

## Research Article

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### Author for correspondence:

Juliette N. Wilms,

Email: [juliette.wilms@trouwnutrition.com](mailto:juliette.wilms@trouwnutrition.com)

# Blood calcium dynamics in cows receiving an aqueous calcium suspension for voluntary consumption or a calcium bolus following parturition

Juliette N. Wilms<sup>1</sup>, Jean-Baptiste Daniel<sup>1</sup>, Javier Martín-Tereso<sup>1</sup>, Arie Klop<sup>2</sup>, Roselinde Goselink<sup>2</sup>, Yanming Han<sup>1</sup> and Sandra van Kuijk<sup>1</sup>

<sup>1</sup>Trouw Nutrition R&D, PO Box 299, 3800 AG, Amersfoort, the Netherlands and <sup>2</sup>Wageningen Livestock Research, PO Box 338, 6700 AH, Wageningen, the Netherlands

## Abstract

The form of oral calcium (Ca) supplement and the Ca source influence Ca absorption dynamics resulting in different postpartum calcemia. The objective of this study was to investigate whether an oral Ca supplement (mainly CaCO<sub>3</sub>) offered for voluntary consumption would maintain or increase postpartum blood Ca to the same degree as a Ca bolus (mainly CaCl<sub>2</sub>) providing an equivalent dose of a Ca. A total of 72 Holstein cows were blocked by expected parturition date and parity. Within each block of 3 animals, cows were randomly assigned to one of three treatments, including an oral Ca supplement offered for voluntary consumption (Ca-drink, *n* = 23), an oral Ca bolus (Ca-bolus, *n* = 24), or an untreated group (CON, *n* = 25). Treatments were administered once within 15 min postpartum. The Ca-drink provided 45 g of Ca (CaCO<sub>3</sub> source) and was mixed in 20 L of lukewarm water and offered to cows for 30 min. The Ca-bolus provided 43 g of Ca (71% from CaCl<sub>2</sub> and 29% from CaSO<sub>4</sub>) and was administered once. Both Ca-bolus and CON cows received 20-l of lukewarm water at parturition to standardize the volume of fluids (Ca-drink or 20-l lukewarm water) offered at parturition. Dairy cows offered Ca-drink had a 28% higher fluid consumption than Ca-bolus and CON cows. Milk yield and milk composition expressed in percentage protein, fat, lactose, and urea did not differ, whilst there was a small but significant increase in DMI in cows receiving the Ca-drink compared to CON, while Ca-bolus did not differ from other groups. This was consistent with reduced BW losses between week 1 and 3 in cows receiving the Ca-drink suspension. Treatment by time interactions were present for blood Ca, glucose, and urea concentrations. Blood Ca was relatively stable in Ca-drink cows, while higher fluctuations were observed in Ca-bolus cows. In Ca-bolus cows, blood Ca increased from 15 min to 6 h, decreased from 6 to 24 h, and finally increased again from 24 to 48 h. At 24 h post administration, blood Ca was greater in cows receiving the Ca-drink than cows receiving the Ca-bolus. Blood glucose was greater in Ca-bolus cows at 15 min after treatment administration compared with Ca-bolus and CON, while blood urea was higher in CON than Ca-drink and Ca-bolus throughout the sampling period. These results indicate that voluntary oral Ca resulted in a relatively stable calcemia, whereas higher fluctuations were observed in cows receiving the Ca-bolus. Due to a lack of differences between Ca-drink and Ca-bolus compared with CON, it is not possible to conclude regarding the efficacy in maintaining postpartum blood Ca.

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Subclinical hypocalcemia is a common metabolic disorder which occurs during the transition period and is characterized by blood calcium (Ca) concentrations below 2 mmol/l (Martín-Tereso and Martens, 2014). While clinical hypocalcemia (blood Ca < 1.4 mmol/l) only affects a small fraction of animals in properly managed herds, subclinical hypocalcemia can affect between 41 and 54% of multiparous cows (Reinhardt *et al.*, 2011). Cows with subclinical hypocalcemia are at a greater risk for other disorders related to parturition (Chapinal *et al.*, 2011; Martínez *et al.*, 2012) and for culling (Roberts *et al.*, 2012). Short-term postpartum interventions (preventive and curative) against hypocalcemia are often integrated into farm protocols and may include oral Ca supplements (Goff and Horst, 1993; Sampson *et al.*, 2009; Wilms *et al.*, 2019), as well as subcutaneous (Goff, 1999) or intravenous Ca infusions (Blanc *et al.*, 2014).

In contrast to intravenous Ca infusions, oral Ca supplementation is unlikely to result in a marked calcemic signal immediately after administration (Blanc *et al.*, 2014; Wilms *et al.*, 2019), unless a high dose ( $\geq 100$  g; Goff and Horst, 1993) or multiple doses are administered (Valdecabres *et al.*, 2018). The source of Ca present in an oral supplement and its physico-chemical characteristics may greatly influence Ca absorption and blood Ca responses

(Oetzel, 2013). Boluses including Ca from CaCl<sub>2</sub> are frequently used as they elevate blood Ca effectively after administration when compared to other sources of Ca such as CaCO<sub>3</sub> or Ca propionate (Goff and Horst, 1993, 1994). For example, supplying 50 g of Ca from CaCO<sub>3</sub> did not induce any noticeable change in blood Ca within the first 6 h after administration, whereas in response to 50 g of Ca from CaCl<sub>2</sub>, blood Ca increased rapidly to reach a peak 30 min after drenching the solution but returned to initial level within 5 to 6 h (Goff and Horst, 1993). These different blood Ca dynamics demonstrate different Ca absorption dynamics between CaCO<sub>3</sub> and CaCl<sub>2</sub>. For these reasons, it may be important to evaluate blood Ca dynamics over a long period (>24 h) when different sources of Ca such as CaCO<sub>3</sub> and CaCl<sub>2</sub> are compared.

Unlike CaCl<sub>2</sub>, CaCO<sub>3</sub> does not represent an irritation risk to the mucous lining of the gastrointestinal tract (Thilising-Hansen *et al.*, 2002), and offers the possibility to avoid drenching, as aqueous suspensions of CaCO<sub>3</sub> may be voluntarily consumed by cows following parturition (Wilms *et al.*, 2019). The hypothesis of this study was that oral CaCO<sub>3</sub> supplement offered for voluntary consumption would maintain a steady blood Ca postpartum whereas providing an equivalent amount of Ca as CaCl<sub>2</sub> through a bolus would induce fluctuations in blood Ca (rapid increase, followed by a decrease). The aim was to evaluate how a single Ca administration, either offered for voluntary consumption as CaCO<sub>3</sub> or orally administered as CaCl<sub>2</sub>, would influence blood Ca dynamics and other blood metabolites in the 48 h after parturition, as well as DMI and production parameters in the first 3 weeks postpartum in dairy cows in the presence of a control group.

## Materials and methods

This study was conducted between January and May 2017 at the experimental dairy farm of Wageningen Livestock Research (Dairy Campus, Leeuwarden, the Netherlands). All procedures described in this article complied with the Dutch Law on Experimental Animals, which complies with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 (<http://data.europa.eu/eli/dir/2010/63/oj>) and were approved by the Ethical Committee on Animal Experiments of the Animal Sciences Group of Wageningen University and Research Centre (Wageningen, the Netherlands). The project approval number was 2016.D-0038, with project title: Dairy cow nutrition to improve lifespan.

## Animals and experimental design

In total, 72 Holstein Friesian cows (14 primiparous and 58 multiparous with median parity of 3 and a range of 2–5) with a mean BW of 690 ± 88 kg in the first week following parturition were used in this randomized block design experiment. Cows deemed suitable for enrollment in the study based on the expected parturition date were included 2 weeks prepartum and remained in the experiment until 3 weeks postpartum. In the event of a cow calving between 2200 h and 0500 h the next day, the animal was not considered for the study. Cows were blocked by expected parturition date and parity (primiparous or multiparous; online Supplemental Table S1). Within each block of three animals, cows were randomly assigned to one of three treatments, including an oral Ca suspension offered for voluntary consumption (Ca-drink, *n* = 23), a Ca bolus which was orally administered (Ca-bolus, *n* = 24), or a group not receiving any Ca

supplementation (CON, *n* = 25). The uneven number of animals in the treatment groups is related to the fact that cows were pre-assigned to a certain treatment based on their expected calving date, however, some animals did not calve as expected leaving incomplete blocks. In addition, one cow receiving the Ca-drink and two cows receiving the Ca-bolus treatment developed clinical hypocalcemia and were not included in the sampling procedures. Whereas this is an outcome of the study, the low number of cows enrolled was incompatible with a statistical analysis evaluating the effect of treatment on clinical cases of hypocalcemia. This led to a final total of 22 cows in Ca-drink and Ca-bolus and 25 cows in CON, resulting in 21 complete blocks with 3 cows, two blocks with 2 cows, and two blocks with only one cow. It was also decided to remove blocks with one animal, resulting in 22 cows in Ca-drink and Ca-bolus and 23 cows in CON. The number of first lactation cows was respectively 5 for CON, 4 for Ca-bolus and 4 for Ca-drink. Ca-drink cows were individually offered a 20-L commercial Ca suspension (45 g of Ca: 93.5% CaCO<sub>3</sub>, 5% Ca formate, 1.8% Ca propionate; Farm-O-San Reviva, Trouw Nutrition, Amersfoort, the Netherlands) for voluntary consumption. Besides the Ca components, the products also included dextrose (31.8%), lactose (15.5%), protein (6.7%), fat (4.7%), others macro-minerals (in g/kg, 51 Cl, 47 Na, 9 K, 5 P, 0.6 Mg), trace minerals (in mg/kg, 200 Fe, 190 Mn, 20 I and 2 Se), and vitamins (in IU, 80 000 Vitamin A, 18 000 Vitamin D and 2700 Vitamin E). The Ca suspension was prepared in a 25-l bucket containing 10 L of warm water (40 to 45°C) to which 1 kg of supplement was added. Once dissolved, 10 l of cold water was added, to achieve a drinking temperature of 25 to 30°C. The bucket was placed in front of the cow within 15 min after parturition and administration of the Ca suspension was always supervised to prevent any other cow from drinking the solution. The Ca suspension was offered once only and cows could voluntarily consume the Ca suspension for 30 min, after which any leftovers would be weighed. In contrast to manufacturer recommendations to repeat the application after 12–48 h, Ca-bolus treatment (43 g of Ca: 71% CaCl<sub>2</sub> and 29% CaSO<sub>4</sub>; Bovikalc bolus, Boehringer Ingelheim, St. Joseph, MO) was orally administered one single time within 15 min postpartum. This was to offer similar amounts of Ca between the Ca-drink and Ca-bolus groups to allow better comparison between the Ca sources. Cows in the Ca-bolus and CON groups were offered 20-l of lukewarm water within 15 min postpartum following the same protocol as described above for the Ca suspension to standardize the amount of fluid offered at parturition to all animals.

## Housing and herd management

Cows were housed indoors throughout the experimental period. During the dry period, cows were kept in a free stall setting. At first signs of parturition, cows were transferred to a deep litter barn with straw bedding (40 m<sup>2</sup>). Cows stayed in the calving pen between 24 and 48 h following parturition and were then moved into the lactation group. The lactating dairy herd at the dairy research facility (20–40 cows) was housed in a free stall barn. Stall floors consisted of concrete with rubber strips, and bedding consisted of rubber mattresses with sawdust. During lactation, cows were milked twice daily at 0600 and 1700 h in a carousel milking parlor. The barn was naturally ventilated through the roof and wall slats. *Ad libitum* water was available through the usual drinking system present in the calving pen, including during treatment administration.

### Diets and feeding

For prepartum and postpartum diets, cows were fed partial mixed rations *ad libitum* in roughage feeder and a restricted amount of concentrates at the concentrate feeder (online Supplementary Table S2). The amount of concentrate offered was 1.5 kg in the dry-period, and 8–9 kg during lactation, and lactating cows were also offered another 0.5 kg of concentrate at each milking in the milking parlor.

### Measurements and sampling

Intakes of liquid treatments, either the Ca suspension or the 20-L lukewarm water offered within 15 min postpartum, were recorded by weighing leftovers 30 min after initiation of administration. Within the first three weeks following parturition, daily individual feed intakes from the partial mixed ration were automatically recorded by feeding bins (RIC stations, Hokofarm, Marknesse, The Netherlands). Individual concentrate intakes were registered by automatic concentrate feeders (Hokofarm, Marknesse, The Netherlands) and in the milking parlor during milking (GEA Farm Technologies, Leeuwarden, The Netherlands). Throughout the three weeks following parturition, individual milk production was recorded at each milking. Milk samples were taken from each cow weekly during four consecutive milking events including two mornings and two evenings. Composites of each day's morning and evening samples were made by weighing each individual sample proportionally to yield per milking. Body weight was automatically recorded twice daily until 3 weeks postpartum at the exit of the milking parlor. Blood samples were collected from the coccygeal vessels (*Vena caudalis mediana*) into one 9-mL serum/gel tubes (Vacuette, type 455010; Greiner Bio-One, Frickenhausen, Germany) and into one 9-mL lithium heparin tube (Vacuette, type 455084; Greiner Bio-One, Frickenhausen, Germany) at –14 d (before expected parturition date), within 15 min after treatment initiation and at 6, 12, 24 and 48 h relative to treatment initiation. The 15 min sampling timepoint was designed to detect differences in cortisol concentrations potentially related to stress following treatment administration. In relation to cortisol metabolism, serum cortisol was shown to be elevated immediately after the mild stressor of claw trimming and returned to baseline after 24 h (Jansen *et al.*, 2016). Inducing a strong stressor such as restraining increased serum cortisol concentrations within 5 up to 40 min (Yavari *et al.*, 2017). These data indicate that the peak in serum cortisol happens during the first hour following a stressor, with the length being dependent on the severity of the stressor. In the current study, the stressor was the administering of Ca supplement and was considered mild, thus justifying the 15 min sampling time point. At 15 min post treatment initiation, it is possible that some animals were still drinking (either the Ca suspension or the lukewarm water) while blood was collected. This is because these solutions were offered for a total of 30 min following treatment administration (oral Ca suspension or Ca-bolus). However, previous data from Wilms *et al.* (2019) showed that the same Ca suspension was consumed within 5 min which is a positive indication that most of the solution was consumed by the time the 15-min blood sample was collected. For the 6, 12, 24 and 48 h sampling time points, samples could be collected with a variation of about 2 h when sampling time would fall during the night (2200–0500 h). Homogeneity of sampling timepoints across the three experimental groups was ensured to allow fair comparisons across treatment groups.

Samples were then stored in 1.5-mL serum and plasma cryotubes at –18°C.

### Chemical analyses

Milk sample composites were analyzed for fat, protein, lactose and urea with a MilkoScan FT6000 infrared analyzer (Foss Electric, Hillerød, Denmark) which was calibrated according to the NEN-ISO 9622 (NEN, 2013) reference methods. Milk analyses were performed by Qlip (Zutphen, the Netherlands). Serum samples were analyzed for urea, Ca, Mg, NEFA, BHBA, and plasma samples for glucose at the NEN-EN-ISO 9001:2015 (NEN, 2015) accredited commercial laboratory of Animal Health Service (Gezondheidsdienst voor Dieren, Deventer, the Netherlands). The laboratory uses internal validation and has been NEN-EN-ISO/IEC 17025:2017 (NEN, 2018) accredited by the Dutch Accreditation Council (RvA, registration number L120, <https://www.gdanimalhealth.com/about-us/quality-standards>) for serum urea, Mg, NEFA, BHBA and plasma glucose. Serum Ca and Mg were analyzed by ICP-MS using a Synchron Chemistry Analyzer (UniCel DxC 600 SN6730, Beckman Coulter, Canada L.P.). Urea, NEFA, BHBA, and glucose were analyzed by enzymatic methods using a Synchron Clinical Analyzer (UniCel DxC 800 SN4764, Beckman Coulter, Canada L.P.). Plasma cortisol was analyzed at the University of Utrecht (Utrecht, the Netherlands) by chemiluminescence technique using an Immulite® 2000 XPI analyzer (Siemens Healthcare, den Haag, the Netherlands).

### Statistical analysis

The total number of cows available for this study was limited to 72 cows. Based on previous experience from our dairy Research Facility of Trouw Nutrition Research and Development (Boxmeer, the Netherlands; Fowers *et al.*, 2015) investigating the efficacy of the present voluntary oral Ca supplement, 22 cows per treatment allowed the detection of differences ( $P < 0.05$  with a power of 0.80) of 0.15 mmol/l with a standard deviation of 0.18 mmol/l in serum tCa between the present oral Ca supplement and an untreated control group at 24 h post treatment initiation. Therefore, minimal sample size was considered to be 24 cows per treatment group. The weekly mean of daily individual feed intake was calculated from the data stored by the RIC stations and concentrate feeders. The fourteen consecutive milkings collected at the milking parlor were used to calculate weekly milk yield. General linear mixed models with repeated measures were used for the analyses of blood parameters using the MIXED procedure of SAS (SAS/STAT(R) 9.4, 2018, SAS Institute Inc., Cary, NC). The experimental unit was the cow and the statistical model was as follows:

$$Y_{ijk} = \mu + T_i + V_j + W_k + T_{W_{ik}} + e_{ijk}$$

where  $Y_{ijk}$  is the dependent variable,  $\mu$  is the overall mean,  $T_i$  is the fixed effect of treatment ( $i = 3$ ),  $V_j$  is the random effect of block ( $j = 23$ ),  $W_k$  is the fixed effect of time used as a repeated measure ( $k = 6$  for all analysis in blood and  $k = 3$  for other variables),  $T_{W_{ik}}$  is the effect of treatment by time interaction, and  $e_{ijk}$  is the residual. The heterogeneous Toeplitz covariance structure was applied to all blood variables as the samples were not equally spaced in time. For all other dependent variables (DMI, milk yield, and milk components yield), the autoregressive



covariance structure was applied as time point were equally spaced. Interactions between treatment and time were explored using the SLICE option of the LSMEANS statement of the MIXED procedure of SAS. Comparisons across treatments at each statistically significant time point were conducted with the PDIF option of the LSMEANS statement of SAS. Blood metabolites measured at 14 d pre-partum were analyzed separately using general linear mixed models including the fixed effect of block and treatment. Refusals of fluids offered after parturition, as well as hypocalcemia incidence at 24 h postpartum were analyzed with the PROC GENMOD procedure in SAS (SAS 9.4M6, SAS Studio, SAS Institute Inc.). Results are reported as mean  $\pm$  SEM and were considered statistically significant at  $P \leq 0.05$ . A non-significant numerical difference is reported for  $0.1 > P > 0.05$ .

## Results

Cows receiving the Ca-drink were individually offered a 20-l commercial Ca suspension (45 g of Ca; Ca-drink) for voluntary consumption. Cows in the Ca-bolus treatment group received a Ca-bolus that was orally administered (43 g of Ca). Additionally, cows in the Ca-bolus and CON groups were offered 20-l of lukewarm water within 15 min postpartum for 30 min to standardize the amount of fluid offered at parturition. Three cows which developed clinical hypocalcemia requiring an i.v. Ca infusion (one Ca-drink and two Ca-bolus), and 2 cows belonging to blocks of only one animal were removed post-inclusion from the study.

### Treatment intakes

Treatment intakes include either the 20-l voluntary oral Ca suspension or the 20-l of lukewarm water offered within 15 min relative to parturition. The Ca-drink solution was consumed in greater amount (18.8 l) compared to the water offered directly after parturition for Ca-bolus and CON cows (14.5 and 14.8 l, respectively;  $P = 0.05$ ). Consistent with the solution intake volumes, non-significant numerical differences were observed for refusal rate of part of the 20-l solution between cows receiving the Ca-bolus cows receiving the Ca-drink (50% vs. 18%;  $P = 0.07$ ), whereas refusal rate from CON cows did not differ (39%).

### Feed intakes and production parameters

Milk yield and milk components measured over 3 weeks postpartum did not differ between cows offered the Ca-drink, the Ca-bolus, and cows in the CON group. However, post-partum DMI was greater in cows receiving the Ca-drink when compared with CON cows (18 vs. 17 kg/d;  $P = 0.04$ ; Table 1). During the first 3 weeks postpartum, cows offered the Ca-drink lost less BW compared with CON and Ca-bolus cows ( $-2.5$  vs.  $-15.0$  kg;  $P < 0.01$ ). Additionally, milk yield and milk composition did not differ between cows offered the Ca-drink, the Ca-bolus, and cows in the CON group.

### Blood minerals (Ca and Mg)

Blood Ca and Mg concentrations measured 14 d prior to expected parturition date did not differ across treatment groups (Fig. 1). The overall effect of treatment on blood Ca was not statistically significant ( $P = 0.36$ ), but there was a treatment by time interaction ( $P = 0.01$ ; Table 1). In cows receiving the Ca-drink

solution, blood Ca increased linearly with time, and the highest value was found at 48 h. Blood Ca measured in cows receiving the Ca-bolus was highest at 6 and 48 h, and lowest at 15 min and 24 h ( $P < 0.01$ ), reflecting a first increase from administration to 6 h, a decrease from 6 to 24 h and a second increase from 24 to 48 h. Cows receiving the Ca-drink had greater blood Ca concentrations (2.11 mmol/l; 95% CI of 2.01 to 2.22 mmol/l) at 24 h after treatment initiation compared with cows receiving the Ca-bolus (1.98 mmol/l; 95% CI of 1.88 to 2.09 mmol/l). At this time point, the fraction of cows that would be considered as suffering from subclinical hypocalcemia (serum tCa  $< 2$  mmol/l; Martín-Tereso and Martens, 2014) was 24% (5/21) in Ca-drink cows, compared with 35% in CON (15/23) and 55% in Ca-bolus cows (12/22) but this was not statistically significant ( $P = 0.11$ ). Besides blood Ca, a small and non-significant treatment by time interaction on blood Mg was detected ( $P = 0.07$ ). This resulted from greater concentrations in cows receiving the Ca-bolus compared to cows receiving the Ca-drink and cows in the CON group at 15 min (95% CI of 1.09 to 1.18 for Ca-bolus, 1.00 to 1.09 for Ca-drink and 1.03 to 1.12 for CON, all mmol/l) and 48 h (95% CI of 0.96 to 1.04, 0.88 to 0.97 and 0.90 to 0.99 mmol/l, respectively) relative to treatment initiation.

### Blood metabolites

Blood concentrations of glucose, urea, NEFA, BHBA and cortisol measured 14 d prior to expected parturition did not differ across treatment groups. While there was no overall effect of treatment on glucose concentrations ( $P = 0.11$ ), a treatment by time interaction was detected ( $P < 0.01$ ). A transitory state of hyperglycemia, in which glucose was the highest for Ca-bolus cows, was observed 15 min after treatment administration (Fig. 2a). Following this glucose peak, blood glucose was higher in CON cows. In addition, a non-significant numerical difference of lower cortisol concentration was present at 15 min after treatment administration for cows receiving the Ca-drink (60 nmol/l; 95% CI of 46 to 73 nmol/l) when compared to cows receiving the Ca-bolus (78 nmol/l; 95% CI of 64 to 91 nmol/l) and cows in the CON treatment group (76 nmol/l; 95% CI of 63 to 90 nmol/l;  $P = 0.10$ ). Differences between treatments were also observed for blood urea and blood BHBA (see Table 1). For blood urea, an effect of treatment ( $P < 0.01$ ) and treatment by time interaction ( $P < 0.01$ ; Figure 2b) were detected with higher concentrations for cows in the CON treatment group compared to cows receiving the Ca-drink and the Ca-bolus. Regarding blood BHBA, cows receiving the Ca-drink had numerically lower concentrations (0.56 mmol/l; 95% CI of 0.52 to 0.61 mmol/l) than cows receiving the Ca-bolus (0.62 mmol/l; 95% CI of 0.58 to 0.66 mmol/l) and cows in the CON treatment group (0.62; 95% CI of 0.58 to 0.67 mmol/l;  $P = 0.07$ ).

## Discussion

Intakes of treatments (20.0 L of either voluntary oral Ca suspension or water) is in line with previous results in which cows would voluntarily consume Ca solutions (mainly from Ca propionate and CaCO<sub>3</sub>, respectively) offered to postpartum dairy cows (Geishauser *et al.*, 2008; Wilms *et al.*, 2019). This demonstrates that such Ca supplementation products formulated with CaCO<sub>3</sub> offer an alternative to products that are drenched or administered orally by husbandry staff. As 18% of Ca-drink cows did not consume the entire solution, the mean Ca intake for that group is 41 g

**Table 1.** Effect of calcium source in oral Ca supplementation products administered at parturition on production parameters during the first three weeks post-partum, as well as blood parameters taken following treatment initiation

Item <sup>1</sup>	Treatment <sup>2</sup>			Pooled SEM	P-value		
	CON	Ca-drink	Ca-bolus		Treat	Time	Treat × Time
<i>n</i>	23	22	22				
DMI post-partum (kg/d)	16.80 <sup>a</sup>	18.40 <sup>b</sup>	17.36 <sup>ab</sup>	0.59	<0.05	<0.01	ns
Milk yield (kg/d)	32.74	32.56	33.27	1.81	ns	<0.01	ns
Milk composition (%)							
Protein	3.80	3.91	3.85	0.08	ns	<0.01	ns
Fat	4.71	4.67	4.91	0.12	ns	<0.01	ns
Lactose	4.44	4.45	4.40	0.03	ns	<0.01	ns
Urea (mg/dl)	20.42	19.03	20.37	0.75	ns	<0.05	ns
BW change (week 3 – week 1; kg)	–15.75 <sup>a</sup>	–2.53 <sup>b</sup>	–14.32 <sup>a</sup>	3.19	<0.01	-	-
Serum minerals							
tCa (mmol/l)	2.05	2.11	2.06	0.04	ns	<0.01	0.01
Mg (mmol/l)	1.02	0.98	1.03	0.02	ns	<0.01	0.07
Blood chemistry							
Glucose (mmol/l)	5.23	4.87	5.25	0.15	ns	<0.01	<0.01
Urea (mmol/l)	4.58 <sup>a</sup>	3.96 <sup>b</sup>	4.20 <sup>b</sup>	0.13	<0.01	<0.01	<0.01
NEFA (mmol/l)	0.54	0.49	0.55	0.04	ns	<0.01	ns
BHBA (mmol/l)	0.62	0.56	0.62	0.02	0.07	<0.01	ns
Cortisol (nmol/l)	76.13	59.77	77.60	6.31	0.10	-	-

<sup>a,b</sup>Means with a different superscript are statistically significantly different ( $P \leq 0.05$ ).

<sup>1</sup>Blood samples were collected at 15 min after administration of treatment (within 30 min relative to parturition), and 6, 12, 24 and 48 h relative to treatment administration.

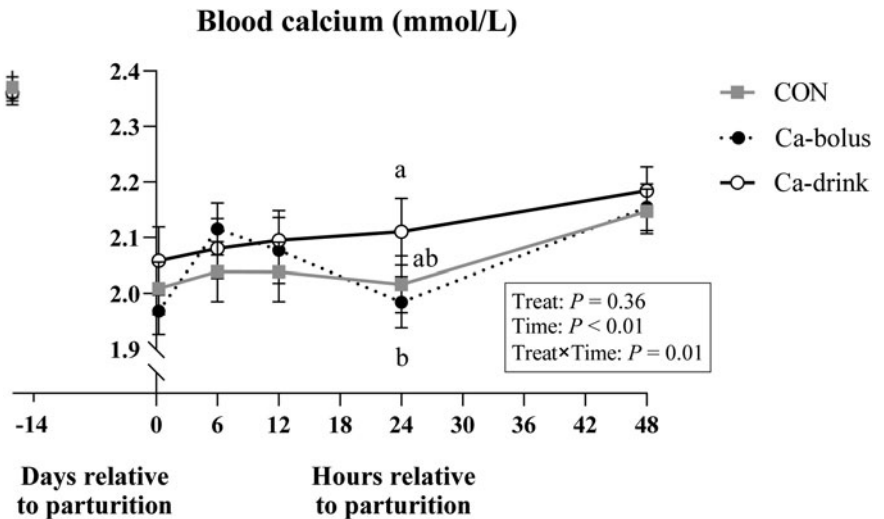
<sup>2</sup>Treatments included an oral Ca supplement offered for voluntary consumption (Ca-drink), an oral Ca bolus (Ca-bolus) or a control (CON).

of Ca (considering a mean intake of  $18.31 \pm 1.09$  per cow), which remains comparable with the Ca-bolus group (43 g of Ca). Ca-drink cows had greater DMI which was in line with lower BW changes between week 1 and 3 after parturition in that group. This could indicate that the oral Ca supplement stimulated DMI after parturition. The reason for the potential beneficial effects of the Ca suspension cannot be ascribed to the Ca content of the product and may also be linked with other nutrients present in the product (e.g., dextrose and lactose; Daniel *et al.*, 2021). However, due to a lack of baseline for BW and DMI measured prepartum, it is possible that non-homogeneous groups were present despite adequate randomization. Milk yield and milk composition did not differ which is in line with previous experiments looking into the effect of oral Ca supplementation at parturition on lactation performance. For instance, Melendez *et al.* (2003) showed that two Ca supplements including a CaCl<sub>2</sub> as a paste (60 g of Ca) or Ca propionate orally (110 g of Ca) administered at parturition did not improve milk yield and fertility when compared with an untreated group.

The Ca-drink cows had a steady increase in blood Ca concentrations from parturition to 48 h later. This result was independent from parity, and removing the primiparous cows did not affect the outcomes in blood Ca. In contrast, blood Ca dynamics were characterized by a greater increase for the first 6 h but followed by a decrease from 6 to 24 h, before increasing again until 48 h. This could be the result of different absorption dynamics between CaCl<sub>2</sub> and CaCO<sub>3</sub>. The magnitude of the differences

between CaCl<sub>2</sub> and CaCO<sub>3</sub> was highest at 24 h post administration, where greater blood Ca concentrations were present in Ca-drink cows than Ca-bolus cows. This highlights the importance of evaluating blood Ca dynamics over a minimum of 24 h when comparing these two Ca sources. Therefore, differences in Ca availability between the two sources may not be as large as what was previously described in literature. Literature by Goff and Horst (1993) showed that supplying 50 g of Ca from CaCO<sub>3</sub> did not lead to noticeable changes in blood Ca within the first 6 h after administration, unlike an equivalent Ca dose from CaCl<sub>2</sub>, which is consistent with the results from the current experiment, where CaCl<sub>2</sub> resulted in a sharp increase within the first 6 h while no differences were observed in Ca-drink cows. In contrast to CaCO<sub>3</sub>, CaCl<sub>2</sub> is a readily soluble and ionizable form of Ca, which may increase soluble ruminal Ca concentrations to levels favoring what appears to be unregulated Ca absorption (Wilkens *et al.*, 2015; Goff, 2018). These effects are, however, short lived. Within the ruminal conditions (high pH and high carbonate concentrations) CaCl<sub>2</sub> may precipitate, although it is not clear quantitatively how much of the Ca may be subject to rapid absorption and how much would precipitate. However, any precipitated fraction of Ca may solubilize in lower segments of the gut where pH is low, such as in the abomasum and the upper part of the duodenum, and as a result become available for further absorption. A non-soluble source of Ca at neutral pH, such as CaCO<sub>3</sub>, could mainly follow such route of absorption which may sustain the availability of Ca over a longer period, as

**Fig. 1.** Blood calcium dynamics relative to treatment initiation in cows at parturition. Treatments included an oral Ca supplement offered for voluntary consumption (Ca-drink,  $n=22$ ), an oral Ca bolus (Ca-bolus,  $n=22$ ) or a control (CON,  $n=23$ ). Blood samples were collected at  $-14$  d (before expected parturition date), within 15 min after treatment initiation, and at 6, 12, 24 and 48 h relative to treatment initiation. Baseline value was not included as covariate in the statistical model. Statistically significant differences for treatment effects are indicated by different superscripts a,b ( $P \leq 0.05$ ).



passage rate delays the release of ruminal contents into the abomasum and duodenum to a small percentage per hour. However, studies evaluating how apparent total tract Ca digestibility may differ between Ca fed as  $\text{CaCO}_3$  or Ca fed as  $\text{CaCl}_2$  are lacking to substantiate this hypothesis.

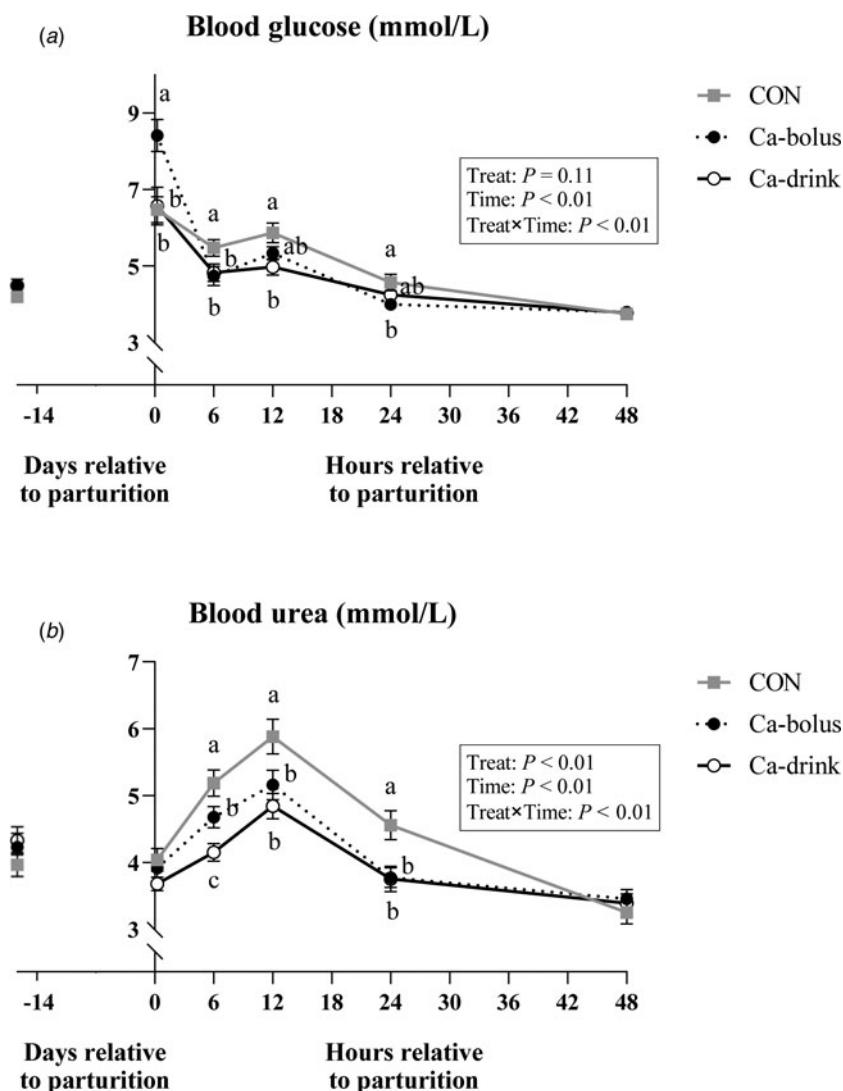
The quick increase in blood Ca induced by feeding a  $\text{CaCl}_2$  (71% of total Ca) based bolus may have been sufficient to generate a positive Ca signal that could be responsible for the subsequent reduction in blood Ca beyond the 6 h post bolus administration. Similar responses are usually observed when high Ca doses (100 g; Goff and Horst, 1993), or multiple Ca doses (Martinez *et al.*, 2016; Valdecabres *et al.*, 2018) are administered. Results of the current study are, however, in contrast with Martinez *et al.* (2016), who showed no drop in blood Ca concentrations at 24 h post treatment administration following the administration of a Ca-bolus containing 43 g of Ca (71%  $\text{CaCl}_2$  and 29%  $\text{CaSO}_4$ ). This may be related to the repeated Ca-bolus administrations (d 0 and 1 post parturition) in the study from Martinez *et al.* (2016). The current study may, therefore, not reflect commercial practices as manufacturers recommend applying the Ca-bolus supplement twice; a first application immediately after parturition and a second application 12 h later. Sampson *et al.* (2009) showed that when this protocol is applied, blood Ca concentrations were higher with a Ca bolus treatment than a negative control at 13 h postpartum (1 h after the second administration) and did not differ with the CON group at 24 h postpartum. If the observed dip was the result of a homeostatic rebound following a high positive Ca signal, repetition of the oral application would not mitigate the problem but rather delay it for another day. Nevertheless, increase of blood Ca in the Ca-drink group was moderate (+0.1 mmol/l) and biological relevance of these differences remain unknown and should be evaluated on a larger number of animals. Whether an oral Ca dose of 43 g mainly from  $\text{CaCl}_2$  induces a Ca signal triggering the main Ca regulatory mechanism to lower blood Ca concentrations deserves further investigation.

The transitory state of hyperglycemia observed in the current study has been previously documented in dairy cows at parturition (e.g., Ingvarthsen and Andersen, 2000; Weber *et al.*, 2016). Cows in the Ca-bolus group had somewhat higher glucose and cortisol concentrations postpartum than Ca-drink cows which could potentially be the consequence of stress induced by the

oral administration of the Ca-bolus (Leroy *et al.*, 2011). However, the cortisol difference did not achieve significance, and since higher cortisol concentrations were also observed for CON cows, the results are not consistent with this hypothesis. Additionally, these cortisol concentrations are within ranges of what is observed in prepartum dairy cows for all three treatment groups (Patbandha *et al.*, 2017). Furthermore, Valdecabres *et al.* (2018) did not observe any differences in blood glucose concentrations measured 1 h after Ca-bolus administration (50 g of Ca from  $\text{CaCl}_2$ ). Besides blood glucose, the study showed higher blood urea concentrations in CON cows compared to Ca supplemented cow. This could be related to protein breakdown due to the high BW losses around parturition. However, cows receiving the Ca-bolus also had high BW losses and their blood urea concentration was the same as cows receiving the Ca-drink treatment. To our knowledge, the effect of Ca supplementation at parturition on blood urea has not been investigated in the literature and requires further work.

It should be noted that the experimental design of the current study presents the limitation that both oral Ca supplements contained other nutrients besides the Ca components. The Ca-drink contained dextrose, as well as other minerals and vitamins, while the Ca-bolus provided gluconate. Thus, the effects of treatment on the studied parameters cannot be entirely attributed to the Ca content and Ca source (Daniel *et al.*, 2021). Although the Ca-drink provides some energy, this represented less than 5% of total gross energy intake for a cow eating 14 kg DM with a gross energy content of 19 MJ/kg of DM on the first day after calving. In addition, the gluconate present in the Ca-bolus treatment could also be relevant for preventing subclinical ketosis (McArt *et al.*, 2011). However, a single application is unlikely to be effective, as such treatments should be repeated in time. Therefore, the observed differences in blood Ca dynamics are most probably linked to the Ca source and the form of treatment administration, although other minerals or vitamins present in the Ca-drink may also have played a role.

In conclusion, this study showed that a single administration of an oral  $\text{CaCO}_3$  supplement offered for voluntary consumption resulted in a steady increase in blood Ca concentrations from parturition to 48 h, whereas administering an equivalent dose of Ca as  $\text{CaCl}_2$  as the main source induced fluctuation in blood Ca dynamics, characterized by a greater increase for the first 6 h



**Fig. 2.** Blood glucose (a) and blood urea (b) dynamics relative to treatment initiation in cows at parturition. Treatments included an oral Ca supplement offered for voluntary consumption (Ca-drink,  $n = 22$ ), an oral Ca bolus (Ca-bolus,  $n = 22$ ) or a control (CON,  $n = 23$ ). Blood samples were collected at  $-14$  d (before expected parturition date), within 15 min after treatment initiation, and at 6, 12, 24 and 48 h relative to treatment initiation. Baseline values were not included as covariate in the statistical model. Statistically significant differences for treatment effects are indicated by different superscripts a,b,c ( $P \leq 0.05$ ).

and followed by a decrease from 6 h to 24 h, before increasing again from 24 h to 48 h. Differences in blood glucose and urea concentrations were also detected and are probably linked to the product compositions, as well as administration route. Unfortunately, it was not possible to draw conclusions regarding the potential stress induced by the Ca-bolus administration. In addition, due to the absence of overall differences between the two oral Ca supplements and the CON treatment, it is also not possible to draw conclusions regarding the efficacy in maintaining blood Ca concentrations postpartum. Although the observed differences most probably resulted from the Ca source, the effect of treatment cannot be isolated to the Ca content of the product as other nutrients were present in both Ca supplements.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S002202992200019X>.

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