Effect of decreasing dietary phosphorus supply on net recycling of inorganic phosphate in lactating dairy cows

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ABSTRACT

Five ruminally cannulated lactating Holstein cows, fitted with permanent indwelling catheters in the mesenteric vein, hepatic vein, portal vein, and an artery were used to study intestinal absorption and net recycling of inorganic phosphate (P_i) to the gastrointestinal tract. Treatments were low P (LP; 2.4 g of P/kg of DM) and high P (HP; 3.4 g of P/kg of DM). The dietary total P (tP) concentrations were obtained by replacing 0.50% calcium carbonate in the LP diet with 0.50% monocalcium phosphate in the HP diet. Diets were fed for 14 d and cows were sampled on d 14 in each period. Cows were fed restrictively, resulting in equal dry matter intakes as well as milk, fat, and protein yields between treatments. Net P_i recycling (primarily salivary) was estimated as the difference between net portal plasma flux (net absorption of P_i) and apparently digested tP (feed – fecal tP difference). Phosphorus intake, apparently digested tP, and fecal tP excretion decreased with LP. An effect of decreased tP intake on net portal plasma flux of P_i could not be detected. However, despite numerically minute net fluxes across the liver, the net splanchnic flux of P_i was less in LP compared with that in HP. Though arterial plasma P_i concentration decreased, net P_i recycling was not decreased when tP intake was decreased, and recycling of P_i was maintained at the expense of deposition of P_i in bones. Data are not consistent with salivary P_i secretion being the primary regulator of P_i homeostasis at low tP intakes. On the contrary, maintaining salivary P_i recycling at low tP intakes indicates that rumen function was prioritized at the expense of bone P reserves.

Key words: dairy cow, phosphorus, absorption, recycling

INTRODUCTION

Phosphorus plays an important role in the production and health of cattle and is essential for rumen

microbial fermentation. Rates of ruminal fermentation and protein synthesis are impaired if the dietary total P (tP) supply is insufficient, and digestibility of OM may be decreased (Müschen et al., 1988; Kincaid and Rodehutschord, 2005). Dietary tP and salivary secretion of inorganic phosphate (P_i) are the major sources of P_i input to the rumen (Kincaid and Rodehutschord, 2005) and a significant part of P_i secreted via saliva is reabsorbed in the digestive tract, thereby creating a system of P_i recycling.

Valk et al. (2002) demonstrated that lowering plasma P_i concentration, through a decrease in dietary tP intake, induced a decrease in salivary P_i concentration. Inorganic phosphate supply to the rumen is, therefore, assumed to be affected by changes in the dietary tP concentration by 1) the direct effect of dietary tP concentration and 2) indirectly through the effect of dietary tP concentration on P_i concentration in plasma and saliva (Kincaid and Rodehutschord, 2005). Reynolds et al. (1991) observed that net portal-drained visceral (PDV) absorption of P_i exceeded total P intake, indicating a P_i recycling via saliva and other digestive secretions. Breves and Schröder (1991) suggested that the daily endogenous secretion of P_i accounts for 30 to 60 g of P in dairy cows. Thus, recycling of P_i is responsible for a substantial part of ruminal microbial P supply. Nonetheless, there is a shortage of quantitative data of salivary secretion of P_i and sparse information on regulation and extent of P_i recycling in lactating dairy cattle.

Inorganic phosphate recycling may generate an endogenous fecal P loss through incomplete reabsorption of salivary P_i because fecal excretion is the most quantitatively important excretion route in cattle (Scott et al., 1985; Bravo et al., 2003). Therefore, the utilization of P by dairy cows is 31% on a production cycle basis (Poulsen, 2009). Total P excretion has been shown to increase in response to increasing dietary tP supply (Scott et al., 1985; Bravo et al., 2003), and a decrease in tP intake is, therefore, expected to decrease fecal tP excretion and increase P utilization by cattle (Wu et al. 2001; Knowlton and Herbein, 2002). The objective of the present study was to quantify the net recycling of P_i to the gastrointestinal tract and the bone tissue

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Table 1. Ingredient and nutrient composition of experimental diets (g/kg of DM if not otherwise noted)

Item	Treatment ¹	
	LP	HP
Ingredient		
Corn silage ²	500	500
Grass clover silage ³	200	200
Sugar beet molasses	105	105
Sugar beet pulp	63.9	63.9
Soybean meal	100	100
Mineral premix ⁴	10	10
Urea	2.5	2.5
Vegetable fat	10	10
Calcium carbonate	5	
Monocalcium phosphate		5
MetaSmart ⁵	2.6	2.6
Chromium(III)oxide	1	1
Nutrient		
DM, %	44.2	43.2
OM	892	899
NDF	302	295
CP	140	148
Crude fat	34.7	34.7
Ca	7.2	6.9
P	2.4	3.4
NE _L , ⁶ MJ/kg of DM	6.76	6.76

¹Experimental diets had the same composition of ingredients with the exception that the LP (low-phosphorus) diet contained 0.5% of DM calcium carbonate, which was substituted by 0.5% of DM monocalcium phosphate in the HP (high-phosphorus) diet.

²Chemical composition of corn silage: DM, 363 (g/kg); ash, 31; CP, 87; NDF, 375; starch, 320; in vitro OM digestibility, 72.5%.

³Chemical composition of grass clover silage: DM, 407 (g/kg); ash, 86; CP, 146; NDF, 363; in vitro OM digestibility, 78.8%.

⁴VM 1 (Vitfoss, Gråsten, Denmark) contained (per kg): Ca, 180 g; P, 4 g; Mg, 65 g; Na, 80 g; S 30 g; vitamin A, 693 kIU; vitamin D, 146 kIU; α -tocopherol, 4,620 mg; Mn, 3,080 mg; Cu, 693 mg; Co, 19 mg; Zn, 3,460 mg; I, 173 mg; Se, 38.5 mg.

⁵2-Hydroxy-4-(methylthio)-butanoic acid isopropyl ester (MetaSmart, Adisseo, Antony, France).

⁶Calculated at DMI level of 20 kg/d according to Norfor feed evaluation system (www.norfor.info).

balance of tP in lactating cows challenged with a low dietary tP concentration. By comparing a low tP with a high tP ration, we aimed at testing if decreased tP intake would be a double challenge to rumen function (decreased P input from both feed and saliva) or if compensatory mechanisms would affect the relationship between tP intake and P_i recycling. We hypothesized that recycling of P_i would be decreased with decreased dietary tP because of a decreased intestinal absorption and subsequent decreased plasma concentration of P_i .

MATERIALS AND METHODS

The present experiment complied with the Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993), concerning experiments with animals and care of experimental animals.

Animals, Design, Feeding, and Samplings

Five ruminally cannulated (#1C; Bar Diamond Inc., Parma, ID) Danish Holstein cows, second parity, 597 ± 31 kg of BW, and 145 ± 55 DIM were used in an experiment with 2 treatments and 2 periods, and 2 or 3 cows on each treatment in each period. Cows were fitted with permanent indwelling catheters in the mesenteric vein, hepatic vein, hepatic portal vein, and an artery at least 6 wk before expected calving. Surgery was performed according to Kristensen et al. (2007). Cows were randomly assigned to one of the 2 treatment sequences. Treatments were low P (**LP**, dietary tP concentration of 2.4 g/kg of DM) or high P (**HP**, dietary tP concentration of 3.4 g/kg of DM). The HP diet meets the recommendations by NRC (2001) as well as current Danish recommendations (Sehested, 2004). The TMR were based on the same grain and forage ingredients (Table 1). The different dietary tP concentrations were obtained by replacing 0.50% calcium-carbonate in the LP diet with 0.50% mono-calcium-phosphate in the HP diet. Chromium(III)oxide (Cr_2O_3) was used as a flow-marker to determine fecal output and total tract digestibility of tP and NDF. To decrease day-to-day variation in rations and to avoid drift in silage composition, the TMR were prepared once, then vacuum packed in portions of 10 to 15 kg (Webomatic I22; Webo A/S, Aarup, Denmark) and stored below -20°C until the day before feeding. Rations stored in vacuum packs were thawed overnight. Cows were fed 20 kg of DM/d in 3 equal-sized portions at 0800, 1600, and 2400 h, which allowed for an 8-h sampling window. Orts were removed and registered daily at 0730 h. Cows were kept in tie stalls with wood shavings as bedding and were milked at 0530 and 1530 h.

The diets were fed for 14 d and cows were sampled at d 14 in each period. Blood plasma flows in the splanchnic tissues were determined by downstream dilution of *p*-aminohippuric acid (**pAH**; 175 mmol/L) continuously infused (28 ± 2 mmol/h) into the mesenteric vein. The infusates were prepared by dissolving pAH in sterile water and pH was adjusted to 7.4. Infusates were filtered (Vacu Cap 0.8/0.2 μm ; Pall Corp., Ann Arbor, MI) and autoclaved. Infusion was initiated 1 h before the first sampling. Sampling of arterial, portal, and hepatic blood was initiated 30 min before morning feeding and conducted simultaneously every hour for 8 h. From each catheter, 1 mL of heparinized whole blood was collected in a 1-mL syringe for immediate blood gas analysis and determination of arterial pH (ABL 520; Radiometer A/S, Copenhagen, Denmark). Then a total of 30 mL of blood was collected and the blood was immediately stabilized in Na-heparin tubes (Greiner BioOne GmbH, Kremsmuenster, Austria). Blood sam-

ples were placed on ice, and plasma was harvested after centrifugation at $3,000 \times g$ for 20 min at 4°C. Ruminal fluid was collected every hour by sampling from the ventral ruminal sac using an extended suction strainer (#RT; Bar Diamond) and a 50-mL syringe.

Ruminal fluid pH was measured immediately after sampling using a combination electrode (PHC2002-8; Hach Lange APS, Brønshøj, Denmark) and a pH meter calibrated at pH 4.005 and 7.000 (PHM 240; Hach Lange APS). Immediately after pH measurement, 2 subsamples of ruminal fluid were stored at -20°C: a sample stabilized with 5% meta-phosphoric acid (MPA; for VFA and ammonia analysis) and an unstabilized sample. Spot samples of urine were collected every hour by hand-stimulation. Feces were collected as grab samples morning (0630 to 0800 h) and afternoon (1400 to 1500 h) on the day before sampling, and on the day of sampling. Milk yield and feed intake were recorded daily. Milk samples were collected morning and afternoon the day before sampling and on the day of sampling. All samples except milk samples were stored at -20°C until further analysis.

Analytical Procedures

The hematocrit was determined on all arterial samples by centrifugation of heparin-stabilized arterial plasma at $13,000 \times g$ for 6 min at ambient temperature. The concentration of inorganic phosphate was determined in heparinized plasma and ruminal fluid using an ammonium molybdate-based kit (ABX Pentra Phosphorus CP kit; Horiba ABX, Montpellier, France) adapted to run on a Cobas Mira Plus autoanalyzer (Triolab A/S, Brøndby, Denmark). The method was calibrated according to standards with the concentrations 0.25, 2.5, and 5 mM phosphate obtained by dissolving disodium hydrogen phosphate in deionized water. Randox Bovine Assayed Sera level 1, 2, and 3 were used as controls (Randox Laboratories Ltd., Crumlin, UK). Before analysis untreated ruminal fluid was centrifuged at $3,000 \times g$ for 15 min at 4°C followed by centrifugation at $20,000 \times g$ for 15 min at 4°C. The supernatant was diluted 1:2 with deionized water.

For tP determination, milk, feces, and feed samples were destroyed by a dry ash method (450°C for 3 h) and urine by a wet ash method (using perchloric and nitric acids). Phosphorus content was determined by the ammonium vanadate method as described by Stufins (1967).

Concentrations of pAH in plasma and urine were determined according to the method described by Harvey and Brothers (1962) using a continuous-flow analyzer (Autoanalyzer 3, method US-216-72 Rev.1; Seal Ana-

lytical Ltd, Burgess Hill, UK), following a deacetylation step (Kristensen et al., 2009).

Ruminal concentrations of VFA were determined by gas chromatography according to Kristensen et al. (1996). For ammonia analysis, MPA-stabilized rumen fluid samples were diluted 1:20 in phosphate buffer (pH 7.8). Ammonia was determined using a glutamate dehydrogenase based kit (AM 1015; Randox laboratories, Ltd.) and Randox Ammonia Ethanol level 1, 2, and 3 as controls.

Feed DM was determined by drying samples at 60°C for 48 h in a forced-air oven. Crude ash was determined after combustion at 525°C for 6 h. Organic matter was determined as the difference between DM and crude ash. Neutral detergent fiber was determined as amylase-treated NDF using the fibertec system as described by Mertens (2002). Nitrogen content in feces was determined according to the Dumas method described by Hansen (1989). Crude fat was determined as petroleum ether extract in an acid hydrolysate according to Stoldt (1952). Content of Cr_2O_3 in feed and feces was determined by spectrophotometric determination according to Schurch et al. (1950).

Calculations and Statistical Procedures

Production of ECM was calculated according to the formula described by Sjaunja et al. (1991). Fecal tP output was calculated as (dietary Cr_2O_3 concentration $\times$ DMI/fecal Cr_2O_3 concentration) $\times$ fecal tP concentration. Phosphorus excretion in urine was calculated by multiplying daily diuresis (infusion rate of pAH/urinary pAH concentration) by tP content in urine. Apparently digested tP was calculated as the difference between tP intake and tP excretion in feces. Balance of tP was calculated as the difference between tP input in feed and tP output in feces, urine, and milk. Calculations of portal and hepatic blood plasma flows, net portal fluxes, net hepatic fluxes, and net splanchnic fluxes of phosphate were done as described by Kristensen et al. (2007). The P_i absorbed to the portal blood in excess of apparently digested tP has been transferred to the gut from an endogenous source (i.e., recycled). Accordingly, P_i recycling was calculated as net portal plasma flux of P_i - apparently digested tP. Data on fluxes and balance are given in g/d using the atomic weight of P (30.97 g/mol).

Data were analyzed by ANOVA using the MIXED procedure in SAS (Statistical Analysis System version 9.2 SAS Institute Inc., Cary, NC). Data on DMI, milk production, tP intake, and tP excretion in feces, urine, and milk, as well as apparently digested tP, tP balance, and P_i recycling, were analyzed using a model including the fixed effects of treatment, sequence, and

Table 2. Dry matter intake, NDF digestibility, and milk production parameters

Item	Treatment ¹		SEM (n = 5)	P-value ²
	LP	HP		
DMI, kg/d	19.8	19.6	0.37	0.67
Digestibility of NDF, %	47.8	52.6	1.77	0.02
Yield, kg/d				
Milk	30.6	30.8	2.69	0.71
ECM ³	29.7	29.2	1.84	0.80
Fat	1.20	1.22	0.072	0.66
Protein	0.87	0.88	0.079	0.88

¹Experimental diets had the same composition of ingredients with the exception that LP (low phosphorus) contained 0.5% of DM calcium carbonate and HP (high phosphorus) contained 0.5% of DM monosodium phosphate.

²P-value for treatment effect.

³Calculated according to Sjaunja et al. (1991).

period, and the random effect of cow within sequence. Data on ruminal fluid, arterial variables, blood flow, and flux measurements were evaluated as repeated measurements using the auto regressive 1 covariance structure. The model included the fixed effects of treatment, sequence, period, sample, and the interaction treatment by sample. The effect of cow within sequence was included as a random effect. Data are presented as least squares means $\pm$ residual standard error of means. Significance was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

RESULTS

Dry Matter Intake, Digestibility of NDF, and Milk Production

The total amount of DM offered was limited to 20 kg/d for both treatments, and the actual DMI was unaffected by treatment ($P = 0.67$; Table 2). Digestibility of NDF was less in LP ($P = 0.02$; 47.8 ± 1.8) compared with HP (52.6 ± 1.8), whereas milk, fat, protein, and ECM yields were unaffected by treatment ($P = 0.66$ to $P = 0.88$).

Digestibility and tP Balance

As planned, tP intake was less in LP ($P = 0.001$) compared with HP, whereas Ca intake was similar among diets (LP, 142 ± 3 ; HP, 134 ± 3 g/d). The tP excretion in feces was decreased with LP ($P = 0.02$; Table 3), but digestibility of tP was not affected by treatment ($P = 0.82$). Nevertheless, apparently digested tP, calculated as difference between tP intake and tP in feces, was less in LP ($P = 0.01$; 26.3 ± 2.1 g/d) compared with HP (36.4 ± 2.1 g/d). The urinary tP excretion was lower in cows fed LP ($P = 0.05$) but the magnitude of the

change was small. The tP excretion in milk tended to decrease ($P = 0.10$) in LP compared with HP. The tP concentration in milk was 0.82 ± 0.02 g/kg and 0.84 ± 0.02 g/kg with LP and HP, respectively (data not shown). The tP balance, calculated as the difference between tP intake and tP excreted in feces, urine, and milk was less in LP (1.0 ± 2.1 g/d) compared with that in HP ($P = 0.02$; 10.4 ± 2.1 g/d). Apparently digested Ca was not affected ($P = 0.13$) by treatment (LP, 36 ± 5 ; HP, 48 ± 5 g/d).

Urinary, Ruminal, and Arterial Variables

No effects of treatment or time on urine pH ($P = 0.24$ to $P = 0.57$; Table 4) were observed. Ruminal pH was unaffected by treatment ($P = 0.20$) and no treatment by time interaction was detected ($P = 0.65$). The P_i concentration in ruminal fluid was decreased with LP ($P = 0.0001$), and no interaction between treatment and time was detected ($P = 0.34$; Figure 1). The ruminal ammonia concentration, total ruminal VFA concentration, and molar percentage of VFA were not affected by treatment ($P = 0.12$ to $P = 0.98$). The molar proportions of propionate, isovalerate, valerate, and caproate increased after feeding ($P < 0.01$), the molar proportion of acetate decreased after feeding ($P = 0.02$), and the molar proportion of isobutyrate was decreased only at the sampling 30 min after feeding ($P < 0.01$).

Arterial whole blood pH was greater in LP compared with that in HP ($P = 0.04$; Table 5), but was not affected by sampling time ($P = 0.16$).

No overall major differences in patterns for arterial hematocrit and oxygen concentration were noted. A

Table 3. Digestibility of total phosphorus (tP), excretion of tP, and tP balances

Item	Treatment ¹		SEM (n = 5)	P-value ²
	LP	HP		
Digestibility of tP, %	54.9	54.0	2.64	0.82
P, g of P/d				
P intake	47.9	67.2	1.18	0.001
tP in feces	21.6	30.8	1.73	0.02
tP in urine	0.22	0.26	0.015	0.05
tP in milk	25.1	25.8	2.10	0.10
Apparently digested tP ³	26.3	36.4	2.08	0.01
tP balance ⁴	0.95	10.4	2.06	0.02

¹Experimental diets had the same composition of ingredients with the exception that LP (low phosphorus) contained 0.5% of DM calcium carbonate and HP (high phosphorus) contained 0.5% of DM monosodium phosphate.

²P-value of treatment effect.

³Apparently digested P denotes the feed – fecal difference.

⁴Balance was calculated as the difference between P input via feed and P output via feces, urine, and milk.

Figure 1. Concentration of inorganic phosphate (P_i) in ventral ruminal fluid in cows fed either a low-phosphorus (P) ration (2.4 g of P/kg of DM; ○) or a high-P ration (3.4 g of P/kg of DM; ■). Samples were collected 0.5 h before morning feeding as well as 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 h after morning feeding. Each data point is the mean of 5 observations $\pm$ standard error. The P_i concentration in ruminal fluid was affected by treatment ($P = 0.0001$) as well as sampling time ($P = 0.006$), but no interaction was detected ($P = 0.34$).

transient increase in hematocrit and oxygen with LP 30 min after feeding caused an interaction between sampling time and treatment ($P = 0.07$ and $P = 0.02$, respectively). In line with the greater blood pH in LP, the arterial carbon dioxide concentration tended to increase in LP ($P = 0.07$). Arterial plasma P_i was less with LP ($P = 0.001$; 1.15 ± 0.10 mmol/L) compared with HP (1.85 ± 0.10 mmol/L) and with both treatments, plasma P_i was observed to increase in samples obtained 30 min after feeding ($P = 0.01$; Figure 2).

Blood Flows, Net Fluxes, and Recycling of P_i

Portal, hepatic, and hepatic arterial blood plasma flows were not affected by treatment ($P = 0.46$ to $P = 0.70$; Table 6). Portal blood plasma flow tended to be affected by sampling time ($P = 0.10$) without showing any meaningful pattern. Hepatic vein and hepatic arterial blood plasma flows were unaffected by sampling time ($P = 0.39$ to $P = 0.78$). Treatment and sampling time was without effect for net portal plasma flux of P_i (net absorption of P_i ; $P = 0.20$ to $P = 0.33$). The net hepatic plasma flux of P_i was not affected by treatment or sampling time ($P = 0.13$ to $P = 0.74$). However, the net splanchnic plasma flux of P_i increased ($P = 0.05$) in HP (68.6 ± 5.4 g/d) compared with that in LP (56.4 ± 5.4 g/d), whereas sampling time was without effect on net splanchnic plasma flux of P_i ($P = 0.37$). Net recycling of P_i was not affected by treatment ($P = 0.86$) and averaged 29.7 ± 4.2 and 28.6 ± 4.2 with LP and HP, respectively.

DISCUSSION

DMI and Milk Production

The DMI was maintained equal among treatments in the present study by restricting feed allowance to 20 kg of DMI/d and with equal intakes, milk, fat, and protein production were unaffected by decreased tP intake. A decrease in DMI, milk yield, and loss of BW are among the initial signs of P deficiency (Call et al., 1987). The present study was not designed to show effects of decreased P intake on production variables. In a study on lactating goats, Müschen et al. (1988) observed a

Table 4. Urinary and ruminal variables

Item	Treatment ¹		SEM (n = 5)	P-value ²		
	LP	HP		Trt	Time	Trt $\times$ Time
Urinary pH	8.09	8.07	0.021	0.41	0.24	0.57
Ruminal pH	6.43	6.35	0.10	0.20	0.001	0.65
Ruminal fluid P_i , ³ mmol/L	3.26	5.33	0.68	0.0001	0.006	0.34
Ammonia, mmol/L	7.04	7.35	0.68	0.73	<0.0001	0.59
Total VFA, mmol/L	105	105	6.21	0.96	0.05	0.72
Acetate, mol/100 mol	61.3	59.9	1.24	0.45	0.02	0.17
Propionate, mol/100 mol	20.3	20.8	1.19	0.61	0.001	0.20
Isobutyrate, mol/100 mol	0.55	0.55	0.045	0.98	0.007	0.65
Butyrate, mol/100 mol	14.2	14.6	0.91	0.61	0.12	0.46
Isovalerate, mol/100 mol	1.35	1.59	0.28	0.12	0.006	0.11
Valerate, mol/100 mol	1.45	1.57	0.065	0.23	0.0003	0.57
Caproate, mol/100 mol	0.87	0.99	0.082	0.30	0.002	0.69

¹Experimental diets had the same composition of ingredients with the exception that LP (low phosphorus) contained 0.5% of DM calcium carbonate and HP (high phosphorus) contained 0.5% of DM monosodium phosphate.

²P-values for effects of treatment (Trt), sampling time (Time), and the interaction (Trt $\times$ Time).

³ P_i = inorganic phosphate.

Figure 2. Arterial plasma inorganic phosphate (P_i) concentration in cows fed either a low-phosphorus (P) ration (2.42 g of P/kg of DM; ○) or a high-P ration (3.43 g of P/kg of DM; ■). Samples were collected 0.5 h before morning feeding as well as 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 h after morning feeding. Each data point is the mean of 5 observations $\pm$ standard error. The arterial plasma P_i concentration was affected by treatment ($P = 0.001$) and sampling time ($P = 0.01$), but no interaction was detected ($P = 0.78$).

decreased milk yield in a single lactation period when tP intake was decreased. Other studies with dairy cows have shown that prolonged feeding of P-depleted diets resulted in a decrease in milk production (Wu et al. 2000) along with decreases in DMI and BW (Valk and Sebek, 1999).

Ruminal Environment

Ruminal fermentation rate and protein synthesis might be decreased if the P_i supply for microbial growth is not sufficient (Komisarczuk et al., 1987; Petri et al., 1988), and this may decrease digestibility of OM

(Kincaid and Rodehutsord, 2005) as well as DMI (Call et al., 1987; Valk and Sebek, 1999). Ruminal fluid and salivary P_i concentrations are highly correlated and a decrease in tP intake has been shown to decrease plasma and ruminal P_i concentration (Tomas et al. 1967). With adequate P supply, plasma concentrations of P_i in ruminants are in the range of 1.5 and 2.5 mmol/L, and concentrations below 1.2 mmol/L may indicate P depletion (NRC, 2001). However, Wu et al. (2000) observed that plasma P_i concentrations were not always good indicators of P status. In the present study, the mean plasma concentration of P_i was 1.15 and 1.85 mmol/L with LP and HP, respectively. With LP, cows were not depositing P in bone tissues and the present study, therefore, adds to the pool of evidence that a plasma P_i concentration below 1.2 mmol/L is an indication of insufficient P supply. The ruminal P_i concentration decreased from 5.33 to 3.26 mmol/L in LP. The required P_i concentration for microbes was evaluated in vitro and reported to be between 0.7 and 2.6 mmol/L (Breves and Schröder, 1991). However, the requirements for ruminal P_i concentrations in vivo have not been confirmed in lactating cows.

Digestibility of NDF decreased in response to decreased tP intake, whereas digestibility of tP was similar among treatments. Thus, a decreased NDF digestibility might indicate an alteration in microbial degradation of feedstuffs with decreased dietary tP supply. Petri et al. (1988) detected an increase in ruminal pH (6.86 vs. 6.11) and decreased ammonia concentrations; however, these effects accompanied decreases in DMI and might, therefore, be indirect effects of decreased tP intake. Cows in the present study were kept in tie stalls with wood shavings as bedding and although we did not observe any excessive intake of bedding with LP, we cannot rule out the possibility that the observed effect of treatment on NDF digestibility was affected by intake

Table 5. Arterial variables

Item	Treatment ¹		SEM (n = 5)	P-value ²		
	LP	HP		Trt	Time	Trt $\times$ Time
Whole blood						
pH	7.45	7.43	0.0064	0.04	0.16	0.19
Hematocrit, %	25.7	26.0	0.014	0.07	0.44	0.07
Oxygen, mmol/L	5.03	5.09	0.35	0.18	0.73	0.02
Carbon dioxide, mmol/L	24.9	24.1	0.66	0.18	0.07	0.87
Blood plasma, mmol/L						
p-Aminohippuric acid	0.072	0.086	0.007	0.15	0.31	0.91
Arterial plasma P_i ³	1.15	1.85	0.099	0.001	0.01	0.78

¹Experimental diets had the same composition of ingredients with the exception that LP (low phosphorus) contained 0.5% of DM calcium carbonate and HP (high phosphorus) contained 0.5% of DM monosodium phosphate.

²P-values for the effects of treatment (Trt), sampling time (Time), and the interaction (Trt $\times$ Time).

³ P_i = inorganic phosphate.

Table 6. Blood plasma flows, net plasma fluxes, and recycling of inorganic phosphate (P_i)

Item	Treatment ¹		SEM (n = 5)	P-value ²		
	LP	HP		Trt	Time	Trt × Time
Blood plasma flows, L/h						
Portal vein	1,149	1,172	104	0.60	0.10	0.28
Hepatic vein	1,314	1,297	97.9	0.70	0.39	0.22
Hepatic artery	167	126	72.0	0.46	0.78	0.38
Net plasma fluxes of P_i , ³ g of P/d						
Portal	57.1	65.5	4.75	0.20	0.21	0.33
Hepatic	0.39	3.03	3.65	0.43	0.74	0.13
Splanchnic	56.4	68.6	5.36	0.05	0.37	0.10
Net recycling of P_i , ⁴ g of P/d	29.7	28.6	4.21	0.86	—	—

¹Experimental diets had the same composition of ingredients with the exception that LP (low phosphorus) contained 0.5% of DM calcium carbonate and HP (high phosphorus) contained 0.5% of DM monosodium phosphate.

²P-value for effects of treatment (Trt), sampling time (Time), and the interaction (Trt × Time).

³ P_i = inorganic phosphate.

⁴Net recycling of P_i was calculated as the difference between net portal flux of P and apparently digested P.

of bedding. The observed effect on NDF digestibility might have been related to intake of bedding, and the lack of change in milk yield, ruminal pH, VFA concentrations, and ammonia concentrations suggests that the decrease in NDF digestibility had little meaningful effect. If LP had been associated with decreased carbohydrate fermentation, we would have expected to find increased ammonia and decreased VFA concentrations with LP. The present study did not provide evidence for any changes in rumen function when ruminal P_i concentrations were decreased to 3.3 mmol/L by decreasing tP intake.

Salivary Secretion and Recycling of P_i

Collection of saliva samples and especially quantification of mixed saliva secretion in conscious ruminants is difficult and subject to considerable errors, whether based on sponge technique (Valk et al., 2002), cardial collection (Cassida and Stokes, 1986), or permanent parotid duct fistulas (Tomas, 1973). It has been established that the salivary P_i concentration is positively correlated to plasma P_i concentrations (Challa and Braithwaite, 1988; Valk et al., 2002) and increasing dietary tP intake leads to increased plasma P_i concentrations due to an increased absorption (Lueker and Lofgreen, 1961; Scott et al., 1985). However, the accumulation of P_i in different salivary glands differs substantially (Breves and Schröder, 1991), which limits what can be inferred from measurements on saliva from single glands over short periods of time.

Contrasting theories on regulation of salivary P_i secretion exist. The “rumen support theory” suggests that salivary P_i secretion is upregulated with low tP intakes to maintain high ruminal P_i concentrations necessary

for optimal microbial growth (Clark, 1953; Dias et al., 2009). The “homeostatic theory” suggests that the primary function of regulating salivary P_i secretion is to maintain plasma P_i homeostasis (Horst, 1986; Kebreab et al., 2005). Data from the present study support the view that salivary P_i secretion is maintained at a disproportionately high concentration with low tP intakes in cattle in the short-term and, therefore, that salivary P_i has a limited role in maintaining P_i homeostasis at low intakes. However, this does not preclude that salivary P_i secretion is crucial in maintaining plasma P_i homeostasis at high tP intakes.

Quantitative data on P_i recycling in dairy cattle are sparse. Breves and Schröder (1991) estimated total P_i recycling to be in the range of 30 to 60 g of P/d and Kebreab et al. (2005) observed that duodenal P fluxes exceeded tP intake by 48 to 64 g of P/d in cows fed at relatively high tP intakes compared with diets used in the present study. It has previously been observed that net portal P_i fluxes exceeded tP intake (Reynolds et al., 1991). Recycling, estimated from net portal absorption of P_i , was 10 to 75 g of P/d in cows at variable planes of nutrition (Kristensen et al., 2005). Available data point toward a total P_i recycling in the same range as we have observed in the present study. However, available data from dairy cattle do not allow for evaluation of the recycling response to marginal undersupply of tP. In sheep, recycling of P_i exceeded tP intake by a factor of 1.6 at high tP intake, whereas recycling exceeded intake by a factor of 3.9 at low intake (Scott et al., 1985). These data on sheep agree with the observation of the present study that P_i recycling is not downregulated at low tP intakes. Thus, P_i recycling cannot be the sole homeostatic mechanism in maintaining plasma P_i concentrations. To the contrary, data in both sheep and dairy

cattle indicate that salivary P_i uptake is maintained at the expense of the plasma P_i concentration. This might serve as an important adaptation to maintain ruminal P_i supply during periods of dietary tP shortage.

Phosphorus Homeostasis

We suggest that the role of salivary P_i secretion in P_i homeostasis differs in situations with under- and oversupply of P in dairy cattle. In a situation with oversupply of P_i , upregulation of salivary secretion might be an important homeostatic response, ensuring a limited increase in plasma P_i . On the other hand, in situations with undersupply of P_i , the salivary secretion is maintained at a relatively high level, which forces other organs such as bones to increase P_i supply. The constancy of milk tP secretion (Valk et al. 2002) suggests that the homeostatic adaptations to low tP intake must involve absorption or bone tissue balance of P_i .

Absorption. It is generally accepted that the main site of P_i absorption is in the small intestine (Horst, 1986; Breves and Schröder, 1991). However, questions remain about the importance of endocrine control of absorption (Scott et al., 1985) and whether the intestinal absorption can respond to the increased demand for P_i caused by relatively high salivary secretion at low tP intakes. Horst (1986) as well as Breves and Schröder (1991) suggested that, at least in monogastrics, the transport of P_i by the small intestine involves an active Na^+ -coupled mechanism as well as a passive, non-saturable H^+ -coupled transport. The active transport system appears to be readily saturable, which signifies that the passive transport may predominate in times of higher luminal P_i concentrations (Breves and Schröder, 1991). In situations with low dietary tP intake, the active transport is upregulated by the vitamin D pathway, which results in an increase of $1,25-(OH)_2D_3$ synthesis via kidneys stimulating intestinal P_i absorption (Horst, 1986). However, the role of vitamin D in ruminant P_i absorption has been questioned by Field et al. (1983), who claimed that the absorptive mechanism is not subject to any regulation, and considered absorptive efficiency to be largely independent of dietary tP intake and that variation in fecal tP excretion is achieved through changes in salivary P_i secretion alone.

Existing models on P_i metabolism in dairy cows predict true P_i absorption to be a hyperbolic function of intestinal P_i concentration (Kebreab et al. 2004; Hill et al. 2008) reflecting that the dairy cow maintains a relatively constant apparent P_i digestibility across a wide range of intestinal P_i supplies. These previous observations, as well as the minute effects of treatment on apparent P_i digestibility, as well as net portal P_i flux relative to tP intake + recycled P_i in the present study,

indicate that regulation of intestinal P_i absorption is not sufficient to maintain P_i homeostasis in dairy cows challenged with decreased dietary tP intake.

Bone Metabolism and Phosphorus Balance. Phosphorus in the skeleton is mainly found in hydroxyapatite (Ternouth, 1990), and net bone resorption usually occurs in early lactation due to the rapid increase in milk production exceeding the rate of increase in DMI and the concomitant need for mobilization of Ca (Ramberg et al. 1970; Wu et al., 2000). Thus, skeletal reserves of Ca and P are normally decreased during early lactation and restored in late lactation and the dry period (Horst, 1986). Short-term negative tP balances cannot be regarded as detrimental if corrected later in lactation. In moderate P deficiency, such as occurred in the present study and others (Valk and Sebek, 1999; Wu et al. 2000), milk production is maintained due to the animal's ability to draw on its skeletal reserves (Karn, 2001).

Phosphorus balance, estimated by the use of total collection, does not allow for differentiation between P_i resorption from bone and from soft tissue (Knowlton and Herbein, 2002). By using ^{32}P as a tracer, Braithwaite (1980) showed that P_i mobilization primarily occurred from bone. It has been suggested by Blair-West et al. (1992) that not only rate of bone P_i resorption, but also rate of bone P_i accretion, is affected during dietary tP deficiency, as plasma concentration of osteocalcin, a marker of bone formation, was lowered in P-depleted beef cows compared with that in control animals. Together, data indicate that bone balance of tP plays a major role in P_i homeostasis in dairy cows both in the postpartum transition period and during feeding of a P-deficient diet.

Urinary P Excretion. Urinary excretion of P_i or tP in ruminants is usually insignificant due to efficient renal reabsorption of filtered P_i , but may be excessive in case of high dietary tP concentrations (Scott et al., 1985) or if saliva secretion is inhibited by feeding diets low in physical fiber (Scott and Buchan, 1988). Single animals have been reported to excrete significant amounts of P_i via urine (Breves and Schröder, 1991). In the present experiment, the urinary tP excretion was negligible in both treatment groups (0.22 vs. 0.26 g/d), though slightly lowered with LP ($P = 0.05$). The small magnitude of the change supports the view that regulation of urinary P_i excretion is without significance for the homeostatic adaptation to low tP intake in dairy cows.

Meeting the Low P Intake Challenge

Data from the present experiment point toward the need for considering situations with tP intake below

and above recommendations separately, when reflecting on the most important regulatory mechanisms in maintaining P_i homeostasis. In situations with high tP intake, the intestinal absorption can most probably not be decreased enough to limit P_i absorption to the requirement. Bone accretion will account for a small fraction of the absorbed P_i and salivary secretion will be the quantitatively most important excretion route. The exception would be situations with impaired salivation, where kidney reabsorption of P_i decreases and urinary excretion functions as a second-level control. This is in contrast to monogastric animals where urinary excretion will dominate.

Because P_i recycling did not decrease with decreased tP intake in the present study, despite a decreased plasma P_i concentration, data are not in agreement with salivary P_i secretion being of primary importance to P_i homeostasis in the situation with low tP intake. Because intestinal absorption cannot be upregulated sufficiently to supply P_i for recycling, bone resorption must balance the salivary P_i flux maintained at a disproportionately high concentration. It is obvious that sufficient P_i supply of rumen microbes is critical in ruminants (Muschen et al., 1988; Kincaid and Rodehutschord, 2005) and data indicate that P_i supply to the rumen is highly prioritized in lactating dairy cows. Previous studies indicate that the cow will continue the investment in rumen function to the extent where it endangers chances to successfully initiate a new lactation (Valk and Sebek, 1999). It seems as if the cow invests in maximizing rumen fermentation in the short-term at the expense of the long-term consequences of depleting the bone pool.

The implications of the fact that dairy cows will prioritize ruminal P_i supply over bone balance are that rumen function is partly protected from negative effects of low tP intakes, but also that this mechanism could be an important component of the inevitable endogenous loss of P in feces (Spiekers et al., 1993; Meschy, 2003). Therefore, it is difficult to fine tune P utilization of dairy cattle by manipulating dietary tP supply without a simultaneous attempt to manipulate salivary P_i fluxes and more precisely define the ruminal need for P_i .

CONCLUSIONS

Net recycling of P_i was not decreased in lactating dairy cows when tP intake was decreased despite a decrease in arterial plasma P_i concentration. Recycling of P_i was maintained at the expense of deposition of P_i in bones and the tP balance decreased. Data are not consistent with salivary P_i secretion being the primary regulator of P_i homeostasis at low tP intakes. On the

contrary maintaining salivary P_i recycling at low tP intakes indicate that the cow prioritizes to maximize rumen function at the expense of long-term consequences of depletion of bone P. This might contribute to the relatively high inevitable P_i loss in dairy cattle.

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