused into the abomasum on rumen inorganic P (P$_i$) parameters$^1$.

<table>
<thead>
<tr>
<th>Variables</th>
<th>P infusion (g/d)</th>
<th>SEM</th>
<th>Statistical significance, $P&gt;F^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14.4</td>
<td>28.8</td>
</tr>
<tr>
<td>$P_i$ flow from rumen (mmol/h)</td>
<td>71</td>
<td>91</td>
<td>102</td>
</tr>
<tr>
<td>$P_i$ concentration of rumen fluid (mmol/l)</td>
<td>14</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>$P_i$ content of rumen fluid (mmol)</td>
<td>1232</td>
<td>1345</td>
<td>1518</td>
</tr>
</tbody>
</table>

$^1$Data are least square means and standard errors of means (SEM) ($n = 3$).

$^2$ Linear = linear effect of P infusion level.

The amount of $P_i$ entering the small intestine increased in response to increased abomasal infusion of P (Table 4). There was a tendency for increased $P_i$ absorption as P supply increased. $P_i$ absorption efficiency, on the other hand, tended to decline as the level of infused P increased. Total fecal P excretion increased with P infusion. There was also an increase in fecal $P_i$ excretion with P infusion (Table 4).

Table 4. Effect of different amounts of P infused into the abomasum on body inorganic P (P$_i$) kinetics$^3$.

<table>
<thead>
<tr>
<th>Variables</th>
<th>P infusion (g/d)</th>
<th>SEM</th>
<th>Statistical significance, $P&gt;F^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14.4</td>
<td>28.8</td>
</tr>
<tr>
<td>$P_i$ outflow from rumen (g/d)</td>
<td>53</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td>$P_i$ entering small intestine (g/d)</td>
<td>53</td>
<td>82</td>
<td>105</td>
</tr>
<tr>
<td>$P_i$ excreted in feces (g/d)</td>
<td>5</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>Total $P_i$ excreted in feces (g/d)</td>
<td>15</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>$P_i$ absorption (g/d)</td>
<td>48</td>
<td>68</td>
<td>82</td>
</tr>
<tr>
<td>$P_i$ absorption efficiency</td>
<td>0.90</td>
<td>0.82</td>
<td>0.79</td>
</tr>
</tbody>
</table>

$^1$Data are least square means and standard errors of means (SEM) ($n = 3$).

$^2$ Linear = linear effect of P infusion level.

$^3$ The amount of $P_i$ entering small intestine in each treatment was calculated by adding corresponding infused P (g/d) to $P_i$ outflow from rumen (g/d). $P_i$ absorption was calculated by subtracting fecal $P_i$ from $P_i$ entering small intestine.

The relation between amount of $P_i$ entering into the small intestine and corresponding $P_i$ absorption in different levels of P infusion is presented in Figure 1. Fractional absorption of $P_i$ was considered to be the same as $P_i$ absorption efficiency in each treatment. Thus, fractional absorption of $P_i$ tended to decline in response to increased P supply (Table 4).
Figure 1. Amounts of inorganic P ($P_i$) entering small intestine and corresponding $P_i$ absorption at different levels of P infusion into the abomasum.

The concentrations of plasma and urine $P_i$ (mmol/l) were not affected by P infusion (Table 5). The $P_i$ concentration ranged from 2.09 to 3.02 mmol/l and from 0.55 to 1.11 mmol/l for plasma and urine respectively.

Table 5. Effect of different amounts of P infused into the abomasum on plasma and urine $P_i$ concentrations.\(^1\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>P infusion (g/d)</th>
<th>SEM</th>
<th>Statistical significance, P&gt;F(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14.4</td>
<td>28.8</td>
</tr>
<tr>
<td>Plasma $P_i$ (mmol/l)</td>
<td>2.09</td>
<td>3.02</td>
<td>2.89</td>
</tr>
<tr>
<td>Urine $P_i$ (mmol/l)</td>
<td>0.55</td>
<td>0.94</td>
<td>1.11</td>
</tr>
</tbody>
</table>

\(^1\)Data are least square means and standard errors of means (SEM) (n = 3).

\(^2\) Linear = linear effect of P infusion level.

The correlation between plasma and urine $P_i$ concentrations was relatively weak (Figure 2). An exponential function ($r^2 = 0.47$) fitted better to the correlation than a linear function ($r^2 = 0.28$) did (Figure 2). When $P_i$ concentration in plasma exceeded 3 mmol/l, $P_i$ excretion from urine rose (Figure 2).
Discussion

Dietary P supply covered the P requirement of the animals according to NRC (2001). Therefore, phosphate infusion supplied P well beyond the requirements. In addition, it was assumed that there was a negligible net accretion of P in these adult non-lactating non-pregnant animals. The supplemented P was infused into the abomasum not into the rumen so that P\textsubscript{i} outflow from the rumen only contained P\textsubscript{i} coming from saliva and diet. Since, dietary P supply was constant, any changes in P\textsubscript{i} outflow from the rumen were then results of changes in the salivary P\textsubscript{i}.

In modern dairy cow productions, wet feces can be regarded as a hygienic problem. It was reported that P supply beyond requirements results in wet feces in ruminants (Pffefer et al., 2005). This is in agreement with results of the current study. A tendency for reduced fecal DM (Table 2) indicates that as P supply increased, feces were prone to be wet.

A tendency for an increase of P\textsubscript{i} absorption in response to P infusion was accompanied by a tendency for a decline of P\textsubscript{i} absorption efficiency (Table 4). This could imply that P\textsubscript{i} absorption increased but at a reducing rate. In other words, there was a tendency for a negative relation between fractional absorption of P\textsubscript{i} and the increasing amounts of P\textsubscript{i} entered into the small intestine. This was reasonably in agreement with the hypothesis of the current study. It should be taken into account that there were only two levels of P infusion in this experiment. Perhaps, with a higher number of treatments and an infusion period that was slightly longer, the results would have been more conclusive.

It appears that there were mechanisms in small intestine regulating the extent of P\textsubscript{i} absorption. These mechanisms could play significant roles in maintenance of P balance when P intake changes (Mogodiniyai Kasmaei and Holtenius, 2010). One of the most likely
mechanisms which could explain the results of current study would be the carrier facilitated diffusion. It was reported that saturation of this mechanism would terminate continuous increase of $P_i$ absorption (Care et al., 1980 cited by Scott, 1988). Saturation of carrier facilitated diffusion is due to the limited number of available carriers in relation to increasing concentration of external substrate (Harth, 1983). The tendency for the decline of $P_i$ absorption efficiency might suggest the existence of such a mechanism. However, $P_i$ absorption tended to increase with $P$ infusion (Table 4). This would oppose with the concepts of the carrier facilitated diffusion. It appears that the carrier facilitated diffusion could not be the only responsible mechanism that played a role in $P_i$ absorption. If it is accepted that the concentration gradient of $P_i$ between lumen of intestine and blood was the driving force of $P_i$ uptake, the role of passive diffusion of $P_i$ cannot be ruled out.

It is generally accepted that the passive diffusion of a solute proceeds until the concentrations of the solute in two adjacent solutions are equalized (Harth, 1983). However, it has been found that passive diffusion of some ions such as $Na^+$ in digestive tract could be regulated (Johnson, 2001). The ions from the lumen of digestive tract are transferred to the blood from transcellular and paracellular pathways of intestinal epithelial cells (Johnson, 2001). The passive movement of ions from paracellular pathways is regulated by permeability of tight junctions of the intestinal epithelial cells (Johnson, 2001). The passive transcellular movement of ions occurs through the pores (Johnson, 2001). These pores are apparently larger in the proximal small intestine (7 to 8 Å) than in the distal part (3 to 4Å) which in turn could restrict the passive diffusion of solutes in ileum (Johnson, 2001). Therefore, due to the dynamic characteristics of digesta in the lumen of digestive tract, the possible pores size differences alongside the small intestine could be regarded as one of the regulatory mechanisms of $P_i$ passive diffusion. Furthermore, it is worthwhile to assume that the number of transcellular pores responsible for passive $P_i$ diffusion could also be regulated according to the physiological status. Such a regulatory mechanism has been identified for $Na^+$ restricted diffusion in colon. Aldosterone enhances the number of $Na^+$ channels in colon and increases the $Na^+$ passive uptake (Johnson, 2001). In addition, the passive diffusion of $P_i$ through transcellular pores could also be limited when $P_i$ concentration in the lumen of the intestinal tract exceeds the transport capacity of pores. It should be taken into consideration that the suggested regulatory mechanisms of $P_i$ passive diffusion have not been previously addressed nor been investigated. This opens the opportunities for further research in this area.

Virtually the entire absorbed $P_i$ reentered into the gut through saliva because urine $P_i$ excretion was negligible (Table 5). Therefore, the amount of salivary $P_i$ output in each treatment was assumed to be the same as corresponding $P_i$ absorption. Accordingly, a tendency for the increase of salivary $P_i$ secretion in response to $P$ infusion could be expected. In ruminants, the amount of salivary $P_i$ output is well balanced with the extent of intestinal $P_i$ absorption (Breves and Schröder, 1991; Schröder et al., 1995; Huber et al., 2002; Busche et al., 2007). The amount of salivary $P_i$ secretion is determined by two parameters; the volume of salivary output and salivary $P_i$ concentration. Salivary output depends on the diet characteristics such as DM, neutral detergent fibre (NDF) and particle size (Beauchemin et al., 2008). In the current study, the diet and its characteristics were similar in all treatments.
Thus, the volume of salivary output most likely remained unchanged. This was in agreement with our findings that no changes in the rumen fluid volume and outflow rate occurred. A tendency for an increase of salivary \( P_i \) output, then, was due to the increased salivary \( P_i \) concentration. Increased salivary \( P_i \) concentration in response to increased \( P \) supply was also reported in growing calves (Challa and Braithwaite, 1988a). It seems that salivary glands are able to play a role in \( P \) balance by regulating the extent of salivary \( P_i \) output. An evidence for the active role of salivary glands in \( P \) balance could be obtained from the status of plasma \( P_i \) in the current study.

It is generally accepted that the plasma \( P_i \) concentration determines the extent of salivary \( P_i \) secretion (Challa et al., 1989; Breves and Schröder, 1991). However, while plasma \( P_i \) concentration stayed stable (Table 5), the salivary \( P_i \) output increased. This would suggest that salivary glands actively transported plasma \( P_i \), so that the increased \( P \) supply to the blood stream was counteracted by an increased secretion. This could be regarded as a response of \( P \) homeostatic mechanisms to increasing rates of \( P_i \) absorption so that the hyperphosphatemia and its subsequent symptoms were prevented. Such an active transport mechanism has been indentified in parotid glands of small ruminants (Shirazi-Beechey et al., 1991; Shirazi-Beechey et al., 1996). It was reported that a \( Na^+ \) coupled transporter could be responsible for \( P_i \) transport from plasma to the parotid saliva (Shirazi-Beechey et al., 1991).

Urinary \( P_i \) excretion played a negligible role in \( P \) balance because less than 1 \% of \( P_i \) entered into the blood stream was excreted in urine (Table 5). This finding is in agreement with previous studies in small ruminants (Dias et al., 2006a; Dias et al., 2006b). However, when plasma \( P_i \) concentration reached the renal threshold (3 mmol/l), there was an increase in urinary \( P \) excretion (Figure 2). Such a renal threshold (2.3 mmol/l) has been previously reported in growing calves (Challa et al., 1989).

In dairy cows, there is an inevitable loss of \( P \) even if \( P \) supply is below the requirement. The inevitable \( P \) loss in dairy cows is regarded as the maintenance requirement of \( P \) (NRC, 2001). This part of fecal \( P \) is mainly originated from \( P \) in microbial residues (Bannink et al., 2010). Another fraction of fecal \( P \) could be of the endogenous origin. The sloughing of intestinal epithelial cells and digestive tract secretion are the main sources of endogenous losses of \( P \) (Bannink et al., 2010). Dietary \( P \) supply would also contribute into the fecal \( P \) excretion. However, in ruminants, the contribution of dietary \( P \) could be rather small (Wu et al., 2000). This is mainly due to the rumen microbial activities which make the ingested \( P \), in principle, available for absorption (Bannink et al., 2010). \( P_i \) fraction of fecal \( P \) originates from unabsorbed \( P_i \), entering into the small intestine (Hill et al., 2008). The results suggest that a large proportion of \( P_i \) entered into the small intestine originated from salivary \( P_i \) secretion (Table 4). Total fecal \( P \) and fecal \( P_i \) increased in response to increased \( P \) supply (Table 4). This indicates that there was a direct relation between \( P \) supply and \( P \) excretion in feces. The ratio of fecal \( P_i \) to total fecal \( P \) was also increased by \( P \) infusion (Table 4). This ratio, therefore, could be used as a simple tool for the determination of nutritional status of \( P \) in dairy cows.
In a typical dairy diet (concentrate and forages), phytic acid P ($P_P$) is the most widespread forms of dietary organic P ($P_o$) (Toor et al., 2005). Owing to the rumen microbial activities, the majority of dietary $P_P$ is transformed into $P_i$ (Bannink et al., 2010). Part of this $P_i$ is utilized by rumen microbes (Bannink et al., 2010) and the rest enters into the rumen $P_i$ pool. Undegraded dietary $P_P$ in the rumen is virtually undigestible in the lower tract and excreted in feces (Hill et al., 2008). From the results it appears that the contribution of dietary P in $P_i$ pool of rumen was approximately 5 g/d (Table 4). The rest of dietary P (~10 g/d) could have been partly incorporated into the microbial P and partly escaped the rumen undegraded.

The animals used in this experiment had a very low P requirement. Since urinary $P_i$ excretion was negligible, it was expected that the infused P in both levels of 14.4 (g/d) and 28.8 (g/d) excreted in feces. However, there was not a complete recovery of infused P in feces in those treatments (Table 4). This suggests that P homeostatic mechanism did not reach the steady state when 14.4 (g/d) and 28.8 (g/d) of P infused into the abomasum. This was probably due to the short experimental periods. In the following section, schematic modules of $P_i$ kinetics in different levels of P infusion at the last infusion day are presented. The module of P kinetics for lactating dairy cows proposed by Hill et al. (2008) was used as the reference module. Appropriate changes in that module were applied in order to make the module more applicable with the circumstances of current experiment. Dietary P supply was set to 15 g/d. Daily P requirement of animals and urinary $P_i$ excretion were assumed to be zero. Since animals were neither pregnant nor lactating, the net bone turnover of $P_i$ and $P_i$ secretion in milk were also set to zero. In addition, the small intestine and large intestine were merged into one compartment because the turnover of $P_i$ in large intestine is very small (Hill et al., 2008).
Figure 4. Schematic modules of Pi kinetics in 0 (g/d) P infusion at the last infusion day. Boxes with dash lines and solid lines represent pools and compartments respectively. Arrows represent fluxes. Bold data associate with prospective steady state. An asterisk indicates the slight modification of actual data in order to fit data in the module. Data given in the module are in g/d. P_d= dietary P; P_i= inorganic P; P_o= undigested organic P including P in microbial residues, undigested dietary P and endogenous P losses (modified from Hill et al., 2008).
**Figure 5.** Schematic modules of Pi kinetics in 14.4 (g/d) P infusion at the last infusion day. Boxes with dash lines and solid lines represent pools and compartments respectively. Arrows represent fluxes. Bold data associate with prospective steady state. An asterisk indicates the slight modification of actual data in order to fit data in the module. Data given in the module are in g/d. PD = dietary P; Pi = inorganic P; PO = undigested organic P including P in microbial residues, undigested dietary P and endogenous P losses (modified from Hill et al., 2008).
Figure 6. Schematic modules of Pi kinetics in 28.8 (g/d) P infusion at the last infusion day. Boxes with dash lines and solid lines represent pools and compartments respectively. Arrows represent fluxes. Bold data associate with prospective steady state. An asterisk indicates the slight modification of actual data in order to fit data in the module. Data given in the module are in g/d. $P_d =$ dietary P; $P_i =$ inorganic P; $P_o =$ undigested organic P including P in microbial residues, undigested dietary P and endogenous P losses (modified from Hill et al., 2008).
As can be seen from Figures 5 and 6, there were \( P_i \) accumulations of 6 (g/d) and 11 (g/d) in rumen \( P_i \) pool in 14.4 (g/d) \( P \) infusion and 28.8 (g/d) \( P \) infusion, respectively. As a result, \( P_i \) outflow from rumen did not increase (Table 3). \( P_i \) accumulation in rumen, then, could be considered as a feedback response to excessive \( P \) supply so that the abrupt rise of \( P_i \) flow within gut was prevented. It appears that at steady states of \( P \) balance in 14.4 (g/d) \( P \) infusion and 28.8 (g/d) \( P \) infusion, the inflow and outflow of \( P_i \) from rumen will be equalized otherwise the accumulation of \( P_i \) in rumen will be proceeded. Whether \( P_i \) accumulation in rumen \( P_i \) pool would have been continued if the experimental periods were longer and then at which level of \( P_i \) accumulation in rumen, the steady state of \( P \) balance would have been established remain unanswered. However, \( P_i \) accumulation in rumen resulted only in 9 % and 23% numerically increase of rumen \( P_i \) content in 14.4 (g/d) \( P \) infusion and 28.8 (g/d) \( P \) infusion respectively. These results highlight the adaptability of \( P \) homeostatic mechanism of dairy cows to markedly excessive \( P \) supply (Mogodiniyai Kasmaei and Holtenius, 2010). A schematic module of rumen \( P_i \) dynamics in different levels of \( P \) infusion at the last infusion day is presented in Figure 7. It should be taken into account that \( P \) balance did not reach the steady state at 14.4 (g/d) and 28.8 (g/d) \( P \) infusion.
Figure 7. Schematic modules of the dynamics of $P_i$ pool of rumen in different levels of $P$ infusion at the last infusion day. $P_i$ inflow into the rumen $P_i$ pool was calculated by adding the $P_i$ of dietary origin (~5 g/d) to salivary $P_i$ secretion. Salivary $P_i$ secretion was assumed to be the same as the $P_i$ absorption in each treatment (see Table 4). Data are in g/d.
The results clearly demonstrated that P homeostasis was controlled within gut through regulation of P$_i$ absorption, salivary P$_i$ secretion and fecal P$_i$ excretion. There was a P$_i$ recycling through salivary P$_i$ secretion and subsequent P$_i$ reabsorption in small intestine. The extent of P$_i$ recycling was increased with P infusion due to increased P$_i$ absorption and following rise in salivary P$_i$ secretion. However, owing to the negative relation between fractional absorption of P$_i$ and the amount of P$_i$ entering into small intestine, increased P$_i$ absorption had a reducing rate. Accordingly, at steady state of P balance, any P beyond the requirement would be eventually excreted in feces. A simplified module of P$_i$ kinetics in current study is presented in Figure 8. The P$_i$ recycling in dairy cows could be due to the great needs of rumen microbes for this element and the buffering role of P$_i$ against volatile fatty acids produced in the rumen by microbial fermentation. It appears that dairy cows have developed an efficient P homeostatic mechanism in order to supply the P required for both rumen microbes and phosphate buffer irrespective to the dietary P supply.

Figure 8. Inorganic phosphorus (P$_i$) kinetic within gut of non-pregnant non-lactating cow (modified from Scott, 1988).
Conclusions

Phosphorus infusion increased the $P_i$ flow within the gut, resulting in a tendency for an increased $P_i$ absorption. On the other hand, there was also a tendency for a decline in $P_i$ absorption efficiency. This could imply that there was a negative relation between the amounts of $P_i$ entering into the small intestine and the fractional rate of $P_i$ absorption. It appeared that $P_i$ uptake in the small intestine was regulated by yet not fully understood mechanisms. Plasma $P_i$ concentration did not change in relation to increased $P_i$ absorption. This was probably due to the active drainage of absorbed $P_i$ into the saliva regulated by the salivary glands. The concentration of salivary $P_i$ increased with $P$ infusion, resulting in an elevated amount of salivary $P_i$ secretion. Urinary $P_i$ excretion had a negligible role in $P$ homeostasis. It appeared that at steady state of $P$ balance, virtually the entire infused $P$ ($P$ supply beyond the requirements) would be excreted in feces. These results imply that $P$ balance in dairy cows is regulated within gut through regulation of $P_i$ absorption, salivary $P_i$ secretion and fecal $P_i$ excretion.

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References


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