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A dairy cow trial on phosphorus metabolism in the transition period

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Samenvatting NL In deze studie is het effect van verschillende fosfor (P) niveaus in melkveerantsoenen onderzocht gedurende de transitieperiode, voor wat betreft de productieprestaties en diergezondheid. Het overvoeren van P in de droogstand (185% vs. 100% van de behoeftenorm) had geen invloed op de voeropname of melkproductie postpartum, maar verlaagde plasma P en Ca concentraties en verhoogde juist het risico op hypocalcemie. Een reductie van de hoeveelheid P in lactatierantsoenen (70% vs. 100%) verlaagde de plasma P concentratie, verhoogde de plasma Ca concentratie en de P mobilisatie en verlaagde de excretie van P met de mest. Desondanks zijn er geen negatieve gevolgen geconstateerd van een lactatierantsoen met een P gehalte onder de berekende behoefte, voor wat betreft de productieprestaties of de diergezondheid in de eerste acht weken na kalven.

Summary UK The present study evaluates the effect of different dietary phosphorus (P) levels in dairy cattle during the transition period, focussing on animal performance and animal health. Overfeeding P in the dry period (185% vs. 100% of requirements) did not affect feed intake or milk yield postpartum, but decreased plasma P and Ca concentrations and increased the risk for periparturient hypocalcemia. A reduction of the dietary P content in lactation diets (70% vs. 100%) reduced the average plasma P concentration, increased plasma Ca concentration and P mobilisation and reduced faecal excretion of P. However, no negative effects on cow performance or animal health were found for diets with a P content below calculated requirements in the first eight weeks postpartum.

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Foreword

This research project was conducted within the research programme “Feed4Foodure”: a public-private partnership between the Dutch Ministry of Economic Affairs and a consortium of various organizations within the animal feed industry and the animal production chain. Feed4Foodure aims to contribute to sustainable and healthy livestock farming in the Netherlands, simultaneously strengthening our competitive position on the global market.

The research programme comprises three main research lines: socially responsible livestock farming; nutrition, gut health and immunity; and “more-with-less” by efficient nutrient use. The aim of this third research line, “More with Less”, is to reduce the footprint of the Dutch livestock sector in the field of phosphate, nitrate, copper, zinc, ammonia and greenhouse gases. New nutritional models and measurement techniques will be developed to improve efficient use of nutrients in livestock farming.

The present study entitled “Phosphorus in transition; a dairy cow trial on phosphorus metabolism in the transition period” was performed within research line “More with Less”, theme 4: “Reduction of phosphorus losses”. Main aim of this subproject is to gain insight in the dynamics and regulation of phosphorus absorption, mobilisation and utilization in farm animals to update the current phosphorus requirements for farm animals, thereby improving phosphorus efficiency.

For dairy cattle, much work has been done to understand phosphorus digestion, absorption and metabolism, but the regulation of phosphorus homeostasis, especially during the transition period, is not fully understood. In the present study we investigated the effect of different levels of phosphorus in dry cow and lactation rations on dairy cow health, performance and phosphorus efficiency in the transition period.

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Summary

The regulation of phosphorus (P) in dairy cattle is still not fully understood, especially during the transition period with low P requirements in the dry period and high P requirements directly after calving due to the output of P with milk. In the present trial the effect of varying P intake in the dry period (starting 6 weeks before calving; 185% and 100% of P requirement) as well as the first 8 weeks of lactation (100% vs. 70% of P requirement) was evaluated in a randomised block design with 60 multiparous dairy cows.

Overfeeding P in the dry period (3.6 g P/kg DM) did not affect feed intake, milk yield or milk composition after calving compared to a diet fed at requirements (2.2 g P/kg DM). Monitoring P excretion with faeces showed that the additional P ingested by overfeeding was excreted and did not accumulate in the body. On the contrary, overfeeding P in the dry period negatively affected plasma calcium concentration postpartum, and increased the risk for periparturient hypocalcemia.

A reduction of the dietary P content in lactation diets below the calculated requirements (2.9 g P/kg DM) reduced average plasma P concentration and increased plasma Ca concentration in the first 8 weeks postpartum compared to a diet at requirements (3.8 g P/kg DM). Cows on both diets mobilised P from body reserves postpartum; mobilisation was highest for the low P diet (14 g/d compared to 9 g/d) and faecal excretion was reduced (34 g/d compared to 47 g/d). Feeding around 70% of calculated P requirements in the first 8 weeks postpartum with a diet containing 2.9 g P/kg did however not affect feed intake, feed digestibility, milk yield, milk composition or animal health. The maximal duration of this period of P mobilisation requires further research.

1 Introduction

1.1 Background

In dairy cattle physiology, the regulation of phosphorus (P) is still not fully understood, especially during the transition period. The Dutch requirements as defined by the Product Board Animal Feed are internationally the lowest for high yielding dairy cattle. Most Dutch dairy rations contain sufficient P to match the P requirements for the largest part of the lactation cycle. During the dry period P requirements are low, relative to the P content of the rations. Directly after calving, the risk for a P deficiency is largest, as the feed intake is still low while the milk P content (and the animal's P requirement) is high. Experience of farmers and veterinarians in practice suggests that this P deficiency may play a role in health disorders immediately after calving. The scientific base for this relationship is however still insufficient.
Looking at the required reduction in the P excretion of the dairy sector, knowledge of P metabolism in dairy cattle gains importance to further improve P efficiency without harming digestion, production or animal health.

1.2 Research objectives

The present trial investigates the effect of varying P content in dairy cow rations during the transition period to answer the following questions:

- What is the effect of a **high P intake in the dry period** (185% relative to requirement) on animal health, feed intake, milk yield and milk composition of cows after calving?
- What is the effect of a **low P intake during the first 8 weeks** of lactation (70% relative to requirement) on animal health, feed intake, milk yield and milk composition of cows after calving?
- What is the effect of total P intake in the dry period and the lactation on the **digestibility of the rations**?
- What is the calculated **mobilisation of P from body reserves** at different levels of P intake in the dry period and the lactation?

2 Materials and Methods

2.1 Animals and housing conditions

The trial was performed at Dairy Campus, Boksumerdyk 11, 9084 AA Leeuwarden between 16 January and 4 September 2017. All experimental protocols and interventions were approved by the Ethics Committee on Animal Experiments of the Animal Sciences Group of Wageningen University and Research Centre, the Netherlands.

A total number of 60 pregnant Holstein Friesian dairy cows were selected from the 500-head herd, based on their parity (at least two previous calvings) and expected calving date. All cows entered the trial 39-45 days before expected calving date and were monitored until 8 weeks after calving. All cows are housed in two groups in the nutrition barn: a dry-cow group, and a postpartum lactation group. Close to calving, animals were moved to the transition barn; after calving the animals returned to the nutrition barn as soon as permitted by their physical condition.

All animals were fed a roughage-based ration *ad libitum*, fed in feeding bins of the Roughage Intake Control system (RIC; Hokofarm Group, Marknesse, the Netherlands) to measure individual feed intake. Freshly mixed rations were provided automatically four times during the day by an automatic feeding system (Trioliet, Oldenzaal, the Netherlands). Additionally concentrates were fed individually by transponder-controlled concentrate feeders. Drinking water was provided ad libitum. All animals were milked twice daily with an inter milking interval of 11 to 13 hours.

2.2 Experimental design

2.2.1 Experimental groups

Before entering the trial, animals were grouped in 15 blocks of 4 animals based on similarity in expected calving dates, parity and milk production in the previous lactation. Individual animals from each block were randomly assigned to one of four treatment groups: D100/L100, D185/L100, D100/L70 and D185/L70.

GROUP		**Dry period (6 weeks)**			**Lactation (8 weeks)**	
D100	**L70**	**Dry100** **2.0** g P /kg DM	30 cows	**calving**	**Lac70** **2.6** g P /kg DM	15 cows
D100	**L100**				**Lac100** **3.7** g P/kg DM	15 cows
D185	**L70**	**Dry185** **3.7** g P/kg DM	30 cows		**Lac70** **2.6** g P /kg DM	15 cows
D185	**L100**				**Lac100** **3.7** g P/kg DM	15 cows

Each treatment group received a different combination of rations varying in P content relative to the calculated P requirements, according to the calculations of the Dutch Product Board Animal Feed (COMV, 2005). Calculations were based on the expected dry matter intake, requirements for gestation (in the dry period), the expected milk yield (in early lactation) and an average net absorption of 75%.

This resulted in the following treatment groups:

- **D100/L100** (n=15): both the dry cow ration and the lactation ration aimed at a P intake equal to the P requirements (100%).
- **D185/L100** (n=15): the dry cow ration contained a higher level of P relative to the P requirements (185%), while the lactation ration aimed at a P intake equal to the P requirements (100%).
- **D100/L70** (n=15): the dry cow ration aimed at a P intake equal to the P requirements (100%), while the P content of the lactation ration was below the P requirements (70%).
- **D185/L70** (n=15): the dry cow ration contained a higher level of P relative to the P requirements (185%), while the P content of the lactation ration was below the P requirements (70%).

2.2.2 Ration composition

The basal dry cow ration was composed of maize silage, grass silage, wheat straw and a mineral and vitamin premix. Additionally, all cows received 1 kg of compound concentrate with TiO_2 added as an indigestible marker fed in individual concentrate dispensers (10 g Ti per kg). During lactation, the basal ration was composed of maize silage, grass silage, soy bean meal and a mineral and vitamin premix. During lactation, all cows received 2 kg of compound concentrate with TiO_2 as an indigestible marker (5 g Ti per kg) and a second compound concentrate starting at 1 kg the day after calving, gradually increasing up to 7.3 kg at day 21 postpartum.
The level of P in the rations was varied by adding inorganic P (Na_2PO_4) to the low P premix and the low P lactation concentrate, in exchange for $NaCO_3$ in the high P premix and the high P lactation concentrate, to compensate for a potential difference in sodium intake. This resulted in two dry cow diets (Dry100 and Dry 185) and two lactation diets (Lac70 and Lac100).

Table 2.1 *Average ration composition.*

Ingredient	Dry100	Dry185	Lac70	Lac100
Ingredients of feed mixture in % of DM				
Maize silage	39.7	39.3	69.0	70.0
Grass silage	23.7	24.1	21.3	21.0
Wheat straw	32.0	31.5	-	-
Soy bean meal	-	-	6.4	5.4
Premix low P	4.5	-	3.3	-
Premix high P	-	5.1	-	3.6
Compound concentrates in kg/cow per day				
Marker dry period	1.0	1.0	-	-
Marker lactation	-	-	2.0	2.0
Lactation concentrate low P	-	-	7.3*	-
Lactation concentrate high P			-	7.3*

*Lactation concentrate was gradually increased from 1.0 kg/d at d1 postcalving until 7.3 kg/d at d21 postcalving.

2.2.3 Data collection

General performance data
Total intake of the roughage diet was registered automatically by the RIC feeding bins (kg/cow/day).

Milk yield was registered at each milking and combined to a daily milk yield (kg/d). Live weight was registered once a week during the dry period; after calving, all cows were weighed at each milking after leaving the milking parlour. Body condition was scored weekly on a 1 to 5 scale with 0.25 increments.

Feed samples
For the determination of dry matter content, maize and grass silage were sampled daily while wheat straw, soy bean meal and the premixes fed in the roughage diet were sampled weekly. Dry matter content was determined by calculating the weight difference before and after oven drying at 104°C during 36 hours. Every day the relative contribution of each of the ration components to the mixture

was registered (kg product of each component mixed) and with this information, average DM content of the mixture was calculated. Dry matter intake per individual cow was calculated by multiplying dry matter content of the mixture with daily intake of fresh product.
Next to the dry matter determination, all ingredients and compound concentrates were sampled once per week and frozen for chemical analysis.

Milk samples
Milk was sampled weekly for the determination of milk composition. During four consecutive milkings, milk was sampled and pooled in two tubes: one for the two morning milkings, and one for the two evening milkings. Samples were analysed fresh by mid-infrared spectrometry (Qlip, Zutphen, The Netherlands) for fat, protein, lactose and urea concentration, and somatic cell count.
Two separate milkings (one morning and one evening) were sampled and stored frozen (-20°C). These two milk samples were pooled and analyzed by ICP-MS for P content (Qlip, Zutphen, The Netherlands).

Blood samples
Blood was sampled according to a standardized schedule relative to the (expected) day of calving. Before calving, three samples were taken on Thursdays in week -4, -2 and -1 relative to the expected calving date. If the actual calving date was later than expected, additional blood samples were taken weekly until calving. Immediately after calving, the fourth sample was taken (d0). After that, 8 samples were taken during lactation: on d1, d2, d3, d7, d14, d21, d28 and d56. Samples planned for weekend days were taken on the closest weekday (Saturday to Friday; Sunday to Monday).
At each sampling time, blood was collected in serum separator tubes and samples were left 30 min at room temperature to coagulate. Tubes were then centrifuged for 15 min at 3,000 RPM and plasma was transferred to plasma tubes and stored at -20°C. All samples were analysed for plasma P concentration (with ammonium molybdate) and Ca concentration (with Arsenazo III) using an automatic analyser (ABX Pentra 400, Horiba, Europe GmbH, Langenhagen, Germany). Non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHB) concentration were also determined by an automatic analyser (Cobas Mira Plus System from Roche Diagnostica Ltd, Basel, Switzerland) using commercial test kits (NEFA: HR(2) R1+R2 Set, WAKO Chemicals GmbH, Neuss, Germany; BHB: RANBUT, RB 1008, Randox Laboratories GmbH, Wülfrath, Germany). Analysis of bone resorption marker CTX, parathyroid hormone PTH, and 25-hydroxyvitamin D_3 were conducted by means of commercially available ELISA kits (CTX: Serum CrossLaps ELISA, Immundiagnostic Systems (ids) GmbH, Frankfurt am Main, Germany; PTH: Bovine Intact PTH ELISA Kit; Immuntopics Inc., San Clement, CA; 25-hydroxyvitamin D_3: 25(OH)-Vitamin D direct day ELISA, Immundiagnostik AG, Bensheim, Germany).

Faecal samples
Faeces was sampled at biweekly intervals, except for the week of calving, in week -4, -2, 2, 4, 6 and 8 relative to the (expected) calving date. In each sampling week, faeces was sampled three days in a row at different time intervals to create a representative sample of average excretion. Timing was around 8.00 and 14.00h on day 1, around 10.00 and 16.00h on day 2 and around 12.00 and 18.00h on day 3, after which these samples (pooled per cow) were stored frozen at -20°C.
Recovery of Ti in faecal samples was used to estimate total faecal production (and P excretion) per day.

2.2.4 Calculations and statistical analysis

Daily feed intake, milk yield, live weight and calculated energy balance were averaged per cow per week relative to calving.
Data was analysed using the software package Genstat 18th edition (2015) by repeated measures ANOVA for the prepartum period and the postpartum period separately. The dry cow diet (Dry100 or Dry185), lactation diet (Lac70 or Lac100) and their interactions were included as fixed factors, and subject within block as random factors. All factors were kept in the model and significant factor effects were determined by the significance value set at $P < 0.05$ and the tendency level at $P < 0.10$.
A chi-square analysis was used to test the difference in the incidence of diseases between dietary treatments.

3 Results and Discussion

3.1 General

One cow (1206) in group D185/L70 received the high P concentrate during lactation with the Lac70 basal diet, due to an administrative mistake. This did not affect total P coverage much, and calculated P balance was negative during the full 8 weeks of lactation (increasing from 75 to 90%). For correct analysis of dietary effects, performance and blood results of this cow in week 1 to 8 postpartum was excluded from all statistical analyses.
One cow (7048) in group D100/L70 suffered from a teat injury in week 7 of lactation. Her results were excluded for week 7 and 8. All other cows completed the trial.

3.2 Rations

During the experimental period of 8 months, grass and maize silage composition varied due to natural variation in harvesting conditions and conservation which affected the dietary P concentration of the roughage mixture.
Next to the roughage mixture which was fed ad libitum, compound concentrates were supplied individually at restricted levels: 1 kg/d marker concentrate precalving, 2 kg/d marker concentrate postcalving, and a lactation concentrate increasing from 1 kg/d to 7.3 kg/d in the first 21 days after calving.
The average P content of the dietary ingredients throughout the study is shown in Table 3.1. Total chemical analysis of the dietary ingredients is reported in Appendix 1.

Table 3.1 *Average P content of dietary ingredients and additional compound concentrates.*

Ingredient	P content
Roughage mixture ingredients (g/kg DM)	
Maize silage	2.3
Grass silage	4.0
Wheat straw	0.7
Soy bean meal	6.8
Premix low P	2.2
Premix high P	31.2
Compound concentrates (g/kg)	
Marker dry period	2.9
Marker lactation	2.9
Lactation concentrate low P	2.3
Lactation concentrate high P	3.2

Chemical analysis of the ration ingredients and actual intake of the roughage mixture combined with the compound concentrates resulted in an average chemical composition and feeding value of the total diets as shown in Table 3.2.

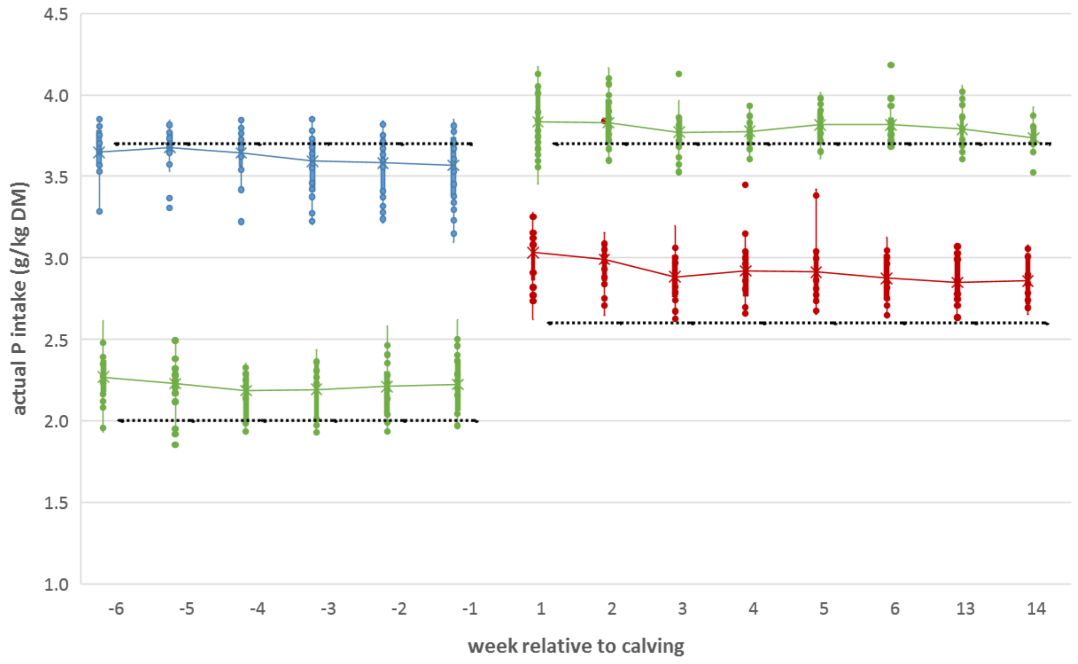
Table 3.2 *Average chemical composition and feeding value of total diet (roughage mixtures and compound concentrates).*

Total diet	Dry100	Dry185	Lac70	Lac100
Parameter				
Chemical composition (g per kg of DM)				
P	**2.2**	**3.6**	**2.9**	**3.8**
OM	926	924	930	931
CP	105	106	150	149
Crude fibre	269	268	183	183
NDF	525	521	374	366
ADF	301	298	214	214
ADL	35	35	25	23
Crude fat	27	27	32	33
Starch	136	136	206	206
Sugars	21	21	41	39
Na	1.6	1.9	1.6	1.7
K	17.5	17.4	16.3	16.2
Mg	2.2	2.4	3.6	3.6
Ca	3.6	3.6	6.2	6.1
S	1.9	1.9	2.2	2.2
Cl	3.5	3.6	3.7	3.3
DCAD (mEq/kg DM)	299	307	248	263
Feeding value (g per kg of DM)				
Digestible OM	631	629	733	730
Fermentable OM	505	503	557	556
NE_L (MJ/kg DM)	5.59	5.58	6.77	6.76
NE_L (VEM/kg DM)	810	808	981	979
DVE* 1991	57	57	93	92
OEB# 1991	-7	-6	7	7
DVE* 2007	49	49	91	90
OEB# 2007	8	10	11	11

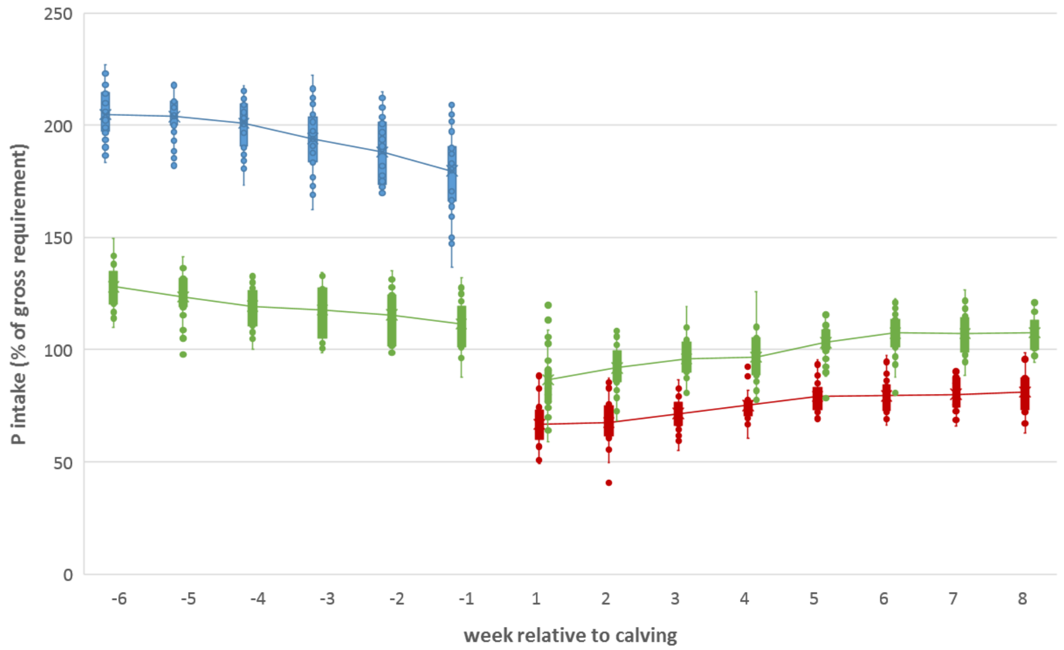
*DVE (Darm Verteerbaar Eiwit): intestinal digestible protein according to CVB.
#OEB (Onbestendig Eiwit Balans): rumen digestible protein balance according to CVB.

As shown in Table 3.2, the dry cow ration at requirement level (Dry100) contained on average 2.2 g/kg DM (planned: 2.0 g/kg DM), while the high P ration (Dry185) contained 3.6 g/kg DM (as planned). The lactation ration at requirement level (Lac100) contained 3.8 g/kg DM (planned: 3.7 g/kg DM) and the ration below P requirement (Lac70) had on average 2.9 g/kg DM (planned: 2.6 g/kg DM).

The average P intake per kg of DM for each individual cow during the trial is displayed in Figure 3.1A, together with the individual P intake relative to the calculated requirements in Figure 3.1B.



A



B

Blue	cows on dry cow ration with P-level above calculated requirement (Dry185)
Green	cows on dry cow and lactation ration with P-level at calculated requirement (Dry100 and Lac100)
Red	cows on lactation ration with P-level below calculated requirement (Lac70)
Black	dotted lines - intended dietary P-levels per ration

Figure 3.1 *Average actual P intake per cow per week, expressed as g/kg DM (A) and as a % of calculated gross P requirement (B). Each dot is an individual cow's week average.*

In Figure 3.1B total average P intake per cow per week is expressed as a percentage of calculated gross P requirement (COMV, 2005). The difference in treatment groups on the calculated P coverage is clearly present, even though dietary intake of P in g/kg DM was higher than planned as displayed in Figure 3.1A.

On average cows on Dry185 received 197% of gross P requirement and Dry100 cows 119%. For lactating animals, P intake was below calculated requirements for all cows on Lac70 diet, receiving on average 74% of gross P requirement; for cows on Lac100 diet, P intake was below calculated requirements in the first 4 weeks (on average 91% coverage) and turned positive in week 5 to 8 (on average 105% coverage).

3.3 Precalving performance

Precalving, a dietary P content of 3.6 vs. 2.2 g/kg DM did not seem to affect dry matter intake or the development of body weight (Table 3.3). In the study of Puggaard et al. (2014), feed intake was not different between dry cows fed a diet with 1.7, 2.1 or 2.5 g P/kg DM. Valk and Šebek (1999) showed a 0.9 kg decrease in feed intake of dry cows fed a dry cow ration with 1.6 g P/kg DM compared to cows fed 2.6 g P/kg DM. This level of P, at 67% of calculated requirements, may affect diet digestibility; in the present study however, the lowest dietary P level was still well above 1.6 g P/kg DM and a difference in feed intake or body weight development is not expected.

Table 3.3 *Performance precalving.*

	Week relative to calving						SED	P-value		
	-6	**-5**	**-4**	**-3**	**-2**	**-1**		**Dry**	**Week**	**Dry×W**
DMI (kg/d)										
Dry185	14.2	14.2	14.8	14.3	13.5	12.1	0.55	0.778	<0.001	0.611
Dry100	14.6	14.2	14.4	14.0	13.2	11.9				
Body weight										
Dry185	783	794	802	814	814	833	16.1	0.363	<0.001	0.113
Dry100	770	777	792	797	810	809				
P intake (% of calculated gross requirement)										
Dry185	205	204	201	194	188	180	3.1	<0.001	<0.001	0.006
Dry100	128	123	119	118	115	112				

Dry185: dry cow ration with high P content (3.6 g/kg DM)
Dry100: dry cow ration with P content at requirement (2.2 g/kg DM)
SED: standard errors of differences of means for Dry×Week
Dry: effect of dry cow diet
Dry×W: effect of interaction of dry cow diet with week

3.4 Postcalving performance

Dry matter intake tended to be affected by the P content of the lactating cow ration over time (Lac x week interaction, P=0.058), due to a slower increase in feed intake in the first 3 weeks for cows on the Lac70 diet (Figure 3.2 or Table 3.4).
Body weight also tended to be affected by the interaction of Lactation diet with week (P=0.094; Table 3.4), with a faster decrease for the Lac70 diet in the first 3 weeks postpartum. Body condition score was unaffected. Milk yield was unaffected by dietary P content during the trial in the first 8 weeks of lactation (Figure 3.3), as well as the calculated energy balance (Table 3.4).

The dietary treatments resulted as expected in a P intake below calculated requirements for the Lac70 diet with 2.9 g P/kg DM; cows receiving the 3.8 g P/kg DM diet (Lac100) also experienced a calculated negative P balance for the first 4 weeks postpartum turning positive in week 5.

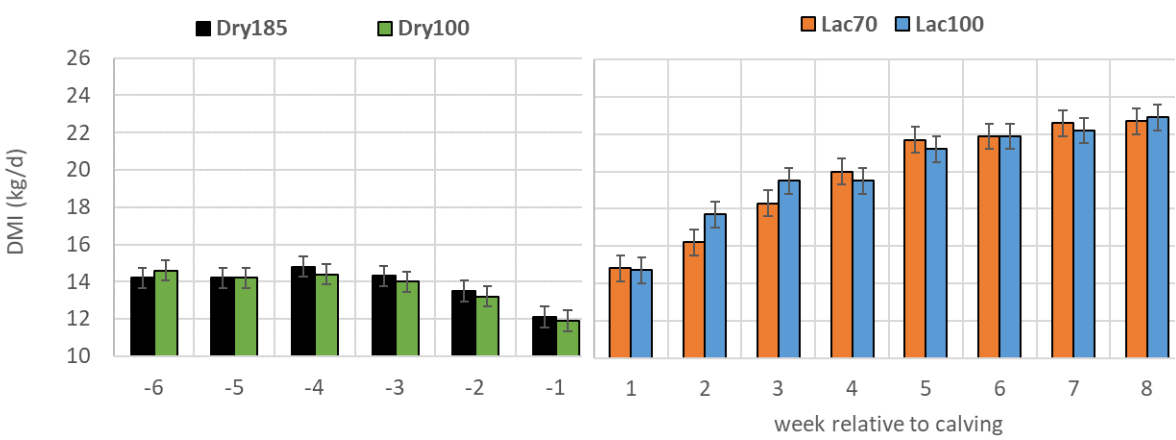


Figure 3.2 *Dry matter intake averaged per ration group (kg DM/cow per day) during dry period (week -6 to -1) and early lactation (week 1 to 8, see also Appendix 2). Error bars representing SED.*

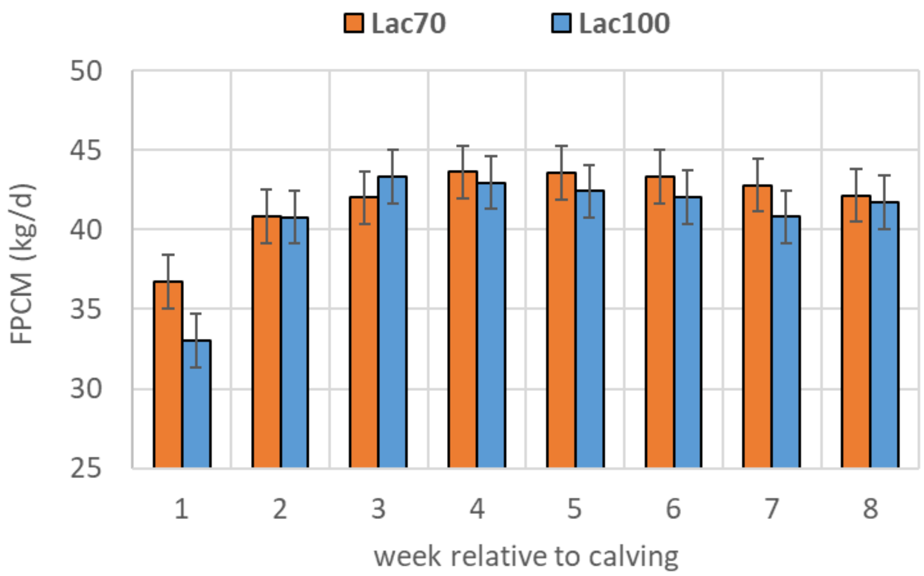


Figure 3.3 *Fat- and protein corrected milk yield averaged per lactation ration group (kg/cow per day) in week 1 to 8 after calving (see also Appendix 2). Error bars representing SED.*

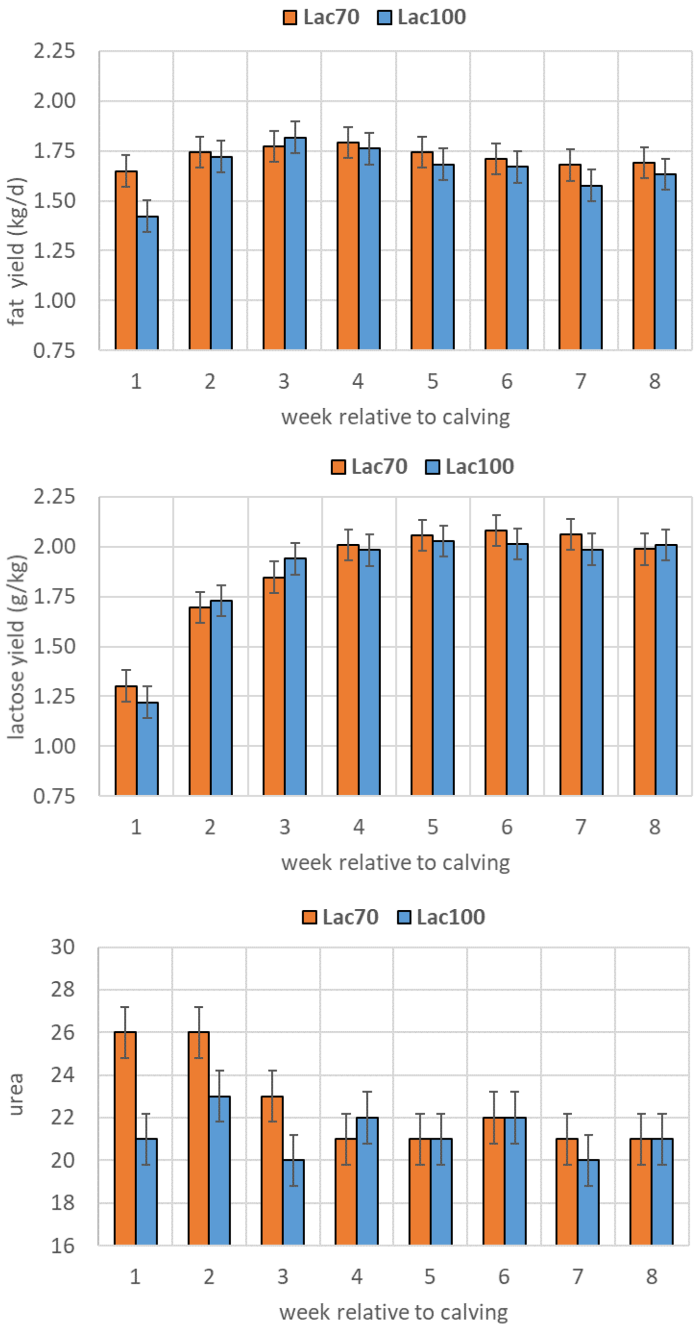
Table 3.4 *Performance postcalving.*

	Week relative to calving								SE	P-value				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	D	**Dr**	**Lac**	**We**	**Dry**	**Lac**
DMI (kg/d)														
	14	15	17	19	20	21	22	22	0.	0.1	0.70	<0.	0.83	0.05
	14	17	19	19	21	22	22	22	97	93	6	001	4	8
	15	16	18	20	22	22	23	23						
	14	17	20	20	21	21	22	23						
Body weight (kg)														
	71	68	67	67	66	67	67	67	19	0.5	0.12	<0.	0.60	0.09
	74	72	71	70	70	70	70	70	.1	57	3	001	8	4
	71	69	68	67	68	68	68	68						
	71	70	69	68	68	67	68	68						
BCS														
	3.	2.	2.	2.	2.	2.	2.	2.	0.	0.4	0.39	<0.	0.25	0.17
	3.	3.	3.	2.	2.	2.	2.	2.	22	08	5	001	6	3
	3.	2.	2.	2.	2.	2.	2.	2.						
	2.	2.	2.	2.	2.	2.	2.	2.						
Milk yield (kg/d)														
	29	37	39	42	43	43	43	42	2.	0.3	0.86	<0.	0.67	0.12
	27	38	41	41	43	43	42	42	37	15	4	001	5	6
	30	38	41	44	45	45	45	43						
	28	38	42	44	44	44	44	44						
FPCM (kg/d)														
	36	40	40	42	42	42	42	41	2.	0.5	0.49	<0.	0.84	0.04
	32	41	44	42	42	42	40	41	36	05	1	001	6	4
	37	41	43	44	44	43	43	43						
	33	40	42	43	42	42	41	42						
Calculated energy balance (kJ/kg$^{0.75}$)														
	-	-	-	-	-	-	-	-	54	0.7	0.26	<0.	0.70	0.11
	-	-	-	-	-	-	-	-	.2	74	5	001	5	4
	-	-	-	-	-	-	-	-						
	-	-	-	-	-	-	-	-						
P intake (% of calculated gross requirement)														
	65	66	71	74	78	77	79	82	3.	0.6	<0.	<0.	0.58	0.03
	88	92	92	96	10	10	10	10	6	06	001	001	4	6
	68	69	72	76	80	81	81	82						
	85	92	10	98	10	10	10	10						

D185/L70: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D185/L100: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
D100/L70: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D100/L100: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
Dry: effect of P content of dry cow ration
Lac: effect of P content of lactation cow ration
Interaction of Dry x Lac and Dry x Lac x Week were not significant for any of the parameters, but were kept in the model

3.5 Milk composition

Fat, protein and lactose content were not significantly affect by the dietary treatments (Table 3.5). Daily yield of fat and lactose was affected (Lac × week interaction, P<0.05; Table 3.5), mainly due to a difference in the first week of lactation (Figure 3.4).
Milk urea content was higher in milk of cows on the diet with 2.9 g P/kg DM compared with the diet with 3.8 g P/kg DM (Table 3.5; Figure 3.4C). This may be related to the lower dry matter intake in the same period.



A

B

C

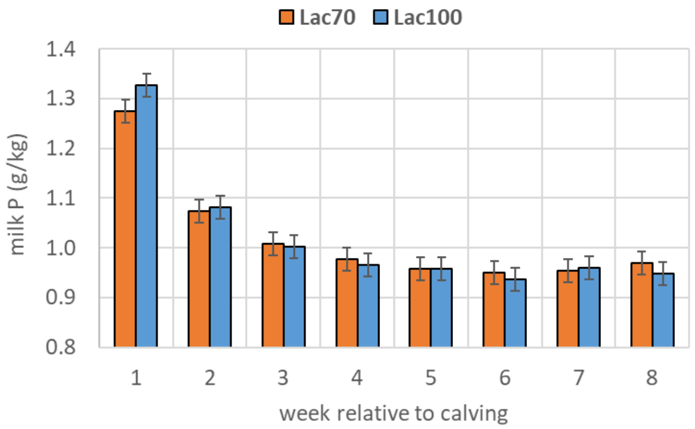
Figure 3.4 *Fat yield (kg/cow per day, A), lactose yield (kg/cow per day, B) and milk urea content (mg/dl, C) averaged per lactation ration group in week 1 to 8 after calving (error bars representing SED).*

Table 3.5 *Milk composition*

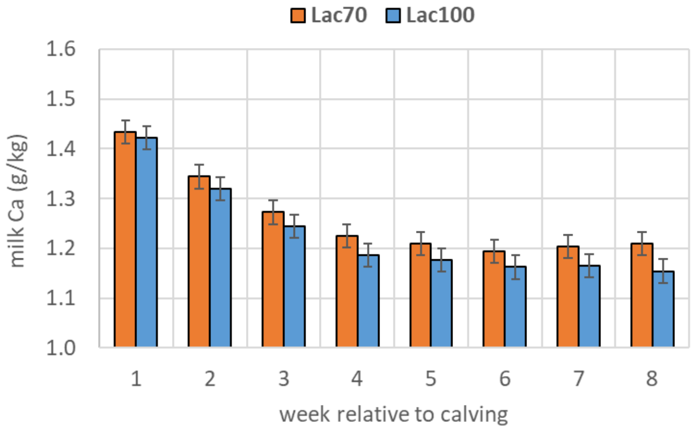
	Week relative to calving								SED	P-value				
	1	2	3	4	5	6	7	8		Dry	Lac	Week	Dry×W	Lac×W
Fat content (g/kg)														
D185/L70	54.2	46.2	45.2	42.3	39.7	39.9	38.6	39.6	2.05	0.229	0.173	<0.001	0.523	0.119
	51.4	46.0	45.2	43.1	39.1	38.7	36.8	38.3						
D100/L70	55.1	47.7	43.7	40.6	39.4	36.8	37.4	39.4						
	49.2	44.0	41.5	39.6	37.7	37.8	36.3	36.8						
Fat yield (kg/d)														
D185/L70	1.63	1.70	1.73	1.77	1.70	1.72	1.68	1.65	0.111	0.807	0.339	<0.001	0.867	0.036
	1.41	1.75	1.88	1.76	1.68	1.68	1.55	1.63						
D100/L70	1.66	1.79	1.81	1.81	1.79	1.70	1.68	1.73						
	1.43	1.69	1.75	1.76	1.68	1.66	1.61	1.64						
Protein content (g/kg)														
D185/L70	39.7	35.1	32.3	31.8	31.4	31.2	31.9	31.6	0.95	0.673	0.358	<0.001	0.779	0.303
	42.0	35.0	32.5	31.4	31.1	30.6	31.0	31.0						
D100/L70	41.0	35.4	33.2	31.8	31.6	31.0	31.0	31.4						
	40.3	34.5	32.0	31.0	30.6	29.9	30.5	30.7						
Protein yield (kg/d)														
D185/L70	1.20	1.31	1.27	1.33	1.35	1.36	1.39	1.32	0.079	0.383	0.491	<0.001	0.774	0.266
	1.17	1.33	1.36	1.31	1.34	1.32	1.30	1.31						
D100/L70	1.24	1.34	1.37	1.41	1.44	1.44	1.40	1.38						
	1.16	1.32	1.35	1.37	1.37	1.32	1.35	1.37						
Lactose content (g/kg)														
D185/L70	42.8	45.2	45.8	46.3	46.3	46.3	46.5	46.1	0.53	0.915	0.617	<0.001	0.714	0.070
	43.2	45.3	46.4	45.9	46.1	45.8	45.8	46.0						
D100/L70	42.7	44.9	45.9	46.4	46.4	46.7	46.4	46.6						
	43.1	45.3	45.9	46.3	46.1	46.0	45.9	45.9						
Lactose yield (kg/d)														
D185/L70	1.30	1.68	1.79	1.94	1.99	2.01	2.03	1.94	0.111	0.276	0.806	<0.001	0.438	0.048
	1.19	1.72	1.94	1.91	1.99	1.99	1.94	1.97						
D100/L70	1.31	1.71	1.90	2.08	2.12	2.15	2.09	2.04						
	1.25	1.74	1.94	2.05	2.07	2.04	2.03	2.05						
Urea (mg/dl)														
D185/L70	25	27	22	20	20	22	21	22	1.7	0.998	0.125	<0.001	0.677	<0.001
	21	23	21	23	22	22	20	21						
D100/L70	27	25	24	22	21	22	21	19						
	20	22	20	22	20	21	21	22						
P content (g/kg)														
D185/L70	1.26	1.06	1.00	0.98	0.97	0.96	0.96	0.97	0.033	0.615	0.905	<0.001	0.218	0.181
	1.30	1.08	1.02	0.98	0.96	0.95	0.98	0.96						
D100/L70	1.29	1.09	1.01	0.97	0.95	0.94	0.94	0.97						
	1.35	1.08	0.98	0.96	0.95	0.92	0.94	0.93						
Ca content (g/kg)														
D185/L70	1.45	1.34	1.28	1.24	1.23	1.21	1.22	1.23	0.033	0.593	0.086	<0.001	0.240	0.526
	1.39	1.31	1.24	1.19	1.18	1.17	1.17	1.17						
D100/L70	1.42	1.35	1.26	1.21	1.19	1.18	1.19	1.19						
	1.46	1.33	1.25	1.18	1.17	1.16	1.16	1.14						
Mg content (g/kg)														
D185/L70	0.12	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.004	0.976	0.106	<0.001	0.084	0.070
	0.13	0.11	0.11	0.10	0.10	0.10	0.10	0.10						
D100/L70	0.13	0.11	0.10	0.10	0.10	0.10	0.10	0.10						
	0.14	0.11	0.11	0.10	0.10	0.10	0.10	0.10						

D185/L70: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D185/L100: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
D100/L70: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D100/L100: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
Dry: effect of P content of dry cow ration
Lac: effect of P content of lactation cow ration
Interaction of Dry x Lac and Dry x Lac x Week were not significant for any of the parameters

The concentration of the three minerals P, Ca and Mg in milk was highest in week 1 postpartum and decreased in 3-4 weeks to a relatively stable level. The average concentration of P in milk was 0.97 g/kg milk in week 5 to 8 postpartum; milk P concentration was not affected by the dietary concentration of P (Table 3.5, Figure 3.5). Milk Ca however tended to be higher for cows receiving the low P diet (Lac70) as shown in Figure 3.5 and Table 3.5 (P=0.086).



A



B

Figure 3.5 *Milk P concentration (A) and Ca concentration (B) in g/kg milk, averaged per lactation ration group in week 1 to 8 after calving (error bars representing SED).*

3.6 Blood parameters

In the dry period, plasma P concentration did not differ between cows fed a dry cow diet containing 2.2 g P/kg DM (Dry100) compared with a high P diet with 3.6 g P/kg DM (Dry185). The Ca concentration however was slightly lower for animals on the high P diet, resulting in a the lower Ca to P ratio (P<0.05; Table 3.6) Ca concentration was still within the reference range for normocalcemia. Plasma NEFA and BHB are relatively low, suggesting little lipid mobilisation prepartum (Table 3.6). Concentrations of vitamin D_3 and PTH were not different between treatments, but CTX concentration increases towards calving for cows on a high P diet (Dry185), indicating bone mobilisation (Dry × week interaction, P<0.01; Table 3.6)

Table 3.6 *Blood results prepartum.*

	Week relative to calving			SED	P-value		
	-4	-2	-1		Dry	Week	Dry×W
P (mmol/L)							
Dry185	1.75	1.74	1.75	0.052	0.146	0.603	0.468
Dry100	1.65	1.70	1.69				
Ca (mmol/L)							
Dry185	2.34	2.32	2.35	0.040	0.046	0.817	0.363
Dry100	2.42	2.42	2.40				
Ca:P ratio							
Dry185	1.34	1.35	1.35	0.048	0.017	0.751	0.576
Dry100	1.47	1.44	1.44				
NEFA (mmol/L)							
Dry185	0.09	0.12	0.22	0.028	0.372	<0.001	0.520
Dry100	0.08	0.11	0.18				
BHB (mmol/L)							
Dry185	0.49	0.52	0.53	0.038	0.402	0.670	0.067
Dry100	0.57	0.53	0.50				
Vitamin D_3 (ng/mL)							
Dry185	20.1	24.4	22.0	2.64	0.494	0.237	0.194
Dry100	24.9	24.3	21.0				
PTH (pg/mL)							
Dry185	320	331	368	143.8	0.487	0.011	0.565
Dry100	214	247	258				
CTX (ng/mL)							
Dry185	0.31	0.47	0.53	0.136	0.726	0.238	0.008
Dry100	0.44	0.36	0.37				

Dry185: dry cow ration with high P content (3.6 g/kg DM)
Dry100: dry cow ration with P content at requirement (2.2 g/kg DM)
Dry: effect of P content of dry cow ration

Interestingly, the dry cow diet also affected plasma parameters after calving. Cows on the dry cow diet with 3.6 g P/kg DM (Dry185) had on average a lower plasma P concentration (1.31 vs. 1.40 mmol/L) and a lower plasma Ca concentration (2.20 vs. 2.29 mmol/L) postpartum than cows on the dry cow diet Dry100 with 2.2 g P/kg DM (P<0.05, Table 3.7 and Figure 3.6).
Feeding a high P diet in the dry period seems to be negatively affecting the Ca and P homeostasis compared with the normocalcemic dry cow ration, as was also shown in the study of Cohrs et al. (2018).

Plasma P gives an indication of the non-bone, extracellular P status but about 99% of non-bone P is present intracellular (Grünberg, 2014). Changes in the balance between intracellular and extracellular P may occur suddenly and affect plasma P. The decrease in plasma P around parturition as found in this study (from 1.7 mmol/L precalving to 1.2 mmol/L at d0) is a common phenomenon in dairy cattle. It is not only due to the loss of P through milk production, as the same drop around parturition also occurs in mastectomized cows (Goff et al., 2002). The decrease 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 (Grünberg, 2014).

Table 3.7 *Blood results postpartum*

	Day relative to calving									SED	P-value				
	0	**1**	**2**	**3**	**7**	**14**	**21**	**28**	**56**		**Dry**	**Lac**	**Week**	**Dry×W**	**Lac×W**
P (mmol/L)															
D185/L70	1.32	1.38	1.36	1.29	0.75	0.98	1.24	1.34	1.31	0.104	0.026	<0.001	<0.001	0.306	<0.001
	1.17	1.21	1.30	1.34	1.49	1.54	1.47	1.52	1.51						
D100/L70	1.23	1.28	1.22	1.14	1.11	1.27	1.39	1.36	1.35						
	1.16	1.41	1.63	1.64	1.54	1.54	1.60	1.64	1.64						
Ca (mmol/L)															
D185/L70	1.85	1.89	2.12	2.38	2.34	2.42	2.40	2.28	2.47	0.091	0.004	0.010	<0.001	0.388	0.321
	1.81	1.74	2.02	2.20	2.30	2.34	2.33	2.33	2.29						
D100/L70	2.05	2.16	2.25	2.37	2.39	2.38	2.44	2.44	2.44						
	1.93	1.88	2.07	2.39	2.28	2.40	2.45	2.40	2.44						
Ca:P ratio															
D185/L70	1.47	1.45	1.71	2.03	3.49	2.75	2.02	1.73	2.23	0.191	0.079	<0.001	<0.001	<0.001	<0.001
	1.66	1.58	1.64	1.69	1.59	1.54	1.60	1.58	1.53						
D100/L70	1.69	1.71	1.90	2.14	2.26	1.91	1.84	1.84	1.86						
	1.95	1.45	1.30	1.50	1.47	1.58	1.55	1.50	1.51						
NEFA (mmol/L)															
D185/L70	0.94	0.93	0.67	0.65	0.59	0.44	0.37	0.25	0.14	0.110	0.272	0.611	<0.001	0.504	0.527
	0.99	0.74	0.67	0.69	0.56	0.39	0.38	0.32	0.17						
D100/L70	0.78	0.66	0.71	0.65	0.62	0.39	0.38	0.26	0.21						
	0.92	0.67	0.66	0.59	0.47	0.28	0.29	0.25	0.13						
BHB (mmol/L)															
D185/L70	0.67	0.87	1.07	0.79	0.90	0.68	0.69	0.70	0.71	0.150	0.774	0.260	0.004	0.365	0.156
	0.62	0.80	0.88	0.83	0.82	0.71	0.86	0.99	0.80						
D100/L70	0.53	0.65	0.73	0.82	1.01	0.66	0.71	0.66	0.81						
	0.54	0.83	0.89	0.80	0.80	0.93	0.82	0.90	0.99						
Vitamin D_3 (ng/L)															
D185/L70	20.2	21.3	19.8	23.0	22.6	20.6	19.6	18.3	16.0	4.65	0.673	0.621	0.060	0.557	0.433
	21.5	25.5	24.0	23.4	21.1	20.5	19.2	20.1	19.1						
D100/L70	21.3	34.1	20.8	34.0	21.2	20.1	18.6	18.0	17.3						
	20.2	22.0	22.1	21.3	20.0	20.0	18.2	18.1	18.6						
PTH (pg/mL)															
D185/L70	399	374	235	211	127	83	85	102	147	138.7	0.632	0.909	<0.001	0.305	0.770
	611	520	501	405	250	210	176	216	355						
D100/L70	492	502	422	363	280	196	160	150	202						
	265	269	233	150	138	112	103	105	95						
CTX (ng/mL)															
D185/L70	1.08	1.26	1.19	1.28	1.99	2.64	2.97	3.14	3.12	0.340	0.160	0.007	<0.001	0.122	<0.001
	0.92	0.95	1.27	1.24	1.16	1.55	1.62	1.92	2.11						
D100/L70	0.95	1.15	1.00	1.14	1.42	2.42	2.52	2.52	2.07						
	0.71	1.00	0.99	0.88	1.11	1.46	1.75	1.77	1.64						

D185/L70: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D185/L100: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
D100/L70: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D100/L100: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
Dry: effect of P content of dry cow ration
Lac: effect of P content of lactation cow ration
Interaction of Dry x Lac is not significant for any of the parameters; interaction Dry x Lac x Week is only significant for plasma P (P=0.002)

The dietary P concentration of the lactation diet also affected plasma P and Ca concentration. Cows consuming the low P lactation diet (Lac70) with 2.9 g P/kg DM had on average a lower plasma P concentration (1.24 vs. 1.46 mmol/L) and a higher plasma Ca concentration (2.28 vs. 2.20 mmo/L) in the first 8 weeks of lactation than cows consuming the high P lactation diet with 3.8 g P/kg DM (Lac100). For plasma P, the interaction of the dry cow diet × lactation diet × week was significant with the strongest decrease after calving for cows receiving the Dry185 with Lac100 combination, and most

stable plasma P for cows receiving the Dry100 with Lac100 combination (see also Appendix 2, Figure A2.3).
The plasma Ca to P ratio was affected most clearly with a relatively stable ratio for cows on the high P diet, while cows on the low P diet had an increased Ca to P ratio with highest level 7 days postpartum.

Vitamin D_3 and PTH do not seem to be good indicators for changes in P metabolism postpartum. In contrast to the changes in plasma P and Ca, vitamin D_3 and PTH concentration are not affected by the dietary treatments prepartum or postpartum (Table 3.7). In the study of Cohrs et al. (2018), PTH was significantly higher in control cows compared with P-deprived cows. In this study however, P-deprivation was started in the dry period and continued into the lactation, which may be different in our study were all dry cows were fed above calculated P requirements.

The effect of the dietary changes on bone mobilisation is shown in CTX concentration, which is increased for animals on Lac70, the low P diet postpartum (Table 3.7).

Postpartum, plasma NEFA and BHB do not seem to be affected by the dietary treatments (Table 3.7). This was expected, as energy balance does not seem te be affected either (Table 3.3), suggesting no strong differences in lipid mobilisation due to the dietary treatments.

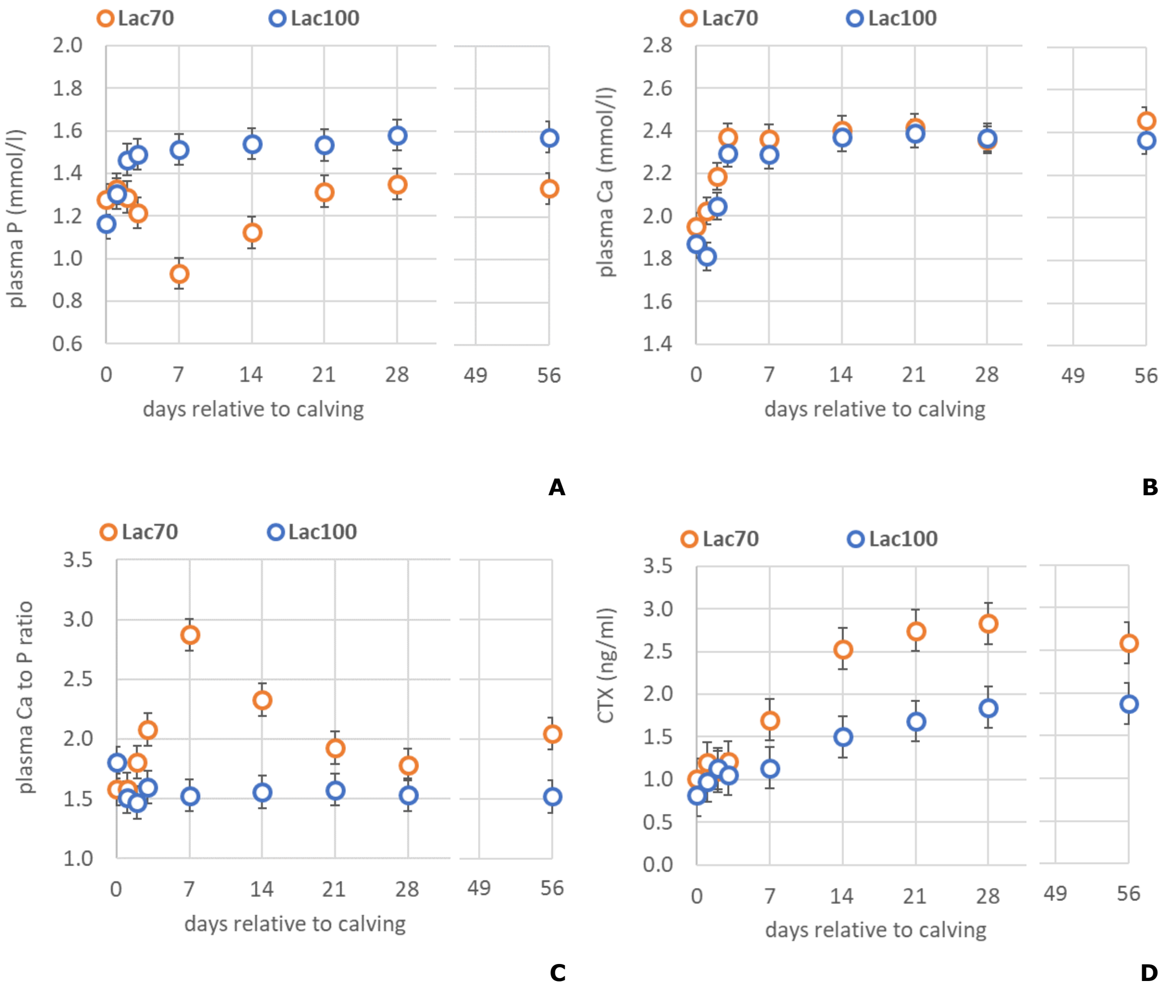


Figure 3.6 *Plasma P concentration (mmol/L; A), Ca concentration (mmol/L; B), Ca to P ratio and plasma CTX concentration (ng/mL, D), averaged per lactation ration group for blood sampling moments on day 0, 1, 2, 3, 7, 14, 21, 28 and 56 after calving (error bars representing SED).*

3.7 Health

In case a cow showed at least 2 out of 3 symptoms of clinical hypocalcemia around calving (i.e. 'recumbency / not able to stand', 'cold ears' and 'low rumen fill with no contractions'), animal caretakers treated that cow intravenously with a CaMg infusion (450 mL 1.65% Ca and 0.95% Mg).

During the trial, 15 cows were treated for clinical hypocalcemia (Table 3.8a) and all treatments were successful; cows recovered after single treatment. The incidence of clinical hypocalcemia in the four treatment groups (Table 3.8a) or between the experimental diets (Table 3.8b) was not significantly different. The total incidence of cows treated for clinical symptoms of hypocalcemia in this trial (25%) seems relatively high for common dairy practice; it is however comparable to the average incidence in multiparous cows at the research farm in 2016 and 2017 (22%).

When looking at the blood plasma Ca levels measured at d0 and d1, a total of 21 cows (36%) had Ca levels below 1.8 mmol/L – with or without clinical signs of hypocalcemia (Table 3.8). The number of animals with low plasma Ca values (either below 2.0, 1.9 or 1.8 mmol/L) was significantly lower in treatment group D100/L70 compared to the other three treatment groups. This suggests that a low level of P in dairy cow rations in the transition period (in the dry period as well as lactation) reduces the risk for hypocalcemia (Table 3.8a; $P < 0.05$). The same result was found for the incidence of low plasma Ca on the two dry cow diets as well as the incidence with the two lactation diets (Table 3.8b). The effect of the lactation diets cannot have been large however, as cows were provided the dry cow diets until calving. Hypocalcemia was defined at d0 and d1, when cows had consumed the lactation diets for only 1 to 2 days.

Table 3.8a *Hypocalcemia incidence per treatment group including P-value for chi-square analysis. For the various cut-off levels of plasma Ca defining hypocalcemia, the number of cows that was treated for clinical signs within this group of animals is shown between brackets.*

	D185/L70 (n = 14)	D185/L100 (n = 15)	D100/L70 (n = 15)	D100/L100 (n = 15)	P-value
Clinical hypocalcemia					
Animals treated for clinical signs	4	5	2	4	0.629
Total hypocalcemic animals					
Plasma Ca < 2.0 mmol/L	12 (4)	13 (5)	5 (1)	12 (4)	0.003
Plasma Ca < 1.9 mmol/L	9 (3)	11 (5)	3 (1)	11 (4)	0.007
Plasma Ca < 1.8 mmol/L	5 (3)	7 (4)	1 (1)	8 (2)	0.040

D185/L70: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D185/L100: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
D100/L70: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D100/L100: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)

Table 3.8b *Hypocalcemia incidence per diet including P-value for chi-square analysis. For the various cut-off levels of plasma Ca defining hypocalcemia, the number of cows that was treated for clinical signs within this group of animals is shown between brackets.*

	Dry100 (n = 30)	Dry185 (n = 29)	P-value	Lac70 (n = 29)	Lac100 (n = 30)	P-value
Clinical hypocalcemia						
Animals treated	6	9	0.330	6	9	0.412
Total hypocalcemic animals						
Plasma Ca < 2.0 mmol/L	17 (5)	25 (9)	0.012	17 (5)	25 (9)	0.036
Plasma Ca < 1.9 mmol/L	14 (5)	20 (8)	0.083	12 (4)	22 (9)	0.013
Plasma Ca < 1.8 mmol/L	9 (3)	12 (7)	0.361	6 (4)	15 (6)	0.019

Dry100: dry cow ration with P content at requirement (2.2 g/kg DM)
Dry185: dry cow ration with high P content (3.6 g/kg DM)
Lac70: lactation ration with low P content (2.9 g/kg DM)
Lac100: lactation ration with P content at requirement (3.8 g/kg DM)

As a common finding, plasma P is reported to be low in cows with clinical signs of hypocalcemia in practice. When comparing plasma P concentrations of the 15 treated cows around calving with the non-treated animals, a low plasma P may be noted at d0 (Figure 3.7B). This is likely related to the reduced feed intake accompanying the clinical hypocalcemia around d0 (Figure 3.7A) and does not seem to be involved in the mechanism of periparturient paresis. Precalving, plasma Ca is already decreasing while plasma P is stable or even higher for the group of cows with clinical hypocalcemia.

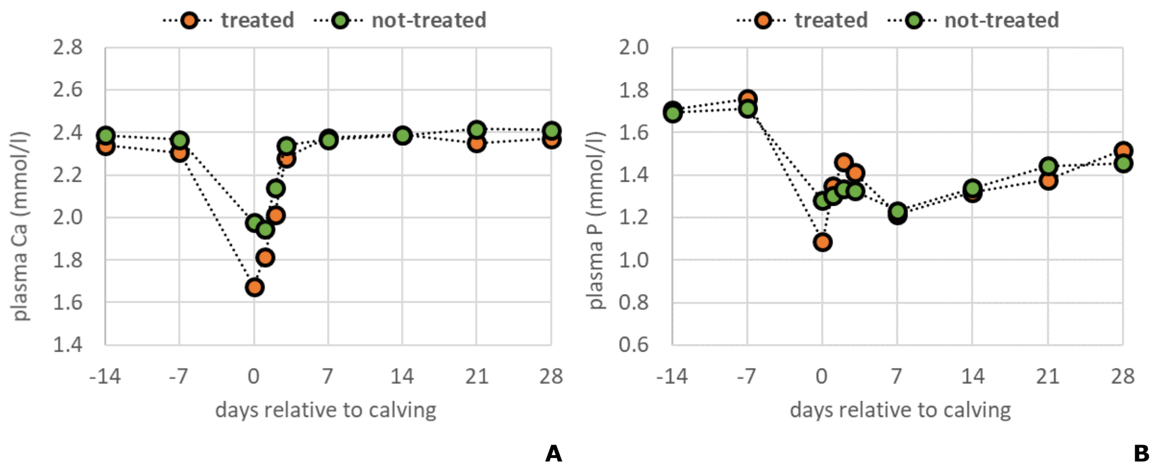


Figure 3.7 *Plasma P concentration (mmol/L; A) and Ca concentration (mmol/L; B) averaged for cows treated for clinical hypocalcemia compared with the non-treated cows for blood sampling moments 2 and 1 week before calving as well as on day 0, 1, 2, 3, 7, 14, 21 and 28 after calving.*

For other diseases, no differences were found between treatment groups (P > 0.10; Table 3.9).

Table 3.9 *Incidence of diseases other than hypocalcemia, per treatment group (n = 15)*

	D185/L70	D185/L100	D100/L70	D100/L100
Generalized conditions				
Fever	1	2	2	1
Udder health				
Clinical mastitis	1	1	1	2
*Reproduction**				
Retained placenta	1	1	2	0
Acute endometritis (<14d, fever)	3	1	1	0
Chronic endometritis (>14d)	7	6	4	8
Pyometra	3	2	1	0
Cystic Ovaries	1	1	0	1
Claw health				
Laminitis	1	0	0	1
Digital dermatitis	0	2	0	2
Interdigital dermatitis	0	0	0	1
Interdigital phlegmona	0	1	0	0
White line disease	0	0	0	1
Sole ulcer	0	1	0	0
Intestines				
Abomasal displacement	0	1	0	0
Hardware disease	1	0	0	0
Diarrhea	0	0	1	0
Indigestion	0	0	0	1

D185/L70: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D185/L100: dry cow ration Dry185 (high P content, 3.6 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
D100/L70: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac70 (low P content, 2.9 g/kg DM)
D100/L100: dry cow ration Dry100 (P content at requirement, 2.2 g/kg DM) and lactation ration Lac100 (P content at requirement, 3.8 g/kg DM)
*Reproduction: retained placenta and acute endometritis were determined in the clinical phase. Other diagnoses (chronic endometritis, pyometra, cystic ovaries) were subclinical and diagnosed at the regular examination of fresh cows, 4 to 8 weeks after calving.

3.8 Feed digestibility and P excretion

The apparent faecal digestibility of NDF, OM and P were determined as well as the apparent P uptake (daily P intake minus faecal P excretion) and the apparent P uptake relative to the calculated net P requirements. Averages are shown for the different diets in Table 3.10 (prepartum diets) and Table 3.11 (postpartum diets).

Table 3.10 *Apparent faecal digestibility of the two dry cow diets, P excretion and apparent retention prepartum.*

	week		SED	P value		
	-4	-2		Dry	Week	Dry×W
NDF digestibility (%)						
Dry185	55	50	3.3	0.499	0.066	0.487
Dry100	52	50				
OM digestibility (%)						
Dry185	59	56	2.8	0.389	0.277	0.429
Dry100	55	55				
P digestibility (%)						
Dry185	13	12	6.5	0.719	0.115	0.116
Dry100	4	16				
P excretion (g/d)						
Dry185	47	42	2.8	<0.001	<0.001	0.875
Dry100	30	24				
Gross retention: P intake – P excretion (g/d)						
Dry185	7	7	2.6	0.161	0.308	0.286
Dry100	2	4				
Net retention: gross retention – calculated net P requirement for gestation (g/d)						
Dry185	2	1	2.6	0.161	0.482	0.286
Dry100	-3	-1				

Dry185: dry cow ration with high P content (3.6 g/kg DM)
Dry100: dry cow ration with P content at requirement (2.2 g/kg DM)
Dry: effect of P content of dry cow ration

During the dry period, there was no effect of dietary P content on the apparent faecal digestibility of NDF or OM, suggesting that a reduced P content of 2.2 vs. 3.6 g P/kg DM did not negatively affect fermentation or digestion processes. For both dietary treatment groups, apparent P digestibility was relatively low (on average 11%); there was no effect of dietary P content on P digestibility. In a long term study with reduced P intake in the dry period and following lactation, even a negative P digestibility were found in the last four weeks before calving (Elizondo Salazar et al., 2012).
The daily excretion of P with faeces was, as expected, higher on a high P diet (3.6 g P/kg DM) compared to the low P diet (2.2 g/kg DM) and decreased towards calving parallel to the decrease in dry matter intake.

Daily P intake in the same weeks was on average 51 g/d for the Dry185 diet containing 3.6 g P/kg DM and 30 g/d for the Dry100 diet containing 2.2 g P/kg DM. The difference between daily P intake and P excretion was on average 5 g P per day and comparable between diets. When the calculated net P requirement for gestation (COMV, 2005) was subtracted from the gross retention, the calculated net retention was on average 0 g/kg DM for both groups. This suggests the high P diet with 3.6 g P/kg DM (Dry185) did not result in an additional accumulation of P in body reserves in the cows selected for our trial, which may have already been in a positive P balance at the start of the experiment.

Table 3.11 *Apparent faecal digestibility postpartum.*

	week				SED	P value		
	2	4	6	8		Lac	Week	Lac×W
NDF digestibility								
Lac70	58	58	51	52	2.7	0.901	0.014	0.236
Lac100	55	57	55	54				
OM digestibility (%)								
Lac70	66	66	61	62	1.9	0.623	0.070	0.097
Lac100	65	66	66	65				
P digestibility (%)								
Lac70	50	49	40	45	3.4	0.110	<0.001	0.398
Lac100	46	41	40	40				
P excretion (g/d)								
Lac70	25	33	38	38	2.9	<0.001	<0.001	0.371
Lac100	39	43	51	55				
Gross retention: P intake – P excretion (g/d)								
Lac70	26	30	26	30	2.1	0.070	0.094	0.356
Lac100	31	31	33	35				
Net retention: gross retention – milk P excretion (g/d)								
Lac70	-16	-13	-16	-12	4.1	0.008	0.079	0.355
Lac100	-10	-10	-8	-6				

Lac70: lactation ration with low P content (2.9 g/kg DM)
Lac100: lactation ration with P content at requirement (3.8 g/kg DM)
Lac: effect of P content of lactation ration
The effect of dry cow diet Dry nor its interactions with the other factors (Dry x Week, Dry x Lac, Dry x Lac x Week) are significant (P > 0.10)

After calving, the apparent digestibility of NDF and P decreased significantly over time (Table 3.11). This is likely related to the increasing dry matter intake in the same period, from 17 kg DM in week 2 up to 23 kg DM in week 8 (Table 3.3). There was no effect of diet on digestibility, suggesting the reduction in P content from 3.8 to 2.9 g P/kg DM did not negatively affect fermentation or digestion processes. This is in agreement with the study of Elizondo Salazar (2012), showing no differences in diet digestibility at 4.3, 3.6 or 2.9 g P/kg DM.

The daily excretion of P with faeces was in the first 8 weeks of lactation higher on the Lac100 diet compared to the Lac70 diet (47 vs. 34 gram per day) and increased with time, parallel to the increase in dry matter intake. The difference between daily P intake and P excretion was on average 30 g P per day; cows on the Lac70 diet with 2.9 g P/kg DM tended to have a lower gross P retention (28 vs. 32 g P per day, P=0.070).

When the calculated net P excretion with milk was subtracted from the gross retention, the calculated net retention was negative suggesting cows on both diets must have mobilized P body reserves (Table 3.11). Net retention was on average lower for the Lac70 diet compared to Lac100 (-14 vs. -9 g P per day) and tended to increase in both groups towards the 8th lactation week (P=0.079).

The P excretion with milk was also expressed as a relative value of total P intake, as an indicator for P efficiency. As expected, the P efficiency was clearly higher for cows fed the Lac70 diet (P<0.001, Figure 3.8). The P content of the dry cow diet did not affect P efficiency (P>0.10).

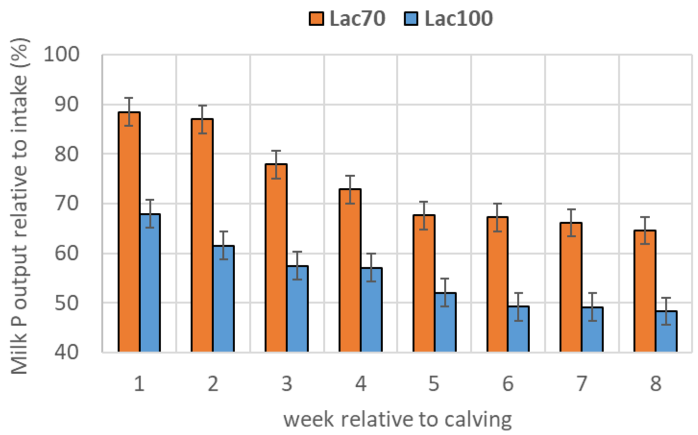


Figure 3.8 *Milk P output relative to dietary P intake (P efficiency ,%) averaged per lactation ration group in week 1 to 8 after calving (error bars representing SED).*

4 Conclusions

In the dry period (final 6 weeks before calving) a dietary P content above calculated requirements (3.6 g P/kg DM) does not affect feed intake, milk yield or milk composition of dairy cows in the first 8 weeks after calving compared to a dry cow diet at P requirements (2.2 g P/kg DM). Extra P intake does not result in any additional accumulation of P in body reserves, but it may negatively affect calcium homeostasis and increase the risk for (subclinical) hypocalcemia postpartum.

A reduction of the dietary P content in lactation diets below the calculated requirements (2.9 g P/kg DM) reduced average plasma P concentration and increased plasma Ca concentration in the first 8 weeks postpartum compared to a diet at requirements (3.8 g P/kg DM). There were no effects of the reduced P intake on feed intake, milk yield, milk composition or animal health.

A reduction of the P content of dry cow or lactation diets does not seem to negatively affect rumen fermentation or digestibility of the diets, as the apparent NDF and OM digestibility remained unaffected.

The P requirements for dry cows may be reconsidered, as the apparent P digestibility still seem to be rather low when feeding at the current requirements. The calculated net retention of P (corrected for the net P requirements for gestation) was around 0, suggesting no body mobilisation or accumulation during the dry period. A further reduction of P in dry cow diets may be possible to reduce environmental P excretion without negatively affecting health or cow performance. Moreover, overfeeding P during the dry period may negatively affect calcium homeostasis increasing the risk for hypocalcemia during early lactation.

In the first 8 weeks postpartum, calculated body mobilisation of P was on average 14 gram per day for the low P diet (2.9 g P/kg DM) and 9 gram per day for the diet aimed at requirements (3.8 g P/kg DM) to support lactation. The efficiency of P utilisation for milk P production was 19 % units higher for the low P diet (74% vs. 55%).

Even though P balance was negative and body mobilisation was on average 14 gram P per day in cows fed the low P lactation diet (2.9 g P/kg DM), the excretion with faeces was still around 34 gram P per day. This rate of P mobilisation hence seems to be possible without negatively affecting health or performance, but it requires accumulation of P later in lactation to replenish the body P reserves. The preferred maximal duration of a period with significant P mobilisation requires further research.

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Appendix 1 Chemical analysis and feeding value of dietary ingredients

Table A1.1 *Grass silages per batch, including relative contribution (intake per batch relative to total grass silage intake) during the trial.*

Batch	GK_2016_12	GK_2016_13	GK_2016_4	GK_2016_16	GK_2016_14	GK_2016_1B
Feed management						
Period fed (2017)	Jan	Feb-Mar	Apr	May-Jun	Jul-Aug	Sep
Batch contribution relative to total intake	3%	24%	19%	34%	17%	3%
Parameter (g/kg DM, unless stated differently)						
DM (g/kg)	317	284	257	468	302	429
Chemical analysis						
P	3.0	3.5	3.6	4.1	4.7	5.4
OM	900	899	897	876	873	870
CP	84	99	97	119	130	152
Crude fibre	337	325	345	292	278	225
NDF	603	600	623	527	512	469
ADF	359	350	374	326	301	260
ADL	44	32	36	45	28	23
Crude fat	29	34	38	40	48	52
Starch	-	-	-	-	-	-
Sugars	36	20	16	60	26	49
Na	1.5	1.5	1.8	1.8	3.0	3.4
K	28.2	30.6	30.2	32.4	34.4	33.2
Mg	1.4	1.5	1.5	1.7	2.1	2.4
Ca	4.4	4.7	4.8	6.1	7.0	6.1
S	2.1	2.5	2.9	3.3	3.8	5.4
Cl	10.8	11.3	10.7	10.0	12.2	8.5
DCAD (mEq/kg DM)	352	374	369	421	427	421
Feeding value						
Digestible OM	642	653	613	625	659	667
Fermentable OM	531	523	469	514	515	528
NE_L (MJ/kg DM)	5.6	5.7	5.4	5.6	6.1	6.3
NE_L (VEM/kg DM)	805	833	776	805	877	906
DVE* 1991	39	45	37	54	53	67
OEB# 1991	-26	-9	5	-5	14	24
DVE* 2007	30	33	28	47	43	56
OEB# 2007	3	21	29	17	39	50

*DVE (Darm Verteerbaar Eiwit): intestinal digestible protein according to CVB.
#OEB (Onbestendig Eiwit Balans): rumen digestible protein balance according to CVB.

Table A1.2 *Maize silages per batch, including relative contribution (intake per batch relative to total maize silage intake) during the trial; as well as the average composition of soy bean meal and wheat straw during the total trial.*

Batch	MK_2016_15	MK_2016_3	MK_SLURF	MK_BAAL	Soy bean meal	Wheat straw
Feed management						
Period fed (2017)	Jan-Mar	Apr-May	Jun-Aug	Sep		
Batch contribution relative to total intake	20%	25%	51%	3%		
Parameter (g/kg DM, unless stated differently)						
DM (g/kg)	366	336	348	342	867	883
Chemical analysis						
P	2.5	2.5	2.1	2.1	6.9	0.7
OM	952	948	965	964	931	932
CP	52	48	63	61	494	22
Crude fibre	186	192	194	197	57	410
NDF	379	392	380	374	143	775
ADF	201	214	214	221	81	450
ADL	29	25	31	31	11	48
Crude fat	34	34	34	34	19	11
Starch	358	341	334	339	35	-
Sugars	-	-	-	-	112	-
Na	0.1	0.1	0.1	0.1	0.2	0.5
K	13.5	13.4	12.4	12.4	24.4	14.4
Mg	1.1	1.3	1.1	1.1	3.8	0.8
Ca	1.8	2	1.9	1.9	3.6	3.2
S	1	1	1.0	1	4.3	1.1
Cl	2.4	2.9	2.1	2.1	0.5	0.0
DCAD (mEq/kg DM)	220	203	200	200	350	321
Feeding value						
Digestible OM	740	726	719	725	847	481
Fermentable OM	529	522	517	519	626	460
NE_L (MJ/kg DM)	6.8	6.7	6.6	6.7	8.0	3.8
NE_L (VEM/kg DM)	993	970	954	964	1157	549
DVE* 1991	48	45	48	48	271	14
OEB[#] 1991	-44	-46	-35	-36	177	-57
DVE* 2007	47	45	48	48	256	-7
OEB[#] 2007	-43	-45	-35	-37	193	-25

*DVE (Darm Verteerbaar Eiwit): intestinal digestible protein according to CVB.
[#]OEB (Onbestendig Eiwit Balans): rumen digestible protein balance according to CVB.

Table A1.3 *Ingredients and composition of premixes (added to the mixed roughage diets) and compound concentrates (fed individually in transponder-controlled automatic feeders).*

Batch	Premix_High	Premix_Low	Lactation_Norm	Lactation_Low	Marker_dry	Marker_lact
Ingredients (%)						
potato protein	63.30	67.87	-	-	-	9.45
sugar beet pulp	-	-	27.41	27.41	45.41	22.50
soya bean hulls	-	-	24.82	25.00	-	22.65
soya bean meal, solvent extracted	-	-	16.44	16.44	34.01	-
wheat	-	-	-	-	-	28.39
maize	-	-	20.00	20.00	-	
urea	13.19	14.14			-	3.75
palm kernel expeller	-	-	3.92	3.93	-	-
molasses, sugarcane	3.56	3.77	5.45	5.45	13.83	5.00
sugar	-	-	-	-	3	-
palm oil	-	-	0.94	0.94	1.77	-
MgO	2.67	2.90	0.24	0.24	-	2.01
chalk	-	-	0.27	0.27	-	5.00
NaCl	-	-	0.10	0.32	-	-
vitamin and mineral premix	4.45	4.71	-	-	1.97	0.81
Na_2CO_3	-	6.60	-	-	-	-
NaH_2PO_4	12.84	-	0.41	-	-	0.45
Parameter (g/kg DM, unless stated differently)						
DM (g/kg)	896	891	867	866	876	884
Chemical analysis						
P	31.2	2.2	3.7	2.7	3.3	3.3
OM	819	848	936	934	912	870
CP	863	942	166	164	238	285
Crude fibre	5	5	159	156	86	128
NDF	152	183	321	338	200	298
ADF	68	115	207	201	117	168
ADL	13	22	10	14	9	15
Crude fat	10	12	36	32	41	13
Starch	22	17	132	138	98	164
Sugars	22	23	93	96	196	67
Na	26.2	22.9	2.5	2.6	1.5	1.4
K	8.3	8.7	14.6	14.8	17.4	12.1
Mg	24.8	24.0	3.9	4.3	3.0	12.9
Ca	6.7	6.7	6.8	7.4	7.9	27.5
S	7.7	7.7	2.6	2.6	4.2	2.8
Cl	1.2	1.3	2.2	3.7	3.0	1.3
DCAD (mEq/kg DM)	834	702	260	219	162	154
Feeding value						
Digestible OM	563	606	800	802	790	717
Fermentable OM	373	403	627	628	677	646
NE_L (MJ/kg DM)	5.3	5.8	7.6	7.6	7.6	6.6
NE_L (VEM/kg DM)	774	835	1098	1100	1106	953
DVE* 1991	350	376	134	135	148	128
OEB[#] 1991	545	588	-18	-18	28	106
DVE* 2007	350	376	134	135	148	128
OEB[#] 2007	545	588	-18	-18	28	106

*DVE (Darm Verteerbaar Eiwit): intestinal digestible protein according to CVB.
[#]OEB (Onbestendig Eiwit Balans): rumen digestible protein balance according to CVB.

Appendix 2 Additional figures

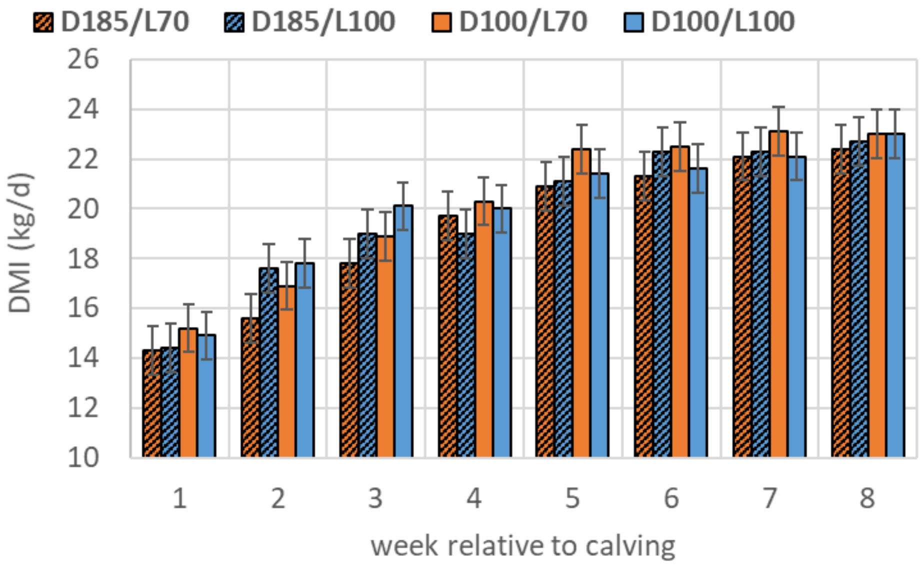

Figure A2.1 *Dry matter intake in early lactation (week 1 to 8), averaged per treatment group (kg DM/cow per day, error bars representing SED).*

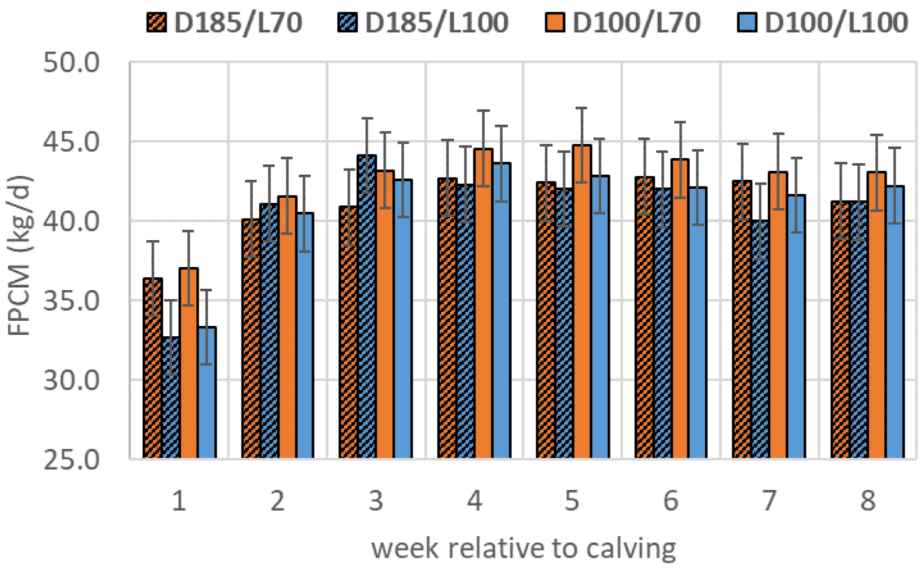

Figure A2.2 *Fat and Protein Corrected Milk (FPCM) yield in early lactation (week 1 to 8), averaged per treatment group (kg/cow per day, error bars representing SED).*

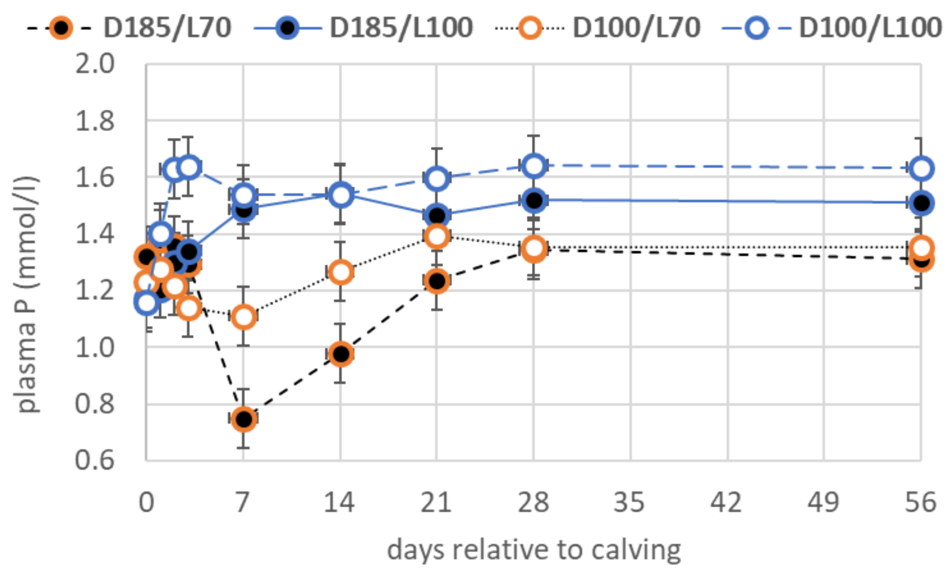

Figure A2.3 *Plasma P concentration in early lactation (week 1 to 8), averaged per treatment group (kg/cow per day, error bars representing SED).*

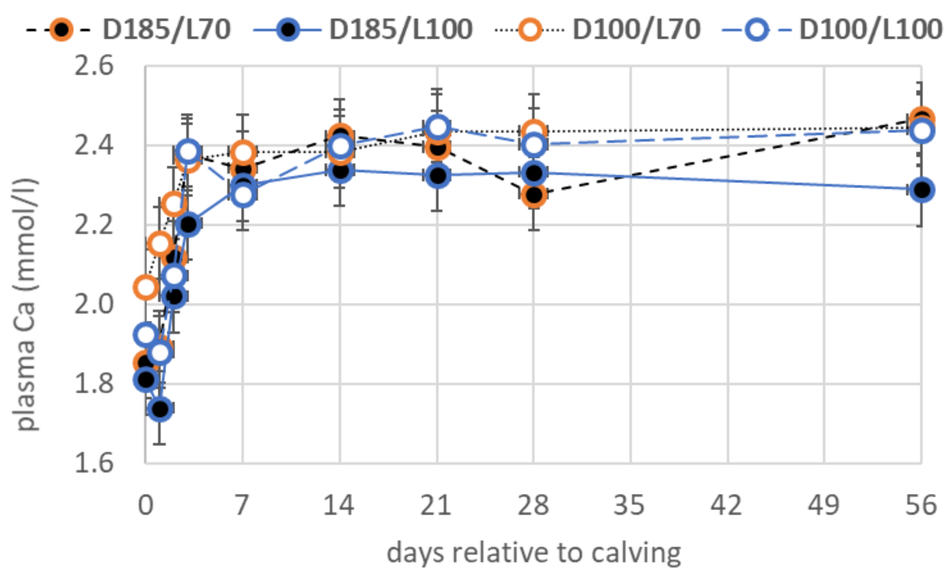

Figure A2.4 *Plasma Ca concentration in early lactation (week 1 to 8), averaged per treatment group (kg/cow per day, error bars representing SED).*

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