Animal 20 (2026) 101753

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Arterial blood ionised calcium activity in periparturient Holstein cows fed an alkaline low-energy density or acidifying high-energy density close-up prepartum rations

M.B. Samarasinghe [a,*], L.E. Hernández-Castellano [a,2], N.B. Kristensen [b,1], M. Larsen [a]

[a] Department of Animal and Veterinary Sciences, Aarhus University Viborg, 8830 Tjele, Denmark
[b] SEGES Innovation P/S, Agro Food Park 15, 8200 Aarhus N, Denmark

ARTICLE INFO

Article history:
Received 21 July 2025
Revised 19 December 2025
Accepted 19 December 2025
Available online 23 December 2025

Keywords:
Acidifying salts
Ca metabolism
Metabolic acidosis
Parturient paresis
Phase feeding

ABSTRACT

Periparturient dairy cows often experience metabolic and health challenges due to impaired Ca homeostasis. Therefore, improving Ca metabolism and monitoring functional Ca status are essential during this critical transition phase. The objective was to test the effects of different feeding strategies in the close-up dry period on arterial blood ionised Ca concentration (**iCa**) of periparturient Holstein cows. A total of 28 Holstein dry cows were fed a common far-off dry cow ration (**FAR**; grass silage and barley straw-based, dietary cation–anion difference (**DCAD**) of +300 mEq/kg DM) and randomly allocated to four experimental treatments at the beginning of the close-up period (−21 days relative to expected parturition). The treatments were (1) continuation of FAR ration (FAR; n = 7), (2) high-energy acidifying ration with $MgCl_2$ (**MGC-70**; maize silage and mechanically expelled rapeseed cake based, DCAD of −70 mEq/kg DM, n = 6), (3) high-energy acidifying ration with $MgCl_2$ and NH_4Cl (**MGC-100**; maize silage and mechanically expelled rapeseed cake based, DCAD of −100 mEq/kg DM, n = 7), (4) a 7 days transition diet (grass silage:MGC-70 ratio of 20:80 DM basis, DCAD of 0 mEq/kg DM), followed by 14 days of MGC-100 feeding (**OVE**; n = 8). During the close-up period, urine samples were collected weekly. Arterial blood samples were collected on −12, +0.5, +1.5, and +2.5 days relative to parturition by puncturing the arteria auricularis caudalis. On −7 day relative to parturition, urine pH in MGC-70, MGC-100 and OVE was lower than in FAR, indicating metabolic acidosis. Furthermore, MGC-100 and OVE had lower blood pH than FAR on −12 day relative to parturition. Although having higher postpartum arterial blood pH, cows fed acidifying close-up diets had a higher postpartum iCa than FAR. Tendency for an increased arterial iCa was detected in MGC-100 already on +0.5 day postpartum and MGC-70 tended to have an increased iCa on +2.5 day relative to parturition. Plasma total Ca concentration (**tCa**) was greater in cows fed acidifying close-up diets compared with FAR on +2.5 day, but tCa was not affected by treatments on +0.5 day and +1.5 day relative to parturition. Therefore, the present results indicate that the determination of physiologically active Ca status in periparturient dairy cows can differ depending on the chosen biological indicator (iCa vs tCa). Overall, feeding maize silage-based acidifying close-up rations improved iCa status in periparturient cows. Sampling of arterial blood from the arteria auricularis caudalis is a method to be considered in future studies evaluating functional Ca status.

Implications

Improved Ca metabolism and monitoring functional Ca status are important for dairy cows having difficulties in maintaining normocalcemia in the periparturient period. Feeding maize silage-based, high-energy, acidifying close-up prepartum rations can induce metabolic acidosis in close-up dry cows, thereby increasing arterial blood ionised Ca status during the first 2.5 days postpartum. Determination of functional Ca status of the periparturient dairy cows can differ when evaluated using either arterial plasma

* Corresponding author.
E-mail address: bhagya.samarasinghe@anivet.au.dk (M.B. Samarasinghe).
[1] Present address: Department of Animal and Veterinary Sciences, Aarhus University Viborg, 8830 Tjele, Denmark.
[2] Present address: IUSA-ONEHEALTH 4. Animal Production and Biotechnology group, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, 35413 Arucas, Spain.

https://doi.org/10.1016/j.animal.2025.101753
1751-7311/

total Ca or arterial ionised Ca concentrations. Furthermore, sampling arterial blood from a superficial ear artery is a method to be considered in future studies investigating functional Ca status.

Introduction

One of the peculiarities of high-yielding dairy cows is the apparent lack of efficient homeorhetic and homeostatic mechanisms to maintain normocalcemia in the periparturient period. If plasma or serum total calcium (**tCa**) is measured, a large proportion of cows enter a transient hypocalcemia in the first few days after parturition (Reinhardt et al., 2011). This metabolic disorder is apparently caused by the increased need of Ca by the mammary gland for milk synthesis, which is higher than the sensitivity and speed of the homeostatic response to compensate for Ca uptake from the bloodstream. Blood tCa includes ionised calcium (**iCa**), protein-bound Ca (mostly bound to albumin) and complexed Ca bound to anions such as phosphate. However, it is important to consider that cows do not regulate the tCa concentration in blood. The biologically functional Ca is represented by the activity of iCa, which is tightly regulated via extracellular sensors with high abundance in the parathyroid gland as well as in other tissues, e.g., kidney and bones (Brown et al., 1993). In addition, the periparturient cow faces several metabolic challenges (Ingvartsen and Andersen, 2000), including the reduced pool and concentration of plasma albumin (Larsen et al., 2017), which can reduce measured tCa levels even when functional iCa remains unaffected. Albumin is an important modulator of Ca activity as it binds Ca; thus, variation in tCa has to be corrected for co-occurring changes in albumin, phosphate, and other modulators of Ca activity to present a meaningful measure in a physiological transition state (Kovacs, 2011). Therefore, measuring arterial blood iCa can provide a more accurate indication of functional Ca status, especially in periparturient cows where albumin fluctuations can mask blood tCa measurements. Furthermore, blood pH impacts the activity of blood Ca. Under reduced blood pH conditions, a greater proportion of tCa is active (i.e. ionised), and vice versa (Burritt et al., 1980). Hypocalcemia only exists from a functional perspective if Ca activity in the blood is depressed. Consequently, Ca activity in blood can only be described by tCa if other factors are in steady state (e.g. pH, phosphate, and blood albumin level). Therefore, the assessment of hypocalcemia based on a transient decrease in plasma or serum tCa might not be a suitable biomarker in transition cow programmes (Hernandez and McArt, 2023).

Decreased blood Ca activity induces a rapid parathyroid response with release of parathyroid hormone that, in combination with effects of calcitonin, 1,25-dihydroxyvitamin D3, estrogens, androgens, growth hormone, and thyroxin, control complex multi-organ responses to reestablish blood Ca activity (Garel, 1987; Hernández-Castellano et al., 2020). Parathyroid hormone response to reduced Ca activity is fast and apparent in a few minutes (Blum et al., 1978). However, the main effect of parathyroid hormone on Ca absorption is mediated through 1,25-dihydroxyvitamin D3 (Wilkens and Muscher-Banse, 2020) and this response is delayed by 24–48 h (Goff et al., 1991). Intestinal Ca absorption is therefore not likely to be induced within the first few hours after calving, thus the primary source of extra Ca in the immediate postpartum period is mobilisation from the skeleton. Several nutritional strategies have been used to increase the resilience of periparturient cows to the sudden Ca drain driven by lactogenesis. Some of these strategies are based on feeding low calcium rations (Jönsson et al., 1980), feeding negative dietary cation–anion difference (**DCAD**) rations (Tucker et al., 1992), or feeding a phosphate adsorbent (Thilsing-Hansen and Jørgensen, 2001). Although these strategies appear different, they all target activation of skeletal Ca mobilisation. Feeding negative DCAD rations can result in decreased DMI, as an effect of reduced palatability and/or discomfort created by the acidosis itself. Therefore, the benefits of inducing metabolic acidosis must be balanced against the potential adverse effects due to reduced prepartum DMI (Charbonneau et al., 2006).

As the postpartum Ca status is a balance between increasing Ca use for lactogenesis, and the effectiveness of the homeorhetic responses to counterbalance the Ca use, we hypothesised that the evaluation of Ca status postpartum would differ when evaluated as iCa in arterial blood compared with tCa in arterial blood. In addition, we hypothesised that feeding a high-energy density close-up diet in combination with acidification to activate skeletal Ca mobilisation in the periparturient period would result in greater plasma functional Ca status within the first three days of lactation. It was further hypothesised that a 2-week acidification protocol, preceded by a 1-week transition diet with intermediary acidification, would result in functional calcium status comparable to that achieved through a continuous 3-week intense acidification period. The aim of the study was to investigate the effects of phase-feeding maize silage-based, high-energy, negative DCAD close-up rations with varying intensities of acidification, fed with or without a transitioning diet, on improving arterial blood iCa status of the cows. In addition, an uncommon experimental method was used for sampling arterial blood to monitor functional Ca status in these cows.

Material and methods

Experimental design, animals, and feeding

Experimental procedures used in this study were approved by the Danish Animal Experiments Inspectorate. The animal experimental procedures and care of animals under study were carried out in accordance with the Ministry of Food, Agriculture and Fisheries, and the Danish Veterinary and Food Administration under Act 474 of 15 May 2014 and executive order 2028 of 14 December 2020.

A total of 28 multiparous single-pregnant Danish Holstein cows, dried-off from first (n = 20) and second (n = 8) lactation, were included in a randomised block design with repeated measurements during the close-up dry-period, i.e., –21 ± 3 (mean ± SD) days relative to parturition, and first 3 d after parturition.

Cows were dried off on Wednesdays of the week when cows were at least 56 days from their expected calving date. During the far-off dry-period, all cows were fed a common grass-silage and barley straw-based far-off ration. The close-up period was defined to begin Wednesdays of the week corresponding to 21 days before expected calving, where cows were randomly allocated to four experimental treatment rations (Fig. 1; Tables 1 and 2). Control group continued far-off ration (**FAR**, n = 7). The other three groups were fed a maize silage and rapeseed cake (i.e., mechanically expelled, 10.5% crude fat) based close-up rations acidified with; (1) $MgCl_2$ (**MGC-70**, n = 6), (2) MGC-70 ration with added NH_4Cl to reduce the DCAD value (**MGC-100**, n = 7), or (3) a 7 days neutral transition diet (grass silage diluted MGC-70 ration; grass: MGC-70 ratio of 20:80 DM basis) followed by feeding MGC-100 in last 14 days of close-up period (**OVE**; n = 8). After calving, cows received a standard lactation ration optimised using the Nordic Feed Evaluation System (NorFor; Volden, 2011). Some of the cows were used in another experiment, which involved feeding high-protein diets during the early lactation. While 14 cows were receiving the standard lactation total mixed ration, 14 cows were fed with a partially mixed lactation ration plus a protein concentrate. Five cows in the MGC-70, four cows in MGC-100 and five cows in OVE received the protein concentrate during the first 3 d postpartum. Those cows which were fed with the protein concen-

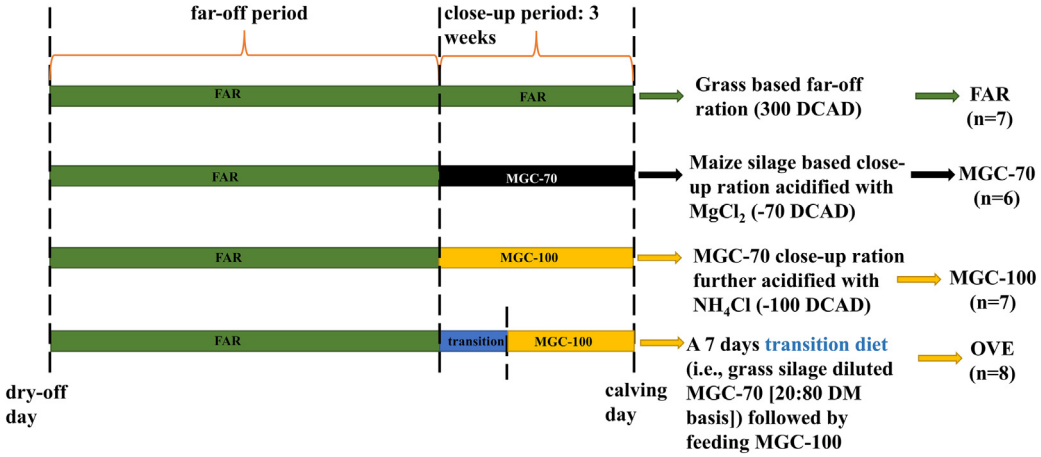

Fig. 1. Schematic representation of the non-acidifying and acidifying rations fed to the experimental cows during the close-up dry period, along with the duration of the respective treatments. The grass silage and barley straw-based far-off diet was continued as the close-up ration for the control group (FAR; formulated composition: net energy for lactation (NEL) 4.7 MJ/kg DM, Dietary Cation-Anion Difference (DCAD) +300 mEq/kg DM; n = 7). The other three treatment groups were fed maize silage and mechanically expelled rapeseed cake based close-up rations, either acidified with $MgCl_2$ (MGC-70; formulated composition: NEL 6.4 MJ/kg DM, DCAD −70 mEq/kg DM; n = 6), or the MGC-70 ration supplemented with NH_4Cl to further reduce the DCAD value (MGC-100; formulated composition: NEL 6.4 MJ/kg DM, DCAD −100 mEq/kg DM; n = 7), or a 7 days transition diet (grass silage:MGC-70, 20:80 DM basis; formulated composition: NEL 6.2 MJ/kg DM, DCAD 0 mEq/kg DM) followed by feeding MGC-100 during the last 14 days of close-up period (OVE; n = 8).

Table 1
Ingredient composition (% of DM) of the treatment and lactation rations fed to the experimental cows during the close-up dry and the postpartum periods.

	Treatments[1]				
	Control	Acidifying close-up rations			lactation1[2]
Ingredient	FAR	MGC-70	MGC-100	Transition diet of OVE	
Maize silage		66.1–71.2	63.5–68.4	52.9–57.0	29.5
Grass-clover silage	51.9–68.1			20.0	20.2
Barley straw	27.2–42.0				
Rapeseed cake, mechanically expelled		23.1	22.2	18.5	5.8
Rapeseed meal, solvent-extracted					12.4
Soybean meal, 54% CP	0–5.3				0.8
Maize grain, ground					15.5
Sugar beet pulp, dried		3.7–8.9	3.6–8.5	3.0–7.1	14.0
Mineral premix type-3[3]	0.8				0.8
Mineral premix close-up[4]		0.4	0.4	0.3	
Magnesium chloride hexahydrate		1.5–1.8	1.4–1.7	1.2–1.4	
Ammonium chloride premix[5]			0.4		
Sodium bicarbonate					1
Limestone-urea mix, 0.2:0.8					0.2
Hygiene4Feed[6]	0–0.1	0–0.1	0–0.1	0–0.1	

[1] The FAR diet was formulated to provide a positive Dietary Cation-Anion Difference (DCAD) that averaged +300 mEq/kg DM, and the acidifying close-up rations, i.e., MGC-70, MGC-100 and transition diet of the OVE treatment were formulated to provide negative DCAD values of −70, −100 and 0 mEq/kg DM, respectively (Fig. 1).

[2] After calving, 14 cows received lactation1 diet, which was a partially mixed ration, formulated to contain 15% of CP content on DM basis. The lactation1 diet was supplemented with a protein concentrate. The composition (as-is basis) of the protein concentrate was Soypass (DLG, Copenhagen, Denmark), 50.1%; maize gluten meal (DLG, Copenhagen, Denmark), 27.4%; solvent-extracted rapeseed meal, 11%; mechanically expelled rapeseed cake, 4%; wheat, 2.3%; molasses, 1.9%; oil, 1.1% and vitamin mineral mix. Rest of the cows received a total mixed ration which was a standard lactation ration comprising (% of DM), maize silage, 20.5–27.2; grass-clover silage, 22.3–33.1; sugar beet pulp, 8.34–18.3; rolled barley, 7.22–15.2; mechanically-expelled rapeseed meal, 7.96–11.6; solvent-extracted rapeseed meal, 7.96–14.2; maize gluten meal (DLG, Copenhagen, Denmark), 10.04; soybean meal, 9.94; Sodium bicarbonate, 0.16–3.54; Mineral premix type-3, 0.7–1.06; limestone, 0.08–0.2; NaCl, 0.16–0.26.

[3] Mineral premix Type-3 (ViloFOSS, Gråsten, Denmark). Composition (% of DM): calcium carbonate, 36.9; sodium chloride, 30.1; magnesium oxide, 25.7; molasses, 2.9; calcium magnesium carbonate, 1. Analytical composition (%): Ca, 14.6; Mg, 14.1; Na, 11.6. Additives (per kg): Vitamin A, 6×10^5 IE; Vitamin D3, 19×10^4 IE; Vitamin E, 4 000 IE; Cu, 1 500 mg; Mn, 4 000 mg; Zn, 4 500 mg, I, 225 mg; Se, 50 mg; Co, 25 mg.

[4] Vilomin 9 941 850 close–up basis (Vilomix, Mørke, Denmark). Composition (% of DM): sodium chloride 66.7; wheat 10. Analytical composition (%): Ca, 0.1; Na, 25.9; Mg, 0.1; Crude ash, 80.7. Additives (per kg): Vitamin A, 4.17×10^5 IE; Vitamin D3, 4.17×10^5 IE; Vitamin E, 55 883 IE; Cu, 833 mg; Mn, 5 000 mg; Zn, 5 000 mg, I, 17 mg; Se, 42 mg; Co, 66.7 mg.

[5] Vilomin 9 941 851 Ammon NaCl (Vilomix, Mørke, Denmark). Composition (% of DM): sodium chloride, 20.0; wheat, 15.0. Analytical composition (%): Ca, 0.1; P, 0.1; Na, 7.8; Mg, 0.1; Crude ash, 33.3. Additives (per kg): ammonium chloride, 5×10^5 mg.

[6] Hygiene4Feed (Biochem Zusatzstoffe Handels- und Produktionsgesellschaft mbH, Lohne, Germany). Additives (per kg): Potassium sorbate, 9.55×10^5 mg.

trate had mean (±SD) protein concentrate intakes of 1.65 ± 1.3, 2.24 ± 0.7 and 2.17 ± 0.8 kg DM/d on 1, 2 and 3 days postpartum, respectively. The composition (as-is basis) of the protein concentrate was 50.1% Soypass (DLG, Copenhagen, Denmark), 27.4% maize gluten meal (DLG, Copenhagen, Denmark), 11% solvent-extracted rapeseed meal, 4% mechanically expelled rapeseed cake, 2.3% wheat, 1.9% molasses, 1.1% oil and vitamin mineral mix (containing 44.7% CP, 4.5% crude fat, and 6.1% crude ash on DM basis). The protein concentrate intakes, considering above mentioned quantities, were assumed not to have substantial effects on the acid-base status; thus, the Ca activity of the cows in the immediate postpartum period.

Table 2
Nutrient composition (g/kg of DM unless otherwise noted) of treatment and lactation rations fed to the experimental cows during the close-up dry and the postpartum periods.

	Treatments[1]				
	Control	Acidifying close-up rations			lactation1[2]
Nutrient composition[3]	FAR	MGC-70	MGC-100	Transition diet of OVE	
DM, g/kg	378	392	395	375	488
CP	118	135	137	138	153
Crude fat	25	47	47	45	25
NDF	543	342	337	369	337
Starch	18	211	207	165	203
AAT20, g/kg DM[4]	70	91	91	87	96.9
NEL20, MJ/kg DM[5]	4.7	6.4	6.4	6.2	6.5
Minerals					
Ca	8.2	4.7	4.7	5.7	6.3
P	3.2	4.5	4.3	4.5	4.3
Mg	3.1	4.0	4.0	3.9	3.2
Na	2.3	1.5	1.8	1.6	4.3
K	28.3	11.6	10.6	14.5	13.4
S	2.4	2.6	2.6	2.8	2.6
Mn (mg/kg DM)	105	61	63	68	75
Zn (mg/kg DM)	77	68	72	68	71
Cu (mg/kg DM)	18	9	9	9	17
Cl (mg/kg DM)	13.4	8.5	10.2	9.6	4.2
DCAD[6] (mEq/kg DM)	299	−39	−96	−4	200–227

[1] The FAR diet was formulated to provide a positive Dietary Cation-Anion Difference (DCAD) that averaged +300 mEq/kg DM, and the acidifying close-up rations, i.e., MGC-70, MGC-100 and transition diet of the OVE treatment were formulated to provide negative DCAD values of −70, −100 and 0 mEq/kg DM, respectively (Fig. 1).
[2] After calving, 14 cows received a lactation1 diet which was a partially mixed ration formulated to contain 15% of CP content on DM basis. All nutrient values in the lactation1 diet were calculated using the NorFor system (Åkerlind and Volden, 2011) except for DM, CP, NDF, and starch content, which were determined through chemical analysis. The lactation1 diet was supplemented with a protein concentrate providing 44.7% CP, 4.5% crude fat, and 6.1% crude ash on a DM basis. Rest of the cows received a total mixed ration, which had a nutrient composition of: DM, 417 g/kg; CP, 160.9 g/kg DM; crude fat, 36.4 g/kg DM; NDF, 313.6 g/kg DM, starch, 140.6 g/kg DM; AAT20, 96.9 g/kg DM; NEL20, 6.5MJ/ kg DM.
[3] DM was determined after drying in a forced air oven at 60 °C for 48 h. Other nutrients were determined by proxy methods (i.e., NIR analysis) according to the Nordic Feed Evaluation System (NorFor; Volden, 2011). The lactation rations' nutrient compositions are formulated values from the Nordic Feed Evaluation System (NorFor; Volden, 2011).
[4] Amino acids absorbed in the small intestine at 20 kg of DM intake per day, calculated according to NorFor (Åkerlind and Volden, 2011).
[5] Net energy for lactation at 20 kg of DM intake per day, calculated according to NorFor (Åkerlind and Volden, 2011).
[6] Dietary Cation-Anion Difference (DCAD) was calculated as (K + Na) − (Cl + 2 × S).

During the experimental period, total mixed rations were adjusted weekly based on 3-week running means of silage DM content. The FAR ration was formulated for an organic matter digestibility of 65%. Soybean meal was included in the FAR ration to maintain a CP concentration of at least 110 g/kg DM. The basal acidogenic close-up ration (MGC-70) was formulated for a starch concentration of 200 g/kg of DM and a minimum CP concentration of 130 g/kg DM. The starch concentration was balanced by substituting sugar beet pulp for maize silage. All total mixed rations were mixed daily using an auger mixer.

Body condition score of the experimental cows was recorded on a weekly basis as described by Ferguson et al. (1994), and the minimum increment scored was 0.25. Before parturition, the mean (±SD) body condition score of the FAR, MGC-70, MGC-100 and OVE cows was 3.2 ± 0.1, 3.3 ± 0.1, 3.4 ± 0.1 and 3.4 ± 0.1, respectively. After parturition (i.e., 7.8 ± 2.8 d), the mean (± SD) body condition score of the FAR, MGC-70, MGC-100 and OVE cows was 3.2 ± 0.1, 3.3 ± 0.1, 3.3 ± 0.2, and 3.3 ± 0.1, respectively.

Housing and management

Cows entered the dry-off pen −56 ± 4 d relative to expected parturition. When the cows were dried off and during the subsequent far-off period, they were loose housed in pens with slatted floors and cubicles bedded with mattresses and fine wood shavings. Feed was offered in gated weighing feed stations (Hokofarm Group, Emmeloord, the Netherlands). The FAR cows were kept in the same pens during the close-up period and were moved to a straw-bedded calving pen approximately 1 week before calving. The OVE group was moved to a separate free-stall pen during the first week, where they were fed with the transition ration. Afterwards, OVE cows were housed in a straw-bedded pen similar to the other three treatment groups, and they were fed with the MGC-100 ration. All other cows were housed in a straw-bedded pen during the entire close-up period. All cows in MGC-70, MGC-100 and OVE groups were moved to straw-bedded individual calving pens at signs of calving. All the close-up rations were offered in gated weighing stations with restricted access. The gated weighing stations were filled with respective total mixed rations twice daily at 1000 and 1700 h. The feed bins were emptied every Monday, Wednesday, and Friday. All feeding stations were validated weekly using a 10-kg test weight. At all times, cows had free access to water from either water troughs or bowls.

Considering postpartum management, the first milking was done in the calving pen within 2.7 ± 1.9 h (mean ± SD) postpartum, using a portable milking machine. Calves were kept with cows for at least 12 h and fed with colostrum and milk following standard protocols. Then, fresh cows were transferred to a fresh cow pen and were kept in free-stalls bedded with rubber mattresses and fine wood shavings. The fresh cows were milked twice daily.

Sampling and analysis

The total mixed rations and ingredients were sampled weekly and stored at −20 °C and composited monthly to analyse for nutrients. These samples were dried at 60 °C for 48 h in a forced-air oven for weekly DM determination. The ration compositions (CP, CF, NDF, and starch) were analysed by Fourier Transform Near Infrared spectroscopy (Bruker MPA, Bruker Optics GmbH, Ettlingen, Germany) at Kvægbrugets Forsøgslaboratorium, Skejby, Den-

mark, and are reported in Table 2. In addition, the total mixed ration samples were analysed for mineral composition (Ca, P, Mg, Na, K, S, Mn, Zn, and Cu; Table 2) using inductively coupled plasma optical emission spectrometry and Cl was analysed by silver nitrate titration at a commercial laboratory (Eurofins Agro Testing Denmark A/S). Milk samples were analysed for fat and protein concentration (Eurofins Agro Testing Denmark A/S) using an infrared analyzer (MilkoScan 7RM FT+, Foss Analytical, Hillerød, Denmark).

During the close-up period, urine samples were collected weekly as mid-stream urine. Urine samples were analysed approximately 4 h after collection. The urine pH was determined by inserting a glass electrode in urine samples diluted 1:1 with deionised water. Net acid excretion (mmol/d) in urine was determined as the product of titratable net acid concentration and rate of urine production (L/d). Rate of urine production was calculated as 114/urinary creatinine concentration (mmol/L) (Røjen et al., 2011). The titratable net acid concentration in urine (mEq./L) was determined by Near-Infrared Spectroscopy (Bruker MPA with LSM module, Bruker Optics GmbH, Ettlingen, Germany) calibrated against titration of acidified urine in the presence of formaldehyde (Jørgensen, 1957). Urinary excretion of buffer base is designated by negative values. Urinary Ca concentration (mg/L) was analysed using a colorimetric method with O-cresolphthalein (RX-CA 590, Randox, Crumlin, UK), and urinary Ca excretion (g/d) was determined as the product of urinary Ca concentration and rate of urine production (L/d).

Blood sample collection and analyses

Arterial blood samples were collected by puncturing the arteria auricularis caudalis (superficial ear artery) 12 days before expected parturition, and after parturition between 0700 and 0800, 1400 and 1500, or 2100 and 2200 h to obtain samples closest possible to +0.5, +1.5, and +2.5 days relative to parturition. The mean (± SD) sampling days were −9.4 ± 3.6 days, +0.4 ± 0.5 days, +1.4 ± 0.5 days, +2.4 ± 0.5 days relative to parturition.

Prior to puncture, the cow was fixated in a self-locking barrier and the head was held using a VINK Headlock for Cows (VINK-ELST B.V., Bemmel, the Nertherlands). Backside of the ear was shaved, and the located artery was desensitised using a lidocaine/prilocaine creme (Tapin-crème, 25 mg lidocaine/g + 25 mg prilocaine/g, Orifarm Group A/S, Odense, Denmark). Five minutes afterwards, the artery was punctured using a 23G × 3/4″ hypodermic butterfly needle (BD Precisionglide, E-vet A/S, Haderslev, Denmark) and a blood sample was collected into 5-mL Na-heparin vacutainers (Greiner Bio-One GmbH, Kremsmuenster, Austria), gently turned and immediately placed on wet ice.

Arterial blood pH, iCa concentration, oxygen saturation (*s*O2) and partial pressure of O_2 (*p*O2) in the sample were measured using an ABL90 flex Blood Gas Analyzer (Radiometer A/S, Copenhagen, Denmark) within 10 min. Blood samples, which were chosen for further analysis, had *s*O2 of 99.80 ± 0.68% (mean ± SD) and *p*O2 of 141.8 ± 37.1 (mean ± SD). Thereafter, blood plasma was harvested by centrifugation at 3 000 × *g* for 15 min at 4 °C and stored at −20 °C until analysis. Blood plasma concentrations of glucose, tCa, inorganic phosphate, and Mg were determined according to standard procedures in an ADVIA 1800 clinical chemistry analyzer (Siemens Healthcare A/S, Denmark). Plasma tCa was measured using the Arsenazo method (Siemens CA_2; Siemens Healthcare Diagnostics Manufacturing Ltd., Dublin, Ireland), inorganic phosphate using the Molybdate method (Siemens IP; Siemens Healthcare Diagnostics Manufacturing Ltd., Dublin, Ireland), and total Mg using the Xylidyl Blue method (Siemens MG; Siemens Healthcare Diagnostics Manufacturing Ltd., Dublin, Ireland), following the manufacturer's guidelines. In addition, blood plasma non-esterified fatty acid concentrations were determined using the Wako, non-esterified fatty acids C ACS-ACOD assay method (Fujifilm NEFA-HR (2); TriChem ApS, Skanderborg, Denmark), whereas plasma β-Hydroxybutyrate concentrations were determined according to Harano et al. (1985) as an increase in absorbance at 340 nm due to the production of NADP at slightly alkaline pH in the presence of β-Hydroxybutyrate dehydrogenase (Hydroxybutyrate dehydrogenase HBD-301; Sorachim, Lausanne, Switzerland). Furthermore, this method used Oxamic acid in the media to inhibit the activity of lactate dehydrogenase (Harano et al., 1985). Both analyses were performed using the ADVIA 1800 clinical chemistry analyzer (Siemens Healthcare A/S, Denmark). Tartrate-Resistant Acid Phosphatase activity in plasma was analysed using a spectrophotometric assay as described by Lau et al., 1987.

Calculations and statistical analyses

Feed intake and milk yield were calculated for each cow on a 24 h basis (cut-off 1000 h). Dry matter intake was calculated using the weekly mean DM of feed rations. Energy and protein values in total mixed rations; net energy for lactation and AA absorbed in the small intestine at 20 kg DMI/day, were calculated according to NorFor (Åkerlind and Volden, 2011).

Statistical analyses were performed using R 4.0.2 (R Core Team, 2020). The effect of feeding acidifying close-up rations on prepartum and postpartum (i.e., separately) DMI and CP intakes, and postpartum milk yields were analysed using a linear mixed model using treatment, time relative to parturition and their interaction as fixed effects, and cow as the random effect. The model was fitted with REML, and the "lmer" function from the "lme4" package was used (Bates et al., 2015). Milk protein and fat concentrations were analysed using a one-way ANOVA, considering treatment as the fixed factor.

The effect of feeding acidifying close-up rations on whole blood pH, iCa, plasma tCa concentrations, ratio of iCa:tCa and other blood plasma variables (i.e., Tartrate-Resistant Acid Phosphatase, inorganic phosphate, Mg, glucose, non-esterified fatty acids, β-Hydroxybutyrate concentrations) were analysed using a linear mixed model. The model included treatment, time relative to parturition (i.e., as a repeated measurement with cow as subject) and their interaction as fixed effects and cow as the random effect. Several covariance structures were tested for the repeated measurements, and the one with the lowest Akaike information criterion, i.e., continuous AR (1) correlation, was selected. The model was fitted with REML, and the "lme" function from the "nlme" package was used (Pinheiro et al., 2023). The data from acidifying treatments were pooled (**ACID**; pooled data from MGC-70, MGC-100 and OVE groups) and analysed using the same model for the comparison between positive and negative DCAD rations (i.e., FAR vs ACID). For the urinary parameters (i.e., urine pH, urinary net acid concentration and excretion, urinary Ca concentration and excretion), the same statistical model but with an autoregressive order (1) correlation structure was used. To achieve a normal distribution of the residuals, data were transformed for statistical analysis when it was necessary (i.e., non-esterified fatty acids, β-Hydroxybutyrate). Variance heterogeneity was addressed by grouping the variation by day of sampling, using the varIdent function.

Results are presented as least square means, and statistical significance was declared at $P \leq 0.05$, and tendencies were considered when $0.05 < P \leq 0.10$.

Results

Metabolic acidosis

Prepartum DMI and CP intakes were affected by the treatments (Table 3). However, DMI and CP intakes during the first 0–3 d post-

partum did not differ among treatments (P = 0.27 and P = 0.23, respectively; Table 3).

Urinary pH was reduced in all three acidifying close-up treatments compared with FAR at −14 and −7 days relative to parturition (P < 0.01; Table 4), accompanied by a higher net acid excretion in these acidifying close-up treatments (P < 0.01). At day −7 relative to parturition, all three acidifying close-up groups had elevated urinary Ca concentrations ($P \leq 0.03$). Consequently, urinary Ca excretion was greater in MGC-70 (P < 0.01), MGC-100 (P = 0.04), and OVE (P = 0.02) compared with FAR at day −7 relative to parturition.

Calcium mobilisation

Arterial blood pH was affected by a strong treatment-by-day interaction effect (P < 0.001; Table 5). On day −12 relative to parturition, MGC-100 (P = 0.02) and OVE (P < 0.01) had reduced blood pH compared with FAR, with MGC-70 showing a tendency (P = 0.10). No differences in blood pH were observed on day +0.5 postpartum (P > 0.69), but MGC-100 had higher blood pH than FAR on day + 1.5 (P = 0.02), which persisted on day +2.5 postpartum. When pooled (Fig. 2), ACID had reduced prepartum arterial pH compared with FAR (P < 0.01) but increased arterial pH on days +1.5 (P < 0.01) and +2.5 (P < 0.01) relative to parturition.

Arterial blood iCa was affected by a treatment-by-day interaction (P = 0.02; Table 5). When pooled (Fig. 3), ACID had lower iCa than FAR on day −12 (P = 0.03), but persistently increased iCa concentrations on days +0.5, +1.5, and +2.5 relative to parturition (P = 0.02).

Arterial plasma tCa was affected by a treatment (P < 0.01; Table 5) and a treatment-by-day interaction effect (P < 0.01). Arterial plasma tCa was higher in FAR compared with all three acidifying close-up treatments on day −12 prepartum. No differences in plasma tCa were observed among treatments on days +0.5 and +1.5 postpartum. On day +2.5 postpartum, OVE had higher plasma tCa than FAR (P = 0.03), while MGC-70 (P = 0.07) and MGC-100 (P = 0.08) tended to have a higher plasma tCa. When pooled (Fig. 4), ACID had reduced plasma tCa than FAR on day −12 (P < 0.001), but it was increased on days +1.5 (P = 0.03) and +2.5 (P < 0.01) relative to parturition.

The arterial blood iCa:tCa ratio was neither affected by treatment (P = 0.34) nor by treatment by day interaction effect (P = 0.12, Table 5). When pooled (Fig. 5), the arterial blood iCa:tCa ratio was affected by the treatment by day interaction effect (P < 0.01), and on day +2.5 relative to parturition, FAR had a higher iCa:tCa ratio (P = 0.01) than ACID.

Other variables related to bone resorption and fat mobilisation

On day +0.5 postpartum, OVE had higher arterial plasma Tartrate-Resistant Acid Phosphatase concentration than FAR (P = 0.02; Table 5). Arterial plasma Mg concentration was affected

Table 3
Least square means for DMI, CP intakes, milk yield, milk protein and fat composition of the cows fed close-up dietary treatments FAR, MGC-70, MGC-100 or OVE starting on −21 days relative to expected parturition until parturition.

	Treatments[1]				SEM	Fixed effects, *P*-values		
Variable[1]	FAR	MGC-70	MGC-100	OVE		TRT	D	TRT × D
DMI before calving, kg/day								
−21	13.6	15.9	11.5	13.0	1.47	0.006	0.491	0.046
−14	11.3	15.6	15.4	12.0	1.47			
−7	9.8^{bB}	18.5^{a}	15.1^{a}	14.8^{aA}	1.47			
DMI after calving, kg/day								
0	10.7	11.1	7.3	9.1	1.55	0.269	<0.001	0.230
+1	10.3	16.2	15.0	14.6	1.43			
+2	13.8	15.1	15.1	14.6	1.43			
+3	13.1	15.9	15.1	15.3	1.43			
CP intake before calving[2], g/day								
−21	1 587	1 911	1 418	1 564	202	0.004	0.055	0.018
−14	$1\ 360^{cB}$	$2\ 040^{aA}$	$2\ 095^{ab}$	$1\ 553^{ac}$	202			
−7	$1\ 173^{b}$	$2\ 432^{a}$	$2\ 075^{a}$	$2\ 024^{a}$	202			
CP intake after calving[3], g/day								
0	1 454	1 616	1 072	1 320	245	0.232	<0.001	0.287
+1	1 632	2 609	2 401	2 358	226			
+2	2 222	2 437	2 433	2 352	226			
+3	2 106	2 555	2 437	2 469	226			
Milk yield, L/day								
+1	16.4	23.0	20.8	19.5	3.26	0.332	<0.001	0.671
+2	22.8	29.3	26.3	27.7	3.26			
+3	25.4	35.2	29.6	34.0	3.09			
Milk protein, %	3.5	3.6	3.7	3.6	0.16	0.921		
Milk fat, %	4.3	4.0	4.6	4.8	0.33	0.277		

Abbreviations: TRT = treatment; D = Days relative to parturition; DMI = DM intake.
The means within a row with different lowercase letter superscripts differ significantly ($P \leq 0.05$) and uppercase letter superscripts indicate tendencies ($0.05 < P \leq 0.01$).
[1] The grass silage and barley straw–based far-off diet was continued as the close-up ration for the control group (FAR; formulated composition: net energy for lactation (NEL) 4.7 MJ/kg DM, Dietary Cation-Anion Difference (DCAD) +300 mEq/kg DM; n = 7). The other three treatment groups were fed maize silage and mechanically expelled rapeseed cake based close-up rations, either acidified with $MgCl_2$ (MGC-70; formulated composition: NEL 6.4 MJ/kg DM, DCAD −70 mEq/kg DM; n = 6), or the MGC-70 ration supplemented with NH_4Cl to further reduce the DCAD value (MGC-100; formulated composition: NEL 6.4 MJ/kg DM, DCAD −100 mEq/kg DM; n = 7), or a 7 days transition diet (grass silage:MGC-70, 20:80 DM basis; formulated composition: NEL 6.2 MJ/kg DM, DCAD 0 mEq/kg DM) followed by feeding MGC-100 during the last 14 days (OVE; n = 8) of the close-up period (Fig. 1).
[2] CP intake before calving was calculated as: CP concentration in respective close-up total mixed ration (g/kg DM) × DMI from the respective close-up total mixed ration (kg/day).
[3] CP intake after calving was calculated as: [CP concentration in lactation total mixed ration (g/kg DM) × DMI from the lactation total mixed ration (kg/day)] + [CP concentration in the protein concentrate (g/kg DM) × DMI from the protein concentrate (kg/day)].

Table 4
Least square means of metabolic urinary indices, at −14 and −7 days relative to parturition in cows fed close-up dietary treatments FAR, MGC-70, MGC-100 or OVE starting on −21 days relative to expected parturition until parturition.

	Treatments[2]				SEM	Fixed effects, *P*-values		
Variable[1]	FAR	MGC-70	MGC-100	OVE		TRT	D	TRT × D
Urine pH								
−14	8.23^{a}	5.74^{bc}	5.53^{b}	6.54^{c}	0.321	<0.001	<0.001	0.021
−7	8.36^{a}	5.64^{b}	5.82^{b}	5.78^{b}	0.321			
Urinary net acid concentration, mEq./L								
−14	−116.5^{b}	61.2^{a}	81.3^{a}	66.4^{a}	21.46	<0.001	0.005	<0.001
−7	−148.3^{b}	84.4^{a}	84.8^{a}	94.1^{a}	19.75			
Urinary net acid excretion, mmol/day								
−14	−1 847^{b}	675^{a}	876^{a}	327^{a}	453.2	<0.001	0.003	0.082
−7	−2 545^{b}	651^{a}	979^{a}	1 037^{a}	453.2			
Urinary Ca concentration, mg/L								
−14	35^{b}	247^{ab}	667^{a}	440^{ab}	207.5	<0.001	0.274	0.119
−7	30^{b}	560^{a}	853^{a}	690^{a}	237.8			
Urinary Ca excretion, g/day								
−14[3]	0.6^{bB}	2.9^{a}	7.4^{Aa}	4.8^{ab}	2.33	<0.001	0.002	0.027
−7[4]	0.7^{b}	3.9^{a}	9.4^{a}	9.3^{a}	2.68			

Abbreviations: TRT = treatment; D = Days relative to parturition.
The means within a row with different lowercase letter superscripts differ significantly ($P \leq 0.05$) and uppercase letter superscripts indicate tendencies ($0.05 < P \leq 0.01$).
[1] Urine samples were collected on −14 and −7 days relative to parturition.
[2] The grass silage and barley straw–based far-off diet was continued as the close-up ration for the control group (FAR; formulated composition: net energy for lactation (NEL) 4.7 MJ/kg DM, Dietary Cation-Anion Difference (DCAD) +300 mEq/kg DM; n = 7). The other three treatment groups were fed maize silage and mechanically expelled rapeseed cake based close-up rations, either acidified with $MgCl_2$ (MGC-70; formulated composition: NEL 6.4 MJ/kg DM, DCAD −70 mEq/kg DM; n = 6), or the MGC-70 ration supplemented with NH_4Cl to further reduce the DCAD value (MGC-100; formulated composition: NEL 6.4 MJ/kg DM, DCAD −100 mEq/kg DM; n = 7), or a 7 days transition diet (grass silage:MGC-70, 20:80 DM basis; formulated composition: NEL 6.2 MJ/kg DM, DCAD 0 mEq/kg DM) followed by feeding MGC-100 during the last 14 days (OVE; n = 8) of the close-up period (Fig. 1).
[3] SEM for FAR, MGC-70, MGC-100, and OVE were ±0.435, ±0.495, ±2.327, and ±2.173, respectively.
[4] SEM for FAR, MGC-70, MGC-100, and OVE were ±0.435, ±0.495, ±2.675, and ±2.421, respectively.

by treatment ($P < 0.01$; Table 5), with higher concentrations in MGC-70 ($P < 0.01$) and OVE ($P = 0.05$) than in FAR on day +0.5 postpartum. These differences persisted on day +1.5.

On day −12 relative to parturition, arterial plasma glucose concentration was higher in MGC-100 ($P < 0.01$) and OVE ($P = 0.02$) than FAR (Table 6). Furthermore, arterial plasma non-esterified fatty acid concentrations were affected by the treatments ($P = 0.05$; Table 6). On day −12 prepartum, MGC-70 had a lower plasma non-esterified fatty acid concentration than FAR ($P = 0.05$), and OVE tended to have a reduced plasma non-esterified fatty acid concentration ($P = 0.09$). No differences in plasma glucose or non-esterified fatty acid concentrations were observed among treatments on days +0.5, +1.5, or +2.5 postpartum ($P > 0.60$).

Discussion

This study aimed at phase feeding acidifying rations during the close-up dry period, specifically evaluating the effects on physiologically active arterial iCa status during the immediate postpartum period (i.e., 0 to 3 days of lactation). The study did not aim to evaluate milk production or other postpartum performance parameters of the cows. None of the cows in the study were treated for clinical parturient paresis.

Metabolic acidosis

Feeding acidifying close-up dry cow diets had effects on blood pH, urinary pH, renal acid excretion, and renal Ca excretion compared with non-acidified cows. These effects were expected and in agreement with the response to metabolic acidosis (Ahn et al., 2012). Monitoring urine pH is commonly used for monitoring the animal response to negative DCAD rations (Goff, 2008). Hence, a urine pH ranging from 5.5 to 6.2 is commonly used as an indicator of successful administration of anions (Thilsing-Hansen et al., 2002; Oetzel, 2022), which was also observed in the present study. Feeding a transition close-up ration to OVE was a practical strategy to avoid the risk of prepartum DMI depression, which may occur due to abrupt shifts from alkaline far-off to acidic close-up rations. This intermediate acidification effect in the first week of close-up period was evident via intermediate urinary pH of OVE group at −14 day relative to parturition (Table 4). In addition, the urine pH from cows in the OVE group was acidified to a higher degree within a shorter period compared with those animals in the MGC-70 and MGC-100 groups. This indicates that homeorhetic mechanisms of Ca mobilisation due to acidification can be activated at parturition by feeding a negative DCAD ration during the last 2 weeks before parturition. Furthermore, only minor effects were observed on plasma nutrient metabolites prepartum with lower plasma glucose and greater non-esterified fatty acid concentrations in the FAR group compared with acidifying rations fed groups. Carryover effects were not apparent for the postpartum plasma glucose concentrations, which are similar to those described in other studies (Zhang et al., 2022; Grünberg et al., 2011).

Arterial blood pH and ionised calcium activity

Blood pH impacts the activity of blood Ca, with a lower pH, a greater proportion of tCa is active i.e., ionised (Burritt et al., 1980). Similar to Zhang et al. (2022) and Wilkens et al. (2020), cows fed an alkaline diet throughout the dry period had higher blood pH prepartum compared with cows fed an acidifying close-up diet. The pH reduction ranged from −0.05 to −0.08 pH units, and prepartum blood pH of the cows fed acidifying close-up diets remained within the normal biological range of 7.35–7.45

Table 5
Least square means for blood acid-base balance related indices, blood ionised Ca and blood plasma total Ca concentrations from −12 to +2.5 days relative to parturition in cows fed close-up dietary treatments FAR, MGC-70, MGC-100 or OVE starting on −21 days relative to expected parturition, until parturition.

	Treatments[2]				SEM	Fixed effects, *P*-values		
Variable[1]	FAR	MGC-70	MGC-100	OVE		TRT	D	TRT × D
Blood pH								
−12	7.50^{Aa}	7.45^{Bc}	7.43^{bc}	7.42^{bc}	0.015	0.059	<0.001	<0.001
+0.5	7.48	7.50	7.50	7.48	0.015			
+1.5	7.48^{b}	7.51^{ab}	7.53^{a}	7.52^{ab}	0.013			
+2.5	7.47^{b}	7.50^{Bbc}	7.54^{aA}	7.52^{ac}	0.011			
Blood ionised Ca, mmol/L								
−12	1.22	1.18	1.17	1.18	0.020	0.985	<0.001	0.016
+0.5	0.96^{B}	1.07	1.12^{A}	1.07	0.047			
+1.5	1.01	1.11	1.10	1.08	0.033			
+2.5	1.06^{B}	1.16^{A}	1.14	1.14	0.030			
Plasma total Ca, mmol/L								
−12	2.51^{a}	2.38^{b}	2.34^{b}	2.34^{b}	0.023	<0.001	<0.001	<0.001
+0.5	2.10	2.23	2.32	2.22	0.087			
+1.5	2.16	2.35	2.34	2.31	0.071			
+2.5	2.29^{bB}	2.46^{aA}	2.45^{aA}	2.48^{a}	0.046			
Ratio ionised Ca: total Ca								
−12	0.49	0.49	0.50	0.50	0.009	0.335	<0.001	0.116
+0.5	0.48	0.48	0.48	0.48	0.011			
+1.5	0.48	0.47	0.47	0.47	0.006			
+2.5	0.48	0.47	0.47	0.46	0.005			
Tartrate-resistant acid phosphatase concentration, U/l								
−12	10.1	10.6	11.9	11.9	1.18	0.477	0.785	0.007
+0.5	8.3^{b}	10.1^{ab}	10.7^{ab}	13.7^{a}	1.26			
+1.5	11.8	11.1	10.7	11.5	1.39			
+2.5	12.4	11.2	14.3	9.4	1.72			
Inorganic phosphate, mmol/L								
−12	2.03	2.11	2.14	2.19	0.105	0.422	<0.001	0.754
+0.5	1.71	1.72	1.73	1.62	0.154			
+1.5	1.63	1.79	1.55	1.77	0.161			
+2.5	1.71	2.06	1.72	1.93	0.130			
Mg, mmol/L								
−12	0.86^{b}	0.97^{ab}	0.96^{ab}	1.00^{a}	0.033	0.001	<0.001	0.002
+0.5	0.89^{b}	1.13^{a}	1.01^{ab}	1.05^{a}	0.044			
+1.5	0.88^{b}	1.06^{a}	0.94^{bc}	1.01^{ac}	0.031			
+2.5	0.84	0.83	0.83	0.87	0.035			

Abbreviations: TRT = treatment; D = Days relative to parturition.
The means within a row with different lowercase letter superscripts differ significantly ($P \leq 0.05$) and uppercase letter superscripts indicate tendencies ($0.05 < P \leq 0.01$).
[1] Blood samples were collected on −12 and +0.5, +1.5, +2.5 days relative to parturition.
[2] The grass silage and barley straw–based far-off diet was continued as the close-up ration for the control group (FAR; formulated composition: net energy for lactation (NEL) 4.7 MJ/kg DM, Dietary Cation-Anion Difference (DCAD) +300 mEq/kg DM; n = 7). The other three treatment groups were fed maize silage and mechanically expelled rapeseed cake based close-up rations, either acidified with $MgCl_2$ (MGC-70; formulated composition: NEL 6.4 MJ/kg DM, DCAD −70 mEq/kg DM; n = 6), or the MGC-70 ration supplemented with NH_4Cl to further reduce the DCAD value (MGC-100; formulated composition: NEL 6.4 MJ/kg DM, DCAD −100 mEq/kg DM; n = 7), or a 7 days transition diet (grass silage:MGC-70, 20:80 DM basis; formulated composition: NEL 6.2 MJ/kg DM, DCAD 0 mEq/kg DM) followed by feeding MGC-100 during the last 14 days (OVE; n = 8) of the close-up period (Fig. 1).

(Charbonneau et al., 2006). In the current trial, postpartum blood pH increased in the ACID cows when acidifying close-up rations were shifted to an alkaline lactation ration at calving. Similarly, Joyce et al. (1997) and Zhang et al. (2022) observed that postpartum blood pH increases more rapidly and to a greater extent in cows which were abruptly transitioned from a negative DCAD prepartum diet to a positive DCAD lactation diet, compared with cows that were fed a positive DCAD diet both before and after calving.

Interestingly, while having higher postpartum arterial blood pH, the ACID cows were able to maintain higher postpartum iCa concentration compared with the FAR cows, which reflects the effectiveness of the acidification strategy in priming the skeletal Ca mobilisation at parturition. The results are in agreement with the hypothesised differences in postpartum Ca activity of non-acidified close-up cows compared with cows fed acidifying close-up diets. According to Thilsing-Hansen et al. (2002), increased dietary Ca absorption in the intestines requires at least 24 h of 1,25-dihydroxyvitamin D stimulation. Similarly, increased bone Ca resorption requires 48 h of parathyroid hormone stimulation. Therefore, the time needed for activation of Ca absorption and resorption to fulfil the high Ca pull at the onset of lactation appears prolonged in FAR as compared with acidifying strategies. Furthermore, the increased arterial blood iCa concentration of the ACID cows within the first 0.5 to 2.5 days postpartum is of major interest, as hypocalcemia is of major concern within the first 3 days after parturition (Goff, 2008; Caixeta et al., 2015).

It is evident that Ca metabolism of dairy cows is challenged around parturition (Reinhardt et al., 2011). However, it is also becoming evident that the assessment of the physiological impact of Ca status via monitoring tCa in blood plasma or serum has its own limitations (Hernandez and McArt, 2023) as iCa is the physiologically regulated trait (Garel, 1987; Brown et al., 1993). Therefore, this study had interests to investigate whether conclusions

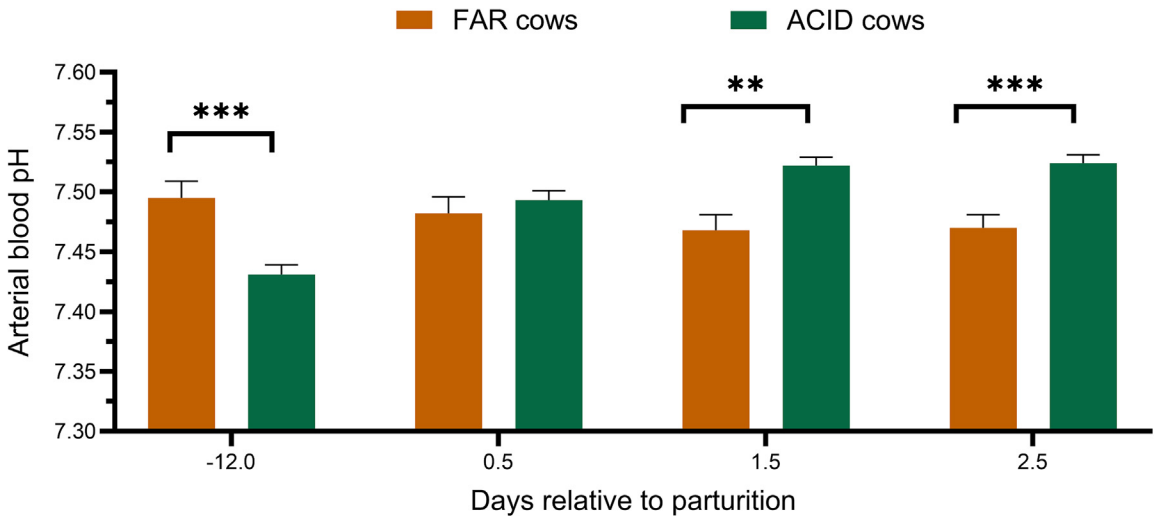

Fig. 2. Arterial blood pH on −12 to +2.5 days relative to parturition in cows fed far-off diet (i.e., FAR, n = 7) versus the acidifying close-up rations (i.e., ACID, n = 21). The FAR group was fed with grass silage based far-off diet, which was formulated to offer a positive Dietary Cation-Anion Difference (DCAD) that averaged +300 mEq/kg DM. The other three treatment groups which were pooled to create the ACID group, were fed with maize silage-based close-up rations with $MgCl_2$ and, with or without added NH_4Cl to offer a negative DCAD of either −70 mEq/kg DM or −100 mEq/kg DM. Data are presented as least square mean ± SEM. **, denotes, $P < 0.01$, ***, denotes, $P < 0.001$, between FAR and ACID. The *P*-values; *P*(TRT) = 0.11, *P*(D) = <0.01, *P*(TRT × D) = <0.01 where, *P*(TRT) = *P*-value for treatment effect, *P*(D) = *P*-value for days relative to parturition effect, *P* (TRT × D) = *P*-value for interaction effect of treatment and days relative to parturition.

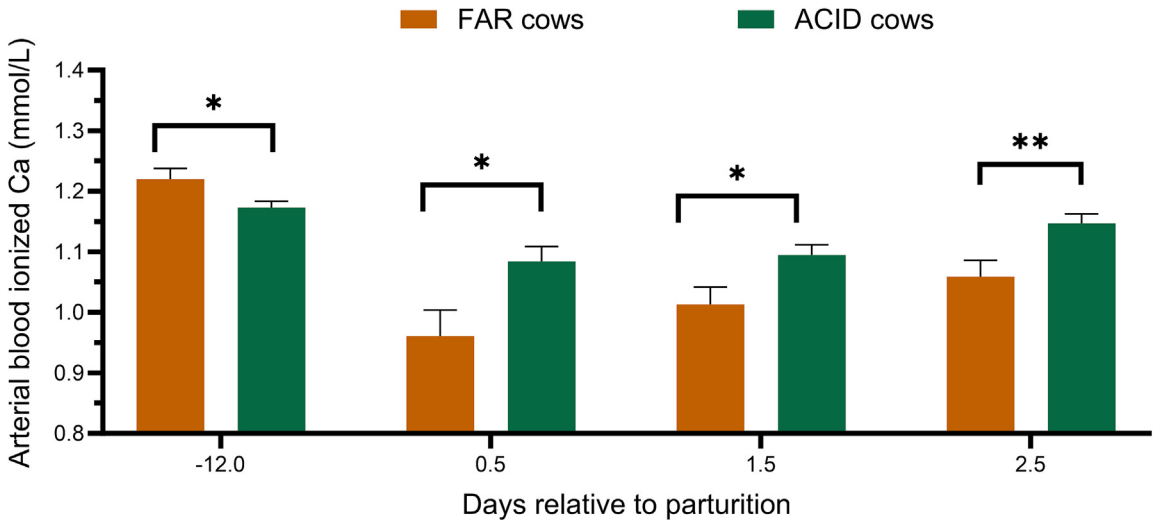

Fig. 3. Arterial blood ionised Ca concentration (iCa) on −12 to +2.5 days relative to parturition in cows fed far-off diet (i.e., FAR, n = 7) versus the acidifying close-up rations (i.e., ACID, n = 21). The FAR group was fed with a grass silage–based far-off diet which was formulated to offer a positive Dietary Cation-Anion Difference (DCAD) that averaged +300 mEq/kg DM. The other three treatment groups, which were pooled to create the ACID group, were fed with maize silage-based close-up rations with $MgCl_2$ and, with or without added NH_4Cl to offer a negative DCAD of either −70 mEq/kg DM or −100 mEq/kg DM. Data are presented as least square mean ± SEM. *, denotes, $P < 0.05$; **, denotes, $P < 0.01$, between FAR and ACID. The *P*-values; *P*(TRT) = 0.83, *P*(D) = <0.01, *P*(TRT × D) = <0.01 where, *P*(TRT) = *P*-value for treatment effect, *P*(D) = *P*-value for days relative to parturition effect, *P*(TRT × D) = *P*-value for interaction effect of treatment and days relative to parturition.

on functional Ca status of transition dairy cows would differ if these were based on either tCa or iCa traits. Considering individual treatment differences (Table 5), arterial iCa did not differ prepartum but it differed among treatments already on 0.5 day and 2.5 day postpartum; however, tCa differed before parturition and subsequently on 2.5 day postpartum. The iCa concentration reflects the functional Ca status of the cows; therefore, our results indicate that conclusions based on tCa concentrations cannot be directly translated into the functional Ca status of periparturient cows.

According to Kurosaki et al. (2007), increased Tartrate-Resistant Acid Phosphatase activity indicates the activation of mature osteoclasts that can be induced by acidosis. In addition, tartrate-resistant acid phosphatase is highly expressed in the bone-resorbing osteoclasts. Consequently, tartrate-resistant acid phosphatase is commonly used as a cytochemical marker indicating osteoclast activity (Minkin, 1982; Kurosaki et al., 2007). Therefore, the increased postpartum tartrate-resistant acid phosphatase activity in the OVE group and numerically higher tartrate-resistant acid phosphatase activity in the MGC-70 and MGC-100 groups compared with the FAR group may indicate the increased osteoclast activity due to induced metabolic acidosis already at +0.5 day relative to parturition, which might have led to the increased arterial blood iCa concentrations. In addition, the plasma concentration of inorganic phosphate was not affected by treatments. However, plasma inorganic phosphate was decreased postpartum. This effect is in line with a parathyroid hormone response

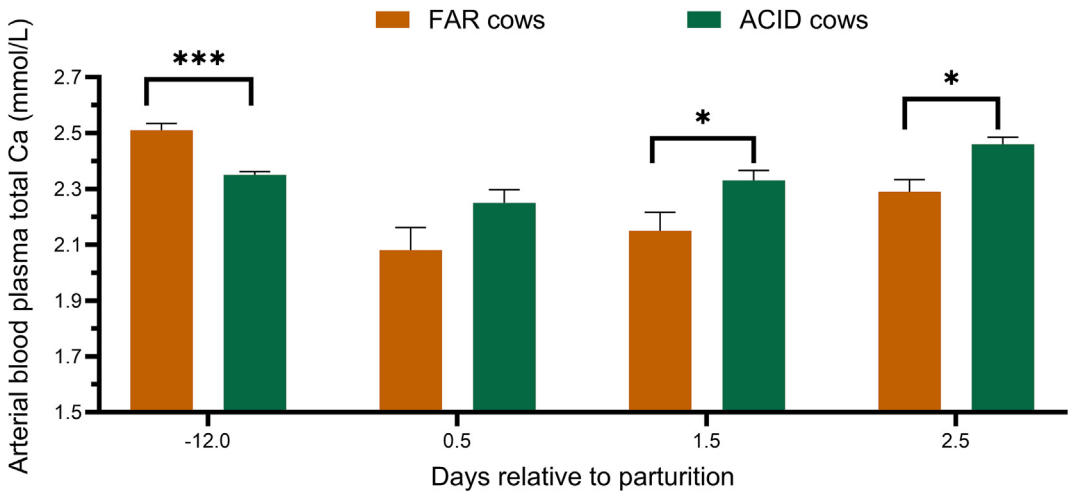

Fig. 4. Arterial blood plasma total Ca (tCa) concentration on −12 to +2.5 days relative to parturition in cows fed far-off diet (i.e., FAR, n = 7) versus the acidifying close-up rations (i.e., ACID, n = 21). The FAR group was fed with grass silage–based far-off diet, which was formulated to offer a positive Dietary Cation-Anion Difference (DCAD) that averaged +300 mEq/kg DM. The other three treatment groups, which were pooled to create the ACID group, were fed with maize silage-based close-up rations with $MgCl_2$ and, with or without added NH_4Cl to offer a negative DCAD of either −70 mEq/kg DM or −100 mEq/kg DM. Data are presented as least square mean ± SEM. *, denotes, $P < 0.05$; ***, denotes, $P < 0.001$, between FAR and ACID. The *P*-values; P(TRT) = <0.01, P(D) = <0.01, P(TRT × D) = <0.01 where, P(TRT) = *P*-value for treatment effect, P(D) = *P*-value for days relative to parturition effect, P(TRT × D) = *P*-value for interaction effect of treatment and days relative to parturition.

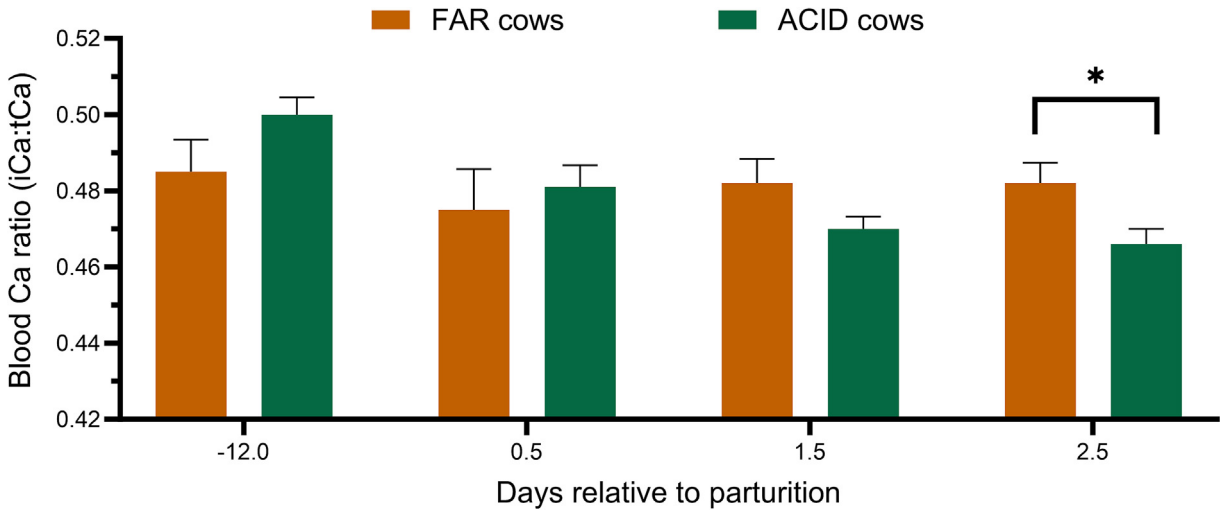

Fig. 5. Arterial blood Ca ratio; blood ionised Ca: plasma total Ca (iCa:tCa) on −12 to +2.5 days relative to parturition in cows fed far-off diet (i.e., FAR, n = 7) versus the acidifying close-up rations (i.e., ACID, n = 21). The FAR group was fed with grass silage–based far-off diet, which was formulated to offer a positive Dietary Cation-Anion Difference (DCAD) that averaged +300 mEq/kg DM. The other three treatment groups, which were pooled to create the ACID group, were fed with maize silage-based close-up rations with $MgCl_2$ and, with or without added NH_4Cl to offer a negative DCAD of either −70 mEq/kg DM or −100 mEq/kg DM. Data are presented as least square mean ± SEM. *, denotes, $P < 0.05$, between FAR and ACID. The *P*-values; P(TRT) = 0.11, P(D) = <0.01, P(TRT × D) = <0.01 where, P(TRT) = *P*-value for treatment effect, P(D) = *P*-value for days relative to parturition effect, P(TRT × D) = *P*-value for interaction effect of treatment and days relative to parturition.

to hypocalcemia postpartum, increasing salivary inorganic phosphate secretion for increasing blood Ca activity (Clark et al., 1975). The effect on plasma Mg postpartum might be an indication of increased bone resorption coupled with carry-over effects of acidifying close-up rations having $MgCl_2$ as the anion salt (Simesen, 1980; Littledike and Goff, 1987), which was also observed in cows fed acidifying close-up diets compared with non-acidified cows in the present study.

Most of the literature dealing with hypocalcemia in dairy cows had used blood samples collected from either the coccygeal vein or the jugular vein to determine blood pH, iCa and tCa concentrations (Grünberg et al., 2011; Glosson et al., 2020; Zhang et al., 2022). However, blood sample collection from these veins can cause masking effects due to the possible mixing of venous and arterial blood and, therefore, underestimation of the actual blood iCa concentrations (Sadariya et al., 2021). Although, venous blood sampling is frequently used in clinical practice due to ease of sampling, reduced risk for arterial hematoma, dissection, and thrombosis. Therefore, the arterial sampling technique we applied in our study, i.e., by puncturing arteria auricularis caudalis (superficial ear artery), could be relevant for future experiments aiming at studying calcium metabolism of periparturient cows.

Conclusion

Feeding maize silage-based, high-energy, acidifying close-up rations with a DCAD value of −39 to −96 mEq/kg DM induced metabolic acidosis in close-up cows, increasing arterial blood iCa

Table 6
Least square means for mobilisation-related plasma metabolites, from −12 to +2.5 days relative to parturition in cows fed close-up dietary treatments FAR, MGC-70, MGC-100 or OVE starting on −21 days relative to expected parturition until parturition.

	Treatments[2]				SEM	Fixed effects, *P*-values		
Variable[1]	FAR	MGC-70	MGC-100	OVE		TRT	D	TRT × D
Glucose, mmol/L								
−12	3.77^{b}	3.83^{bc}	4.04^{a}	3.99^{ac}	0.051	<0.001	<0.001	0.985
+0.5	4.07	3.98	4.17	4.13	0.191			
+1.5	3.72	3.56	3.76	3.67	0.165			
+2.5	3.38	3.32	3.55	3.55	0.141			
Non-esterified fatty acid concentration, mEq./L								
−12	0.28^{aA}	0.17^{b}	0.20^{ab}	0.19^{bB}	0.035	0.046	<0.001	0.281
+0.5	0.52	0.43	0.47	0.36	0.129			
+1.5	0.45	0.47	0.37	0.45	0.094			
+2.5	0.63	0.42	0.46	0.40	0.138			
β-Hydroxybutyrate concentration, mmol/L								
−12	0.60	0.58	0.52	0.63	0.067	0.476	<0.001	0.054
+0.5	0.72	0.80	0.83	0.75	0.069			
+1.5	0.85^{ab}	0.95^{a}	1.00^{a}	0.71^{b}	0.078			
+2.5	1.00	0.97	0.98	0.80	0.087			

Abbreviations: TRT = treatment; D = Days relative to parturition
The means within a row with different lowercase letter superscripts differ significantly ($P \leq 0.05$) and uppercase letter superscripts indicate tendencies ($0.05 < P \leq 0.01$).
[1] Blood samples were collected on −12 and +0.5, +1.5, +2.5 days relative to parturition.
[2] The grass silage and barley straw–based far-off diet was continued as the close-up ration for the control group (FAR; formulated composition: net energy for lactation (NEL) 4.7 MJ/kg DM, Dietary Cation-Anion Difference (DCAD) +300 mEq/kg DM; n = 7). The other three treatment groups were fed maize silage and mechanically expelled rapeseed cake based close-up rations, either acidified with $MgCl_2$ (MGC-70; formulated composition: NEL 6.4 MJ/kg DM, DCAD −70 mEq/kg DM; n = 6), or the MGC-70 ration supplemented with NH_4Cl to further reduce the DCAD value (MGC-100; formulated composition: NEL 6.4 MJ/kg DM, DCAD −100 mEq/kg DM; n = 7), or a 7 days transition diet (grass silage:MGC-70, 20:80 DM basis; formulated composition: NEL 6.2 MJ/kg DM, DCAD 0 mEq/kg DM) followed by feeding MGC-100 during the last 14 days (OVE; n = 8) of the close-up period (Fig. 1).

concentration during the first 2.5 days after parturition. The assessment of physiologically active Ca status of the periparturient dairy cows can differ when evaluated from either arterial blood plasma tCa or arterial blood iCa concentrations. In addition, arterial blood sampling from puncturing the arteria auricularis caudalis (superficial ear artery) is suggested as a method with relevance for future studies evaluating functional Ca status in periparturient dairy cows.

Ethics approval

Experimental procedures used in this study were approved by the Danish Animal Experiments Inspectorate. The animal experimental procedures and care of animals under study were carried out in accordance with the Ministry of Food, Agriculture and Fisheries, and the Danish Veterinary and Food Administration under act 474 of 15 May 2014 and executive order 2028 of 14 December 2020.

Data and model availability statement

None of the data were deposited in an official repository. The data are available from the corresponding author upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

M. B. Samarasinghe: https://orcid.org/0000-0003-4946-1150
L. E. Hernández-Castellano: https://orcid.org/0000-0003-2729-0434.
N. B. Kristensen: https://orcid.org/0000-0003-0214-2929.
M. Larsen: https://orcid.org/0000-0003-3767-0908.

CRediT authorship contribution statement

M.B. Samarasinghe: Writing – review & editing, Writing – original draft, Visualisation, Project administration, Formal analysis, Data curation. **L.E. Hernández-Castellano:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualisation. **N.B. Kristensen:** Writing – review & editing, Visualisation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualisation. **M. Larsen:** Writing – review & editing, Visualisation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualisation.

Declaration of interest

None.

Acknowledgements

The authors acknowledge the technical staff at Aarhus University-Viborg for their contribution during the animal experimentation period.

Financial support statement

Funding support to conduct this research was provided by the Danish Milk Levy Fund (Mælkeafgiftsfonden, Aarhus, Denmark).

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