Blood calcium concentration and performance in periparturient and early lactating dairy cows is influenced by plant bioactive lipid compounds

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ABSTRACT

Previous studies ex vivo suggested that plant bioactive lipid compounds (PBLC) can increase ruminal calcium absorption. Therefore, we hypothesized that PBLC feeding around calving may potentially counteract hypocalcemia and support performance in postpartum dairy cows. The corresponding aim of the study was to investigate the effect of PBLC feeding on blood minerals in Brown Swiss (BS) and hypocalcemia-susceptible Holstein Friesian (HF) cows during the period from d −2 to 28 relative to calving and on milk performance until d 80 of lactation. A total of 29 BS cows and 41 HF cows were divided each into a control (CON) and PBLC treatment group. The latter was supplemented with 1.7 g/d menthol-rich PBLC from 8 d before expected calving to 80 d postpartum. Milk yield and composition, body condition score and blood minerals were measured. Feeding PBLC induced a significant breed × treatment interaction for iCa, supporting that PBLC increased iCa exclusively in HF cows; the increase was 0.03 mM over the whole period and 0.05 mM from d 1 to 3 after calving. Subclinical hypocalcemia was seen in one BS-CON and 8 HF-CON cows and 2 BS-PBLC and 4 HF-PBLC cows. Clinical milk fever was detected only in HF cows (2 HF-CON and one HF-PBLC). Other tested blood minerals, such as sodium, chloride, and potassium, as well as blood glucose, were neither affected by PBLC feeding nor breed, nor were their 2-way interactions, except for higher sodium levels in PBLC cows on d 21. Body condition score showed no effect of treatment, except for a lower body condition score in BS-PBLC compared with BS-CON at d 14. Dietary PBLC increased milk yield, milk fat yield, and milk protein yield at 2 consecutive dairy herd improvement test days. As indicated by treatment × day interactions, energy-corrected milk yield and milk lactose yield were increased by PBLC on the first test day only, and milk protein concentration decreased from test d 1 to test d 2 in CON only. The concentrations of fat, lactose, and urea, as well as somatic cell count, were not affected by treatment. The weekly milk yield over the first 11 wk of lactation was 29.5 kg/wk higher for PBLC versus CON across breeds. It is concluded that the applied PBLC induced a small but measurable improvement of calcium status in HF cows in the study period and had additional positive effects on milk performance in both breeds.

Key words: calcium absorption, hypocalcemia, transition dairy cow, phytogenic compounds

INTRODUCTION

The requirement for calcium increases abruptly from ~80 mg/kg$^{0.75}$ during late pregnancy to 500 mg/kg$^{0.75}$ at the beginning of lactation (Horst et al., 2005). This massive increase in demand exerts overt challenges on calcium homeorhesis that regularly lead to a decrease in blood calcium levels. If severe enough, hypocalcemia can lead to clinical milk fever with signs such as hyperesthesia, anorexia, weakness, or cold extremities and, finally, downer cow syndrome (Oetzel, 1988).

Subclinical hypocalcemia has also health consequences for the cow. It is associated with higher culling rates, poorer fertility and increased probability of suffering from other diseases, such as metritis or ketosis (Houe et al., 2001; Rodríguez et al., 2017; Venjakob et al., 2018). The risk of subclinical hypocalcemia increases from about 6 to 25% during first lactation to 29 to 60% in multiparous cows (Reinhardt et al., 2011; Venjakob et al., 2017). In addition to parity, breed also seems to be a decisive risk factor. An increased susceptibility to hypocalcemia in the Jersey breed (Erb and Grohn, 1988; Lean et al., 2006; Roche and Berry, 2006) could be due to a reduced expression of the receptor for 1,25(OH)$_2$D$_3$ in the intestine (Goff et al., 1995). The Holstein Friesian (**HF**) breed is known for a higher risk of developing hypocalcemia compared with Brown Swiss (**BS**; Erb and Grohn, 1988; Saborío-Montero et al., 2017), although no difference could be found in vi-

tamin D receptor expression in the intestine of mid- to late-lactation cows (Liesegang et al., 2008).

Various prophylactic treatments have been developed for hypocalcemia, including the provision of calcium compounds in larger amounts (5–125 g of calcium/dose). Surplus calcium is mainly absorbed by passive enteric diffusion, thereby preventing the deficit due to insufficient active absorption (Goff and Horst, 1993; Pehrson et al., 1998; Goff, 2008). The active absorption of calcium in the intestinal tissue occurs mainly through vitamin $1,25(OH)_2D_3$-stimulated channels of the transient receptor potential (**TRP**) family, especially TRPV6 and possibly TRPV5 (den Dekker et al., 2003; van Goor et al., 2017). By contrast, active absorption from the rumen is not sensitive to stimulation by vitamin $1,25(OH)_2D_3$ (Schröder et al., 2001; Wilkens et al., 2009) and likely involves TRPA1 and TRPV3 (Rosendahl et al., 2016; Liebe et al., 2020). The latter channels can be activated by various plant bioactive lipid compounds (**PBLC**). In vitro, an agonistic effect of the PBLC menthol has been demonstrated for TRPA1, TRPV3, and TRPM8 (Macpherson et al., 2006; Bautista et al., 2007; Vogt-Eisele et al., 2007) and validated for the bovine isoform of TRPV3 (Schrapers et al., 2018). Furthermore, an increased absorption of calcium could be detected in isolated ruminal epithelia of sheep ex vivo after luminal addition of menthol (Rosendahl et al., 2016; Geiger et al., 2021). Finally, feeding PBLC enriched with menthol increased blood calcium levels in mid- to late-lactating dairy cows (Braun et al., 2019) with a similar trend in growing sheep (Patra et al., 2019b).

The cited facts led to the hypothesis that feeding a diet containing menthol-rich PBLC may have a positive influence on calcium homeorhesis during the periparturient phase in dairy cows. We further suspected that this benefit may be dependent on the breed's susceptibility to hypocalcemia and may imply positive influences on lactation performance. Therefore, the aim of the present study was to compare the influence of a commercial mix of menthol-rich PBLC on blood calcium level and lactation performance among the high-performing HF breed, which is highly susceptible to hypocalcemia, and the BS breed, which has a lower milk yield and is less prone to hypocalcemia.

MATERIALS AND METHODS

Animals, Diets, and Feeding

The experiment was conducted on a dairy farm in Bavaria in compliance with the German legislation on the welfare of experimental animals and with the approval of the District Government of Upper Bavaria (Refer-

ence number: ROB-55.2–2532.Vet_03–18–35). The trial began in July 2018 and ran until January 2020. Due to breakdown of the milking robot, the trial had to be interrupted for 3 mo, which divided the experiment into 2 runs. The farm housed ~110 cows. The experiment was performed with cows of multiple parity from the BS (29 animals) and HF breeds (41 animals) in a randomized block design. The cows of each breed were divided into a PBLC-treated group and an untreated control (**CON**) group by lactation number, calving date (to exclude seasonal effects) and the 305-d milk yield of the previous lactation. Final group sizes were 14 BS-CON, 15 BS-PBLC, 23 HF-CON, and 18 HF-PBLC. Sample size estimation proceeded from the intention to detect a 0.12 mM difference of iCa between treatments upon an assumed standard deviation of 0.22 mM. Each cow entered the trial 8 d before the expected calving date and remained in the trial until 80 d postpartum.

Cows received a TMR antepartum ad libitum (Table 1). A partial mixed ration (**PMR**) was provided ad libitum postpartum together with 2 different types of concentrated feed supplied by the milking robot as follows: concentrate A increased linearly from 2 to 4 kg/d from d 1 to 15 and remained at 4 kg/d until d 30. Concentrate B was fed at 1 kg/d from d 0 to 30, except for the first run where it increased linearly from 1 to 3 kg/d from d 16 to 30. After d 30, concentrate allocation followed a farm-specific scheme, supplying a total of 0.5 to 10 kg/d of concentrate A and B per cow according to milk yield. The composition and chemical analysis of the feed components can be found in Table 1.

The cows were moved from a barn area with all-season pasture to a separate straw-bedded box 8 d before the calculated calving date. From this time on, each cow in the PBLC group received a commercial PBLC mixture (100 g/d of BTX14; PerformaNat GmbH), containing ground corn cobs mixed with 1.7 g of a PBLC mixture of propylene glycol as carrier and monoterpenes with menthol as the lead compound. To increase the acceptance of the additive, especially during the prepartum phase, 150 g of brewer's yeast was added per day which was also fed to the CON group. Correct dosing during the prepartum period until 2 d after calving was ensured by manual allocation once a day separately from TMR; cows were fixed in a feeding fence during this time and released after intake of the additive. On the third day after parturition, cows were moved to a freestall barn. The barn was divided into 2 areas, which were served by a central double box milking robot (Boumatic Robotics GmbH). The 2 barn areas and the 2 automatic milking systems were equally accessible to all cows. During automatic milking, the concentrated feed mix containing the brewer's yeast was allocated via a dosing unit to each cow. A

Table 1. Feed ingredients and chemical composition of diets fed prepartum and postpartum; the concentrated feed was administered postpartum via the automatic milking system

Item	TMR prepartum	Partial mixed ration postpartum	Concentrate A	Concentrate B
Ingredient (% of DM)				
Hay	19.5	11.0	0	0
Maize silage	21.4	23.7	0	0
Grass silage	34.4	38.0	0	0
Maize	4.3	4.8	35	33
Wheat	0	0	10	16
Barley	4.3	4.8	0	14.5
Rapeseed meal	5.8	6.4	20	22.5
Soy extraction meal	0	0	9	10
Maize gluten meal	0	0	7	0
Dried molasses	3.8	4.1	6	0
Malt sprouts[1]	0	0	5	0
Biscuit flour	0	0	3	0
Sugar beet vinasse	0	0	2	2.6
Sugar beet pulp	5.3	5.9	1	0
Mineral mix	1.2[2]	1.3[2]	0.1[3]	0.1[3]
Calcium carbonate	0	0	1.5	0.9
Sodium carbonate	0	0	0	0.4
Sodium chloride	0	0	0.4	0
Chemical composition (g/kg DM)				
DM	401.7	380.7	899.7	901.2
Crude ash	81.1	82.2	76.2	79.6
Crude fiber	235.8	218.7	79.2	72.4
CP	143.6	150.6	211.5	209.8
Crude fat	31.9	33.9	54.3	45.0
Starch	145.4	156.7	323.2	340.0
Sugar	29.3	30.6	73.2	58.8
aNDFom[4]	432.3	402.8	261.0	248.4
ADFom[4]	260.2	241.9	113.2	106.4
ADL	28.3	25.4	18.5	23.2
Calcium	6.08	6.40	9.87	8.42
Magnesium	2.48	2.63	2.97	2.86
Phosphorus	3.68	3.98	7.22	7.14
DCAD (mEq/kg)	+238	+248	+72	+70

[1]In the second run, malt sprouts were replaced by 2.5% dextrose and 2.5% palm oil.

[2]The mineral mix contained (per kg): 44 g of calcium carbonate, 19 g of sodium chloride, 16 g of calcium sodium phosphate, 12 g of magnesium oxide, 3 g of magnesium sulfate, 400,000 IU of vitamin A, 65,000 IU of vitamin D_3, 4,000 mg of vitamin E, 250 mg of vitamin C, 80 mg of vitamin B_1, 40 mg of vitamin B_2, 20 mg of vitamin B_6, 200 µg of vitamin of B_{12}, 5,000 mg of niacinamide, 80 mg of calcium D-pantothenate, 16,600 µg of biotin, 16,500 mg of choline chloride, 5,000 mg of zinc [4,500 mg as solid glycine zinc chelate hydrate (solid) and 500 mg as zinc oxide], 5,000 mg of manganese [1,500 mg as glycine-manganese chelate hydrate and 3,500 mg as manganese(II)-oxide], 1,000 mg of copper [750 mg as copper(II) glycine chelate hydrate (solid) and 250 mg as copper(II) sulfate pentahydrate], 150 mg of iodine as calcium iodate (anhydrous), 15 mg of cobalt as coated cobalt(II) carbonate granules, 25 mg of selenium [3 mg as selenium yeast from *Saccharomyces cerevisiae* NCYC R397 (inactivated) and 22 mg as sodium selenite].

[3]The mineral mix contained (per kg): 9,000 IU of vitamin A, 1,000 IU of vitamin D_3, 10 mg of vitamin E, 13 mg of copper-(II)-sulfate, pentahydrate, 0.75 mg of iodine as calcium iodate (anhydrous), 0.15 mg of cobalt as coated cobalt(II) carbonate granules, 30 mg of manganese as manganese(II)-oxide, 45 mg of zinc as zinc oxide, and 0.12 mg of selenium as sodium selenite.

[4]aNDFom = neutral detergent fiber corrected for residual ash and analyzed with amylase; ADFom = acid detergent fiber corrected for residual ash.

second dosing unit added the PBLC premix to cows of the treatment group. The daily allocation of the PBLC additive was distributed over 2 or more robot visits, taking care to ensure complete eating under consideration of individual milking frequency. Based on the design of the study with manual allocation of the PBLC-containing concentrate before calving, no effective blinding was possible during the study.

Animal health was checked daily by visual inspection and through data evaluation of the milking robot. In case of a disease, the cow was treated by a veterinarian and the incident was recorded. Calcium infusion was administered to downer cows with clinical hypocalcemia that was classified if the cow was unable to stand up and the ionized calcium (**iCa**) value was ≤0.8 mM. Subclinical hypocalcemia was assumed if the iCa

value was ≤1.0 mM (Oetzel et al., 1988; Martinez et al., 2018). During the whole trial, 3 HF cows developed milk fever with recumbency (1 cow fed PBLC, 2 cows fed CON). These cows were removed from further analyses, because serum calcium concentration as the key target variable was modified by calcium infusions.

Blood Sampling and Analyses

Blood samples were targeted for d −2 before calving and d 1, 3, 5, 7, 14, 21, and 28 after calving. For practical reasons, sampling was always performed on Mondays, Wednesdays, and Fridays between 1300 and 1500 h, implying a variation in sampling day by ± 1 d and, in less than 2% of samples, by ± 2 d. Blood samples were taken from coccygeal vessels using a 9-mL lithium-heparin vacutainer (Vacuette, Greiner Bio-One GmbH) and were processed within 30 min. Sodium, potassium, chloride, and iCa were analyzed with ion-selective electrodes and glucose was determined by the glucose oxidase test (Stat Profile Prime blood gas analyzer, NOVA Biomedical GmbH).

Body Condition Score

Body condition score was determined on each sampling day according to Edmonson et al. (1989); BCS was visually evaluated always by the same person. Assessment was performed using the following anatomical locations: the thoracic and spinal regions (chin, loin, and trunk), the ribs, spinous processes (loin), tuber sacrale (hip or hook bones), tuber ischii (pin bones), anterior coccygeal vertebrae (tail head), and femoral regions. The classification was based on a scale of 0 (highly emaciated) to 5 (very fat) with increments of 0.5.

Milk Yield and Milk Composition

The total daily milk yield of each cow was recorded by the software of the milking robot. The milk components fat, CP, lactose, urea and somatic cell count were determined by the monthly DHI testing (Milchprüfring, Wolnzach, Germany). Fat, CP, lactose, and urea were measured by infrared spectrophotometry (MilkoScan 7 RM, FOSS GmbH), whereas SCC was determined by flow cytometry (Fossomatic 7 and Fossomatic FC, FOSS GmbH). To calculate the ECM on measurement days, the 7-d median milk yield was used and extrapolated to 40 g/kg fat and 34 g/kg CP according to the formula of German herd testing organizations (Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH, LKV): ECM (kg/d) = milk yield (kg/d) × [0.038 × fat (g/kg) + 0.021 × protein (g/kg) + 1.05]/3.28.

Feed Analysis

Samples of TMR and PMR were taken every month and analyzed for feed components. Feed concentrates were sampled from each batch and analyzed as one combined sample per concentrate. The chemical composition of feed was determined by an external laboratory (Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH, Niederwiesa, Germany) according to standard protocols (Naumann et al., 2004). The DM was determined in a drying cabinet (UF55, Memmert GmbH + Co. KG; VDLUFA MB III 3.1). Crude fiber, CP, crude fat, crude ash, starch, sugar, NDF after amylase treatment and exclusive of residual ash (**aNDFom**), ADF after ashing (**ADFom**), and ADL were measured by near infrared spectroscopy (VDLUFA MB III 31.2 and 31.3). Calcium, phosphorus, and magnesium were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES; DIN EN ISO 11885:2009–09).

Statistical Analysis

The statistical evaluation of the randomized block design was performed with the software SPSS Statistics 26 (SPSS Inc.) using the MIXED procedure with cow as the experimental unit. During data analysis, group allocation was blinded. Data were tested for normal distribution and homogeneity of variances and, in the case of potassium, a Box-Cox transformation was performed to achieve normality and homogeneity criteria (Box and Cox, 1964). Potassium values were then back transformed for data summary and presentation. Exclusion criteria for further analysis were the administration of calcium infusion and severe disease such as *Escherichia coli* mastitis, PBLC feeding for less than 3 d antepartum due to premature calving, as well as missing milk data caused by the interruption due to the milking robot breakdown. The statistical model for repeated measurements with day as repeated effect included breed, treatment, and day as fixed factors, parity as block (2, 3, ≥4), the last 305-d milk yield as block and BCS, as well as all 2- and 3-way interactions between breed, treatment, and day as fixed factors. Cow nested within treatment and run were included in the model as random factors. Because hypocalcemia is essentially relevant in the early postpartum period, iCa in blood was additionally compared between treated and untreated cows for the critical period from d 1 to 3 after calving. For DHI data, 2 tests were included in the analysis. The first test included all cows in the period from d 4 to 40, and the second test from d 41 to 72 postpartum. The 2 tests were analyzed as repeated measures and are given as least squares means over the

whole period from 4 to 72 d. Daily milk yields were summed to weekly yields and evaluated until wk 11 after calving. The covariance structure was selected according to the smallest information criterion of Bayes. To determine the approximate denominator degrees of freedom for the F-tests in the models, the Satterthwaite method was used. For post hoc tests, a Bonferroni adjustment was chosen, and statistical significance was assumed at $P < 0.05$. Data are given as least squares means and standard error of the mean.

RESULTS

Whole Blood Concentrations of Minerals and Glucose

During the PBLC feeding period, blood values were monitored from d −2 to 28 relative to calving. Feeding a ration with PBLC induced a significant breed × treatment interaction for iCa ($P = 0.038$). Follow-up of the interaction with Bonferroni post hoc test evidenced a positive effect of PBLC treatment on iCa in HF cows only ($P = 0.015$) whereas iCa of BS cows was not affected by treatment ($P = 0.51$) and not different to that of HF-CON cows (Table 2). Additionally, an effect of day was found with iCa being lower at d 1 compared with all other days ($P < 0.001$; Figure 1). When comparing CON versus PBLC for the critical period d 1 to 3 after calving, iCa values were different only for HF cows (1.14 vs. 1.19 mM; $P = 0.046$) but not for BS cows (1.13 vs. 1.12 mM; $P = 0.97$).

Other tested blood minerals, such as sodium, chloride, and potassium, as well as blood glucose, were affected by neither PBLC feeding nor breed, nor their 2-way interaction ($P \geq 0.05$). However, an effect of day was observed for all of them ($P < 0.05$). In addition, a significant interaction of treatment × day was found for sodium where sodium levels were increased by PBLC treatment exclusively on d 21 ($P = 0.018$).

Calcium Status

Of the 70 animals examined (14 BS-CON, 15 BS-PBLC, 23 HF-CON, and 18 HF-PBLC), 15 animals developed subclinical hypocalcemia (21.4%). This affected one BS-CON (7.1%), 2 BS-PBLC (13.3%), 8 HF-CON (34.8%), and 4 HF-PBLC cows (22.2%). Clinical milk fever was detected only in HF cows [2 HF-CON (8.7%); one HF-PBLC (5.6%)].

Body Condition Score

Body condition evaluated on each sampling day showed differences among breeds, with BS hav-

Table 2. Effect of feeding plant bioactive lipid compounds (PBLC) on concentrations of selected minerals [ionized calcium (iCa), sodium, chloride, potassium] and glucose in whole blood, as well as BCS in Holstein Friesian and Brown Swiss dairy cows[1,2]

Item	Brown Swiss		Holstein Friesian		SEM[3]	P-value[4]						
	CON (n = 14)	PBLC (n = 15)	CON (n = 21)	PBLC (n = 17)		B	T	D	B × T	B × D	T × D	B × T × D
iCa (mM)	1.19[b]	1.19[b]	1.19[b]	1.22[a]	0.01	0.073	0.26	<0.001	0.038	0.20	0.90	0.86
Na (mM)	140.9	140.8	140.4	140.9	0.35	0.39	0.38	<0.001	0.26	0.74	0.042	0.67
Cl (mM)	106.7	107.0	106.9	106.9	0.39	0.84	0.59	<0.001	0.66	0.21	0.80	0.24
K (mM)	4.52	4.45	4.38	4.44	0.05	0.10	0.94	0.014	0.16	0.24	0.47	0.071
Glucose (mM)	3.15	3.18	3.11	3.10	0.08	0.40	0.87	<0.001	0.77	0.99	0.83	0.26
BCS	3.47	3.29	3.10	3.20	0.08	0.01	0.58	<0.001	0.083	<0.001	0.85	0.047

a,b Different letters indicate significant ($P < 0.05$) differences among groups.
[1] Feeding cows with the PBLC additive started 3 to 8 d before calving. Whole blood samples were measured on d −2, 1, 3, 5, 7, 14, 21, 28 (±1) relative to calving.
[2] CON = control without PBLC.
[3] Pooled SEM.
[4] B = breed; T = treatment; D = test day.
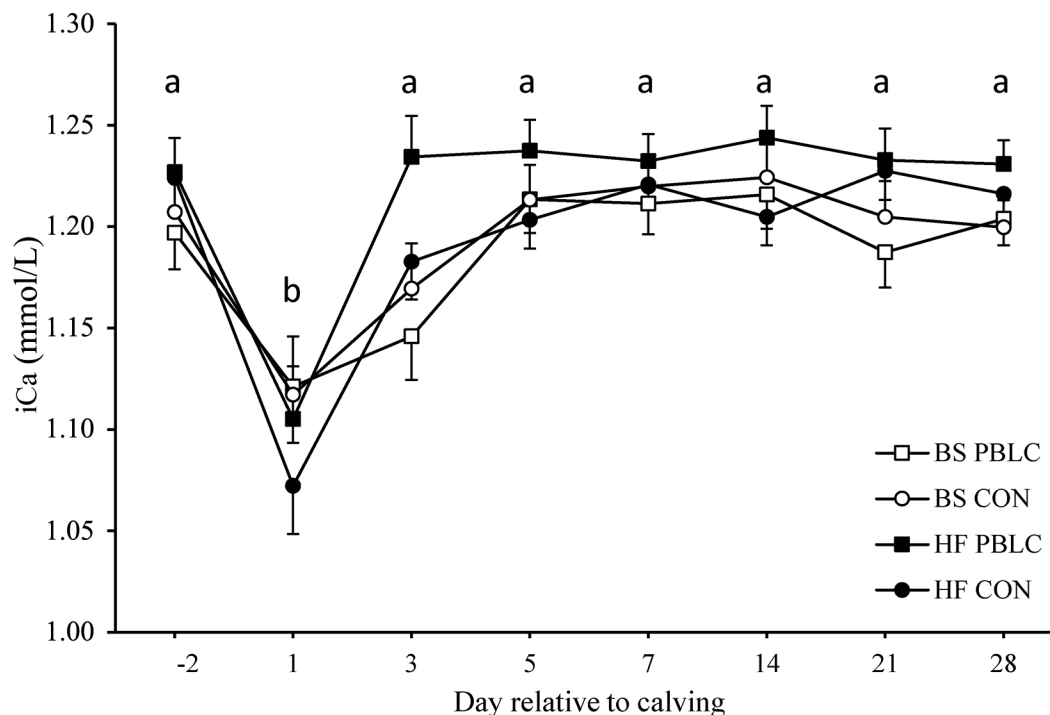
Figure 1. Concentration of ionized calcium (iCa) in whole blood of Holstein Friesian (HF) or Brown Swiss (BS) cows fed a standard ration without (CON) or with additional plant bioactive lipid compounds (PBLC) around calving. Error bars represent SEM. *P*-values are listed in Table 2. Different letters (a, b) indicate that data differ ($P < 0.05$) on different days across groups.

ing higher scores than HF (3.38 vs. 3.15 ± 0.066; P = 0.011) but no effect of treatment (3.24 vs. 3.29 ± 0.063 for PBLC vs. CON; $P = 0.58$; Table 2, Figure 2). The effect of day ($P < 0.001$) and the interactions breed × day ($P < 0.001$) and breed × treatment × day ($P = 0.047$) were also significant. Follow-up of these interactions identified that BS had consistently higher BCS than HF from d 1 to 28 within CON ($P < 0.05$), except for d 3 ($P = 0.052$) and d 5 ($P = 0.21$). Within PBLC-fed groups, no difference among breeds was detected. The PBLC-treated BS cows showed a lower BCS only at d 14 compared with CON (3.12 vs. 3.44 ± 0.107 for BS-PBLC vs. BS-CON; $P = 0.031$). Treatment in HF cows did not affect BCS (Figure 2).

Milk Yield and Milk Composition

The dietary treatment with PBLC affected daily milk yield at the 2 DHI test days ($P = 0.006$; Table 3). It also affected all other values related to milk quantity, such as ECM ($P = 0.018$), milk fat yield ($P = 0.049$), milk protein yield ($P = 0.026$), and milk lactose yield ($P = 0.006$), whereas the concentrations of individual milk components (fat, protein, and lactose) were not affected by treatment ($P \geq 0.05$). The average increase by PBLC of milk yield was 4.3 and 5.4 kg/d in BS

and HF cows, respectively, which penetrated to proportionate increases in yields of milk fat and milk protein (Table 3). No treatment effects were found for SCC and urea concentrations of milk.

As expected, milk yield was affected by breed ($P < 0.001$), with HF cows producing on average 11.6 ± 0.90 kg/d more milk compared with the BS cows. This further resulted in breed effects for ECM ($P < 0.001$), milk fat yield ($P < 0.002$), milk protein yield ($P < 0.001$) and milk lactose yield ($P < 0.001$) with distinctly higher values in HF cows, because milk constituent concentrations were not affected by breed except for milk protein concentration (3.49 vs. 3.24 ± 0.132% in BS vs. HF; $P = 0.002$; Table 3).

Regarding the 2 DHI test days, there were significant differences in fat yield (1.71 vs. 1.58 ± 0.62 kg/d; $P = 0.032$) and fat concentration (4.26 vs. 3.90 ± 0. 16%; $P = 0.005$), as well as in urea concentration (218.3 vs. 190.7 ± 23.4 mg/kg at DHI test d 1 vs. 2; $P = 0.030$) across breeds. There was also a significant difference in protein concentration at the 2 DHI test days with a treatment × test day interaction, pointing to an effect of DHI test day on protein concentration exclusively in CON cows (3.50 vs. 3.29 ± 0.133 at test d 1 vs. 2; $P < 0.001$). A treatment × test day interaction for ECM showed an increased ECM yield
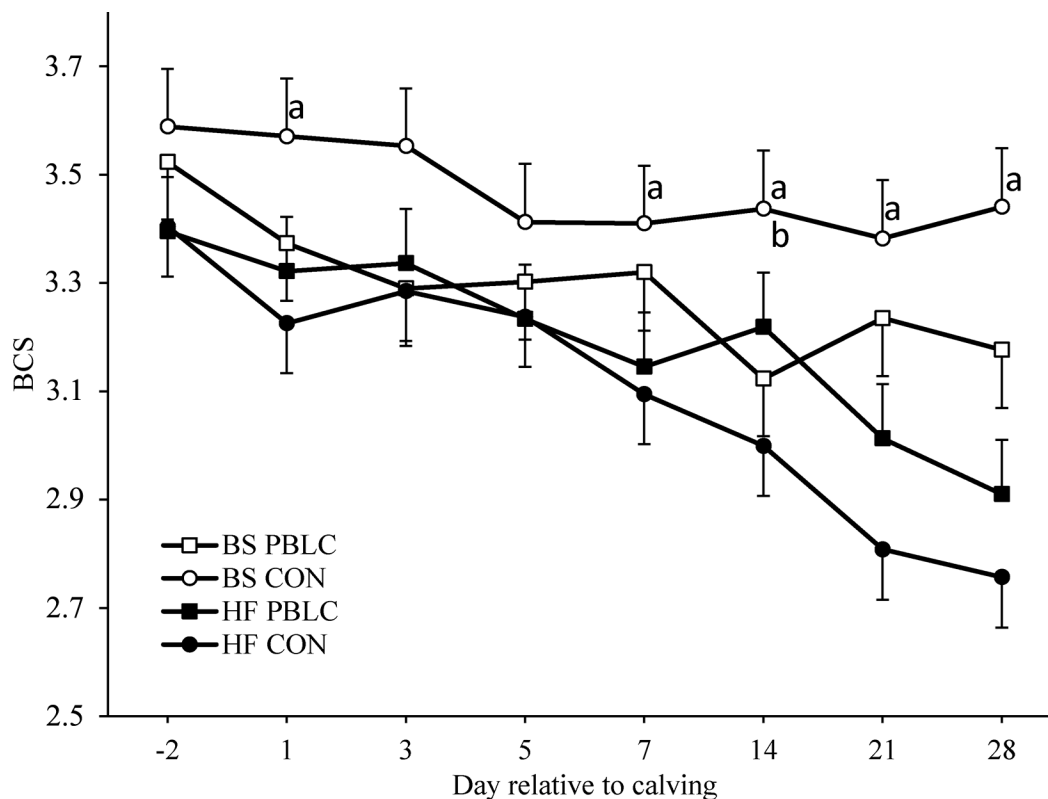
Figure 2. Development of BCS in Holstein Friesian (HF) or Brown Swiss (BS) cows fed a standard ration without (CON) or with additional plant bioactive lipid compounds (PBLC) around calving. Error bars represent SEM. P-values are listed in Table 2. The letter a indicates a significant difference between BS-CON and HF-CON ($P < 0.05$), and b indicates a significant difference between BS-CON and BS-PBLC ($P < 0.05$). No significant differences were identified for PBLC versus CON within HF cows or between breeds in PBLC-fed cows. Differences among different days within each group are not shown for reasons of clarity.

as a result of treatment on the first DHI test day (44.6 vs. 38.4 ± 1.46 kg/d, $P = 0.002$) but not on the second DHI test day (41.3 vs. 38.8 ± 1.59 kg/d for PBLC vs. CON; $P = 0.23$). Similarly, a treatment effect on the yield of lactose was also observed only on the first (2.11 vs. 1.80 ± 0.73 kg/d; $P = 0.002$) but not the second DHI test day (2.03 vs. 1.92 ± 0.070 kg/d for PBLC vs. CON; $P = 0.25$).

To exclude the possibility that the difference in DHI-recorded milk yield among PBLC and CON was biased by collection at different days after calving, the weekly milk yield was additionally recorded over the first 11 wk of lactation (Figure 3). Significant effects of breed ($P < 0.001$) and week ($P < 0.001$) with a breed × week interaction showed that HF cows (321.8 kg/wk) produced on average more milk than BS cows (239.4 ± 12.9 kg/wk). As a result of PBLC treatment, an increase in milk production of 29.5 kg/wk was observed during the measurement period (295.4 vs. 265.9 ± 12.2 kg/wk for PBLC vs. CON; $P = 0.009$) across breeds.

DISCUSSION

With the onset of lactation, dairy cows need large amounts of calcium to support calcium homeorhesis and milk production (Horst et al., 2005; Wilkens et al., 2020). Homeorhesis is the coordinated control of the metabolism of body tissues to support a physiological state. In extension of the homeostatic concept, homeorhesis takes special reference to flexible adaptation and metabolic prioritization to cover changing metabolic needs (Bauman and Bruce Currie, 1980). Given the variety of physiological tasks that require calcium, even small changes in calcium bioavailability can have negative effects on, for example, muscle, endocrine, and immune functions. The main intention of the present field study was to elucidate whether a menthol-rich PBLC supplement could support calcium homeorhesis precisely during the critical period around calving. To proof this, feeding of the supplement commenced 8 d before the predicted calving date. We were able to show a PBLC-induced increase of blood iCa level in

Table 3. Effect of feeding plant bioactive lipid compounds (PBLC) on milk composition and total milk yield in Holstein Friesian and Brown Swiss cows[1,2]

Item	Brown Swiss CON (n = 13)	Brown Swiss PBLC (n = 14)	Holstein Friesian CON (n = 21)	Holstein Friesian PBLC (n = 13)	SEM[3]	P-value[4] B	T	D	B × T	B × D	T × D	B × T × D
Milk yield (kg/d)	32.6	36.9	43.7	49.1	1.82	<0.001	0.006	0.56	0.77	0.47	0.12	0.71
ECM (kg/d)	34.0	38.0	43.2	48.0	1.95	<0.001	0.018	0.13	0.85	0.59	0.048	0.78
Fat (kg/d)	1.39	1.56	1.71	1.91	0.10	0.002	0.049	0.032	0.91	0.87	0.076	0.62
Protein (kg/d)	1.15	1.26	1.42	1.56	0.059	<0.001	0.026	0.38	0.77	0.61	0.38	0.65
Lactose (kg/d)	1.56	1.76	2.16	2.38	0.092	<0.001	0.018	0.56	0.91	0.11	0.013	0.78
Fat (%)	4.28	4.21	3.87	3.97	0.21	0.106	0.96	0.005	0.64	0.69	0.40	0.55
Protein (%)	3.58	3.39	3.21	3.27	0.14	0.002	0.29	0.003	0.070	0.30	0.030	0.22
Lactose (%)	4.80	4.79	4.86	4.83	0.038	0.28	0.55	0.25	0.81	0.29	0.21	0.29
Urea (mg/kg)	215	204	210	189	27.04	0.60	0.32	0.030	0.79	0.049	0.60	0.50
Cell count (cells/mL)	304	263	240	186	119.1	0.56	0.69	0.29	0.96	0.67	0.54	0.71

[1]Milk composition values and daily milk yields are LSM computed from 2 consecutive samples per cow on DHI test days in the periods 4 to 40 d and 41 to 80 d of lactation.

[2]CON = control without PBLC.

[3]Pooled SEM.

[4]B = breed; T = treatment; D = test day.

HF cows but not in BS cows. The observed increase of iCa in HF cows is in congruence with previous studies from our group, which had evidenced positive effects of a similar PBLC mixture on total calcium in blood serum of HF cows in mid to late lactation (Braun et al., 2019) and, as a trend, in growing sheep (Patra et al., 2019b). In companion experiments to the latter study, we were further able to demonstrate that PBLC feeding increased absorption of calcium from the rumen via TRP channels (Geiger et al., 2021).

It has been well established in monogastric animals, as well as in ruminants, that transcellular calcium absorption in the intestine occurs mainly via TRPV5 and TRPV6 channels (Schröder et al., 2015; Christakos et al., 2016; van Goor et al., 2017). In ruminants, however, calcium absorption in the rumen seems to be of particular importance (Schröder et al., 1999; Leonhard-Marek et al., 2010; Wilkens et al., 2016). Unlike the intestine, TRPV5 and TRPV6 are not or only weakly expressed in the ruminal tissue, implying that ruminal calcium absorption must operate by a different mechanism (Wilkens et al., 2009, 2011; Schröder et al., 2015). In addition to TRPV5 and TRPV6 (Nilius et al., 2002; Hoenderop, 2003; Woudenberg-Vrenken et al., 2012), other nonselective TRP channels are also able to transport cations such as calcium (e.g., TRPV3, TRPM8, or TRPA1; Gees et al., 2010). The mRNA expression of TRPA1 and TRPV3 has been detected in the rumen of sheep and cows (Rosendahl et al., 2016; Geiger et al., 2021) and TRPV3 was also detected at the protein level by western blot and immunohistochemistry in the bovine rumen (Liebe et al., 2020). Furthermore, bovine TRPV3 expressed in HEK cells was shown to be activated by menthol (Schrapers et al., 2018). It was thus proposed that TRPV3 may play a central role in ruminal calcium absorption (Geiger et al., 2021). The PBLC stimulation increased calcium absorption in isolated ruminal epithelium elicited by substances such as menthol (Rosendahl et al., 2016; Geiger et al., 2021). It may thus also explain the PBLC-induced increases in blood calcium levels in mid- to late-lactating HF cows in our previous study (Braun et al., 2019). An outstanding and new finding of the present study is that menthol-rich PBLC also are effective to increase blood calcium level during early lactation in HF cows. The increase in blood calcium may appear quantitatively small. However, it has to be considered that cows experiencing milk fever and requiring calcium infusions were removed from the study, which applied to only one HF-PLBC but 2 HF-CON cows. Furthermore, baseline calcium levels were rather high in the study herd. Therefore, any surplus calcium provision would likely initiate homeorhetic counter-regulation (Martín-Tereso and Verstegen, 2011), especially with prolonged
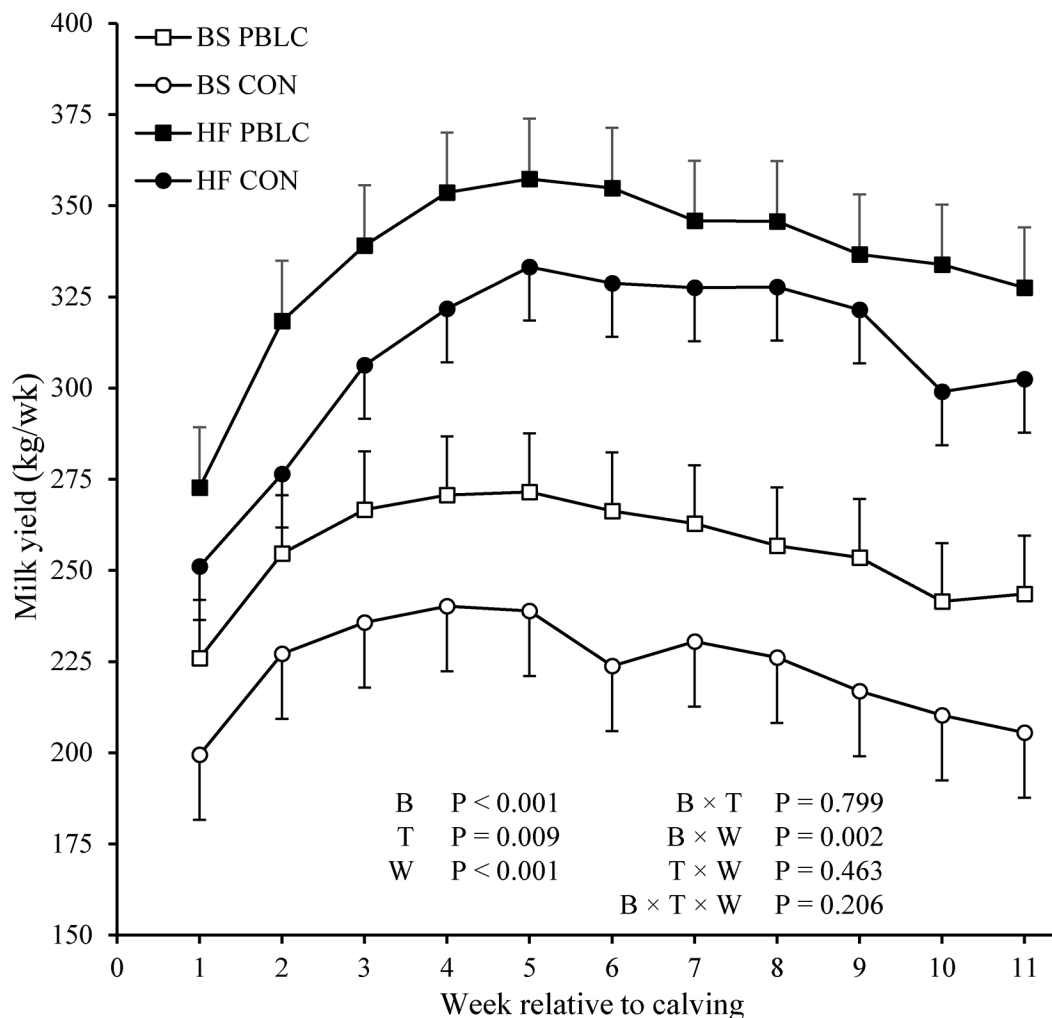
Figure 3. Weekly milk yields of Holstein Friesian (HF) or Brown Swiss (BS) cows fed a standard ration without (CON) or with additional plant bioactive lipid compounds (PBLC). Error bars represent SEM. *P*-values for main effects and interactions are shown in the diagram. Individual differences for the interaction B × W are not indicated by letter code for reasons of clarity (B = breed; T = treatment; W = week).

application toward the end of the study. Vice versa, this also means that animals in need of surplus calcium would initiate less counter-regulation and benefit from the surplus calcium, especially in the critical days around calving. As outlined above, the source of surplus calcium provision to the system is likely increased ruminal absorption via TRPV3 (and possibly TRPA1) by the menthol-containing PBLC. As TRPV3 and TRPA1 also conduct sodium (Rosendahl et al., 2016; Schrapers et al., 2018), an activation of such channels may additionally explain the increase in plasma sodium at 21 DIM. Alternatively or additionally, it may be speculated that HF cows increased DMI during PBLC supplementation as previously observed in growing sheep (Patra et al., 2019a). Although a similar increase in DMI was not observed in late-lactating cows (Braun et al., 2019), it would include a proportionate increase in calcium intake if present. Unfortunately, DMI could not be recorded in the current field study.

The prevalence of subclinical hypocalcemia in HF cows appeared relatively low in the study herd compared with literature data. In the study of Venjakob et al. (2017) on cows belonging almost exclusively to the HF breed, a prevalence of 29 and 49.4% was reported for animals in the second and third lactation, respectively. The study by Reinhardt et al. (2011) quoted a prevalence of 41% and 49%, respectively, for the second and third lactation. In our study, the average lactation number of HF cows was about 2.75 (BS = 3.5). Therefore, we expected a prevalence of subclinical hypocalcemia of ~40% or higher, especially, because there were no prophylaxis measures against hypocalcemia on the farm except our treatment. In addition, the DCAD of the basal ration was quite high both prepartum (+238

mEq/kg) and postpartum (+248 mEq/kg); therefore, a promoting effect of diet on hypocalcemia prevalence would have been expected (Charbonneau et al., 2006; Lean et al., 2006; Santos et al., 2019). The reasons why hypocalcemia prevalence was so low in our study compared with previous studies may partly relate to the calcium measurement method. We have shown recently that assessment of calcium status from total calcium may cause overestimation of the prevalence of postpartum hypocalcemia because the functionally relevant iCa shows much less variation around calving than total calcium (Ott et al., 2021). Already previous studies had shown that the direct measurement of ionized calcium is preferable over measurement of total calcium (Wilms et al., 2019). The ionized calcium represents the biologically active fraction in the organism. Around the period of calving, there is a change of the proportion of ionized calcium within total calcium (Ballantine and Herbein, 1991; Joyce et al., 1997). Therefore, an accurate prediction of calcium status can only be achieved by measuring ionized calcium. The low (and probably more precisely determined) prevalence of hypocalcemia based on iCa, might possibly explain why a positive effect of PBLC on blood iCa concentration could not be observed in BS cows. Already previous studies indicated that hypocalcemia is less common in the BS breed compared with others such as HF or Jersey (Erb and Grohn, 1988; Saborío-Montero et al., 2017). The more stable calcium level in BS cows around calving may have possibly blunted an effect of PBLC supplementation on blood calcium concentration in BS cows in the present study. In the high-yielding HF breed, however, a positive effect of PBLC could be observed on iCa, including the days immediately after calving (d 1 and 3). It has been shown recently that such fast return to normal blood calcium concentration is associated with markedly lower incidences for other postpartum diseases such as hyperketonemia, metritis, displaced abomasum, or herd removal in the first 60 DIM (McArt and Neves, 2020).

Previous research on the effect of PBLC in ruminants had already considered effects on health status or milk yield (Khiaosa-ard and Zebeli, 2013). Many of these studies explained PBLC effects by their action on microbiota and fermentation, although this often requires PBLC dosages much higher than that used in the present study (Ando et al., 2003; Calsamiglia et al., 2007). When feeding concentrations of a menthol-based PBLC comparable to the present study to growing sheep, only minor changes in ruminal microbiota and fermentation were detected (Patra et al., 2019c). Thus, other effects of PBLC on sensory perception, inflammation or pain, which are mediated by TRP channels among others, could also play a role for the effects of PBLC on animal health and productivity (Holzer, 2011; Nilius and Szallasi, 2014; Oh et al., 2017; Oz et al., 2017).

The milk yield and milk composition of the 2 breeds clearly differed in the present study. Holstein Friesian cows produced >10 kg more milk per day than BS cows; whereas, the percentage of milk protein was higher in BS cows. These differences agree with literature findings (Carroll et al., 2006; De Marchi et al., 2008; Visentin et al., 2018). Feeding the PBLC supplement resulted in a marked increase in milk yield by >10% that was comparable in both breeds, whereas milk composition was not affected by feeding. Such noticeable increase in milk yield appears a rather rare occasion in literature. A meta-analysis by Khiaosa-ard and Zebeli (2013) analyzed milk production data from 43 studies in which PBLC were supplemented to dairy cows. They did not identify a general stimulating effect of PBLC on milk production, although some studies identified such positive effects (Hausmann et al., 2018; Kholif et al., 2020). This suggests that stimulation of milk production appears rather specific for certain compositions and concentrations of PBLC mixtures. Of note, the stimulation of milk production by the menthol-rich PBLC used in the present study reproduced the increase in milk production observed in a previous study using a similar PBLC formulation in dairy cows in mid to late lactation, also without changing the milk composition (Braun et al., 2019). We were not able to determine if increased milk production coincided with increased feed intake because measurement of feed intake was not possible in the present study. As mentioned earlier, a similar PBLC mix as used in the present study was shown previously to increase feed intake in growing sheep (Patra et al., 2019a) but not in mid- to late-lactating dairy cows (Braun et al., 2019).

With progressing DIM, the BCS changed significantly in the present study, which was expectable due to the negative energy balance at the onset of lactation (Coffey et al., 2002). Additionally, 3-way interaction between breed × treatment × day indicated that the loss of body condition in HF-CON cows was significantly higher, especially toward the end of the study, compared with BS-CON cows. This fits the higher milk yields observed in HF-CON versus BS-CON cows because higher milk yields are commonly associated with greater losses of BW (Domecq et al., 1997) and body condition (Carroll et al., 2006). Of note, there was no such difference among HF-PBLC and BS-PBLC cows, indicating that HF-PBLC cows were able to largely compensate the negative effect of higher milk yield on body condition. The most plausible reason for this compensation would be increased PMR intake (Patra et al. 2019a). That such compensation occurred selectively in the HF breed may relate to the fact that HF were bred

for superiority in roughage intake (Gruber et al., 1991) and feed efficiency (Dickinson et al., 1969).

The present report is about the first study that monitored the effect of PBLC on blood ionized calcium in periparturient dairy cattle. The study was designed to compare such effects in 2 breeds with greatly diverging hypocalcemia prevalence in a field setup with high DCAD diet and no prophylactic measures against hypocalcemia. The latter should have led to a high prevalence of hypocalcemia. Unexpectedly, however, hypocalcemia prevalence was rather low. Thus, an effect of the PBLC on hypocalcemia prevalence could not be properly evaluated and the measured effect of PBLC on blood ionized calcium was rather small. A more prominent effect was observed on milk production. Unfortunately, the field study on a cohort of >70 animals did not allow measurement of individual feed intake for deeper mechanistic evaluation of the latter finding. Thus, further studies are needed to investigate the effect of the menthol-rich PBLC on feed intake and feed efficiency in HF cows around calving more closely.

CONCLUSIONS

The present study demonstrated that feeding a menthol-rich PBLC supplement can increase blood ionized calcium in high-yielding dairy cows of the HF breed in the critical peripartal period. Although this increase was relatively small, an elevation of iCa by ~5% around parturition may become relevant in settings where cows are prone to hypocalcemia and secondary diseases promoted by hypocalcemia. We further observed a marked increase in milk yield during early lactation in both HF and BS cows with almost no changes in milk composition and, especially in HF cows, no negative effects on body condition. The improved calcium homeorhesis in HF cows is most likely explainable by enhanced calcium absorption from the rumen. For production effects, other explanations could additionally apply, such as increases in PMR intake and an improved immune or health status, which should be followed up in further studies.

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REFERENCES

Ando, S., T. Nishida, M. Ishida, K. Hosoda, and E. Bayaru. 2003. Effect of peppermint feeding on the digestibility, ruminal fermentation and protozoa. Livest. Prod. Sci. 82:245–248. https://doi.org/10.1016/S0301-6226(03)00012-5.

Ballantine, H. T., and J. H. Herbein. 1991. Potentiometric determination of ionized and total calcium in blood plasma of Holstein and Jersey cows. J. Dairy Sci. 74:446–449. https://doi.org/10.3168/jds.S0022-0302(91)78190-3.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514–1529. https://doi.org/10.3168/jds.S0022-0302(80)83111-0.

Bautista, D. M., J. Siemens, J. M. Glazer, P. R. Tsuruda, A. I. Basbaum, C. L. Stucky, S.-E. Jordt, and D. Julius. 2007. The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 448:204–208. https://doi.org/10.1038/nature05910.

Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. J. R. Stat. Soc. B 26:211–252.

Braun, H.-S., K. T. Schrapers, K. Mahlkow-Nerge, F. Stumpff, and J. Rosendahl. 2019. Dietary supplementation of essential oils in dairy cows: Evidence for stimulatory effects on nutrient absorption. Animal 13:518–523. https://doi.org/10.1017/S1751731118001696.

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90:2580–2595. https://doi.org/10.3168/jds.2006-644.

Carroll, S. M., E. J. DePeters, S. J. Taylor, M. Rosenberg, H. Perez-Monti, and V. A. Capps. 2006. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Anim. Feed Sci. Technol. 131:451–473. https://doi.org/10.1016/j.anifeedsci.2006.06.019.

Charbonneau, E., D. Pellerin, and G. R. Oetzel. 2006. Impact of lowering dietary cation-anion difference in nonlactating dairy cows: A meta-analysis. J. Dairy Sci. 89:537–548. https://doi.org/10.3168/jds.S0022-0302(06)72116-6.

Coffey, M. P., G. Simm, and S. Brotherstone. 2002. Energy balance profiles for the first three lactations of dairy cows estimated using random regression. J. Dairy Sci. 85:2669–2678.

De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit, and M. Cassandro. 2008. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. J. Dairy Sci. 91:4092–4102. https://doi.org/10.3168/jds.2007-0788.

den Dekker, E., J. G. G. J. Hoenderop, B. Nilius, and R. J. M. Bindels. 2003. The epithelial calcium channels, TRPV5 & TRPV6: From identification towards regulation. Cell Calcium 33:497–507. https://doi.org/10.1016/S0143-4160(03)00065-4.

Dickinson, F. N., B. T. McDaniel, and R. E. McDowell. 1969. Comparative efficiency of feed utilization during first lactation of Ayrshire, Brown Swiss, and Holstein cows. J. Dairy Sci. 52:489–497. https://doi.org/10.3168/jds.S0022-0302(69)86593-8.

Domecq, J. J., A. L. Skidmore, J. W. Lloyd, and J. B. Kaneene. 1997. Relationship between body condition scores and milk yield in a large dairy herd of high yielding Holstein cows. J. Dairy Sci. 80:101–112. https://doi.org/10.3168/jds.S0022-0302(97)75917-4.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78. https://doi.org/10.3168/jds.S0022-0302(89)79081-0.

Erb, H. N., and Y. T. Grohn. 1988. Epidemiology of metabolic disorders in the periparturient dairy cow. J. Dairy Sci. 71:2557–2571. https://doi.org/10.3168/jds.S0022-0302(88)79845-8.

Gees, M., B. Colsoul, and B. Nilius. 2010. The role of transient receptor potential cation channels in Ca2+ signaling. Cold Spring Harb. Perspect. Biol. 2:a003962. https://doi.org/10.1101/cshperspect.a003962.

Geiger, S., A. K. Patra, K. T. Schrapers, H. S. Braun, and J. R. Aschenbach. 2021. Menthol stimulates calcium absorption in the rumen but not in the jejunum of sheep. J. Dairy Sci. 104:3067–3081. https://doi.org/10.3168/jds.2020-19372.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176:50–57. https://doi.org/10.1016/j.tvjl.2007.12.020.

Goff, J. P., and R. L. Horst. 1993. Oral administration of calcium salts for treatment of hypocalcemia in cattle. J. Dairy Sci. 76:101–108. https://doi.org/10.3168/jds.S0022-0302(93)77328-2.

Goff, J. P., T. A. Reinhardt, and R. L. Horst. 1995. Milk fever and dietary cation-anion balance effects on concentration of vitamin D receptor in tissue of periparturient dairy cows. J. Dairy Sci. 78:2388–2394. https://doi.org/10.3168/jds.S0022-0302(95)76867-9.

Gruber, L., R. Steinwender, K. Krimberger, and J. Sölkner. 1991. Roughage intake of Simmental, Brown Swiss and Holstein Friesian cows fed rations with 0, 25 and 50% concentrates. Livest. Prod. Sci. 27:123–136. https://doi.org/10.1016/0301-6226(91)90091-4.

Hausmann, J., C. Deiner, A. K. Patra, I. Immig, A. Starke, and J. R. Aschenbach. 2018. Effects of a combination of plant bioactive lipid compounds and biotin compared with monensin on body condition, energy metabolism and milk performance in transition dairy cows. PLoS One 13:e0193685. https://doi.org/10.1371/journal.pone.0193685.

Hoenderop, J. G. J. 2003. Homo- and heterotetrameric architecture of the epithelial Ca2+ channels TRPV5 and TRPV6. EMBO J. 22:776–785. https://doi.org/10.1093/emboj/cdg080.

Holzer, P. 2011. Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. Pharmacol. Ther. 131:142–170. https://doi.org/10.1016/j.pharmthera.2011.03.006.

Horst, R. L., J. P. Goff, and T. A. Reinhardt. 2005. Adapting to the transition between gestation and lactation: Differences between rat, human and dairy cow. J. Mammary Gland Biol. Neoplasia 10:141–156. https://doi.org/10.1007/s10911-005-5397-x.

Houe, H., S. Østergaard, T. Thilsing-Hansen, R. J. Jørgensen, T. Larsen, J. T. Sørensen, J. F. Agger, and J. Y. Blom. 2001. Milk fever and subclinical hypocalcemia – An evaluation of parameters on incidence risk, diagnosis, risk factors and biological effects as input for a decision support system for disease control. Acta Vet. Scand. 42:1–29.

Joyce, P. W., W. K. Sanchez, and J. P. Goff. 1997. Effect of anionic salts in prepartum diets based on alfalfa. J. Dairy Sci. 80:2866–2875. https://doi.org/10.3168/jds.S0022-0302(97)76251-9.

Khiaosa-ard, R., and Q. Zebeli. 2013. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. J. Anim. Sci. 91:1819–1830. https://doi.org/10.2527/jas.2012-5691.

Kholif, A. E., A. A. Hassan, G. M. El Ashry, M. H. Bakr, H. M. El-Zaiat, O. A. Olafadehan, O. H. Matloup, and S. M. A. Sallam. 2021. Phytogenic feed additives mixture enhances the lactational performance, feed utilization and ruminal fermentation of Friesian cows. Anim. Biotechnol. 32:708–718. https://doi.org/10.1080/10495398.2020.1746322.

Lean, I. J., P. J. DeGaris, D. M. McNeil, and E. Block. 2006. Hypocalcemia in dairy cows: Meta-analysis and dietary cation anion difference theory revisited. J. Dairy Sci. 89:669–684. https://doi.org/10.3168/jds.S0022-0302(06)72130-0.

Liebe, F., H. Liebe, S. Kaessmeyer, G. Sponder, and F. Stumpff. 2020. The TRPV3 channel of the bovine rumen: Localization and functional characterization of a protein relevant for ruminal ammonia transport. Pflugers Arch. 472:693–710. https://doi.org/10.1007/s00424-020-02393-2.

Liesegang, A., K. Singer, and A. Boos. 2008. Vitamin D receptor amounts across different segments of the gastrointestinal tract in Brown Swiss and Holstein Frisean cows of different age. J. Anim. Physiol. Anim. Nutr. (Berl.) 92:316–323. https://doi.org/10.1111/j.1439-0396.2007.00782.x.

Macpherson, L. J., S. W. Hwang, T. Miyamoto, A. E. Dubin, A. Patapoutian, and G. M. Story. 2006. More than cool: Promiscuous relationships of menthol and other sensory compounds. Mol. Cell. Neurosci. 32:335–343. https://doi.org/10.1016/j.mcn.2006.05.005.

Martín-Tereso, J., and M. W. A. Verstegen. 2011. A novel model to explain dietary factors affecting hypocalcaemia in dairy cattle. Nutr. Res. Rev. 24:228–243. https://doi.org/10.1017/S0954422411000126.

Martinez, N., R. M. Rodney, E. Block, L. L. Hernandez, C. D. Nelson, I. J. Lean, and J. E. P. Santos. 2018. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses. J. Dairy Sci. 101:2563–2578. https://doi.org/10.3168/jds.2017-13740.

McArt, J. A., and R. C. Neves. 2020. Association of transient, persistent, or delayed subclinical hypocalcemia with early lactation disease, removal, and milk yield in Holstein cows. J. Dairy Sci. 103:690–701. https://doi.org/10.3168/jds.2019-17191.

Naumann, K., R. Bassler, R. Seibold, and K. Barth. 2004. Die Chemische Untersuchung von Futtermitteln: Methodenbuch Band III, Loseblattausgabe Mit Ergänzungen 1983, 1988, 1993, 1997 Und 2004. VDLUFA-Verlag.

Nilius, B., J. Prenen, J. G. J. Hoenderop, R. Vennekens, S. Hoefs, A. F. Weidema, G. Droogmans, and R. J. M. Bindels. 2002. Fast and slow inactivation kinetics of the Ca2+ channels ECaC1 and ECaC2 (TRPV5 and TRPV6): Role of the intracellular loop located between transmembrane segments 2 and 3. J. Biol. Chem. 277:30852–30858. https://doi.org/10.1074/jbc.M202418200.

Nilius, B., and A. Szallasi. 2014. Transient receptor potential channels as drug targets: From the science of basic research to the art of medicine. Pharmacol. Rev. 66:676–814. https://doi.org/10.1124/pr.113.008268.

Oetzel, G. R. 1988. Parturient paresis and hypocalcemia in ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 4:351–364. https://doi.org/10.1016/S0749-0720(15)31053-7.

Oetzel, G. R., J. D. Olson, C. R. Curtis, and M. J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302–3309. https://doi.org/10.3168/jds.S0022-0302(88)79935-X.

Oh, J., E. H. Wall, D. M. Bravo, and A. N. Hristov. 2017. Host-mediated effects of phytonutrients in ruminants: A review. J. Dairy Sci. 100:5974–5983. https://doi.org/10.3168/jds.2016-12341.

Ott, D., K. T. Schrapers, and J. R. Aschenbach. 2021. Changes in the relationship between ionized and total calcium in clinically healthy dairy cows in the period around calving. Animals (Basel) 11:1036. https://doi.org/10.3390/ani11041036.

Oz, M., E. G. El Nebrisi, K.-H. S. Yang, F. C. Howarth, and L. T. Al Kury. 2017. Cellular and molecular targets of menthol actions. Front. Pharmacol. 8:472. https://doi.org/10.3389/fphar.2017.00472.

Patra, A. K., S. Geiger, H.-S. Braun, and J. R. Aschenbach. 2019a. Dietary supplementation of menthol-rich bioactive lipid compounds alters circadian eating behaviour of sheep. BMC Vet. Res. 15:352. https://doi.org/10.1186/s12917-019-2109-0.

Patra, A. K., S. Geiger, K. T. Schrapers, H.-S. Braun, H. Gehlen, A. Starke, R. Pieper, A. Cieslak, M. Szumacher-Strabel, and J. R. Aschenbach. 2019b. Effects of dietary menthol-rich bioactive lipid compounds on zootechnical traits, blood variables and gastrointestinal function in growing sheep. J. Anim. Sci. Biotechnol. 10:86. https://doi.org/10.1186/s40104-019-0398-6.

Patra, A. K., T. Park, H.-S. Braun, S. Geiger, R. Pieper, Z. Yu, and J. R. Aschenbach. 2019c. Dietary bioactive lipid compounds rich in menthol alter interactions among members of ruminal microbiota in sheep. Front. Microbiol. 10:2038. https://doi.org/10.3389/fmicb.2019.02038.

Pehrson, B., C. Svensson, and M. Jonsson. 1998. A comparative study of the effectiveness of calcium propionate and calcium chloride for the prevention of parturient paresis in dairy cows. J. Dairy Sci. 81:2011–2016. https://doi.org/10.3168/jds.S0022-0302(98)75775-3.

Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188:122–124. https://doi.org/10.1016/j.tvjl.2010.03.025.

Roche, J. R., and D. P. Berry. 2006. Periparturient climatic, animal, and management factors influencing the incidence of milk fever in grazing systems. J. Dairy Sci. 89:2775–2783. https://doi.org/10.3168/jds.S0022-0302(06)72354-2.

Rodríguez, E. M., A. Arís, and A. Bach. 2017. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. J. Dairy Sci. 100:7427–7434. https://doi.org/10.3168/jds.2016-12210.

Rosendahl, J., H. S. Braun, K. T. Schrapers, H. Martens, and F. Stumpff. 2016. Evidence for the functional involvement of members of the TRP channel family in the uptake of Na+ and NH4+ by the ruminal epithelium. Pflugers Arch. 468:1333–1352. https://doi.org/10.1007/s00424-016-1835-4.

Saborío-Montero, A., B. Vargas-Leitón, J. J. Romero-Zúñiga, and J. M. Sánchez. 2017. Risk factors associated with milk fever occurrence in grazing dairy cattle. J. Dairy Sci. 100:9715–9722. https://doi.org/10.3168/jds.2017-13065.

Santos, J. E. P., I. J. Lean, H. Golder, and E. Block. 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J. Dairy Sci. 102:2134–2154. https://doi.org/10.3168/jds.2018-14628.

Schrapers, K. T., G. Sponder, F. Liebe, H. Liebe, and F. Stumpff. 2018. The bovine TRPV3 as a pathway for the uptake of Na+, Ca2+, and NH4+. PLoS One 13:e0193519. https://doi.org/10.1371/journal.pone.0193519.

Schröder, B., W. Goebel, K. Huber, and G. Breves. 2001. No effect of vitamin D3 treatment on active calcium absorption across ruminal epithelium of sheep. J. Vet. Med. A Physiol. Pathol. Clin. Med. 48:353–363. https://doi.org/10.1046/j.1439-0442.2001.00369.x.

van Goor, M. K. C., J. G. J. Hoenderop, and J. van der Wijst. 2017. TRP channels in calcium homeostasis: From hormonal control to structure-function relationship of TRPV5 and TRPV6. Biochim. Biophys. Acta Mol. Cell Res. 1864:883–893. https://doi.org/10.1016/j.bbamcr.2016.11.027.

Venjakob, P. L., S. Borchardt, and W. Heuwieser. 2017. Hypocalcemia—Cow-level prevalence and preventive strategies in German dairy herds. J. Dairy Sci. 100:9258–9266. https://doi.org/10.3168/jds.2016-12494.

Venjakob, P. L., L. Pieper, W. Heuwieser, and S. Borchardt. 2018. Association of postpartum hypocalcemia with early-lactation milk yield, reproductive performance, and culling in dairy cows. J. Dairy Sci. 101:9396–9405. https://doi.org/10.3168/jds.2017-14202.

Visentin, G., M. Penasa, G. Niero, M. Cassandro, and M. De Marchi. 2018. Phenotypic characterisation of major mineral composition predicted by mid-infrared spectroscopy in cow milk. Ital. J. Anim. Sci. 17:549–556. https://doi.org/10.1080/1828051X.2017.1398055.

Vogt-Eisele, A. K., K. Weber, M. A. Sherkheli, G. Vielhaber, J. Panten, G. Gisselmann, and H. Hatt. 2007. Monoterpenoid agonists of TRPV3. Br. J. Pharmacol. 151:530–540. https://doi.org/10.1038/sj.bjp.0707245.

Wilkens, M. R., C. Kunert-Keil, H. Brinkmeier, and B. Schröder. 2009. Expression of calcium channel TRPV6 in ovine epithelial tissue. Vet. J. 182:294–300. https://doi.org/10.1016/j.tvjl.2008.06.020.

Wilkens, M. R., C. D. Nelson, L. L. Hernandez, and J. A. A. McArt. 2020. Symposium review: Transition cow calcium homeostasis—Health effects of hypocalcemia and strategies for prevention. J. Dairy Sci. 103:2909–2927. https://doi.org/10.3168/jds.2019-17268.

Wilms, J., G. Wang, J. Doelman, M. Jacobs, and J. Martín-Tereso. 2019. Intravenous calcium infusion in a calving protocol disrupts calcium homeostasis compared with an oral calcium supplement. J. Dairy Sci. 102:6056–6064. https://doi.org/10.3168/jds.2018-15754.

Woudenberg-Vrenken, T. E., A. L. Lameris, P. Weißgerber, J. Olausson, V. Flockerzi, R. J. M. Bindels, M. Freichel, and J. G. J. Hoenderop. 2012. Functional TRPV6 channels are crucial for transepithelial Ca2+ absorption. Am. J. Physiol. Gastrointest. Liver Physiol. 303:G879–G885. https://doi.org/10.1152/ajpgi.00089.2012.

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