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

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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_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 ($NaH_2PO_4.2H_2O$) 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_i absorption tended to increase as P supply increased. This was accompanied by a tendency for the reduction of P_i absorption efficiency suggesting mechanisms in the small intestine regulating P_i absorption. It appeared that the fractional absorption rate of P_i was negatively related to the increasing amount of P supply. In addition, P infusion resulted in increased salivary P_i concentration. It was suggested that salivary glands could have increased P_i secretion in response to increased P_i flux in plasma. This was evidenced from the unchanged plasma P_i concentration in relation to increased P_i absorption. Urinary P_i excretion played a negligible role in P balance. It was concluded that P homeostasis was controlled within gut through regulation of P_i uptake, salivary P_i secretion and fecal P_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-$(OH)_2D_3$) concentration rather than the plasma P_i concentration. 1,25-$(OH)_2D_3$ is a product of vitamin D metabolism in the kidneys (McDonald *et al.,* 2002). Increased plasma concentration of 1,25-$(OH)_2D_3$ 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-$(OH)_2D_3$ 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_i in the intestinal digesta is mainly monovalent dihydrogen phosphate ($H_2PO^-_4$) (Pfeffer *et al.*, 2005). The major proportion of intestinal $H_2PO^-_4$ 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-}_4) (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 Pi 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-$(OH)_2D_3$ 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

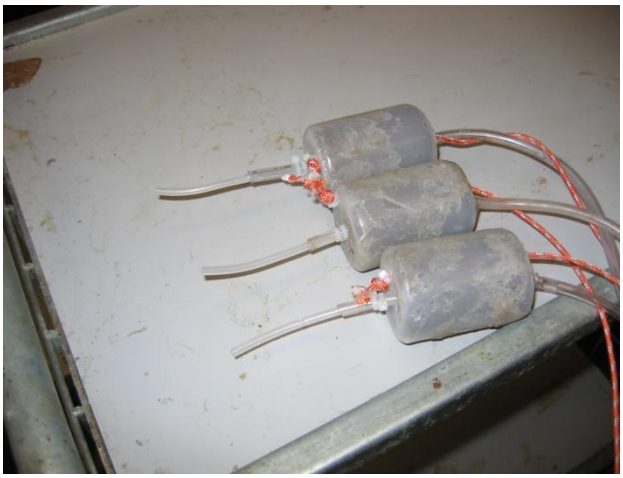
Three non-lactating non-pregnant rumen fistulated cows of the Swedish red and white breed were used. The body weight ranged from 629 to 743 kg. The diet consisted of 4 kg of hay and 1.8 kg of concentrate fed every day at 8:00 h and 16:00 h. Total dry matter (DM) intake was 5.3 kg/d. The feed intake provided 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 ($NaH_2PO_4.2H_2O$) 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.

P infusion (g/d)	$NaH_2PO_4.2H_2O$ (g/l)	P concentration (g/l)	Volume of the phosphate solution (ml/d)	Infusion rate of P (g/d)
0	-	-	4800	Water only
14.4	15.11	3	4800	14.4
28.8	30.22	6	4800	28.8



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 P_i 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 P_i 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 (AAnalyst 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 P_i 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_i outflow rate from rumen (g/h) was calculated by multiplying the P_i concentration of the rumen fluid (g/L) to the rumen fluid outflow rate (L/h). The amount of P_i 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_i 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 2. Effect of different amounts of P infused into the abomasum on digestibility of dry matter (DM) and fecal DM content[1].

	P infusion (g/d)				Statistical significance, $P>F$[2]
Variables	0	14.4	28.8	SEM	Linear
Digestibility of DM (g/kg)	718	719	729	0.02	0.75
Fecal DM content (g/kg)	184	184	168	0.004	0.05

[1]Data 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_i outflow from rumen and P_i content of the rumen fluid. But the P_i 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].

	P infusion (g/d)				Statistical significance, $P>F^2$
Variables	0	14.4	28.8	SEM	Linear
P_i flow from rumen (mmol/h)	71	91	102	13.74	0.18
P_i concentration of rumen fluid (mmol/l)	14	17	18	1.31	0.09
P_i content of rumen fluid (mmol)	1232	1345	1518	147.40	0.24

[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[1].

	P infusion (g/d)				Statistical significance, $P>F^2$
Variables[3]	0	14.4	28.8	SEM	Linear
P_i outflow from rumen (g/d)	53	67	76	9.59	0.18
P_i entering small intestine (g/d)	53	82	105	8.42	0.02
P_i excreted in feces (g/d)	5	13	22	1.21	<0.001
Total P excreted in feces (g/d)	15	22	31	1.43	<0.001
P_i absorption (g/d)	48	68	82	10.69	0.08
P_i absorption efficiency	0.90	0.82	0.79	0.04	0.08

[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).

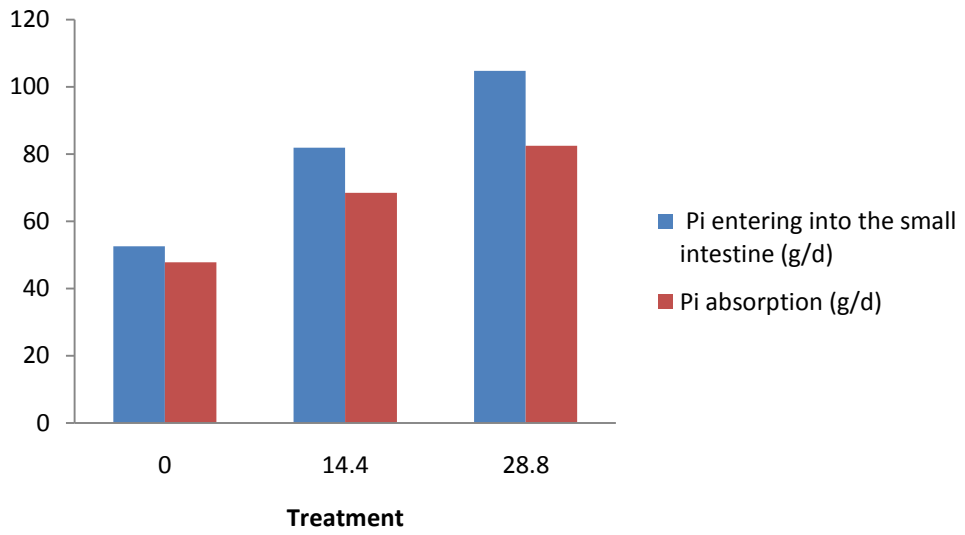


Figure 1. Amounts of inorganic P (P_i) entering small intestine and corresponding P_i absorption at different levels of P infusion into the abomasum.

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]

	P infusion (g/d)				Statistical significance, P>F[2]
Variables	0	14.4	28.8	SEM	Linear
Plasma P_i (mmol/l)	2.09	3.02	2.89	0.67	0.45
Urine P_i (mmol/l)	0.55	0.94	1.11	0.53	0.50

[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 (Figure2). When P_i concentration in plasma exceeded 3 mmol/l, P_i excretion from urine rose (Figure 2).

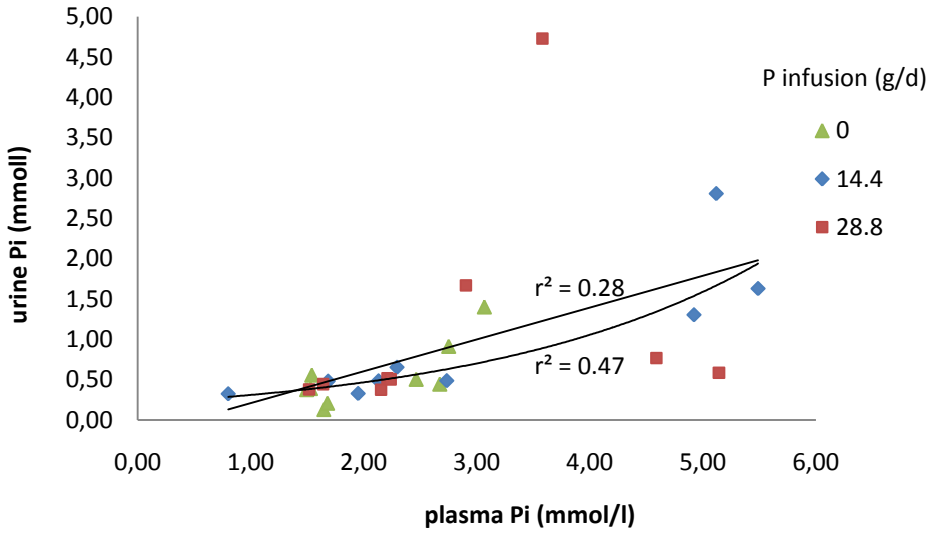


Figure 2. Relation between P_i concentration (mmol/l) in plasma and urine in different levels of P infusion into the abomasum.

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_i outflow from the rumen only contained P_i coming from saliva and diet. Since, dietary P supply was constant, any changes in P_i outflow from the rumen were then results of changes in the salivary P_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_i absorption in response to P infusion was accompanied by a tendency for a decline of P_i absorption efficiency (Table 4). This could imply that P_i absorption increased but at a reducing rate. In other words, there was a tendency for a negative relation between fractional absorption of P_i and the increasing amounts of P_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_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_i 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 when14.4 (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).

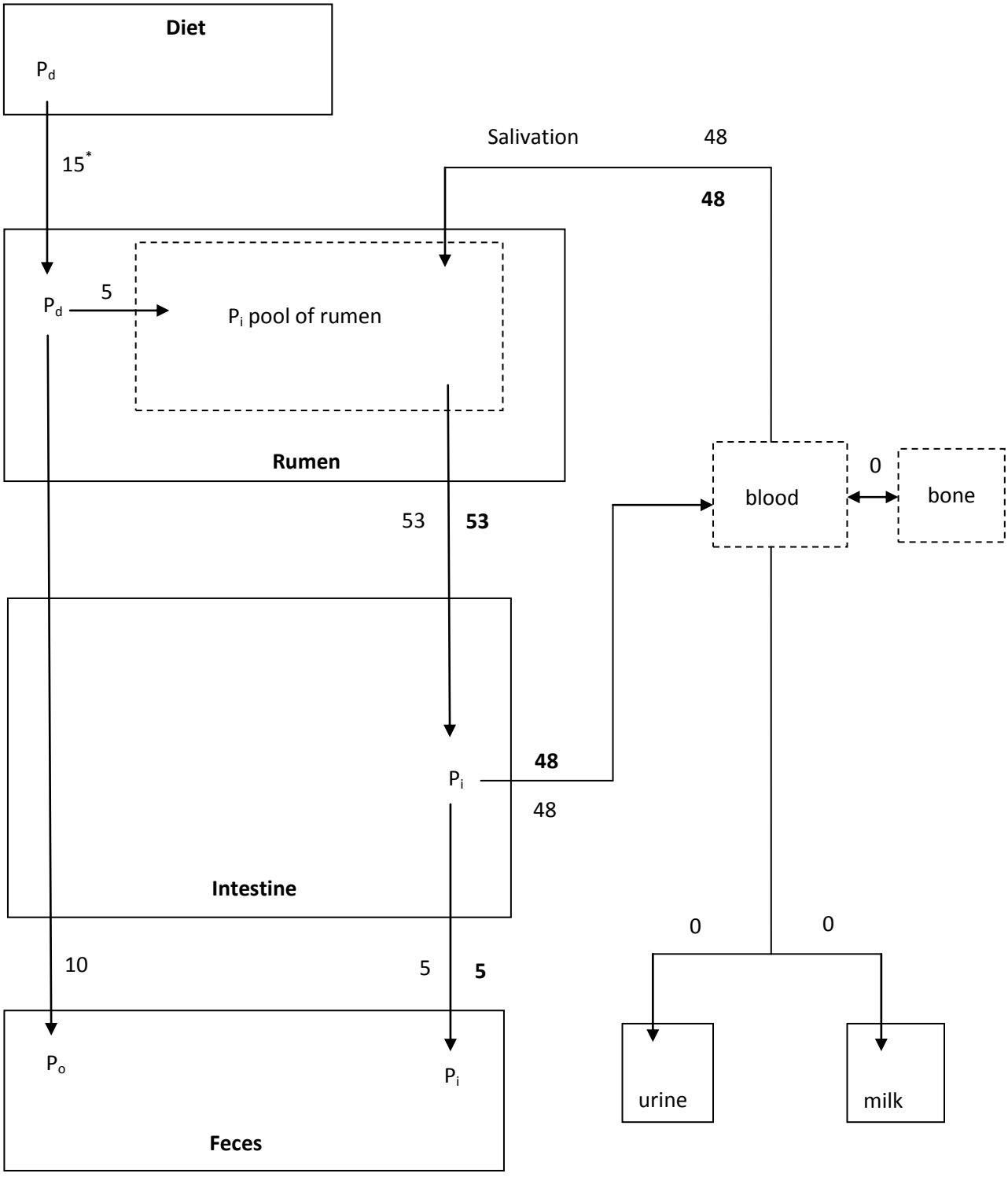


Figure 4. Schematic modules of Pi kinetics in 0 (g/d) P infusion at the last infusion day. Boxes with dash lines and solid lines represent pools and compartments respectively. Arrows represent fluxes. Bold data associate with prospective steady state. An asterisk indicates the slight modification of 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).

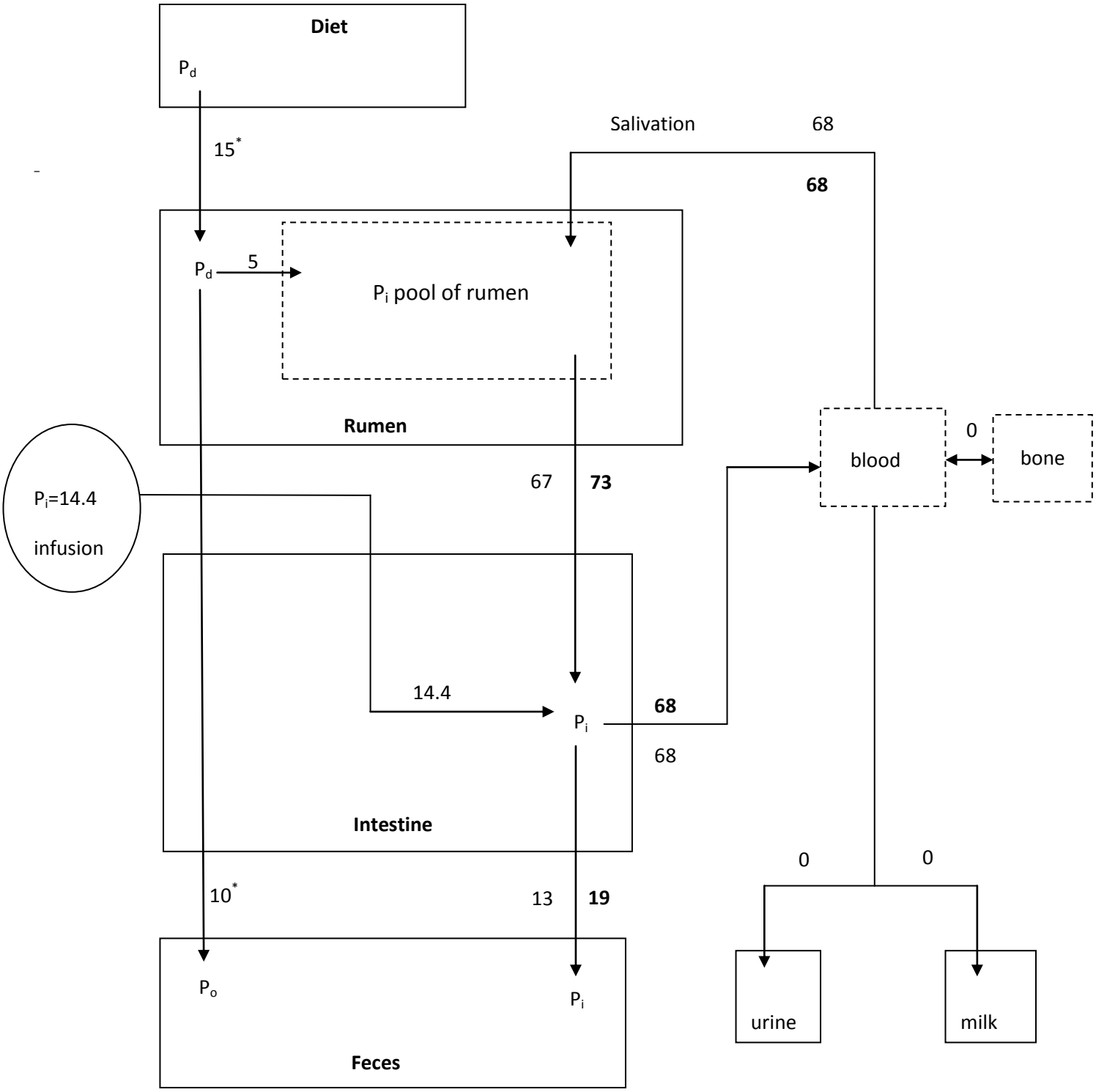


Figure 5. Schematic modules of Pi kinetics in 14.4 (g/d) P infusion at the last infusion day. Boxes with dash lines and solid lines represent pools and compartments respectively. Arrows represent fluxes. Bold data associate with prospective steady state. An asterisk indicates the slight modification of actual data in order to fit data in the module. Data given in the module are in g/d. 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).

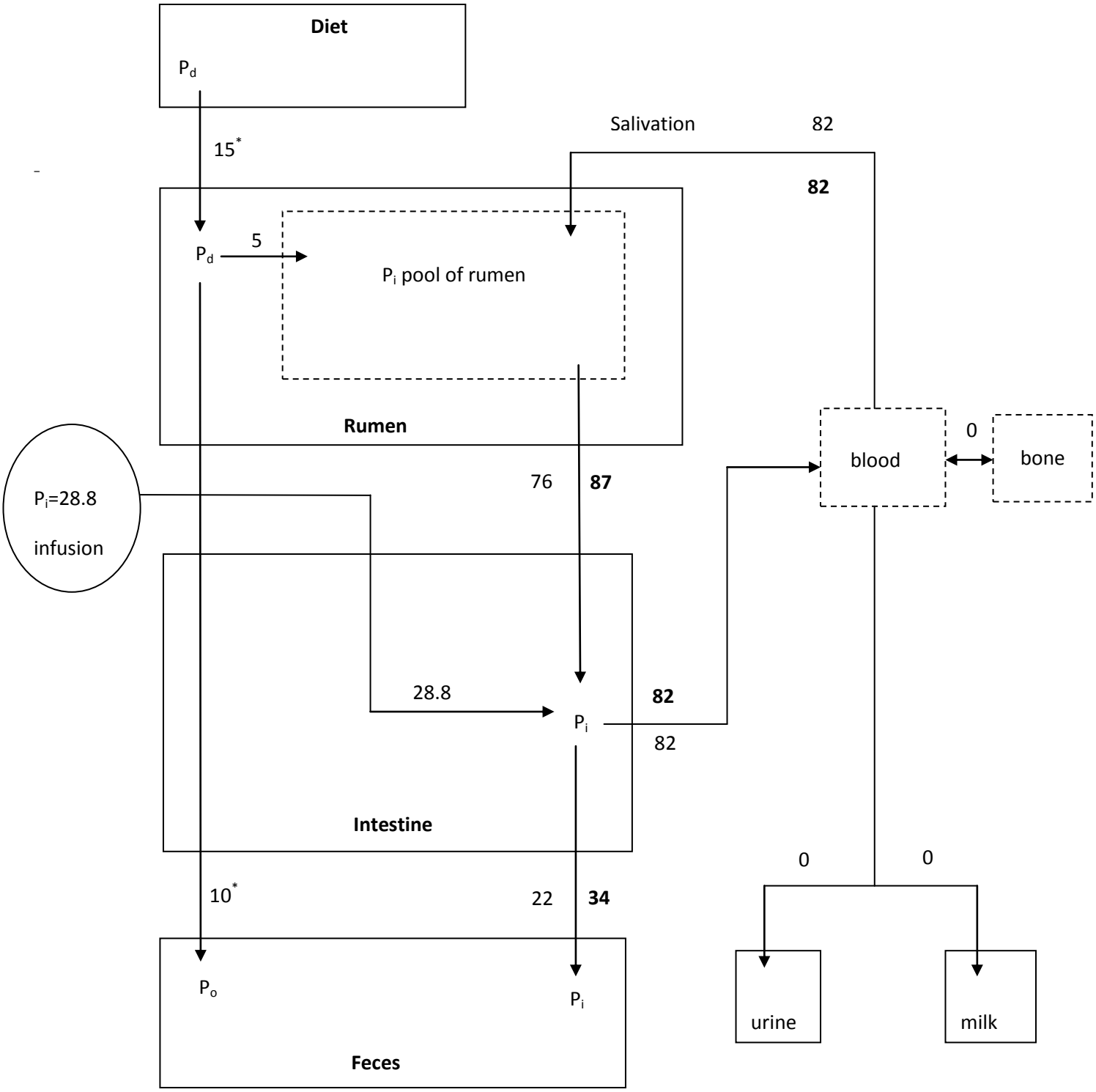


Figure 6. Schematic modules of Pi kinetics in 28.8 (g/d) P infusion at the last infusion day. Boxes with dash lines and solid lines represent pools and compartments respectively. Arrows represent fluxes. Bold data associate with prospective steady state. An asterisk indicates the slight modification 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 Pi 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.

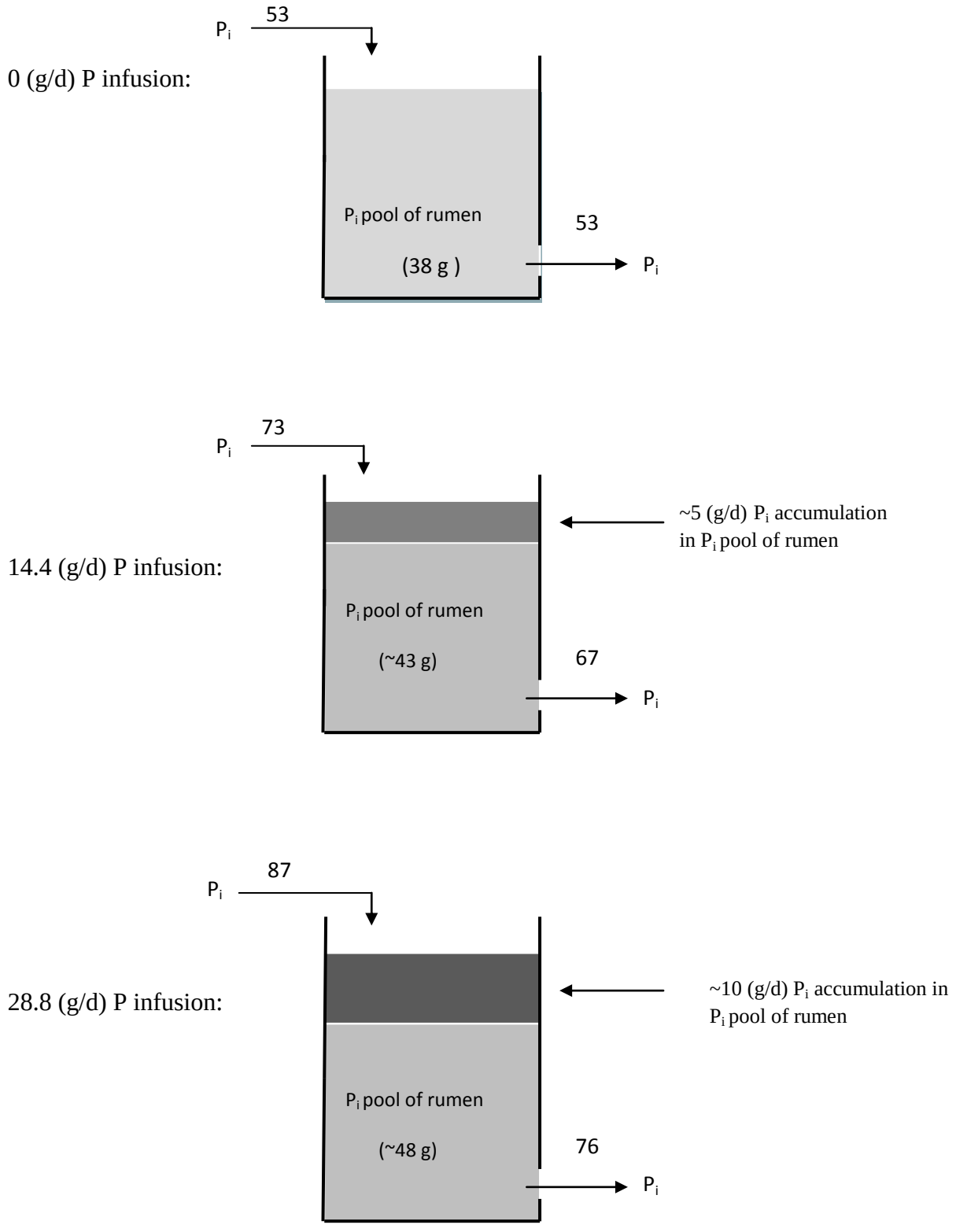


Figure 7. Schematic modules of the dynamics of P_i pool of rumen in different levels of P infusion at the last infusion day. P_i inflow into the rumen P_i pool was calculated by adding the P_i of dietary origin (~5 g/d) to salivary P_i secretion. Salivary P_i secretion was assumed to be the same as the 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.

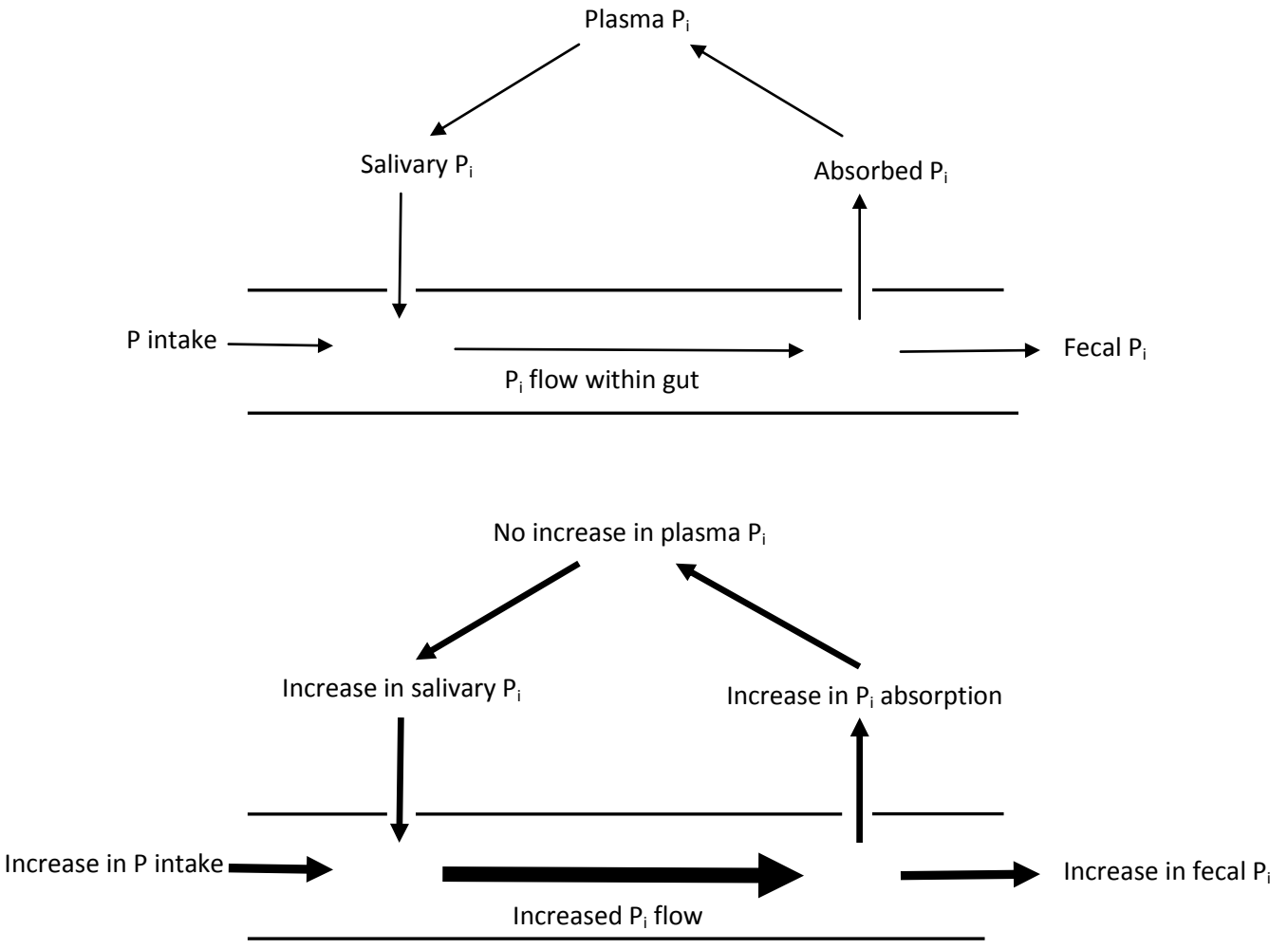


Figure 8. Inorganic phosphorus (P_i) kinetic within gut of non-pregnant non-lactating cow (modified from Scott, 1988).

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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