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Effects of feeding level, milking frequency, and single injection of cabergoline on blood metabolites, hormones, and minerals around dry-off in dairy cows

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ABSTRACT

This study aimed to investigate the effect of the different dry-off strategies based on reducing feeding level (normal vs. reduced energy density), reducing milking frequency (twice vs. once daily), and administration of a dopamine agonist after last milking (i.e. saline vs. cabergoline injection) on blood metabolites, hormones, and minerals around dry-off. In this experiment, 119 Holstein dairy cows were used in a 2 × 2 × 2 factorial arrangement. In the last week before dry-off, cows were allocated to 1 of the 4 possible dry-off strategies based on feeding level and milking frequency. Within 3 h after last milking, cows were injected with either saline or a D_2 dopamine agonist (cabergoline; Velactis, Ceva Santé Animale, Libourne, France; labeled for use only with abrupt dry-off, e.g., no preceding reduction in feeding level or milking frequency before last milking). After dry-off, all cows were fed the same dry cow diet and data collection continued for a week. Blood samples were collected from the coccygeal vein on d −9, −6, −5, −2, 1, 2, 5, and 7 relative to dry-off. Additionally, blood was sampled at 0, 3, and 6 h relative to injection of either cabergoline or saline, equivalent to d 0.125, 0.250, and 0.375 relative to last milking (dry-off). The reduced feeding level before dry-off caused reduced glucose and insulin concentrations as well as increased free fatty acid concentrations, particularly when reduced feeding level was combined with milking the cows 2× daily. The intramuscular injection of cabergoline caused the expected reduction in circulating prolactin concentrations. In addition, dopamine-agonist cabergoline induced an atypical simultaneous pattern of plasma metabolites (i.e., increased glucose and free fatty acid concentrations), hormones (i.e., reduced insulin and increased cortisol concentrations), and minerals (i.e., reduced calcium concentration), indicating that normal metabolic and mineral homeostatic regulations were hindered after the injection of ergot alkaloid cabergoline. In conclusion, reducing milking frequency seems the best management strategy to reduce milk production at dry-off among those tested in this study.
Key words: dopamine agonist, ergot alkaloid, calcium, prolactin

INTRODUCTION

The dry period is important for the mammary parenchyma to regenerate and prepare for the next lactation (Capuco and Akers, 1999), but genetic selection for high milk yield has caused the modern dairy cow to produce considerable amounts of milk when approaching the dry period. Therefore, it is necessary to modulate the metabolic activity of high yielding dairy cows to reduce milk production and thus ensure a 60-d dry period. Gradual dry-off can be practiced by reducing feeding level or milking frequency, or both (Odensten et al., 2005; Tucker et al., 2009). In terms of animal welfare, recent studies have revealed a complex picture of consequences of the different ways dry-off can be managed (Franchi et al., 2021; Larsen et al., 2021). Reduced feeding level reduces the amount of nutrients available to the udder which, in turn, decreases milk synthesis (Jermann et al., 2022). However, reduced feeding level, while not reducing milking frequency, may induce a transient negative energy balance and therefore cause metabolic stress (Odensten et al., 2005). In addition, cows dried off by reducing the feeding level show signs of hunger (Franchi et al., 2021). In contrast, reducing milking frequency at dry-off without concomitant decrease in feed allowance will likely maintain a balanced metabolic condition.

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In addition to these classic dry-off strategies, administration of dopamine agonists such as cabergoline and quinagolide have been tested in dairy cows to inhibit the secretion of prolactin by the pituitary lactotrophs resulting in reduced prolactin concentrations in the bloodstream (Ollier et al., 2014; Bach et al., 2015; Boutinaud et al., 2016). As prolactin has been shown to contribute to galactopoiesis during ongoing lactation (Ollier et al., 2014; Lacasse et al., 2016), reduced blood prolactin concentration will lead to reduced milk secretion by the udder, promoting cessation of milk synthesis and secretion.

The dopamine agonist quinagolide has been observed to increase glucogenic status during dry-off, attributed by the authors to a decrease in mammary glucose uptake (Ollier et al., 2014). However, the metabolic status during dry-off with different combinations of these 3 strategies to cease milk synthesis at dry-off has yet to be elucidated. The major hypotheses were that (1) reduced feeding level during drying-off, particularly without concomitant reduction in milking frequency, will lead to metabolic stress (i.e., decreased blood levels of nutrients), and (2) administration of cabergoline at dry-off will not only reduce circulating prolactin concentrations, but also affect other systemic parameters enhancing any drying-off induced metabolic stress. This study aimed to investigate the combined effects of reduced feeding level, reduced milking frequency, and injection of cabergoline on the metabolic status (i.e., metabolites, hormones, and minerals) of high-yielding dairy cows during dry-off.

MATERIALS AND METHODS

Experimental procedures used in this study were approved by the Danish Animal Experiments Inspectorate (Permit No. 2017–15–0201–01230). The animal experimental procedures and care of animal under study were carried out in accordance with the Ministry of Food, Agriculture and Fisheries, The Danish Veterinary and Food Administration under act 474 of 15 of May 2014 and executive order 2028 of 14 of December 2020. The experimental work was conducted according to Good Clinical Practice Guideline VICH GL19 (VICH, 2001) and the unregistered use of cabergoline (Velactis, Ceva Santé Animale, Libourne, France) was approved by the Danish Medical Agency (Permit No. 2017064040). In countries where Velactis is registered, it is labeled to be used with abrupt dry-off (e.g., no preceding reduction in feeding level or milking frequency before last milking); thus, its use in other dry-off conditions is off-label.

Animals and Experimental Design

The animal experiment is described in detail by Larsen et al. (2021). In brief, 119 (72 primiparous; 47 multiparous) loose-housed, lactating, and pregnant Holstein cows (experimental unit) were used in a randomized block design with repeated measurements. Treatments were arranged in a 2 × 2 × 2 factorial design with the following factors: feeding level, milking frequency, and cabergoline administration. Cows from the resident herd were continuously enrolled into blocks of 8 within parity, 14 d before the day of dry-off. The enrolment occurred every 2 weeks in batches of 1 to 6 cows depending on the availability of cows fulfilling the inclusion criteria (Larsen et al., 2021), resulting in 36 successive batches. A planned sample size of 15 cows per treatment combination was based on the availability of cows in the resident herd and supported by power calculations to identify significant differences at the 5% level with a power of 90% between injection of saline (**SAL**) or cabergoline (**CAB**) for plasma glucose concentration (SD = 0.29 m*M* used; Ollier et al., 2014).

The experimental pen had 10 cubicles, 5 adjacent cubicles in each side (6 cubicles measuring 1.8 m × 1.4 m and 4 cubicles measuring 1.8 m × 1.35 m, length to brisket board), all connected by an alley (8 m × 2.6 m) and a feeding area (8.6 m × 3.25 m). The cubicles were lined with mattresses bedded with a mix of sawdust and finely chopped straw. The alley and feeding area had concrete slatted flooring (slats = 15 cm, slots = 4 cm).

Experimenters were blinded to treatment allocation but could only be partially blinded to the diets because these were visible in the feed bins. From d −14 to −8 relative to dry-off (baseline period), cows were fed for ad libitum intake with a lactation partial mixed ration (**PMR**) diet and milked in an automatic milking system (**AMS**; DeLaval AB). In the AMS, cows were offered 3 kg/d of a commercial pelleted concentrate during milking.

From d −7 to −1 relative to dry-off, cows either continued the normal feeding (**NORM**) level or were fed a reduced feeding (**REDU**) level. The REDU level was obtained by ad libitum allocation of the lactation PMR diluted with chopped barley straw to obtain an energy concentration similar to a standard dry TMR. The diluted PMR was fortified with minerals and vitamins to reach a similar daily supplementation as with the standard lactation PMR. Composition of the different experimental diets were described previously (Larsen et al., 2021). Each cow was individually fed using automated feed bins (Insentec B.V.). On the dry-off day (d 0; last milking), all cows shifted to the standard dry TMR fortified with minerals and vitamins to reach approximately 50% of the daily mineral and vitamin supplementation in the week before dry-off. The concentrate allowance in the AMS was set to maximally 3 and 1 kg/d (as-is form) for NORM and REDU, respectively. As described by Larsen et al. (2021), the planned energy concentration of the whole diets (PMR + concentrate) was 6.75, 5.73, and 5.71 MJ of NE_L/kg of DM for the NORM, REDU, and dry TMR, respectively.

From d −7 to −1 relative to dry-off, cows were milked either twice (2×) or once (1×) daily. All cows were milked in the AMS between 0530 and 0700 h, and cows with 2× milking frequency were milked again between 1530 and 1630 h. Cows were milked for the last time in the morning of d 0. Around 0900 h in the morning of d 0, 3 h after last milking, cows were administered a 5-mL i.m. injection (18 G needle and 5-mL screw-lock syringe) of either saline or cabergoline solution (5.6 mg of cabergoline; Velactis, Ceva Santé Animale) by barn staff.

Blood Sampling and Analysis

Blood was sampled by venipuncture (20 G × 1″ hypodermic needles, BD Precisionglide, Becton Dickinson) into 9-mL Na-heparinized vacutainers (Greiner Bio-One) of the coccygeal vein between 0800 and 0900 h on d −9, −6, −5, −2, 1, 2, 5, and 7 relative to dry-off. Additionally, blood was sampled at 0, 3, and 6 h relative to injection of either cabergoline or saline, equivalent to d 0.125, 0.250, and 0.375 relative to dry-off. Stabilized blood samples were placed on ice after collection and plasma was harvested by centrifugation at 3,000 × g for 20 min at 4°C and stored at −20°C until analysis.

Blood plasma concentrations of glucose, total Ca, inorganic phosphorus (**iP**), and Mg were determined according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1800). Plasma concentrations of free fatty acids (**FFA**) were determined using the Wako, FFA C ACS-ACOD assay method. Plasma concentrations of BHB were determined as an increase in absorbance at 340 nm due to the production of nicotinamide adenine dinucleotide at slightly alkaline pH in the presence of BHB dehydrogenase. The method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by Harano et al. (1985). All analyses were performed using an autoanalyzer, ADVIA 1800 Chemistry System (Siemens Medical Solutions) with Acusera Bovine Chemistry assayed plasma used as high and low controls (Randox Laboratories Ltd.).

Plasma prolactin and insulin concentrations were analyzed by RIA according to the methodology described by Bruckmaier et al. (1992) and Vicari et al. (2008), respectively. Plasma cortisol concentrations was analyzed using an RIA method based on a description by Thun et al. (1981) with modifications as described by Schwinn et al. (2016). The intra-assay coefficients of variation were 7.10, 6.00, and 5.06% and the interassay coefficients of variation were 7.10, 8.90, and 4.86% for prolactin, insulin, and cortisol, respectively. Lyphocheck Immunoassay Plus Control (Bio-Rad) was used as controls with high and low concentrations.

Calculations and Statistical Analyses

All observations for 4 cows were removed due to lameness (n = 2) or mastitis (n = 2) around d −7 relative to dry-off. All observations from just before dry-off and onwards were removed for 4 additional cows due to lameness (n = 1), mastitis (n = 1), or no access to feed for 18 h (Insentec breakdown, n = 2). The number of cows used per experimental group before and after dry-off is given in Table 1.

All blood variables were analyzed with linear mixed effects models using the MIXED procedure of SAS (version 9.4). The baseline (d −9; Table 1) was tested for differences among treatment combinations using a model with parity (primiparous, multiparous) and treatment (8 combinations of feeding level, milking frequency, and cabergoline treatment) as fixed effects. The statistical model to test treatment effects included baseline (d −9) and number of cows in pen as covariates, parity (primiparous, multiparous), feeding level (NORM, REDU), milking frequency (1×, 2×), cabergoline treatment (SAL, CAB), time (from d −6 to 7), and all possible interactions as fixed effects. Cow and batch were considered as random effects, and time within cow was considered as a repeated measurement using the spatial power covariance structure. Denominator degrees of freedom was calculated using the Kenward-Roger method. Normal distribution and homoscedasticity of residuals was graphically assessed. The FFA, prolactin, insulin, and cortisol concentrations were $\log_{10}$ transformed to obtain normal distribution of residuals. Significance was declared at $P \leq 0.05$ and tendencies were considered when $0.05 < P \leq 0.10$.

RESULTS

No differences among treatment combinations were observed in the baseline period ($P > 0.05$; Table 1) for plasma concentrations of glucose, BHB, FFA, prolactin, insulin, Ca, iP, and Mg, but the cortisol concentration did differ among treatment combinations ($P < 0.001$). In addition, none of the studied variables were affected

Table 1. Number of cows used per experimental group, and baseline blood plasma concentrations of glucose, BHB, free fatty acids (FFA), prolactin, insulin, cortisol, Ca, inorganic phosphorus (iP), and Mg at d −9 relative to dry-off for the 8 experimental groups[1]

	Normal feeding level				Reduced feeding level			
	2× milking		1× milking		2× milking		1× milking	
Item	SAL	CAB	SAL	CAB	SAL	CAB	SAL	CAB
Number of cows								
Before dry-off	14	14	15	14	14	15	15	14
After dry-off	13	13	14	13	14	15	15	14
Blood variable								
Glucose, m*M*								
Mean	3.90	3.74	3.99	3.89	3.81	3.72	3.84	3.89
SD	0.193	0.408	0.402	261	0.219	0.256	0.218	0.267
BHB, m*M*								
Mean	0.82	0.66	0.71	0.72	0.68	0.75	0.74	0.74
SD	0.303	0.162	0.241	0.192	0.161	0.188	0.180	0.196
FFA, μ*M*								
Mean	91.0	101.0	104.6	97.1	77.0	67.7	96.2	84.6
SD	48.98	44.73	101.09	110.52	29.28	26.00	49.73	43.41
Insulin, μg/mL								
Mean	30.7	25.2	27.8	32.3	24.5	36.7	31.5	31.2
SD	4.68	4.49	4.40	4.55	4.47	4.38	4.38	4.52
Cortisol, n*M*								
Mean	1.62	2.81	2.63	2.69	1.30	1.52	1.92	1.46
SD	0.41	0.41	0.40	0.41	0.41	0.39	0.39	0.40
Prolactin, ng/mL								
Mean	24.2	38.0	23.6	19.4	18.3	29.3	31.5	20.6
SD	21.65	36.74	20.99	16.53	15.50	28.80	34.69	19.71
Ca, m*M*								
Mean	2.57	2.66	2.61	2.55	2.64	2.64	2.60	2.62
SD	0.132	0.080	0.127	0.129	0.112	0.081	0.107	0.098
iP, m*M*								
Mean	1.72	1.88	1.88	1.82	1.79	1.80	1.86	1.89
SD	0.240	0.211	0.166	0.243	0.201	0.230	0.318	0.194
Mg, m*M*								
Mean	0.97	0.94	0.98	0.97	0.94	0.94	0.98	0.95
SD	0.079	0.083	0.071	0.072	0.099	0.069	0.062	0.068

[1]SAL = saline; CAB = cabergoline.

by either the 4-way interaction between feeding level, milking frequency, cabergoline treatment and day; or the 3-way interaction between feeding level, milking frequency and cabergoline treatment (Table 2).

Plasma glucose concentrations were higher from d −6 to 1 relative to dry-off for the NORM level compared with the REDU level but did not differ between feeding levels from d 2 relative to dry-off ($P_{F \times D} < 0.001$; Table 2 and Figure 1). Plasma glucose concentrations were higher from d −6 to 1 relative to dry-off for 1× milking compared with 2× daily milking but did not differ between milking frequency from d 2 relative to dry-off ($P_{M \times D} < 0.001$). After injection of either cabergoline or saline at dry-off (i.e., after last milking), plasma glucose concentrations increased to a greater extent for CAB compared with SAL but did not differ at d 5 and 7 relative to dry-off ($P_{C \times D} < 0.001$).

Plasma BHB concentrations were higher from d −6 to 0.125 relative to dry-off for the NORM level compared with the REDU level, but after the shift to the dry TMR at dry-off, BHB concentrations were higher for the REDU level compared with the NORM level ($P_{F \times D} < 0.001$; Table 2 and Figure 1). Plasma BHB concentrations were higher for 2× milking compared with 1× daily milking ($P_M = 0.013$). After injection at dry-off, the highest plasma BHB concentrations were observed at d 0.375 relative to dry-off for SAL, and at d 1 relative to dry-off for CAB, but they did not differ for SAL and CAB at d 5 and 7 relative to dry-off ($P_{C \times D} < 0.001$). Indeed, a tendency for a 3-way interaction between feeding level, cabergoline treatment and time ($P_{F \times C \times D} = 0.095$) showed that cows receiving the REDU level before dry-off and treated with CAB at dry-off had the highest BHB concentrations at d 0.375 and 1 relative to dry-off.

Plasma FFA concentrations were higher for the REDU level milked 2× daily from d −6 to 0.250 relative to dry-off compared with the REDU level milked 1× daily and the NORM level, irrespective of the milking frequency ($P_{F \times M \times D} = 0.027$; Table 2 and Figure 1). In addition, the REDU level milked 1× daily had higher FFA concentrations than the NORM level, ir-

Table 2. *P*-values (type-2 *F*-test) for plasma concentrations of glucose, BHB, free fatty acids (FFA), prolactin, insulin, cortisol, Ca, inorganic phosphorus (iP), and Mg in Holstein cows used in a 2 × 2 × 2 factorial arrangement of treatment factors: feeding level (F, normal vs. reduced), milking frequency (M, 2× vs. 1×), and cabergoline treatment (C, SAL vs. CAB) during the experimental period (D, from d −7 to 7 relative to dry-off)

Variable	F	M	C	D	F × M	F × C	M × C	F × D	M × D	C × D	F × M × C	F × M × D	F × C × D	M × C × D	F × M × C × D
Glucose	<0.001	0.001	<0.001	<0.001	0.330	0.601	0.910	<0.001	<0.001	<0.001	0.769	0.275	0.173	0.200	0.881
BHB	0.219	0.013	0.007	<0.001	0.076	0.162	0.458	<0.001	0.141	<0.001	0.893	0.635	0.095	0.104	0.568
FFA	<0.001	0.048	0.003	<0.001	0.812	0.071	0.843	<0.001	<0.001	<0.001	0.455	0.027	0.446	0.040	0.795
Prolactin	0.003	0.636	<0.001	<0.001	0.360	0.350	0.874	0.006	0.089	<0.001	0.276	0.279	0.614	0.947	0.747
Insulin	<0.001	0.084	<0.001	<0.001	0.023	0.719	0.319	<0.001	<0.001	<0.001	0.996	0.729	0.175	0.003	0.801
Cortisol	0.166	0.076	<0.001	<0.001	0.193	0.123	0.552	0.006	0.044	<0.001	0.873	0.112	0.259	0.155	0.241
Ca	<0.001	0.711	0.003	<0.001	0.560	0.237	0.178	<0.001	<0.001	<0.001	0.535	0.381	0.016	0.123	0.951
iP	<0.001	0.140	0.004	<0.001	0.624	0.750	0.459	<0.001	<0.001	<0.001	0.370	0.282	0.007	0.104	0.323
Mg	<0.001	0.060	<0.001	<0.001	0.030	0.205	0.955	<0.001	0.050	<0.001	0.545	0.077	<0.001	0.833	0.219

respective of the milking frequency, from d −6 to 0.250 ($P_{F \times M \times D} = 0.027$). Plasma FFA concentrations did not differ among treatments from d 1 ($P_{F \times M \times D} = 0.027$). The difference in FFA concentrations between milking frequencies observed from d −6 to 0.250 for REDU level was not observed at d 0.375 and 1, although FFA concentrations were higher for CAB than for SAL ($P_{M \times C \times D} = 0.040$). From d 2 to 7, FFA concentrations did not differ among treatment combinations.

Plasma prolactin concentrations were higher from d −6 to 0.375 relative to dry-off for the NORM level compared with the REDU level but did not differ between feeding level from d 1 relative to dry-off ($P_{F \times D} = 0.006$; Table 2 and Figure 2). After injection, plasma prolactin concentrations were lower for CAB compared with SAL from d 0.250 to 7 relative to dry-off ($P_{C \times D} < 0.001$).

Plasma insulin concentrations were higher for NORM compared with REDU from d −6 to 0.250 but did not differ between feeding levels from d 0.375 to 7 ($P_{F \times D} < 0.001$; Table 2 and Figure 2). After injection at dry-off, plasma insulin concentrations decreased to a greater extent for CAB compared with SAL, followed by increases to a level that did not differ at d 5 and 7 relative to dry-off ($P_{C \times D} < 0.001$).

Plasma cortisol concentrations were higher for REDU compared with NORM at d −6 and −5 relative to dry-off ($P_{F \times D} = 0.006$; Table 2 and Figure 2) but did not differ between feeding levels for the rest of the studied period. Plasma cortisol concentrations were higher for 2× milking compared with 1× daily milking at d −6 and −5 ($P_{M \times D} = 0.044$) but did not differ between milking frequencies for the rest of the study period. Plasma cortisol concentrations increased after CAB injection compared with SAL and were higher at d 0.250 and 0.375 ($P_{C \times D} < 0.001$) but did not differ from d 1 to d 7.

Plasma Ca concentrations were higher from d −6 to 0.125 relative to dry-off for the NORM level compared with the REDU level. Plasma Ca were lower for CAB from d 0.250 to 1 compared with SAL irrespective of feeding level before dry-off, did not differ among treatments at d 2, but were higher for CAB at d 5 and 7 compared with SAL ($P_{F \times C \times D} = 0.016$; Table 2 and Figure 3). Plasma Ca concentrations were higher for 1× daily milking at d −6 and −5 relative to dry-off compared with 2× milking, but concentrations were higher for 2× milking from d 2 to 7 ($P_{M \times D} < 0.001$).

Plasma iP concentrations were higher from d −6 to 0.375 relative to dry-off for the REDU level compared with the NORM level, but iP concentrations decreased for CAB at d 0.250 and 0.375 irrespective of feeding level before dry-off compared with stable concentrations for SAL, and from d 1 to 7 concentrations did not differ among treatments ($P_{F \times C \times D} = 0.007$; Table

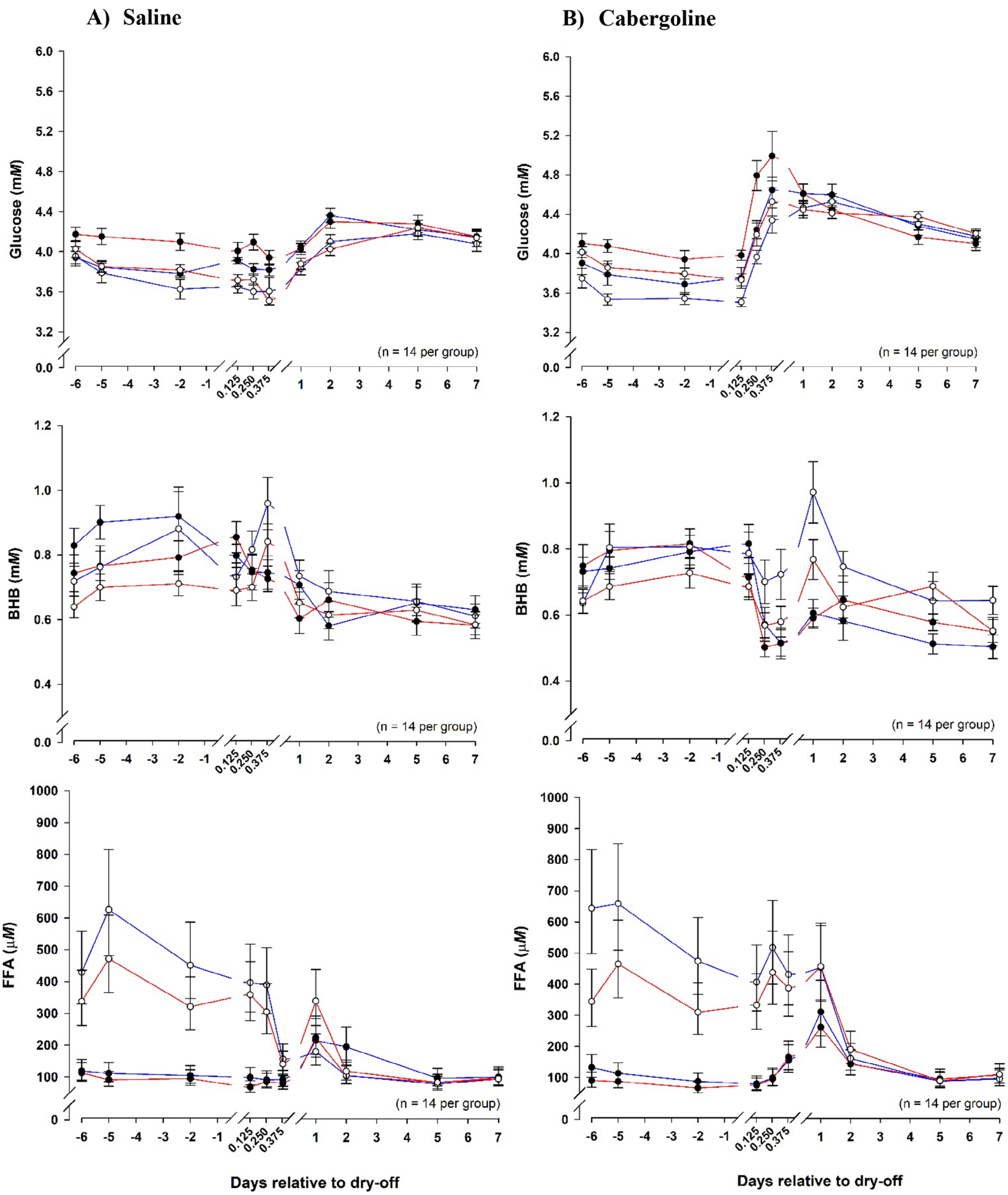

Figure 1. Glucose, BHB, and free fatty acid (FFA) plasma concentrations in Holstein cows from d −6 to 7 relative to dry-off. From d −7 to 0 cows were fed ad libitum either a normal feeding level (black) or a reduced feeding level (white) and either milked twice (solid blue line) or once (solid red line) daily and injected with either saline (panel A) or cabergoline (panel B) 3 h after last milking in a 2 × 2 × 2 factorial arrangement of treatment factors. After dry-off, all cows were fed the same TMR for dry cows. The time points at 0, 3, and 6 h relative to injection of either cabergoline or saline are represented as d 0.125, 0.250, and 0.375 relative to dry-off. Each data point is LSM ± SE for glucose and BHB, but back-transformed LSM and confidence intervals for FFA.

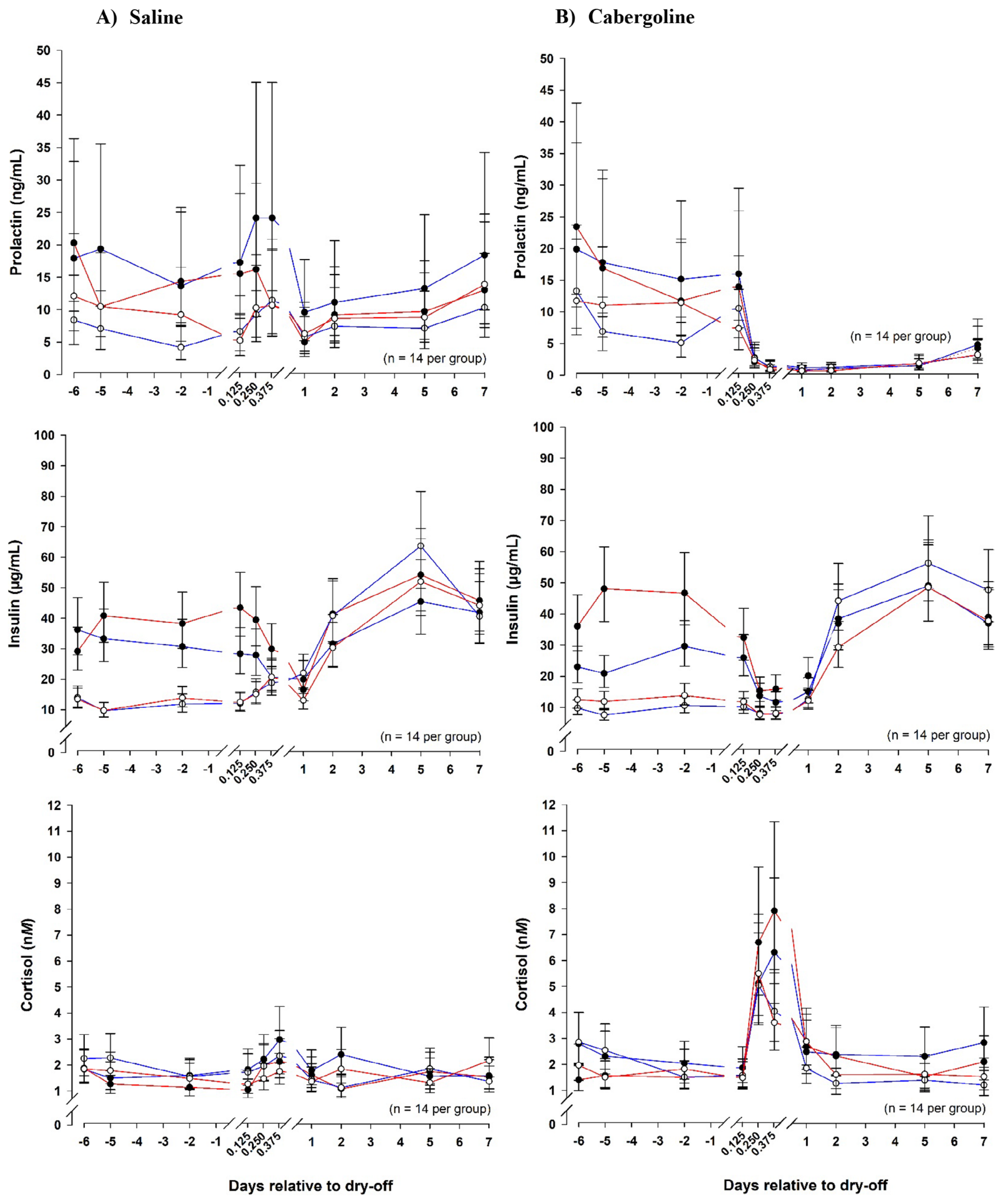

Figure 2. Prolactin, insulin, and cortisol plasma concentrations in Holstein cows from d −6 to 7 relative to dry-off. From d −7 to 0 cows were fed ad libitum either a normal feeding level (black) or a reduced feeding level (white) and either milked twice (solid blue line) or once (solid red line) daily and injected with either saline (panel A) or cabergoline (panel B) 3 h after last milking in a 2 × 2 × 2 factorial arrangement of treatment factors. After dry-off, all cows were fed the same TMR for dry cows. The time points at 0, 3, and 6 h relative to injection of either cabergoline or saline are represented as d 0.125, 0.250, and 0.375 relative to dry-off. Each data point is back-transformed LSM and confidence intervals.

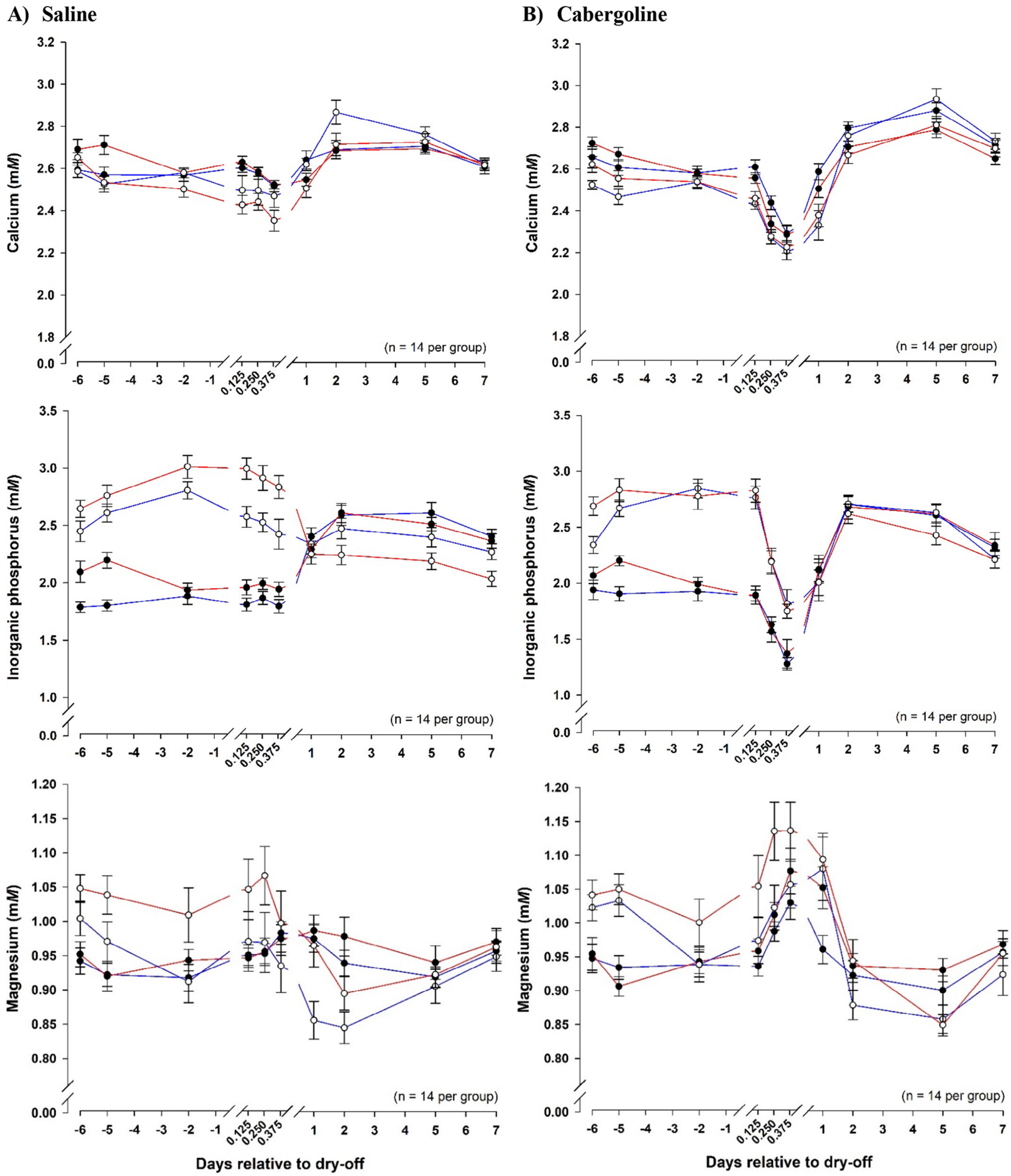

Figure 3. Calcium, inorganic phosphorus, and Mg plasma concentrations in Holstein cows from d −6 to 7 relative to dry-off. From −7 to 0 d cows were fed ad libitum either a normal feeding level (black) or a reduced feeding level (white) and either milked twice (solid blue line) or once (solid red line) daily and injected with either saline (panel A) or cabergoline (panel B) 3 h after last milking in a 2 × 2 × 2 factorial arrangement of treatment factors. After dry-off, all cows were fed the same TMR for dry cows. The time points at 0, 3, and 6 h relative to injection of either cabergoline or saline are represented as d 0.125, 0.250, and 0.375 relative to dry-off. Each data point is LSM ± SE.

2 and Figure 3). Plasma iP concentrations were higher for 1× daily milking at d −6 and −5 relative to dry-off compared with 2× milking, but concentrations were higher for 2× milking at d 5 ($P_{M \times D} < 0.001$).

Plasma Mg concentrations were higher from d −6 to 0.125 relative to dry-off for the REDU level compared with the NORM level, but Mg concentrations increased for CAB at d 0.250 and 0.375 irrespective of feeding level before dry-off compared with stable concentrations for SAL, and Mg concentrations decreased for all treatments from d 1 and did not differ among treatments by d 7 ($P_{F \times C \times D} < 0.001$; Table 2 and Figure 3). Plasma Mg concentrations were higher for 1× daily milking from d −2 to 2 relative to dry-off compared with 2× milking ($P_{M \times D} = 0.050$), but concentrations did not differ between milking frequency at other days.

DISCUSSION

The current data originate from a larger study investigating dry-off strategies. In summary, cows abruptly dried-off yielded around 25 kg/d of milk at drying-off, whereas cows gradually dried off by either feeding REDU level or being milked 1× daily yielded approximately 30% less, whereas cows gradually dried-off by both feeding REDU level and being milked 1× yielded approximately 45% less. In the week before dry-off, REDU cows reduced DMI by 30% less compared with the NORM cows. As a result, the calculated energy balance was most negative in cows fed the REDU level and milked 2× daily compared with the cows fed the NORM level and milked 1× daily. At the day of dry-off, cows administered CAB decreased DMI by 53% compared with the cows treated with SAL, regardless of treatment before dry-off (Larsen et al., 2021). The treatments based on feeding level and milking frequency were applied before dry-off, thus effects of these will be discussed separately from effects of the cabergoline injection that was applied at dry-off.

Feeding Level and Milking Frequency

As described in a companion paper (Larsen et al., 2021), the net energy balance before dry-off of the cows fed the REDU level was lower and more negative than for the cows fed the NORM level. In the present study, plasma glucose concentrations were higher during the week before dry-off in cows fed with the NORM level and milked 1× daily than in cows fed the REDU level and milked 2× daily. This result was expected as cows fed the NORM level had a higher supply of glucose precursors from the diet compared with cows fed the REDU level, while a reduced milking frequency would reduce the demand for glucose by the mammary gland. In addition, the cows fed the NORM level had higher glucose availability, triggering higher insulin secretion by the pancreas, and increasing insulin concentrations in the bloodstream compared with cows fed the REDU level. The more intense negative energy balance in cows fed the REDU level and milked 2× daily was also reflected by the increased FFA concentrations observed in these animals. Similar results have been observed in dairy cows during early lactation when the energy requirements for milk production are higher than the dietary energy intake (Gärtner et al., 2019; Štolcová et al., 2020; Gross et al., 2021). Likewise, Guinard-Flament et al. (2007) showed a similar effect on glucose arterial concentration in cows subjected to a combination of milking frequency and feed restriction. However, other studies have not observed lower plasma glucose concentrations in cows under feed restriction either during lactation (Leduc et al., 2021) or before dry-off (Jermann et al., 2022).

Generally, increased BHB concentrations in blood could either be originating from butyrate produced in the rumen, or from fat mobilization. In the present study, the increased BHB concentrations before dry-off observed in cows fed the NORM level and in cows milked 2× daily could be caused by a combination of higher supply of butyrate from the diet with higher fatty acid mobilization required to meet energy requirements for milk production compared with cows fed the REDU level and cows milked 1× daily.

Prolactin is a hormone which is mainly secreted by lactotrophs located in the anterior pituitary. In the present study, the reduced milking frequency did not reduce prolactin concentrations before dry-off. This finding agrees with results from Lacasse and Ollier (2014), where no differences in prolactin concentrations were observed among cows milked either 2× , 3×, or 7× daily. However, Bernier-Dodier et al. (2010) and Thompson et al. (2015) observed that the gene expression of prolactin receptors was higher in mammary glands that were milked more frequently (3× daily vs. 1× daily). Whereas milking frequency did not affect prolactin concentrations before dry-off, feeding level did. Thus, cows fed the REDU level showed the lowest prolactin concentrations before dry-off. Previous studies have also observed that feed restricted cows before dry-off showed reduced prolactin concentrations around dry-off (Vicini et al., 1988; Kuhla et al., 2010; Ollier et al., 2014). Therefore, it seems that feed restriction, and not milking frequency, can be used to modulate prolactin concentrations in dairy cows around dry-off. However, it should be considered that prolactin is released after milking (Gorewit et al., 1992; Bruckmaier et al., 1993). As blood samples were collected only after morning milking for both milking frequencies (i.e., 1×

and 2×), it may be possible that the similar prolactin concentrations observed in both milking treatments was due to the experimental set up (i.e., morning blood sampling). The observed differences between the treatments in the baseline samples of cortisol means that the results from the initial days of the study are difficult to interpret. However, cortisol concentrations before dry-off may have been affected by feeding level as well as milking frequency. In dairy cows, as in other animals, stressful conditions activate different mechanisms that contribute to adapt the organism to those new conditions (Collier et al., 2017). Among other changes, circulating cortisol concentrations are increased under stressful situations to maintain or re-establish metabolic homeostasis (Collier et al., 2017). As observed by several authors, short and intense reductions in energy intake can induce peaks in circulating cortisol concentrations in dairy cows (Agenäs et al., 2003; Toerien and Cant, 2007; Moyes et al., 2009). Despite cortisol is released after milking (Tančin et al., 2000), no studies have been found regarding the effect of reducing milking frequency on circulating cortisol concentrations, however, the changes observed in the present study can be considered minor and therefore, it can be assumed that the changes observed in the present study are attributed to the metabolic imbalance caused by the shifting the diet from the NORM level to the REDU level.

Plasma minerals (i.e., Ca, iP, and Mg) were affected by both feeding level and milking frequency before dry-off. In this study, the diluted PMR used in the REDU level was fortified with minerals to reach a similar daily supplementation as with the standard lactation PMR assigned to the NORM level. Indeed, the lower Ca and the higher iP and Mg concentrations observed in cows fed the REDU level, could be caused by the different concentration of minerals in the REDU level compared with the NORM level, although those differences were, in part, compensated by the reduced DMI of the cows fed the REDU level compared with those fed the NORM level. Thus, the differences in Ca and Mg concentrations among treatments were minor, but the cows fed the REDU level had higher iP concentrations than those fed the NORM level. In contrast, the lower amount of Ca, iP, and Mg allocated for milk production in cows milked 1× daily could explain the higher Ca concentration observed in these animals compared with cows milked 2× daily, especially during the first days (i.e., d −6 and −5 relative to dry-off). Changes in the concentration of circulating minerals (i.e., Ca, iP, and Mg) caused by either changes in dietary intake or minerals allocated for milk production trigger different mechanisms that regulate those concentrations in the bloodstream, e.g., changes in Ca concentrations experienced at the onset of lactation are commonly compensated within the first 48 h after parturition (Hernández-Castellano et al., 2020). This could explain the differences observed during the first treatment days in cows milked 1× or 2× daily (i.e., d −6 and −5 relative to dry-off), as the different physiological mechanisms would be achieved to regulate those minerals on the following days before dry-off.

Based on the results obtained in the present study before dry-off and taking into consideration the effects of feeding level and milking frequency, it seems that feeding the cows with the NORM level and milking them 1× daily one week before dry-off is the most appropriate strategy to reduce milk yield before dry-off without inducing a considerable metabolic stress. These findings agree with those described in a companion paper (Franchi et al., 2021) showing that, from a hunger perspective, reducing milking frequency rather than feeding level seems to be a less negative management to reduce milk production before dry-off.

Cabergoline Treatment

Treatment with cabergoline after the last milking induced the intended abrupt decrease in plasma prolactin concentrations. Concomitantly, an abrupt increase in plasma glucose concentrations was observed with the highest value measured at 6 h after injections. In normal conditions, plasma glucose concentrations in cows range from 3.3 to 4.4 mmol/L (Stöber and Gründer, 1990). Thus, the observed plasma glucose concentrations within the first 24 h in the cows administered cabergoline were above the normal range. Increased glucose concentrations might be related to the sudden decrease in prolactin concentrations caused by cabergoline injection at dry-off. Reduced prolactin concentrations decreased milk production and, consequently, glucose requirements for lactose synthesis. As described above, increased glucose concentrations are commonly associated with increased insulin concentrations (Sartin et al., 1985). However, insulin concentrations in the present study suddenly decreased in cows treated with cabergoline at dry-off indicating that normal glucose homeostasis mechanisms were apparently overruled. This finding is in agreement with the results described by Contreras et al. (2008) as insulin concentrations were increased in healthy humans treated with metoclopramide, a dopamine antagonist which increases prolactin secretion by a dopaminergic blocking mechanism at the hypothalamic level.

Cortisol concentrations suddenly increased in the animals administered the cabergoline. Similarly, the subcutaneous administration of a dopamine agonist (i.e., Apomorphine) caused increased cortisol concentrations 120 min after administration in humans (Meltzer et al., 2001). It is well-known that cortisol promotes resistance to stress by providing more glucose to the stressed organism (Munck et al., 1984) by, for instance, inhibiting glucose uptake in insulin-sensitive tissues (Reilly and Black, 1973; Munck et al., 1984) or by stimulation of hepatic gluconeogenesis (Baird and Heitzman, 1970) and fat mobilization (Ahmadzadeh et al., 2006). Due to the increased FFA concentrations observed in cows administered cabergoline treatment at dry-off, it seems that these cows had a more intense fat mobilization than those receiving the saline. Based on the previous discussion, it is unlikely that glucose concentrations were exclusively elevated by the reduced milk production observed in the animals administered cabergoline at dry-off. Thus, it seems that the administration of cabergoline did not only cause decreased prolactin concentrations, but also triggered the secretion of cortisol, which in turn promoted gluconeogenesis and lipolysis. Similar results were observed by Ollier et al. (2014), where cows treated with quinagolide showed an increased glucose concentration, although FFA concentrations in those cows were not affected. In addition to being a dopamine D_2 receptor agonist, cabergoline is also an ergot alkaloid (Miyagi et al., 1996). The tetracyclic ergoline ring of ergot alkaloids has structural similarities to the ring structure of the biogenic amine neurotransmitters norepinephrine, dopamine, and serotonin (Klotz, 2015). Consequently, there are many outcomes in terms of ergot alkaloid-receptor interactions. As described by Filipov et al. (1999), the ingestion of ergot alkaloids promotes systemic inflammation and increases the responsiveness to an intravenous lipopolysaccharide challenge. In addition, SCC increase in mammary secretions of cows after cabergoline injection (Boutinaud et al., 2016). Therefore, another possible explanation to the observed results could be that the injection of cabergoline triggers either a systemic inflammation or a local mammary inflammation (or both), increasing cortisol concentrations and causing a transient increased glucose concentration. Thus, these results are in agreement with a recent paper published by this group (Franchi et al., 2022) showing that cows treated with cabergoline had decreased DMI and also rumination time, which are often reduced when cows show increased cortisol concentrations (Bristow and Holmes, 2007).

Regarding the mineral balance, cows receiving the cabergoline injection at dry-off showed decreased Ca and iP concentrations, and increased Mg concentrations. Calcium is essential for milk production and participates in several physiological processes such as muscle contraction. High yielding dairy cows are often reported to be hypocalcemic as the Ca demand by the mammary gland for milk production exceeds the Ca absorbed from the diet or mobilized from the bones (Hernández-Castellano et al., 2017a; Weaver et al., 2017). In the present study, the reduced milk production was expected to be positively correlated with increased circulating Ca concentrations as less Ca was requested by the mammary gland. However, Ca concentration abruptly decreased in cows administered cabergoline although not below 2 mmol/L that is commonly considered the threshold for subclinical hypocalcemia (Hernández-Castellano et al., 2020). As Ca is essential for muscle contraction, the results observed in the present study could explain the reduced gastrointestinal motility observed in cows administered cabergoline described in a companion paper (Franchi et al., 2022). As mentioned above, dopamine agonist administration inhibits the secretion of prolactin by the pituitary lactotrophs by blocking voltage-gated Ca influx through the pertussis toxin-sensitive signaling pathway and by desensitizing Ca secretion coupling through the pertussis toxin-insensitive and protein kinase C-sensitive signaling pathway (Gonzalez-Iglesias et al., 2008). Thus, it has been well-described how increased cortisol concentrations are negatively correlated with circulating Ca concentrations as this hormone inhibits mobilization of Ca from bone (Stern, 1969; Braithwaite et al., 1972) and decreases intestinal absorption of Ca (Kimberg et al., 1971). As described by Horst and Jorgensen (1982), both Ca and iP concentrations in the bloodstream were decreased in dairy goats after the intramuscular administration of cortisol. In the same study, Horst and Jorgensen (1982) also showed that cows with hypocalcemia around parturition had increased circulating cortisol concentrations. These findings agree with the results observed in the present study. Another possibility to explain the reduced circulating Ca concentration in the cows administered cabergoline could be the regulatory role of prolactin on intestinal Ca absorption. As described by Hernández-Castellano et al. (2020) prolactin modulates the expression of diverse Ca transporters at the intestinal level. Therefore, reduced prolactin concentrations will consequently reduce the intestinal absorption of Ca. However, due to the rapid decrease in circulating Ca concentration in the cows administered cabergoline, it is unlikely that the reduced Ca concentration observed was mainly triggered by the reduced prolactin concentration and consequently the reduced intestinal capacity to absorb Ca. As described by Lacasse et al. (2019), the modulation of prolactin (i.e., stimulation or inhibition) does not affect circulating Ca concentrations in dairy cows.

Magnesium participates in the regulation of Ca concentration by increasing the secretion of parathyroid hormone (Suh et al., 1973), as well as decrease tissue responsiveness to parathyroid hormone (Reddy et al., 1973; Hernández-Castellano et al., 2020). As cows injected with cabergoline had decreased circulating Ca concentrations, the increased Mg concentrations observed in these animals after dry-off could be related to the activation of the homeostatic mechanisms aimed to restore circulating Ca concentrations. A similar Mg pattern was observed by Hernández-Castellano et al. (2017b) in cows having reduced circulating Ca concentrations around parturition.

CONCLUSIONS

The current study shows that reduced feeding level before dry-off induced metabolic stress as indicated by the reduced glucose and insulin as well as increased FFA, particularly when associated with milking the cows 2× daily, in contrast to abrupt dry-off and to milking the cows 1× daily with maintained feeding level. Facilitated dry-off by D_2 dopamine agonist cabergoline administration used under the on-label (i.e., abrupt dry-off) or off-label (i.e., reduced feeding level or milking frequency before last milking) prescription, induced the expected reduction in circulating prolactin concentrations but did also induce an atypical simultaneous plasma pattern of metabolites, hormones, and minerals, indicating that normal metabolic and mineral homeostatic regulation was hindered by the administration of ergot alkaloid cabergoline. Therefore, it seems that reducing milking frequency the best management strategy to reduce milk production at dry-off among those tested in this study.

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