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Effects of dietary phosphorus concentration during the transition period on plasma calcium concentrations, feed intake, and milk production in dairy cows

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ABSTRACT

Our aim was to evaluate the effects of a low or high dietary phosphorus (P) concentration during the dry period, followed by either a high or low dietary P concentration during the first 8 wk of lactation, on plasma Ca concentrations, feed intake, and lactational performance of dairy cattle. Sixty pregnant multiparous Holstein Friesian dairy cows were assigned to a randomized block design with repeated measurements and dietary treatments arranged in a 2 × 2 factorial fashion. The experimental diets contained 3.6 (Dry-HP) or 2.2 (Dry-LP) g of P/kg of dry matter (DM) during the dry period, and 3.8 (Lac-HP) or 2.9 (Lac-LP) g of P/kg of DM during 56 d after calving period. In dry cows, plasma Ca concentrations were 3.3% greater when cows were fed 2.2 instead of 3.6 g of P/kg of DM. The proportion of cows being hypocalcemic (plasma Ca concentrations <2 m*M*) in the first week after calving was lowest with the low-P diets both during the dry period and lactation. Plasma Ca concentrations in wk 1 to 8 after calving were affected by dietary P level in the dry period and in the lactation period, but no interaction between both was present. Feeding Dry-LP instead of Dry-HP diets resulted in 4.1% greater plasma Ca values, and feeding Lac-LP instead of Lac-HP diets resulted in 4.0% greater plasma Ca values. After calving, plasma inorganic phosphate (Pi) concentrations were affected by a 3-way interaction between sampling day after calving, and dietary P levels during the dry period and lactation. From d 1 to d 7 postpartum, cows fed Lac-HP had increased plasma Pi concentrations, and the rate appeared to be greater in cows fed Dry-LP versus Dry-HP. In contrast, plasma Pi concentrations decreased from d 1 to d 7 postpartum in cows fed Lac-LP, and this decrease was at a higher rate for cows fed Dry-HP versus Dry-LP. After d 7, plasma Pi concentrations remained rather constant at 1.5 to 1.6 m*M* when cows received Lac-HP, whereas with Lac-LP plasma Pi concentrations reached stable levels (i.e., 1.3–1.4 m*M*) at d 28 after calving. Milk production, DM intake, and milk concentrations of P, Ca, fat, protein, and lactose were not affected by any interaction nor the levels of dietary P. It is concluded that the feeding of diets containing 2.2 g of P/kg of DM during the last 6 wk of the dry period and 2.9 g of P/kg of DM during early lactation increased plasma Ca levels when compared with greater dietary P levels. These low-P diets may be instrumental in preventing hypocalcemia in periparturient cows and do not compromise DM intake and milk production. Current results suggest that P requirements in dairy cows during dry period and early lactation can be fine-tuned toward lower values than recommended by both the National Research Council and the Dutch Central Bureau for Livestock Feeding. Caution however is warranted to extrapolate current findings to entire lactations because long-term effects of feeding low-P diets containing 2.9 of g/kg of DM on production and health needs further investigation.
Key words: dairy cow, phosphorus, hypocalcemia, transition period

INTRODUCTION

Low efficiency of phosphorus use by dairy cattle is associated with excess P excretion into the environment (Klop et al., 2013) and detrimental effects of excess P on water quality, which is of major environmental concern (Kebreab et al., 2008). In addition, phosphate rock reserves are expected to run out in 50 to 100 years (Cordell et al., 2009). Therefore, dietary supply of P should not exceed the minimum amount of P needed to ensure both the production and health of dairy cattle. It is well known that under practical feeding conditions on farms, cows generally ingest an amount of P in excess to their requirement (Puggaard et al., 2014). The

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recommended level of dietary P in dairy cow nutrition, however, is still a matter of debate.

In contrast to diets for lactating cows, research-based recommendations on the minimum dietary P concentration of dry-cow diets are scarce owing to a dearth of studies addressing the issue of dietary P concentration during the periparturient period. Lean et al. (2006) reported that an increase in dietary P concentration from 3 to 4 g/kg of DM during the dry period increases the risk of milk fever by 18%. This result is in line with Peterson et al. (2005), who reported lower serum Ca concentrations at parturition when cows were fed a prepartum diet containing 4.4 versus 2.1 or 3.1 g of P/kg of DM. Moreover, Cohrs et al. (2018) found that the feeding of a prepartum diet containing 2.8 g of P/kg of DM (i.e., adequate dietary P) caused lower plasma Ca concentrations in periparturient cows compared with cows fed for 4 wk with a diet containing 1.5 g of P/kg of DM (i.e., a P-deficient diet). It can thus be suggested that a reduction in dietary P concentration during the dry period prevents hypocalcemia after calving. This suggestion implies that P and Ca metabolism are, at least to a certain extent, linked in dairy cows. The latter notion however, seems not in line with the idea that P and Ca homeostatic regulations are independent of each other (Cohrs et al., 2018). Furthermore, no studies have investigated the possible consequences of interaction between high or low dietary P levels in the dry period and high or low P levels postpartum on plasma P and Ca levels. Further studies are thus warranted to substantiate the aforementioned observations on hypocalcemia and milk fever, as well as effects on feed intake and milk production. Currently, the Dutch (CVB, 2012) recommendations on the P content of dairy diets are about 9% lower compared with the US recommendations (NRC, 2001). However, the Dutch values still contain a safety margin on the recommended dietary P levels thereby implying that, at least potentially, dietary P levels can be further fine-tuned toward lower values. Thus, we formulated low-P diets by moderately lowering (i.e., 30%) the dietary P concentration below the current values recommended by the Dutch Centraal Veevoederbureau (CVB) in early lactation. Low-P diets fed after calving can cause a depression in DMI (Puggaard et al., 2014). It was therefore considered opportune to measure, next to DMI and milk production, selected indices on energy metabolism as well.

The objective of the current experiment was to evaluate the effects of a low or high dietary P concentration during the dry period, followed by either a high or low dietary P concentration during the first 8 wk of lactation, on plasma Ca concentrations, feed intake, and lactational performance of dairy cattle. We hypothesized that the feeding of low- versus high-P diets during both the dry period and thereafter, cause greater plasma Ca concentrations. Furthermore, we hypothesized that feed intake and milk production would not be reduced by moderately lowering the dietary P concentration below requirements from CVB (2012) in early lactation.

MATERIALS AND METHODS

All experimental protocols and interventions were conducted under the Dutch Law on Animal Experimentation and approved (approval no.: AVD401002016653) by the Central Authority for Scientific Procedures on Animals (CCD, The Hague, the Netherlands).

Animals, Experimental Design, and Management

Sixty multiparous (parity ≥3), pregnant Holstein Friesian dairy cows were used in the current study. The animals entered the experiment approximately 6 wk before the expected calving date. The experiment had a randomized block design with repeated measurements and a 2 × 2 factorial arrangement of dietary treatments. Before the start of the experiment, cows were grouped in 15 blocks of 4 animals with comparable expected calving dates, parity, and milk yields during the previous lactation. Within each block, cows were randomly assigned to dry-cow diets containing either 3.6 (**Dry-HP**) or 2.2 (**Dry-LP**) g of P/kg of DM and, within dry-cow treatments, to postcalving diets containing either 3.8 (**Lac-HP**) or 2.9 (**Lac-LP**) g of P/kg of DM. Cows were housed in 2 groups in a freestall barn (i.e., a dry-cow group and a postpartum lactation group). The cubicles were covered with rubber cattle mats with wood shavings as bedding and were cleaned on a daily basis. Throughout the experiment, fresh drinking water was provided ad libitum. Lactating cows were milked twice daily with a milking interval of 11 to 13 h. Animal health was monitored by trained animal caretakers according to standard farm protocols. Symptoms of clinical hypocalcemia around calving (i.e., recumbency or inability to stand, cold ears, and low rumen fill with no contractions) were noted by animal caretakers, and when cows showed at least 2 of these symptoms they were treated intravenously with a CaMg solution (450 mL with 1.65% Ca and 0.95% Mg; Dechra). The cows left the trial after 56 d of lactation.

Feeding and Experimental Diets

Throughout the experiment, the cows received their allocated, freshly mixed diets at regular intervals (3–5 times/d) with the use of an automatic feeding system (Triomatic HP 2 300, Trioliet). This basal diet was composed of corn silage, grass silage, wheat straw (dry-

cow diet only), soybean meal (lactating cows only), and a premix; the premix had either a low or a high P concentration. The basal diet ingredients were stored in bunkers (used for roughages) and silos (used for soybean meal and premix). On a weekly basis and based on the DM concentration of each individual basal diet component, the required amount of diet ingredients was calculated to prepare the experimental diets. The dietary components were taken automatically from their bunker or silo, weighted, and transported into the Trioliet feed mixing robot. Within the Trioliet, the amount of each dietary component was weighted again so the weight actually realized with mixing were recorded. Diets were mixed in the mixing robot for on average 6 min. The mixed basal diets were supplied in weighing troughs with individual transponder-controlled access gates [Roughage Intake Control system (**RIC**), Hokofarm Group]. For every visit of a cow to a RIC bin, the start and end time of the visit as well as the start and end weight of the RIC bin were recorded. Weekly, the RIC bins were calibrated with a standard weight. A total of 32 RIC bins were available, and cows had access to all RIC bins that offered their designated diet. Every morning around 0600 h, feed refusals were removed and individual feed intake was recorded daily. Next to the mixed basal diet, pelleted concentrates were fed individually with the use of transponder-controlled concentrate feeders (Hokofarm Group). Dry cows received 1 kg/d (as fed) of compound concentrate. One day after calving, cows received 3.0 kg/d of compound concentrate gradually increasing up to 9.3 kg/d at d 21 postpartum. The P concentrations of the premix and concentrates were manipulated with the use of disodium phosphate (Na_2HPO_4) and sodium bicarbonate ($NaHCO_3$), thereby aiming to prevent potential confounding effects due to differences in Na intake between the low- and high-P diets. The ingredient and analyzed composition of the experimental diets is shown in Table 1.

Data Collection and Sampling

Body weights were monitored weekly before calving and daily after calving using an appropriate weight scale with automatic registration. For the determination of DM concentration of the basal mixed diet, corn silage and grass silage were sampled daily, whereas wheat straw, soybean meal, and the premixes were sampled weekly. Dry matter concentration was determined by calculating the weight difference before and after oven drying at 104°C during 36 h. All basal diet feed ingredients and concentrates were sampled weekly and stored at −20°C. For grass and corn silage, samples of 5 to 6 consecutive weeks were pooled into 1 composite sample; for premixes, concentrates and wheat straw, all samples were pooled per 16 consecutive weeks (resulting in 2 samples per feedstuff). Once weekly, during 4 consecutive milking events, milk was sampled and pooled in 2 tubes (one tube for morning milkings, the other tube for afternoon milkings) containing sodium azide for preservation and stored no longer than 1 d at 4°C, and analyzed for fat, protein, and lactose. Two separate milkings (one morning and one afternoon) were obtained weekly and stored frozen (−20°C) and (after pooling per cow and week) analyzed for P and Ca. In addition, before calving blood was sampled on a fixed day of the week (Thursday) in wk −4, −2, and −1 relative to calving. During lactation, blood samples were obtained immediately after calving (d 0), and on d 1, 2, 3, 7, 14, 21, 28, and 56 after calving. Blood was collected in serum separator tubes and tubes were centrifuged for 15 min at 3,000 × *g* and 4°C within 1 h after collection. Blood plasma was transferred to 3 plasma tubes and stored at −20°C until analysis of Ca, inorganic phosphate (**Pi**), nonesterified fatty acids (**NEFA**) and BHB.

Chemical Analysis

Feed samples were analyzed by wet chemistry at the MasterLab Laboratory (Boxmeer, the Netherlands). The DM concentration was determined using forced-air oven drying (105°C; ISO, 1999b), crude fat concentration was determined gravimetrically as the ether extract (ISO, 1999a) and crude ash concentration after incineration at 550°C (ISO, 2002). Concentrations of NDF and ADF in samples were determined according to Van Soest et al. (1991) after pretreatment with α-amylase and expressed without residual ash. Acid detergent lignin was analyzed in feed samples using the method of Robertson and Van Soest (1981) using sulfuric acid and expressed without residual ash. Crude protein was calculated as 6.25 × N, where N was determined using the Kjeldahl method with $CuSO_4$ as a catalyst (ISO, 2005). The sugar concentrations were determined as described by van Vuuren et al. (1993). Starch was released by heating in a boiling water bath in the presence of 2 *M* HCl concentration and subsequently starch concentration was determined using the amyloglucosidase method (ISO, 2004). Calcium, sodium, magnesium, potassium, sulfur, chlorine, and P concentrations in feed were determined using inductively coupled plasma atomic emission spectroscopy (Eurofins Agro). The NE_L, intestinal digestible protein (**IDP**) and rumen-degradable protein balance (**RDPB**) were obtained by near-infrared spectroscopy for corn and grass silage (Eurofins Agro). For wheat straw, soybean meal, and concentrates, NE_L, IDP, and

Table 1. Ingredient and chemical composition of the experimental diets[1]; analyzed and calculated composition values of diets are based on the mean DMI during the 6-wk dry period and the subsequent 8-wk lactation period

	Dry period		Lactation period[2]	
Item	Dry-HP	Dry-LP	Lac-HP	Lac-LP
Ingredient composition (% of DM)				
Corn silage	36.8	36.9	44.6	44.9
Wilted grass silage	22.5	22.4	13.5	13.9
Wheat straw	29.5	29.7	—	—
Soybean meal	—	—	3.4	4.2
Concentrates (incl. premixes)				
Low P concentration[3,5]	—	11.0	—	37.0
High P concentration[4,6]	11.2	—	38.5	—
Analyzed composition (g/kg of DM)				
OM	924	926	931	930
CP	106	105	149	150
EE	27	27	33	32
NDF	521	525	366	374
ADF	298	301	214	214
ADL	35	35	23	25
Starch	136	136	206	206
Sugar	21	21	39	41
Ca	3.6	3.6	6.1	6.2
P	3.6	2.2	3.8	2.9
Mg	2.4	2.2	3.6	3.6
Na	1.9	1.6	1.7	1.6
K	17.4	17.5	16.2	16.3
S	1.9	1.9	2.2	2.2
Cl	3.6	3.5	3.3	3.7
DCAD (mEq/kg of DM)[7]	307	299	263	248
Calculated values (/kg of DM)[8]				
NE_L (MJ)	5.6	5.6	6.8	6.8
IDP (g)	57	57	92	93
RDPB (g)	−6	−7	7	7

[1]Dry-HP, Dry-LP, Lac-HP, and Lac-LP = experimental diets fed during the dry period (Dry) or the lactation period (Lac) high in phosphorus (HP) or low in phosphorus (LP) containing either 3.6, 2.2, 3.8, or 2.9 g of P/kg of DM, respectively.

[2]Lactation concentrate was gradually increased from 1.0 kg/d at d 1 postcalving to 9.3 kg/d at d 21 postcalving.

[3]The low-P concentrate in the dry period consisted of (% DM): sugar beet pulp = 28.1, potato protein = 25.8, soybean meal = 21.1, molasses = 10.0, urea = 5.4, vitamins and minerals = 3.0, sodium bicarbonate = 2.5, sugar = 1.9, magnesium oxide = 1.1, palm oil = 1.1.

[4]The high-P concentrate in the dry period consisted of (% DM): sugar beet pulp = 26.5, potato protein = 26.3, soybean meal = 19.9, molasses = 9.6, urea = 5.5, disodium phosphate = 5.3, vitamins and minerals = 3.0, sugar = 1.8, magnesium oxide = 1.1, palm oil = 1.0.

[5]The low-P concentrate in the lactation period consisted of (% DM): sugar beet pulp = 24.8, soyhulls = 23.1, corn = 14.3, soybean meal = 11.7, wheat = 6.6, potato protein = 5.8, molasses = 5.3, palm kernel meal = 2.8, urea = 1.6, calcium carbonate = 1.4, magnesium oxide = 0.8, palm oil = 0.7, vitamins and minerals = 0.4, sodium bicarbonate = 0.4, sodium chloride = 0.2, disodium phosphate = 0.1.

[6]The high-P concentrate in the lactation period consisted of (% DM): sugar beet pulp = 24.7, soyhulls = 22.9, corn = 14.2, soybean meal = 11.7, wheat = 6.6, potato protein = 5.9, molasses = 5.2, palm kernel meal = 2.8, urea = 1.6, calcium carbonate = 1.4, disodium phosphate = 1.1, magnesium oxide = 0.8, palm oil = 0.7, vitamins and minerals = 0.4, sodium chloride = 0.1.

[7]DCAD calculated as $(Na^+ + K^+) - (Cl^- + S^{2-})$ where Na, K, Cl and S are expressed as mEq/kg DM.

[8]The NE_L values were calculated according to the Dutch NE-system (Van Es, 1978). IDP = intestinal digestible protein; RDPB = rumen-degradable protein balance, the latter values are calculated according to the Dutch DVE/OEB-system (Tamminga et al., 1994).

RDPB values were calculated based on table values of ingredients (CVB, 2012).

All plasma samples were analyzed for Pi concentration (with ammonium molybdate) and Ca concentration (with Arsenazo III) using an automatic analyzer (ABX Pentra 400, Horiba Europe GmbH). Nonesterified fatty acids and BHB concentrations were determined with the use of an automatic analyzer (Cobas Mira Plus System from Roche Diagnostica Ltd.) using commercial test kits (NEFA: HR(2) R1+R2 Set, Wako

Chemicals GmbH; BHB: RANBUT, RB 1008, Randox Laboratories GmbH).

Pooled milk samples (2 morning and 2 afternoon milkings weekly) were analyzed for fat, protein, and lactose by mid-infrared spectrometry (ISO, 2013; Qlip). Pooled milk samples (1 morning and 1 afternoon milking weekly) were analyzed by inductively coupled plasma mass spectrometry for P and Ca concentration (Qlip).

Calculations and Statistical Analysis

Before statistical analysis, the daily values on DMI and milk yield were averaged per week. Fat- and protein-corrected milk (**FPCM**) was calculated as follows (CVB, 2012):

$$\text{FPCM (kg/d)} = \text{MY (kg/d)} \times [0.337 + 0.116 \times \text{MF (\%)} + 0.06 \times \text{MP (\%)}],$$

where MY = milk yield, MF = milk fat concentration, and MP = milk protein concentration. The energy balance (**EB**) was defined as the difference between net energy intake and net energy requirements for maintenance and milk production, and EB was calculated accordingly (Heuer et al., 2001). After calving, due to an allocation error, one cow in the Dry-HP, Lac-LP group received the high-P premix in her basal mixed diet, and this cow was excluded from all statistical analyses in the postpartum period. Data were analyzed by repeated measures ANOVA (VSN International, 2018) for the prepartum period and the postpartum period separately. Plasma Ca, Pi, DMI, MY, FPCM, milk concentrations, BW, EB, NEFA, and BHB concentrations were analyzed with repeated measures that accounted for unequal time intervals of blood sampling. The model for variables in the dry period contained main effects of block (block 1–15), dry-period treatment (Dry-LP, 2.2 g of P/kg of DM; Dry-HP, 3.6 g of P/kg of DM), week (wk 4, 2, and 1 before parturition except BW and DMI, which also included wk 6, 5, and 3 before parturition), and the interaction effect dry-period treatment × week. The model for variables in the lactation period contained main effects of block, dry-period treatment, lactation-period treatment (Lac-LP, 2.9 g of P/kg of DM; Lac-HP, 3.8 g of P/kg of DM), week (wk 1–8 after parturition), and the interaction effects lactation-period treatment × week, dry-period treatment × week, lactation-period treatment × dry-period treatment, and lactation-period treatment × dry-period treatment × week. For plasma variables in the lactation period, the effect of week was replaced with effect of sampling day after calving (d 0, 1, 2, 3, 7, 14, 21, 28, and 56). A chi-squared test was used to test for differences in the incidences of clinical and subclinical hypocalcemia between dietary treatments. Differences were considered significant at $P \leq 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

RESULTS

Dry Period

During the dry period, both BW of the cows and DMI were neither affected by a week × dietary P level interaction ($P \geq 0.11$) nor by the level of dietary P ($P \geq 0.36$) but in time BW increased by 3.0%, whereas DMI decreased by 17.8% ($P < 0.01$; Table 2). Plasma Pi values were not affected by dietary P level, week before parturition, or their interaction ($P \geq 0.15$). Plasma Ca concentrations were not affected by a dietary P level × week interaction or by week ($P \geq 0.36$), but in contrast to plasma Pi, plasma Ca concentrations were affected by dietary P supply and were 3.3% greater ($P = 0.05$) when cows were fed 2.2 compared with 3.6 g of P/kg of DM. Plasma NEFA concentrations were neither affected by week × dietary P level interaction ($P = 0.52$) nor by dietary P level ($P = 0.37$), but 1 wk before calving, NEFA concentrations in plasma were 2.4 times greater compared with the values observed 4 wk before calving ($P < 0.01$). Plasma BHB concentrations tended to be affected by dietary P level × week before parturition ($P = 0.07$), indicating increased BHB concentrations with time in the high P group, but decreased BHB concentrations with time in the low P group.

Periparturient Hypocalcemia

No distinct differences between treatment groups were found ($P = 0.81$) in the number of cows treated with intravenous Ca administration (Table 3). However, the number of cows with plasma Ca concentrations <2.0 m*M* during the first week after calving was lowest ($P = 0.02$) when the cows were fed low P diets during both the dry period (Dry-LP) and after calving (Lac-LP). The number of cows having plasma Ca concentrations <2.0 m*M* during wk 2 to 8 was not affected by the level of dietary P ($P = 0.20$).

Postcalving Plasma Pi, Ca, NEFA, and BHB

A sampling day × dry-period P level × lactation-period P level interaction affected postpartum plasma Pi concentration ($P < 0.01$). From d 1 to 7 postpartum, cows receiving the high-P diet had increased plasma Pi concentrations, and the rate of increase from d 1 to 7 greater in cows fed Dry-LP during the dry period

Table 2. Body weight, DMI, and plasma inorganic phosphate, calcium, nonesterified fatty acids (NEFA), and BHB concentrations in cows fed experimental diets differing in dietary phosphorus concentrations[1] during the dry period

	Weeks before parturition							*P*-value		
Experimental diet	6	5	4	3	2	1	SED	Week (W)	Diet (D)	D × R
BW, kg										
Dry-HP	783	794	802	814	814	833	16.1	<0.01	0.36	0.11
Dry-LP	770	777	792	797	810	809				
DMI, kg/d										
Dry-HP	14.2	14.2	14.8	14.3	13.5	12.1	0.55	<0.01	0.78	0.61
Dry-LP	14.6	14.2	14.4	14.0	13.2	11.9				
Inorganic phosphate, m*M*										
Dry-HP	—	—	1.75	—	1.74	1.75	0.052	0.60	0.15	0.47
Dry-LP	—	—	1.65	—	1.70	1.69				
Calcium, m*M*										
Dry-HP	—	—	2.34	—	2.32	2.35	0.040	0.82	0.05	0.36
Dry-LP	—	—	2.42	—	2.42	2.40				
NEFA, m*M*										
Dry-HP	—	—	0.09	—	0.12	0.22	0.028	<0.01	0.37	0.52
Dry-LP	—	—	0.08	—	0.11	0.18				
BHB, m*M*										
Dry-HP	—	—	0.49	—	0.52	0.53	0.038	0.67	0.40	0.07
Dry-LP	—	—	0.57	—	0.53	0.50				

[1]Dry-HP and Dry-LP = experimental diets fed during the dry period (Dry) high in phosphorus concentration (HP) or low in phosphorus concentration (LP) containing either 3.6 or 2.2 g of P/kg of DM, respectively. Inorganic phosphate, Ca, nonesterified fatty acids and BHB were sampled during 4, 2, 1 wk before calving.

compared with those having received the high-P diet during the dry period (Figure 1). In contrast, plasma Pi concentrations decreased from d 1 to 7 postpartum in cows receiving the low-P diet, but this decrease was more pronounced for cows having received the high-P diet during the dry period compared with those having received the low-P diet during the dry period. After d 7, plasma Pi concentrations remained rather constant at 1.5 to 1.6 m*M* for cows receiving the high-P diet irrespective of dietary P level received during the dry period. Similarly, levels of plasma Pi increased in cows fed the low-P diet from d 7 onward and reached stable levels of 1.3 to 1.4 m*M* at d 28. For plasma Ca, BHB, and NEFA concentrations, none of the 2-way or 3-way interactions were significant ($P \geq 0.16$; Table 4). Plasma Ca and BHB concentrations generally increased, and plasma NEFA concentrations decreased, with time postpartum ($P \leq 0.01$). The cows fed high-P diets during the dry period had 4.1% lower plasma Ca concentrations after calving ($P < 0.01$). After calving, feeding the low-P diet resulted on average in 4.0% greater plasma Ca concentrations ($P = 0.01$). Postpartum plasma concentrations of BHB and NEFA were neither affected by dietary P level in the dry period nor in the lactation period ($P \geq 0.26$).

Postcalving DMI, Milk Production, EB, and BW

Total DMI (basal diet plus concentrates) was not affected by any interaction ($P \geq 0.58$), except for a tendency for a week × dietary P level in lactation-period interaction ($P = 0.06$), due to differences in DMI during

Table 3. Numbers of cows associated with hypocalcemia when fed experimental diets differing in dietary P concentrations[1] during both the dry period and lactation[2]

	Dry-HP		Dry-LP		
Treatment	Lac-HP	Lac-LP	Lac-HP	Lac-LP	*P*-value
CaMg i.v. treatment	5/15	4/14	4/15	2/15	0.81
Plasma Ca <2.0 m*M* wk 1 postpartum	14/15	12/14	14/15	5/15	0.02
Plasma Ca <2.0 m*M* during wk 2–8	2/15	2/14	0/15	4/15	0.20

[1]Dry-HP, Dry-LP, Lac-HP, and Lac-LP = experimental diets fed during the dry period (Dry) or lactation period (Lac) with either a high phosphorus (HP) or a low phosphorus (LP) concentration. The diets contained either 3.6 (HP) or 2.2 (LP) g P/kg DM during the dry period and 3.8 (HP) or 2.9 (LP) g P/kg DM during lactation. CaMg i.v. = intravenous treatment with 7.44 and 4.28 g of Ca and Mg, respectively.

[2]Values are presented as no. of cases/no. of total cows.

the first 3 wk (Table 5). Cows fed the low-P diet tended to have a lower rate of increase in DMI in wk 2 and 3 compared with wk 1, than cows fed the high-P diet (i.e., Lac-HP). In wk 8, DMI did not differ and was on average 55% greater than in wk 1. The level of dietary P in the dry-cow diet did not affect DMI ($P = 0.19$) or FPCM ($P = 0.51$). The rate of increase in FPCM yield was greater in cows fed Lac-HP versus Lac-LP ($P = 0.04$) but this effect was mainly restricted to the first 2 wk after calving. Energy balance and BW were not affected by any of the interactions or by dietary P level ($P \geq 0.09$). Energy balance increased and BW decreased from wk 1 to wk 8 postpartum ($P < 0.01$).

Milk Constituents

Except for the lactose concentration of milk, concentrations of P, Ca, fat, and protein in milk (Table 6) were not affected by any interaction ($P \geq 0.42$). The lactose concentration of milk tended to be affected by week × dietary P level ($P = 0.07$). Milk lactose concentration increased with lactation week, mainly from wk 1 to wk 2, and tended to be somewhat greater in cows fed Lac-HP, whereas milk lactose concentration tended to be slightly less in later weeks (i.e., wk 4–8) in cows fed Lac-HP. Dietary P level in the dry period or in the lactation period did not affect milk fat and milk protein concentration ($P > 0.17$). Both milk fat and milk protein concentration decreased with week of lactation, in particular in the first 3 weeks ($P < 0.01$). Dietary P level in the dry period or in the lactation period did not affect milk P concentration ($P > 0.62$). On average, milk P concentrations decreased 25% (from 1.30 to 0.97 g/kg of milk) during the first 4 weeks of lactation ($P < 0.01$) and remained essentially unchanged thereafter. Similarly, milk Ca concentrations dropped 26% (from 1.43 to 1.21 g/kg of milk) during the first 4 wk of lactation and remained stable thereafter ($P < 0.01$). The Ca concentration of milk tended to be greater (i.e., 2.6%; $P = 0.09$) for Lac-LP compared with Lac-HP, whereas dietary P concentration in the dry period did not affect milk Ca concentration ($P = 0.59$).

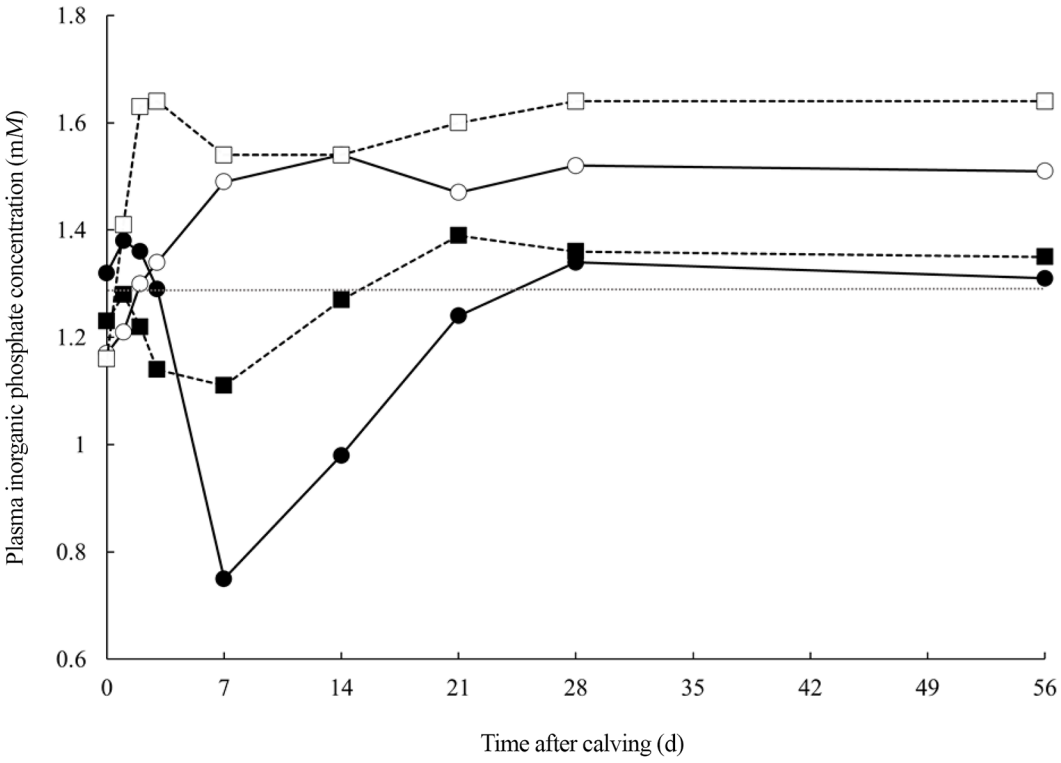

Figure 1. Time courses on plasma inorganic phosphate (Pi) concentrations after calving. The cows were either fed a low-P (LP) or a high-P (HP) diet during the dry period (Dry) and after calving (Lac). High-P diets contained 3.6 g of P/kg of DM during the dry period and 3.8 g of P/kg of DM after calving, and low-P diets contained 2.2 g of P/kg of DM during the dry period and 2.9 g of P/kg of DM after calving. Symbols: solid line, black circle = Dry-HP/Lac-LP; solid line, white circle = Dry-HP/Lac-HP; dotted line, black square = Dry-LP/Lac-LP; dotted line, white square = Dry-LP/Lac-HP, dotted gray line without markers represents the threshold for hypophosphatemia, i.e., 1.3 m*M*. Standard error of the difference is 0.104 m*M*. *P*-values: Sampling day × Dry × Lac = <0.01; Sampling day × Dry = 0.31; Sampling day × Lac = <0.01; Dry × Lac = 0.20; Sampling day = <0.01; Dry-HP vs. Dry-LP = 0.03; Lac-HP vs. Lac-LP = <0.01.

Table 4. Plasma calcium, nonesterified fatty acids (NEFA), and BHB concentrations in cows fed experimental diets differing in dietary P concentrations[1] during both the dry period and lactation period; all values are expressed as m*M*

		Sampling day after calving										*P*-value[2]				
Experimental diet		0	1	2	3	7	14	21	28	56	SED	Sampling day (S)	Dry	Lac	Dry × Lac	S × Lac
Calcium																
Dry-HP	Lac-HP	1.81	1.74	2.02	2.20	2.30	2.34	2.33	2.33	2.29	0.091	<0.01	<0.01	0.01	0.87	0.32
	Lac-LP	1.85	1.89	2.12	2.38	2.34	2.42	2.40	2.28	2.47						
Dry-LP	Lac-HP	1.93	1.88	2.07	2.39	2.28	2.40	2.45	2.40	2.44						
	Lac-LP	2.05	2.16	2.25	2.37	2.39	2.38	2.44	2.44	2.44						
NEFA																
Dry-HP	Lac-HP	0.99	0.74	0.67	0.69	0.56	0.39	0.38	0.32	0.17	0.110	<0.01	0.27	0.61	0.74	0.53
	Lac-LP	0.94	0.93	0.67	0.65	0.59	0.44	0.37	0.25	0.14						
Dry-LP	Lac-HP	0.92	0.67	0.66	0.59	0.47	0.28	0.29	0.25	0.13						
	Lac-LP	0.78	0.66	0.71	0.65	0.62	0.39	0.38	0.26	0.21						
BHB																
Dry-HP	Lac-HP	0.62	0.80	0.88	0.83	0.82	0.71	0.86	0.99	0.80	0.150	<0.01	0.77	0.26	0.55	0.16
	Lac-LP	0.67	0.87	1.07	0.79	0.90	0.68	0.69	0.70	0.71						
Dry-LP	Lac-HP	0.54	0.83	0.89	0.80	0.80	0.93	0.82	0.90	0.99						
	Lac-LP	0.53	0.65	0.73	0.82	1.01	0.66	0.71	0.66	0.81						

[1]Dry-HP, Dry-LP, Lac-HP, and Lac-LP = experimental diets fed during the dry period (Dry) period or lactation period (Lac) with either a high phosphorus (HP) or a low phosphorus (LP) concentration. The diets contained either 3.6 (HP) or 2.2 (LP) g of P/kg of DM during the dry period and 3.8 (HP) or 2.9 (LP) g of P/kg of DM during lactation.
[2]No significant dry × sampling day and lac × dry × sampling day effects were detected for all parameters ($P \geq 0.38$).

DISCUSSION

P Intake, Requirement, and Plasma Pi Concentrations

The absolute intakes of P (g/d) mirrored the various dietary P concentrations of the experimental diets in the current study (Figure 2). During the dry period, when the high-P diet was fed the mean P intake was greater than the P requirement recommended by either the NRC (2001) or CVB (2012) system, whereas P intake was approximately equal to P requirements when the low-P diet was fed. These findings are in line with the observation that hypophosphatemia (i.e., plasma Pi <1.3 m*M*; Goff, 1999) did not occur during the dry period. Plasma Pi concentrations in the dry period were only numerically lower (4%) with LP compared with HP and values are similar to those reported by Peterson et al. (2005) when cows were fed 2.1 g of P/kg of DM. During the first week of lactation, P intakes were lower than those recommended by the NRC (2001) and CVB (2012), irrespective of the P concentration of experimental diets. This observation is most likely explained by the low DMI during the first week of lactation. Phosphorus intakes during wk 3 until 8 of lactation were similar to the P requirements in both the NRC (2001) and CVB (2012) system when Lac-HP was fed. In contrast to the high-P diet, with the low-P diet, P intakes during wk 3 until 8 were ~36% (NRC, 2001) or ~21% (CVB, 2012) lower than requirements. This is largely in line with the current observations on plasma Pi concentrations (Figure 1). Hypophosphatemia did not occur in cows receiving the Lac-HP diet, except for d 0 and 1 after calving. Hypophosphatemia did occur during the first 2 to 3 wk after calving when Lac-LP was fed (except for the first 3 d in cows having received the high P level in the dry period), and plasma Pi values were restored to values ~1.3 m*M* from 4 wk after calving onwards. Plasma Pi concentrations shortly after parturition were considerably lower (1.27 m*M* at d 0 and d 1 postpartum) than in the week before calving (1.72 m*M*). This sharp drop in plasma Pi concentration is a common phenomenon in dairy cattle. The high P losses with milk immediately after calving may have contributed to this decrease in plasma Pi concentration, but it is unlikely to be the sole reason. A similar drop around parturition also occurs in mastectomized cows (Goff et al., 2002). The decrease in plasma Pi concentration may be related to the reduction in feed intake around calving, an increase in plasma corticosteroids, and more generally a redistribution of intracellular and extracellular P, because changes in the balance between intracellular and extracellular P may occur suddenly (Grünberg, 2014).

Table 5. Postcalving feed intake, milk production, BW, and energy balances in cows fed experimental diets differing in dietary P concentrations[1] during both the dry period and lactation

		Week of lactation									*P*-value				
Experimental diet		1	2	3	4	5	6	7	8	SED	Week	Dry	Lac	Dry × Lac	Week × Lac
Total DMI (kg/d)															
Dry-HP	Lac-HP	14.4	17.6	19.0	19.0	21.1	22.3	22.3	22.7	0.97	<0.01	0.19	0.71	0.86	0.06
	Lac-LP	14.3	15.6	17.8	19.7	20.9	21.3	22.1	22.4						
Dry-LP	Lac-HP	14.9	17.8	20.1	20.0	21.4	21.6	22.1	23.0						
	Lac-LP	15.2	16.9	18.9	20.3	22.4	22.5	23.1	23.0						
Milk yield (kg/d)															
Dry-HP	Lac-HP	27.8	38.0	41.5	41.6	43.1	43.4	42.2	42.7	2.37	<0.01	0.32	0.86	0.94	0.13
	Lac-LP	29.9	37.3	39.5	42.5	43.1	43.5	43.4	42.0						
Dry-LP	Lac-HP	28.5	38.3	42.2	44.3	44.8	44.2	44.3	44.7						
	Lac-LP	30.8	38.3	41.6	44.4	45.7	45.2	45.0	43.5						
FPCM yield (kg/d)															
Dry-HP	Lac-HP	32.7	41.1	44.1	42.3	42.0	42.0	40.0	41.2	2.36	<0.01	0.51	0.49	0.85	0.04
	Lac-LP	36.4	40.1	40.9	42.7	42.4	42.8	42.5	41.3						
Dry-LP	Lac-HP	33.3	40.5	42.6	43.6	42.8	42.1	41.6	42.2						
	Lac-LP	37.1	41.6	43.3	44.6	44.8	43.9	43.1	43.1						
Energy balance (kJ/ $kg^{0.75}$ per d)															
Dry-HP	Lac-HP	−358	−399	−403	−350	−238	−181	−126	−141	54.2	<0.01	0.74	0.27	0.73	0.11
	Lac-LP	−446	−479	−391	−335	−245	−242	−207	−152						
Dry-LP	Lac-HP	−358	−374	−305	−329	−242	−211	−174	−155						
	Lac-LP	−439	−455	−397	−336	−242	−202	−167	−164						
BW (kg)															
Dry-HP	Lac-HP	744	728	716	706	703	707	707	709	19.1	<0.01	0.56	0.12	0.22	0.09
	Lac-LP	712	684	672	672	669	673	674	677						
Dry-LP	Lac-HP	718	707	695	687	681	679	681	686						
	Lac-LP	711	690	681	679	683	682	685	684						

[1]Dry-HP, Dry-LP, Lac-HP, and Lac-LP = experimental diets fed during the dry period (Dry) period or lactation period (Lac) with either a high phosphorus (HP) or a low phosphorus (LP) concentration. The diets contained either 3.6 (HP) or 2.2 (LP) g of P/kg of DM during the dry period and 3.8 (HP) or 2.9 (LP) g of P/kg of DM during lactation. FPCM = fat and protein corrected milk. No significant dry × week and lac × dry × week effects were detected for all parameters ($P \geq 0.20$).

Table 6. Concentrations of phosphorus, calcium, protein, fat, and lactose in milk from cows fed experimental diets differing in dietary P concentrations[1] during both the dry period and lactation; all values are expressed as g/kg

		Week of lactation									*P*-value				
Experimental diet		1	2	3	4	5	6	7	8	SED	Week	Dry	Lac	Dry × Lac	Week × Lac
Phosphorus															
Dry-HP	Lac-HP	1.30	1.08	1.02	0.98	0.96	0.95	0.98	0.96	0.033	<0.01	0.62	0.91	0.40	0.18
	Lac-LP	1.26	1.06	1.00	0.98	0.97	0.96	0.96	0.97						
Dry-LP	Lac-HP	1.35	1.08	0.98	0.96	0.95	0.92	0.94	0.93						
	Lac-LP	1.29	1.09	1.01	0.97	0.95	0.94	0.94	0.97						
Calcium															
Dry-HP	Lac-HP	1.39	1.31	1.24	1.19	1.18	1.17	1.17	1.17	0.033	<0.01	0.59	0.09	0.31	0.53
	Lac-LP	1.45	1.34	1.28	1.24	1.23	1.21	1.22	1.23						
Dry-LP	Lac-HP	1.46	1.33	1.25	1.18	1.17	1.16	1.16	1.14						
	Lac-LP	1.42	1.35	1.26	1.21	1.19	1.18	1.19	1.19						
Protein															
Dry-HP	Lac-HP	42.0	35.0	32.5	31.4	31.1	30.6	31.0	31.0	0.95	<0.01	0.67	0.36	0.68	0.30
	Lac-LP	39.7	35.1	32.3	31.8	31.4	31.2	31.9	31.6						
Dry-LP	Lac-HP	40.3	34.5	32.0	31.0	30.6	29.9	30.5	30.7						
	Lac-LP	41.0	35.4	33.2	31.8	31.6	31.0	31.0	31.4						
Fat															
Dry-HP	Lac-HP	51.4	46.0	45.2	43.1	39.1	38.7	36.8	38.3	2.05	<0.01	0.23	0.17	0.42	0.12
	Lac-LP	54.2	46.2	45.2	42.3	39.7	39.9	38.6	39.6						
Dry-LP	Lac-HP	49.2	44.0	41.5	39.6	37.7	37.8	36.3	36.8						
	Lac-LP	55.1	47.7	43.7	40.6	39.4	36.8	37.4	39.4						
Lactose															
Dry-HP	Lac-HP	43.2	45.3	46.4	45.9	46.1	45.8	45.8	46.0	0.53	<0.01	0.92	0.62	0.71	0.07
	Lac-LP	42.8	45.2	45.8	46.3	46.3	46.3	46.5	46.1						
Dry-LP	Lac-HP	43.1	45.3	45.9	46.3	46.1	46.0	45.9	45.9						
	Lac-LP	42.7	44.9	45.9	46.4	46.4	46.7	46.4	46.6						

[1]Dry-HP, Dry-LP, Lac-HP, and Lac-LP = experimental diets fed during the dry period (Dry) period or lactation period (Lac) with either a high phosphorus (HP) or a low phosphorus (LP) concentration. The diets contained either 3.6 (HP) or 2.2 (LP) g of P/kg of DM during the dry period and 3.8 (HP) or 2.9 (LP) g of P/kg of DM during lactation. No significant dry × week and lac × dry × week effects were detected for all parameters ($P \geq 0.31$).

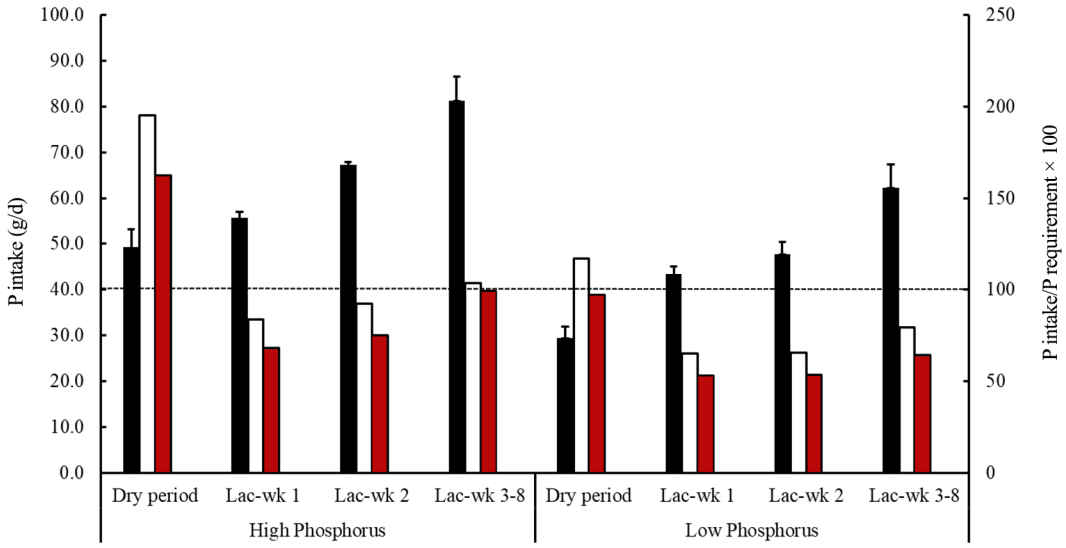

Figure 2. Mean total P intakes expressed as g/d (black bars; left y-axis) and as an index relative to recommended P intake (white bars = CVB, 2012; red bars = NRC, 2001; right y-axis) during the dry period, the first week of lactation (Lac-wk 1), the second week of lactation (Lac-wk 2) and wk 3 to 8 of lactation (Lac-wk 3–8). The cows were either fed a low-P (LP) or a high-P (HP) diet during the dry period (Dry) and after calving (Lac). High-P diets contained 3.6 g of P/kg of DM during the dry period and 3.8 g of P/kg of DM after calving, and low-P diets contained 2.2 g of P/kg of DM during the dry period and 2.9 g of P/kg of DM after calving. The P intakes during lactation (expressed either as absolute values or as an index relative to recommended P intakes) are the mean values of the 2 groups (i.e., Dry-HP and Dry-LP) fed either HP or LP during the lactation period. Recommended P intakes are calculated using the actual DMI and milk yield production (Table 5) and the actual P concentration of milk (Table 6). The dotted black line represents P intake being equal with P requirement. The error bar represents the SD associated with the mean P intake.

After calving, plasma Pi concentrations were affected by a 3-way week × dry period P level × lactation-period P-level interaction. This 3-way interaction is not easy to explain because the current data do not provide clues on the regulation of P absorption (Goff, 2004) and P mobilization from bone (Wu and Satter, 2000; Moreira et al., 2009; Puggaard et al., 2014). It can be speculated that the observed transient hypophosphatemia in cows fed low-P diets after calving has triggered mobilization of P from the skeleton (Puggaard et al., 2014). Mobilization of P from bones may supply significant amounts of P (500–600 g) during the first weeks of lactation (Wu and Satter, 2000). Furthermore, it cannot be excluded that the efficiency of P absorption was affected by the level of dietary P prepartum. It can be suggested that the feeding of high-P versus low-P diets during the dry period caused a low efficiency of P absorption before calving (Shirazi-Beechey et al., 1996). This notion is in line with the observed rapid increase in plasma Pi concentrations when cows switched from Dry-LP to Lac-HP but the initial increase in plasma Pi concentrations when cows switched from Dry-HP to Lac-LP is not in line with the aforementioned reasoning on the inverse relationship between P intake and efficiency of P absorption. Caution, however, is warranted in interpreting plasma Pi concentrations. Although plasma Pi concentration is widely used to diagnose P status of dairy cows, it is an unreliable parameter to diagnose P balance disorders (Grünberg, 2014). Thus, future studies are needed to evaluate if current P recommendations as set by NRC (2001) and CVB (2012) and others may be further lowered.

Periparturient Hypocalcemia

The current results on plasma Ca concentrations clearly indicate that the feeding of low-P diets [i.e., 2.2 g of P/kg of DM; P intake roughly equal to CVB (2012) and NRC (2001) requirements] during the dry period improves plasma Ca concentrations postpartum (wk 1–8) compared with feeding high-P diets (3.6 g of P/kg of DM; above requirements). These results are in line with Lean et al. (2006). In our study, similar to the effect of low-P diets fed during the dry period on plasma Ca levels in wk 1 to 8 after calving, low-P diets fed during the lactation period increased plasma Ca levels. Puggaard et al. (2014) also reported elevated plasma Ca levels in wk 2 to 5 postpartum upon feeding diets containing 2.3 g of P/kg of DM compared with 3.4 g of P/kg of DM. We did not find interactions between sampling day, dietary P level during the dry period, and dietary P level during the lactation period on plasma Ca concentrations. This indicates that the effect on plasma Ca levels of low versus high dietary

P levels in the lactation period did not depend on the dietary P level in the dry period. However, the number of hypocalcemic cows (having plasma Ca levels less than 2 m*M*) in the first week postpartum was smaller when the low dietary P concentration in the dry period was continued in the lactation period. The current data on periparturient plasma Ca concentration warrant the feeding of low-P diets to dairy cows, but they do not identify underlying mechanisms explaining our observations. Phosphorus supply may have interfered with vitamin D_3 metabolism (Köhler et al., 2021) and thus Ca metabolism. In rats, hyperphosphatemia (serum $P > 3$ m*M*) reduced the activity of renal 25-hydroxyvitamin D1 α-hydroxylase leading to a reduced production of 1,25-dihydroxyvitamin D_3 thereby causing hypocalcemia (Tallon et al., 1996; Silver et al., 1999; Masuyama et al., 2000). Vitamin D_3 metabolism may likewise be affected in dairy cows thereby explaining the effect of low-P diets on Ca metabolism (Julien et al., 1977; Kichura et al., 1982; Barton et al., 1987). Indeed, in periparturient P-deprived cows, a typical increase in plasma 1,25-dihydroxyvitamin D concentration and bone resorption occurred despite a less pronounced rise in plasma parathyroid hormone concentrations (Cohrs et al., 2018). These authors suggested either greatly increased sensitivity to parathyroid hormone or bone mobilization independent of parathyroid hormone occurred at low dietary P levels.

DMI, Milk Production, NEFA, and BHB

The current observations on DMI and milk production are corroborated by various studies (Valk and Sebek, 1999; Wu and Satter, 2000; Peterson et al., 2005; Puggaard et al., 2014) who also reported that postpartum diets containing at least 2.8 g of P/kg of DM do not compromise DMI and milk yield. Furthermore, the current results on EB, plasma NEFA, plasma BHB, and BW are in line with our observations on DMI and MY. The lack of effect of the current low-P diets on FPCM yield, and the various indices related to energy status of the cows, is most likely explained by the fact that DMI was hardly affected when the low-P diet was fed. It is well known that fiber digestion is an important determinant of DMI (Oba and Allen, 1999), and various studies have shown that rumen fiber digestion is sensitive to the P supply of the rumen microbes (Hall et al., 1961; Komisarczuk-Bony and Durand, 1991; Puggaard et al., 2011). Komisarczuk-Bony and Durand (1991) reported that a minimum P concentration of 0.5 m*M* in rumen fluid is required for optimum fiber digestion under in vitro conditions. This observation is in line with Rodehutscord et al. (1994) who reported that the feeding of a diet containing 1.4 g of P/kg of DM to goats was associated with rumen P concentrations ~5 m*M* without an adverse effect on DMI. In contrast, Puggaard et al. (2011) did not observe decreased DMI of dairy cattle upon feeding diets with 2.4 g of P/kg of DM, whereas Puggaard et al. (2014) reported that a diet containing 2.3 g of P/kg of DM did compromise DMI compared with 3.4 g of P/kg of DM. However, these authors did not measure rumen P concentrations, which hinders further speculation on cause and effect on their observations on DMI. Overall, at the milk production levels and lactation stage in the current study, our results indicate that a dietary P concentration of 2.9 g of P/kg of DM is sufficient to maintain DMI and FPCM production in dairy cows.

In the current study, P and Ca concentrations of milk did decrease with week of lactation. These minerals have been shown to be positively related to the concentration of milk protein and (for milk P only) to a lesser extent with the concentration of milk lactose (Bijl et al., 2013; Klop et al., 2014). The significant and large decrease in milk protein concentration with week of lactation, and the quantitatively less pronounced increase in lactose concentration, may help to explain the decrease in Ca and P concentrations in milk with lactation week. In the NRC (2001) and CVB (2012) requirement systems for dairy cows, a fixed concentration of 0.9 or 1.0 g of P/kg of milk is assumed to calculate net P requirements. The large variation in milk P concentration (between 0.92 and 1.35 g/kg) observed during the first 8 wk postpartum indicates P requirements of dairy cattle in specific weeks of lactation may be under- or overestimated in current P requirement systems.

CONCLUSIONS

Reduction in dietary P concentration from 3.6 (exceeding P requirements) to 2.2 g/kg of DM (equal to P requirement) during the dry period increased plasma Ca concentrations during the first 8 wk after calving without affecting DMI or milk production. Likewise, a reduction in dietary P concentration from 3.8 g/kg of DM (equal to P requirements in wk 3–8 postpartum) to 2.9 g/kg of DM (below P requirements) after calving also improved plasma Ca levels without compromising DMI and milk production. Our findings indicate that feeding diets low in P concentration in late gestation or early lactation has a positive influence on periparturient Ca homeostasis and may help to prevent (sub-) clinical hypocalcemia during the first week postpartum. Current NRC (2001) and CVB (2012) estimates of the dietary P requirements in early lactating dairy cows may to be too high. The present study was short-term and therefore, long-term effect of this dietary P strat-

egy needs to be investigated before practical recommendations can be made.

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