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Evaluating calcium concentrations in serum of pasture-grazed dairy cows treated with an oral *Solanum glaucophyllum* calcium bolus within 12 h postpartum

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Oral calcium boluses can increase serum calcium after calving, but effects may be short-lived, so repeat dosing is often needed during the highest-risk period for hypocalcaemia. Adding vitamin D glycosides may help sustain the post-treatment rise in calcium. The primary objective was to compare serum calcium, magnesium, and phosphorus concentrations in dairy cows treated with either 1) a new bolus containing calcium salts and vitamin D3 glycosides from *Solanum glaucophyllum*, 2) a commercial calcium bolus without vitamin D3 glycosides, or 3) no treatment.

Methods: During the spring 2024 calving season in New Zealand, we conducted a prospective, randomized, controlled trial in 129 dairy cows from two commercial farms. Within 12 h of calving, cows received either a new formulation (two boluses given simultaneously) containing calcium salts plus vitamin D glycosides (*Solanum glaucophyllum*) (new bolus; n=44), a commercially available calcium-only bolus (two boluses given 12 h apart; n=42), or no treatment (control; n=43). Blood samples were collected at enrolment (0 h) and repeatedly to 72 h post-enrolment to measure serum total calcium, magnesium, and phosphorus concentrations. Associations between treatment and serum mineral concentrations were assessed using mixed linear regression models adjusted for farm, cow age, and sampling time, with an autoregressive structure to account for repeated measures.

Results: Compared with controls, the new bolus increased serum total calcium by up to 0.33 mmol/L from 2 to 72 h post-treatment. Compared with the calcium-only bolus, the new bolus maintained higher serum total calcium across the post-treatment period by up to 0.37 mmol/L, although data at 6, 12, and 14 h were compatible with little to no difference between treatments. The new bolus also increased serum phosphorus from 24 to 72 h compared with both other groups, with the 24 h comparison against controls compatible with no difference. Both bolus treatments reduced serum magnesium compared with controls by up to 0.23 mmol/L (new bolus) and 0.13 mmol/L (calcium-only), with greater and more prolonged reductions for the new bolus.

Conclusion: A calcium bolus containing vitamin D glycosides sustained higher serum total calcium for up to 72 h after treatment in pasture-based dairy cows, supporting its potential to reduce hypocalcaemia risk.

KEYWORDS

calcium, dairy cattle, magnesium, oral bolus, postpartum, *Solanum glaucophyllum*

1 Introduction

Hypocalcemia, which occurs due to the greatly increased demand for calcium resulting from the onset of milk production, is one of the most important metabolic diseases affecting dairy cows (Liang et al., 2017; Mekonnen et al., 2022). If the process for increasing calcium availability in the peripartum period is ineffective, then cows will become hypocalcemic (DeGaris and Lean, 2008). Hypocalcemia can be clinical or subclinical, both of which can have major ongoing effects on subsequent disease risk and productivity (Serrenho et al., 2021; McArt and Oetzel, 2023).

The incidence of clinical hypocalcemia varies considerably by farm (DeGaris and Lean, 2008), but recent data suggest that the average herd incidence is around 3% in both intensive (Husband, 2020; Serrenho et al., 2021) and pasture-based systems (Roberts and McDougall, 2019; Horan et al., 2024). Subclinical hypocalcemia is much more common. In both intensive and pasture-based systems, more than 50% of multiparous cows may be subclinically hypocalcaemic (Goff, 2008; Roberts and McDougall, 2019), however, the prevalence varies markedly depending on the threshold used and the timing of the sample (Serrenho et al., 2021).

Most prepartum strategies for preventing hypocalcemia aim to increase the metabolic flux of calcium prepartum, enhancing the cow's ability to adapt to the increased calcium demands (Glosson et al., 2023). Such strategies include feeding a low-calcium diet prepartum (or reducing available calcium by feeding calcium binders) (Kerwin et al., 2019), feeding an acidifying diet with a negative dietary cation-anion difference (DCAD) (Glosson et al., 2023), and supplementation of the biologically active form of vitamin D ($1,25\text{-}(OH)_2D_{3)}$ 24–72 h prepartum (Vieira-Neto et al., 2024). Particularly in cattle fed temperate pasture dominated by cool-season grasses and clovers, these strategies can be difficult to achieve. Firstly, temperate pastures often have relatively high concentrations of calcium (McNeill et al., 2002) and potassium (Roche, 2023), making low calcium diets and low DCAD difficult to achieve. These issues are exacerbated by the great variability in the composition of pasture fed to dry cows. Additionally, pasture-based farms, such as those in New Zealand, are often not set up for the precise addition of supplements (such as binders or diet acidifiers) to feed or for the precise timing relative to calving required to optimize the effectiveness of supplementation of $1,25\text{-}(OH)_2D_3$ (or its analogues) (Vieira-Neto et al., 2024). Thus, on pasture-based dairy farms, the principal strategy for ensuring that the cow can adapt to increased calcium demands is the supplementation of magnesium (usually by dusting pastures with magnesium oxide pre-grazing) (Roche, 2023).

Alternative means of reducing hypocalcemia risk, especially in high-risk cows, are thus likely to be useful in pasture-based cows. One approach is the supplementation of cattle with oral calcium salts, which have been used for many decades (Parkinson et al., 2010). These typically supply 30 to 45 g of calcium in a highly soluble form (e.g. calcium chloride) to permit passive vitamin D-independent absorption of 3 to 5 g of calcium (Goff and Horst, 1993). While effective, they increase serum calcium concentrations for a short period of time, and there is a need to give a second bolus 12–24 h later (Roberts et al., 2019). Combining oral calcium supplementation with $1,25\text{-}(OH)_2D_3$ could result in an initial increase in calcium concentrations, which are then maintained because of the response to bioactive $1,25\text{-}(OH)_2D_3$. *Solanum glaucophyllum* is a plant that produces significant amounts of glycosides of $1,25\text{-}(OH)_2D_3$. In the rumen, these glycosides are cleaved by rumen bacteria, liberating bioactive,$1,25\text{-}(OH)_2D_3$. Initial studies demonstrated that supplementation with the leaves of *S. glaucophyllum* in a gelatin bolus could improve calcium status (Horst et al., 2003). However, a rebound hypocalcemia occurred in all treated cattle 6–8 days after treatment ended. The development of a product which contained both rapid- and long-acting controlled-release *S. glaucophyllum* extract improved calcium status without resulting in rebound hypocalcemia (Meyer-Binzegger et al., 2022). Preliminary internal, unpublished work on a new oral bolus (Goff-Bol, Contract Manufacturing Services) that contains calcium chloride, calcium acetate, calcium lactate and *S. glycophyllum* extract on multiple commercial dairy farms in the US has shown significant increases in plasma calcium concentrations in the first 4 days after calving when compared to a standard calcium-salt-only bolus. Therefore, the hypothesis was that a bolus which combines this product with soluble calcium salts should maintain blood calcium concentrations for 8–12 h after administration via passive calcium transport across the rumen wall, and then from 12–72 h after calving by stimulating active transcellular intestinal calcium transport.

In all the preliminary, unpublished studies, cattle were fed a total mixed ration and were treated with the bolus within 1–2 h of calving. In New Zealand, cows predominantly calve at pasture, and it is only practical to treat them with a bolus when they are brought to the parlor for milking for the first time after calving. As this occurs only once to twice a day for the majority of farms (Cuttance et al., 2018), bolusing cattle to prevent hypocalcemia within 1–2 h after calving is impractical. Therefore, the primary aim of this work was to compare the serum calcium (tCa), magnesium, and phosphorus concentrations in dairy cows treated with either 1) a

Abbreviations: BCS, Body condition score; C, Control; CB, Commercially available bolus; DCAD, dietary cation-anion difference; ICC, Intraclass correlation coefficient; IVP, Investigational Veterinary Product; NB, New Bolus; tCa, Total Calcium.

new bolus (2 boluses given simultaneously) containing calcium salts and vitamin D_3 glycosides from *Solanum glaucophyllum* (NB); 2) a commercial calcium bolus (2 boluses given twelve h apart) (CB); or 3) no treatment, during the spring 2024 calving season in New Zealand.

2 Materials and methods

This study complied with the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products Good Clinical Practice Principles (VICH GL9, June 2000). All animal manipulations were approved by the AgResearch Ruakura Animal Ethics Committee under application number 2415. The study was also conducted in compliance with ACVM Trial approval A12111–02 and associated Animal Products approval.

This study was a randomized controlled trial conducted on two commercial dairy farms in the Waikato region of New Zealand during the spring calving season of 2024. The study started with the first enrolments on the 15th of July 2024 and was completed on the 1st of September 2024.

A schematic diagram of the methods is presented in Figure 1.

2.1 Power analysis

The power analysis was based on the data at Time 0 from Sampson et al. (2009), who identified a standard deviation of 0.15

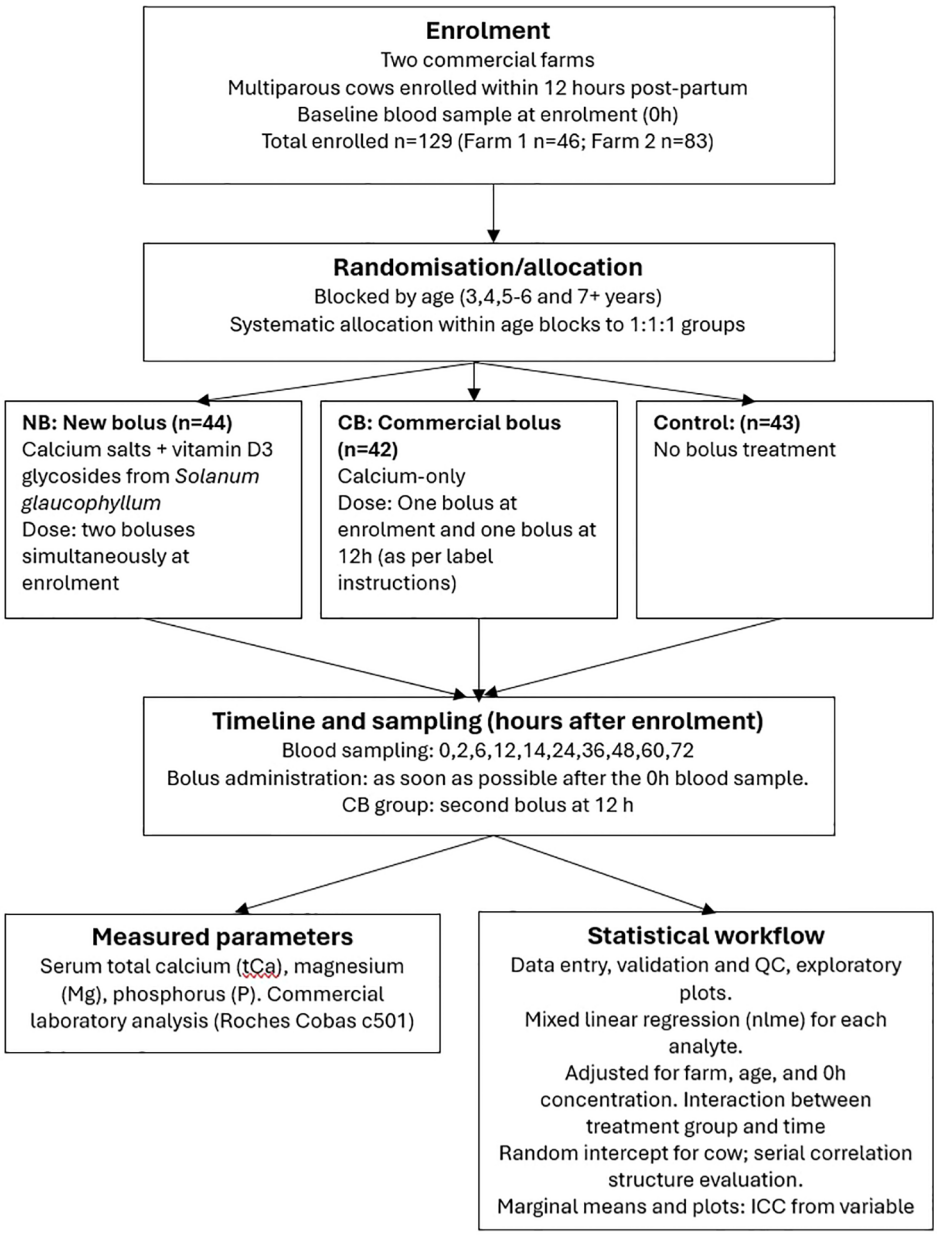

FIGURE 1
Schematic diagram of the methods in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms. C= Control, CB = commercially available bolus, NB = New Bolus.

mmol/L serum calcium. Utilizing this standard deviation, we identified that 20 animals per group would be able to identify a 0.15 mmol/L difference with 80% power and 95% confidence. However, as we anticipated that the differences would become less pronounced over the study period (i.e. the first 3 days after calving), we enrolled two farms to ensure that the sample size was enough to find the 0.15 mmol/L difference with the three treatment groups (new bolus, commercial bolus and negative control) enrolled at a ratio of 1:1:1. This resulted in a sample size of 50 animals per group to be enrolled across the two farms.

2.2 Farm enrolment

Two commercial dairy farms based in the Waikato region of New Zealand were convenience selected based on their herd size, calving pattern, and willingness to participate in the study. Farms were managed in a way that was typical for that farm, but they had to ensure that any minerals provided to calved cows were not dusted on the pasture and only put in the supplementary feed provided in the troughs of the feedpad. Herd productivity was described using milk solids (kg fat + protein per cow per lactation), the standard production metric for New Zealand pasture-based dairy systems (305-day mature-equivalent milk yield is not routinely calculated in this context).

Farm 1 had a planned calving start date of 9 July and expected to calve 620 cows in the spring. The herd comprised predominantly crossbred cows, with 90% being ≥10/16 Friesian (62.5% Friesian), 4% being ≥10/16 Jersey (the highest Jersey proportion being 13/16), and 6% being crossbred, producing a mean of 465 kg of milk solids in the previous season. In addition to pasture, two to three weeks before calving, cows received 2 kg DM maize silage daily, with each 2 kg containing 300 g of a proprietary mineral blend with a negative DCAD (Transition premix, Agvance, Auckland, New Zealand) for 14–21 days before parturition. It included 36 g calcium, 13 g magnesium, 54 g chloride, and 35 g sulfur per 300 kg dose. Freshly calved cows were fed pasture (without dusting of minerals), 2.0 kg DM maize silage, and 2.5 kg DM meal daily. The maize silage was mixed with magnesium oxide and lime flour at a rate that provided approximately 100 g of magnesium oxide and 200 g of lime flour per cow per day. The meal comprised palm kernel expeller (44.8% by weight), citrus pulp pellets (14.0%), tapioca pellets (12.1%), dried distiller's grain (11.2%), a proprietary mineral mixture (6.7%), a proprietary high starch pellet (5.6%), and kibbled maize (5.6%). The proprietary mineral mixture was fed at a rate of 200 g/cow/day and contained 150 g lime flour, 22.2 g magnesium oxide, 22.6 g sodium chloride, and sometimes a sweetener in addition. All cows received trace minerals through drinking water, which provided 920 mg/cow/day of magnesium as a filler. Feed samples were tested daily. Mean feed sample results are summarized in Table 1.

Farm 2 had a planned calving start date of 3 August and expected to calve 770 cows in the spring. The herd was predominantly Friesian (65% of cows ≥10/16 Friesian, 11% ≥10/16 Jersey, 24% crossbred or other) and produced a mean 627 kg milk solids per cow in the previous season. Cows close to calving are usually in the calving mob on this farm for one to two weeks and in this mob received a ration containing 2 kg DM grass, 5 kg DM maize silage, 2 kg DM grass hay, 2 kg DM dried distiller's grain, 200 g of a proprietary supplement (Springer cow balance hi mag, Nutritech, Auckland, New Zealand) for approximately 12 days prepartum. Freshly calved cows were fed *ad libitum* pasture (without dusting of minerals) and a partial mixed ration. The ration was estimated to deliver 4 kg DM maize silage, 2 kg DM dried distiller's grain, 1 kg DM palm kernel expeller, 100 g lime flour and 50 g magnesium oxide per cow daily. The farmer placed 200–300 kg DM of ration into a mobile trough daily, and the cows ate it *ad libitum*. Leftover ration (if any) was consumed by non-study cows. All cows received magnesium sulphate (approximately 60 g/cow/day) and a similar proprietary trace mineral supplement to Farm 1 in the drinking water (containing a trace amount of magnesium for a filler/bulking agent). Sodium chloride was provided *ad libitum* as cows exited the milking parlor. Mean feed sample results are summarized in Table 2.

2.3 Feed samples and analysis

A representative sample of all feeds was collected and analyzed for mineral and nutritional composition every week. A pasture

TABLE 1 Mean (and range) dietary mineral composition of feed samples collected on Farm 1 (n=46 cows), in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms.

Analyte	Pasture	Maize silage[1]	Meal
Ca (g/kg DM)	3.8 (2.9 - 4.3)	12.2 (1.5 - 44.5)	29.8 (20.5 - 52.8)
Mg (g/kg DM)	2 (1.7 - 2.4)	12.9 (1 - 33.2)	9.8 (4.1 - 38.5)
P (g/kg DM)	5.3 (4.8 - 5.8)	1.7 (1.2 - 2.6)	4.1 (2.9 - 5.1)
Na (g/kg DM)	0.7 (0.5 - 1.4)	0.6 (0.1 - 2)	3.2 (2.2 - 4.3)
K (g/kg DM)	43.4 (38.9 - 49.4)	10.2 (7.3 - 11.6)	7.3 (6.3 - 9.5)
Cl (g/kg DM)	11.6 (9.7 - 13.2)	10.3 (2.2 - 33.6)	5.5 (4.3 - 6.5)
K/Na	71.9 (30 - 92)	40.2 (4 - 138)	2.6 (2 - 4)
Ca/P	0.7 (0.5 - 0.9)	7.2 (1.1 - 22)	7.6 (5.6 - 17.7)
DCAD (me/kg DM)	563.9 (518 - 700)	-136.1 (-700 - 153)	39.6 (20 - 69)

C, Control; CB, commercially available bolus; NB, New Bolus.
[1]Maize silage included added magnesium oxide and lime flour that were mixed at a rate that provided approximately 100 g magnesium oxide and 200 g lime flour per cow per day.

TABLE 2 Mean (and range) dietary mineral composition of feed samples collected on Farm 2 (n=83 cows), in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms.

Analyte	Pasture	Meal
Ca (g/kg DM)	3.7 (3.4 - 4.4)	8.4 (7.7 - 9)
Mg (g/kg DM)	1.7 (1.5 - 1.9)	5.8 (4.9 - 7.9)
P (g/kg DM)	4.5 (4.3 - 4.8)	3.7 (3.3 - 4.4)
Na (g/kg DM)	0.8 (0.6 - 1.2)	0.3 (0.2 - 0.6)
K (g/kg DM)	42.7 (39.9 - 45.1)	12.3 (9.5 - 14.7)
Cl (g/kg DM)	10.7 (8.3 - 13.3)	3.5 (2.9 - 4.3)
K/Na	53.7 (33 - 70)	47.8 (23 - 63)
Ca/P	0.8 (0.7 - 0.9)	2.2 (1.9 - 2.4)
DCAD (me/kg DM)	571.2 (550 - 625)	107.8 (57 - 160)

C, Control; CB, commercially available bolus; NB, New Bolus.

sample was collected from the area the cows were grazing on by walking the diagonal of the area and cutting with scissors to a residual of 7.5 cm 30 times to mimic cow grazing behavior. Any feed supplied on the feedpad, in the milking parlor or via a feed trough had a representative sample collected by taking 10 scoops of the feed along the feedpad/parlor/trough. The 10 scoops were collected equally along the row, with every second scoop from the bottom and every other scoop from the top.

All feed samples were sent within a day of collection to Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204 for an extended feed profile via Wet Chemistry (ExtFedWetChem) and minerals.

2.4 Animal enrolment

Cows were enrolled within 12 h of parturition. On both farms, this was practically achieved by checking the due to calve group at approximately 9 pm. Any animals that already had calves at foot were identified and recorded. They were not eligible to be enrolled the following morning. Any multiparous cows that calved between this evening check and the morning check (at ~ 7–8 am) were eligible to be enrolled (i.e., primiparous cows were not eligible). No data were recorded on the interval between calving and treatment, except that they had calved between ~9 pm and the morning enrolment visit.

Eligible cows were assessed for any obvious abnormalities and body condition score (BCS) as measured on a 10-point scale (Roche et al., 2004). Cows were not eligible for enrolment if they had a BCS<4 or if they had any signs of systemic illness or concurrent disease. Cows that had been treated following calving or within the previous 14 days with any antibiotics (systemic or intramammary), corticosteroids, or a calcium product (oral or parenteral) were also excluded. These assessments were made by veterinarians or veterinary research technicians. Finally, if the farmer deemed the cow to have a poor temperament for repeated handling or showed major resistance to swallow the bolus, it was also not eligible to enroll.

Cows were excluded after enrolment if they were distressed or showed significant reluctance to be restrained or blood sampled. It was planned that animals would be removed if they developed any concomitant disease resulting in systemic illness, euthanasia or death (e.g. toxic mastitis), if a bolus was not able to be successfully administered, if they were actually pregnant (not calved yet), or if they developed clinical hypocalcemia that required treatment within 24 h post-enrolment. In addition, cows that were administered systemic antibiotics were also planned for exclusion. Data from samples taken prior to removal were still used in the analysis.

2.5 Randomization

Before treatment group allocation, cows were blocked on age by splitting them into four age groups: 3, 4, 5-6, and ≥7 years. Allocation was a systematic process, with the treatment group of the first cow in each age group selected by drawing from a hat, and the following cows cycling through the three treatment groups in sequence.

The first group (NB) received the new bolus (Goff-Bol, Contract Manufacturing Services, USA). The second group (CB) received a commercially available calcium bolus (Calpro Bolus, Elanco, Auckland, New Zealand). The final group (C) received no additional treatment. Research technicians carried out the randomization and then proceeded to provide the treatment.

2.6 Blood Sampling

Once enrolled, and cows had finished their first milking, blood samples were collected from the coccygeal vein, using a 20 g needle and evacuated serum tubes (Vacutainer, BD, Auckland, New Zealand) by a trained veterinary technician or veterinarian. For all cows, this first blood sample was classified as being collected at 0 h after enrolment, but in addition, the actual time of sampling was recorded for each.

Further blood samples were collected at 2, 6, 12, 14, 24, 36, 48, 60, and 72 h after enrolment. Between samples at 0 and 2 h and 12 and 14 h, cows remained on the yards and were provided with ad libitum maize silage and grass silage feed in feed bins. Between the other sampling times, cows were able to go back to pasture.

Blood samples were allowed to clot and then centrifuged (3,000 RPM for 12 minutes at 1.4 g) to separate the serum. Serum was extracted, split into two vials labelled with the farm, cow ID, sample time and sample A or B, and frozen at -20 °C until analysis. Samples were frozen for between 1–9 weeks, depending on when they were collected during the trial. Serum total calcium, magnesium, and phosphorus are considered stable in frozen separated serum, so the 1–9 week storage at −20 °C is unlikely to have materially affected the measured concentrations.

Frozen serum samples were sent to a commercial laboratory (SVS, Hamilton, New Zealand) for measurement of serum total calcium, magnesium, and phosphorus concentrations. All 3 tests were performed on a Hitachi Cobas c501 analyzer supplied by Roche Diagnostics (Auckland, New Zealand), using Roche Diagnostics recommended reagents and were interpreted as per

the manufacturer's instructions. Results were supplied as electronic files (Excel, Microsoft, Redmond, Washington, USA). The inter-assay precision for tCa was 2.08% at a nominal tCa level of 2.32 mmol/L and 1.70% at a nominal tCa level of 3.03 mmol/L based on the laboratory control data. The intra-assay precision was 2% at 0.60 mmol/L and 0.8% at 2.55 mmol/L based on manufacturer data.

2.7 Treatment administration

As soon as possible following the first blood sample, any boluses were administered to cows using a bolus applicator by a trained veterinarian or veterinary technician.

Cows in the NB group received two boluses weighing 170g each at enrolment consecutively (in two separate actions), comprising calcium chloride, calcium acetate and calcium lactate supplying 80 g (over the two boluses) of readily soluble calcium in the two boluses. The boluses contained *Solanum glaucophyllum* leaf with a known amount of 1,25-$(OH)_2D_3$ glycosides. The second group (CB) received a commercially available calcium bolus (Calpro Bolus, Elanco, Auckland, New Zealand). This bolus contained 114.3 g calcium chloride and 45.7 g calcium sulphate in a 203 g bolus (43 g calcium). Cattle in this group received one bolus at enrolment and the second bolus 12 h later. The final group (C) received no additional treatment.

The timing of the first bolus administration relative to the blood sample was recorded for each cow.

2.8 Statistical analysis

Statistical analysis was performed in RStudio using R version 4.3.2 (R Core Team, 2023). Raw data collected on the farm in data capture forms were entered into an electronic spreadsheet, which was imported along with the laboratory data. A random selection of approximately 10% of the data points were checked against the raw data entry forms. The data were then examined for completeness, duplication, consistency, and spurious values. Exploratory data analysis included generating tables of summary statistics and distributional plots, overall and by farm and age. Relationships between pairs of variables were visualized with frequency tables and plots. Cow breed was taken from farm genetic records and categorized as Friesian (≥12/16 Friesian), Jersey (≥12/16 Jersey), Jersey cross (>0/16 and <12/16 Jersey), "other" and unknown if breed data were unavailable.

The relationships between treatment groups and each of serum tCa, magnesium, and phosphorus concentrations were determined by mixed linear regression using the nlme package (Pinheiro and Bates, 2000), with separate models for each analyte. Candidate variables included treatment group, sample time, farm, age, BCS, breed and concentration at time 0 of tCa, magnesium or phosphorus. In New Zealand, age is synonymous with parity (i.e., parity 1 cows are two years of age). Because repeated samples were collected from cows, serum concentrations were serially correlated within each cow, which was accounted for by including a random intercept for cow and an autocorrelation structure. Compound symmetry, autoregressive, and moving average autocorrelation structure were explored, and the structure that optimized the Akaike information criterion (AIC) was selected. Farm was added to the models as a fixed effect to account for clustering within farms, observe its adjusted association with serum concentration, and check for an interaction with treatment. Farm was only retained if it improved the model fit based on the AIC and/or there was graphical evidence of residual heteroscedasticity between farms. First, separate univariable simple regression models were made for each candidate variable. Then, multiple regression models were constructed in a backwards, stepwise manner, offering all candidate variables regardless of their univariable associations. Interactions were explored between treatment group, pre-treatment (0-h) serum concentration, farm, and age. Final models were tested for the assumptions of independence, linearity, homoscedasticity, and normally distributed residuals by evaluation of diagnostic residual plots. The impact of individual cows and observations (influential observations) on the final model was appraised by observing standardized residuals, performing deletion diagnostics (computing Cook's Distance), and examining leverage values. Model-estimated marginal mean serum tCa, magnesium, and phosphorus concentrations at 2–72 hours were computed from the final model using the emmeans package (Lenth, 2024) and plotted. Model-estimated marginal means were not computed for 0 hours because 0-hour concentrations were model inputs. Intraclass correlations (ICC) were calculated from the models' variance components. The ICC is a measure of the proportion of total variance existing at the cow level, with the rest existing at the observation level within cows and indicates the degree of similarity of observations within cows.

3 Results

A total of 135 cows were presented for enrolment, but 6 were excluded, leaving data from 129 cows, with 44, 42, and 43 allocated to the NB, CB and C groups, respectively. Of the excluded cows, two were excluded for BCS <4, one cow in the CB group was not successfully administered the second bolus (three attempts to administer the bolus, each with a new bolus, failed), one had an adverse event due to a perforation of the esophagus using a bolus applicator, one was later discovered to have not calved, and one had not been milked prior to enrolment. No animals were excluded at any point for clinical hypocalcaemia.

A total of 46 cows were enrolled on Farm 1 between the 15th of July 2024 and the 21st of July 2024, and 83 cows were enrolled on Farm 2 between the 5th of August 2024 and the 29th of August 2024. Fewer cows were enrolled than intended, as the rate at which cows calved was lower than anticipated, so the study ceased prior to 150 cattle being enrolled due to resourcing.

Enrolment data were complete for all variables except age and breed. Age was unknown for one cow on Farm 2. Her plastic herd tag was missing, so her brass birth ID tag was used to obtain her birth year. Her age category (5–6 years) was recorded, but not her exact age. Breed was unknown for six cows due to missing genetic

records (n=3) or use of temporary ear tags that did not match the breed records (n=3). The results of the feed mineral analyses are presented in Tables 1, 2 for Farms 1 and 2, respectively.

Serum results were available for 1,266 blood sample time points and were missing for 24 blood sample time points. When serum results were missing, they were missing for all three analytes (tCa, magnesium, and phosphorus) at that time point because no sample was submitted to the laboratory. The reasons for missing blood samples are summarized in Supplementary Table 1.

The study cows were predominantly Friesian (66/129, 51%), with 49/129 (38%) Jersey cross, 3/129 (2%) Jersey, 5/129 (4%) other and 6/129 (5%) unknown. The three Jersey cows were allocated to groups C (n=2) and NB (n=1) and were all 13/16 Jersey and 3/16 Friesian. Because of the small number of Jersey and "other" breed cows, breed was categorized into Friesian (66/129, 51%), other (57/129, 44%) and unknown (6/129, 5%).

The cows had a median age of five years overall (range = 3–11 years) and a median BCS of 4.5 (range = 4.0-5.5). There were no differences between treatment groups for age, BCS and breed (Table 3), and the treatment groups were balanced for pre-treatment (0 h) serum concentrations of tCa, magnesium, and phosphorus (Table 4). The number of cows enrolled on each day is presented in Supplementary Figure 1.

3.1 Calcium

Farm, age, treatment group, and tCa concentration at 0 h all had associations with serum tCa concentration in the univariable models (P<0.001), but BCS (P = 0.086) and breed (P = 0.7) did not. The final model contained farm, tCa concentration at 0 h, treatment group, sample time, age and an interaction between treatment group and sample time but did not include BCS or breed. Autocorrelation structures did not improve the model fit, so none was included in the final model. The ICC was 0.25. The final model met all diagnostic procedures, and no observations were removed.

Model-estimated marginal mean serum tCa concentrations for cows in each treatment group, after accounting for farm, are presented in Figure 2. At 2 h post bolusing, across both farms, cows in the NB group had serum tCa concentrations that were, on average, 0.33 (95% CI 0.20 to 0.47) mmol/L higher than cows in the C group, while cows in the CB group were, on average, 0.15 (95% CI 0.02 to 0.29) mmol/L higher than cows in the C group. Cows in the NB group were, on average, 0.18 (95% CI 0.04 to 0.31) higher than cows in the CB group. The interaction between treatment and time on serum tCa concentration meant that these differences were not consistent across all time points, as the pattern of change in mean serum tCa concentration over time depended on the treatment group (see Figure 2). To illustrate the interaction between treatment and time on serum tCa concentration, the changes in mean tCa concentration are presented in Table 5 for four time periods (2–72 h; 2–14 h, 14–36 h and 36–72 h). These are presented for each of the three treatment groups, alongside comparisons of those changes for cows in the C group versus cows in the NB group and for cows in the NB group versus cows in the CB group.

For NB and C over the period from 2–72 h after treatment, our data are compatible with no biologically important difference between the two groups in the change of mean tCa over time (i.e. 95% CI do not include differences of more than ±0.2 mmol) (see Table 5). This also applies to the three other time periods. In contrast, for NB and CB over the period from 2–72 h after treatment, our data are compatible with a biologically important difference between the two groups in the change in mean tCa (see Table 5), with the difference in the mean change in tCa between the two groups being most apparent between 14 and 36 h. Thus, while the absolute tCa concentrations were different between cows in the C and NB groups (with the latter being higher throughout the study period), the pattern in change over time was similar for the two

TABLE 3 Balance of cows in treatment groups in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms.

Variable	Overall N = 129	C N = 43	CB N = 42	NB N = 44	p-value
Farm					0.9[1]
Farm 1	46 (36%)	15 (35%)	14 (33%)	17 (39%)	
Farm 2	83 (64%)	28 (65%)	28 (67%)	27 (61%)	
Age (years)					>0.9[1]
3	29 (22%)	9 (21%)	9 (21%)	11 (25%)	
4	28 (22%)	9 (21%)	9 (21%)	10 (23%)	
5-6	31 (24%)	11 (26%)	11 (26%)	9 (20%)	
7+	41 (32%)	14 (33%)	13 (31%)	14 (32%)	
Breed					0.6[1]
Friesian	66 (54%)	25 (60%)	18 (49%)	23 (52%)	
Other	57 (46%)	17 (40%)	19 (51%)	21 (48%)	
Unknown	6	1	5	0	

[1]Pearson's Chi-squared test
C, Control; CB, commercially available bolus; NB, New Bolus.

TABLE 4 Mean (SD) pre-treatment (0 h) serum calcium, magnesium, and phosphorus concentrations in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms.

Characteristic	Overall N = 129	C N = 43	CB N = 42	NB N = 44
Calcium (mmol/L)	1.87 (0.26)	1.85 (0.26)	1.86 (0.23)	1.90 (0.29)
Magnesium (mmol/L)	0.92 (0.15)	0.92 (0.14)	0.90 (0.15)	0.95 (0.14)
Phosphorus (mmol/L)	1.39 (0.42)	1.37 (0.42)	1.44 (0.40)	1.37 (0.46)

C, Control; CB, commercially available bolus; NB, New Bolus.

groups. In contrast, for NB vs CB, the pattern of change over time was different between the two groups, with the response to the second bolus in the CB cows markedly reducing the difference in mean tCa in NB and CB-treated cows at 14 h, but in the period following this increase, the mean tCa concentrations of the two groups diverged, with mean tCa in the CB-treated cows reducing compared to the NB-treated group.

3.2 Magnesium

Farm, age, treatment group, and magnesium concentration at 0 h were all associated with serum magnesium concentration in the univariable models, while BCS (P = 0.4) and breed (P = 0.3) did not have significant associations. Before the development of the multivariable model, serum magnesium concentration was square root transformed due to heteroscedasticity. The final model contained treatment group, sample time, magnesium concentration at 0 h, farm, age and an interaction between treatment group and sample time, but not BCS or breed. Autocorrelation structures did not improve the model fit, so none was included in the final model. The ICC was 0.35. The final model met all diagnostic procedures, and no observations were removed.

Model-estimated marginal mean serum magnesium concentrations for cows in each treatment group, after accounting for farm, are presented in Figure 3. At 2 h post bolusing (after accounting for serum magnesium at time 0), across both farms, no biologically important differences between cows in the NB or CB group and the C group were confirmed, with model-estimated marginal mean differences of -0.03 (95% CI -0.10 to 0.03) mmol/L for NB cows and -0.05 (95% CI -0.12 to 0.01) mmol/L for CB cows. The interaction between treatment and time on serum magnesium concentration meant that these differences were not consistent across all time points, as the pattern of change in mean serum magnesium concentration over time depended on treatment group (see Figure 3). For the C group, mean serum magnesium changed relatively slowly over time, changing by -0.13 (95% CI -0.20 to -0.06) mmol/L from 2 to 72 h after bolusing, although most of that decrease was apparent at 24 h when mean magnesium concentration was 0.11 (95% CI 0.04 to 0.18) mmol/L lower than at 2 h. In contrast, the serum magnesium concentration in both bolus-treated groups decreased at relatively fast rates over the first 24 h after bolusing, with the mean magnesium concentration in CB cows reducing by 0.19 (95% CI 0.12 to 0.25) mmol/L, and that of the

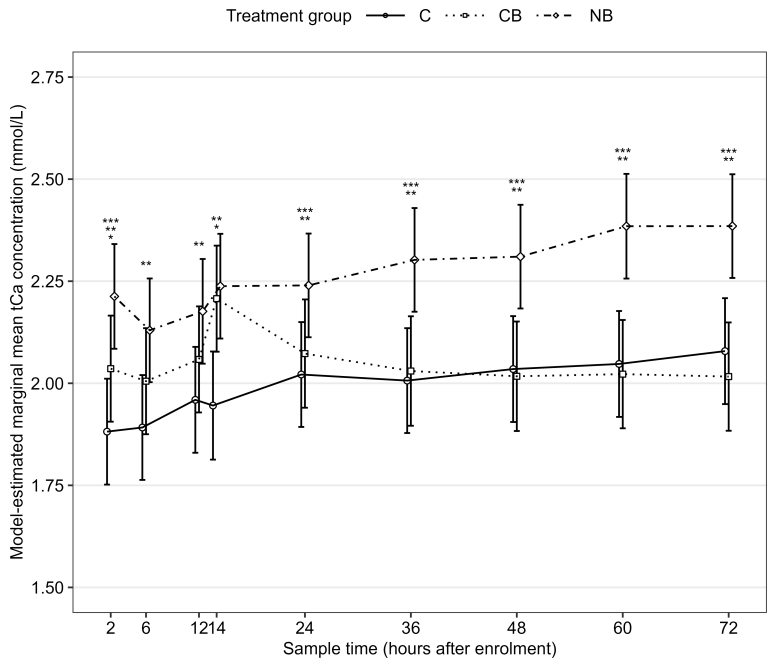

FIGURE 2
Model-estimated marginal mean serum total calcium (tCa) concentrations for cows in each treatment group, margined across farm and age, in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms. Error bars represent 95% CIs. * indicates that for that timepoint lower limit of the CI for mean difference between CP and C was >0, lower limit of the CI for mean difference between NB and C was >0, * lower limit of the CI for mean difference between NB and CP was >0. Note: At all timepoints without a specific asterisk our data were also compatible with the mean difference being the two groups being >0. (CB = commercially available bolus, NB = New Bolus, C = Control). Baseline (0-hour) tCa concentrations are not shown because they were included as covariates in the model.

TABLE 5 **Impact of treatment group on change over time in mean serum total calcium (tCa) concentration for cows in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms.**

Time period after treatment	Change in mean tCa (95%CI)			Difference in change in mean tCa (mmol/L) (95%CI)	
	Control	NB	CB	Control vs NB	NB vs CB
2–72 hrs	0.20 (0.04 to 0.36)	0.17 (0.02 to 0.33)	-0.02 (-0.18 to 0.14)	0.03 (-0.11 to 0.17)	0.19 (0.05 to 0.33)
2–14 hrs	0.06 (-0.10 to 0.23)	0.03 (-0.13 to 0.18)	0.17 (0.01 to 0.33)	0.04 (-0.10 to 0.18)	0.15 (0.01 to 0.29)
14–36 hrs	0.06 (-0.10 to 0.22)	0.06 (-0.09 to 0.22)	-0.18 (-0.34 to -0.01)	-0.00 (-0.14 to 0.14)	-0.24 (-0.38 to -0.10)
36–72 hrs	0.07 (-0.09 to 0.23)	0.08 (-0.07 to 0.24)	-0.01 (-0.18 to 0.15)	-0.01 (-0.15 to 0.13)	-0.10 (-0.24 to 0.05)

C, Control; CB, commercially available bolus; NB, New Bolus.
At 2 hours after treatment, tCa concentrations were higher in NB-treated cows than in CB-treated cows and higher in CB treated cows than in control cows (see Figure 1). NB, new bolus, CB, commercially available bolus.

NB-treated cows by 0.30 (95% CI 0.24-0.36) mmol/L in NB cows. This decrease was then followed by an increase over the next 48 h in mean serum magnesium concentrations of 0.11 (95% CI 0.05 to 0.16) mmol/L for NB-treated cows and 0.09 (95% CI 0.03 to 0.16) mmol/L for CB-treated cows.

3.3 Phosphorus

Farm, age, treatment group, and phosphorus concentration at 0 h had clear associations with serum phosphorus concentration in the univariable models, while BCS (P = 0.5) and breed (P = 0.12) did not. The final model contained treatment group, sample time, phosphorus concentration at 0 h, farm, age and an interaction between treatment group and sample time, but not BCS or breed. Autocorrelation structures did not improve the model fit, so none was included in the final model. The ICC was 0.39. The final model met all diagnostic procedures, and no observations were removed.

Model-estimated marginal mean serum phosphorus concentrations for cows in each treatment group are presented in Figure 4. At 2 h post bolusing, differences in phosphorus concentrations were small; compared to cows in the C group, serum phosphorus concentrations were, on average, 0.02 (95% CI -0.18 to 0.23) mmol/L higher in NB cows and 0.02 (95% CI -0.19 to 0.23) mmol/L higher in CB cows. The interaction between treatment and time on serum phosphorus concentration meant that these differences were not consistent across all time points, as

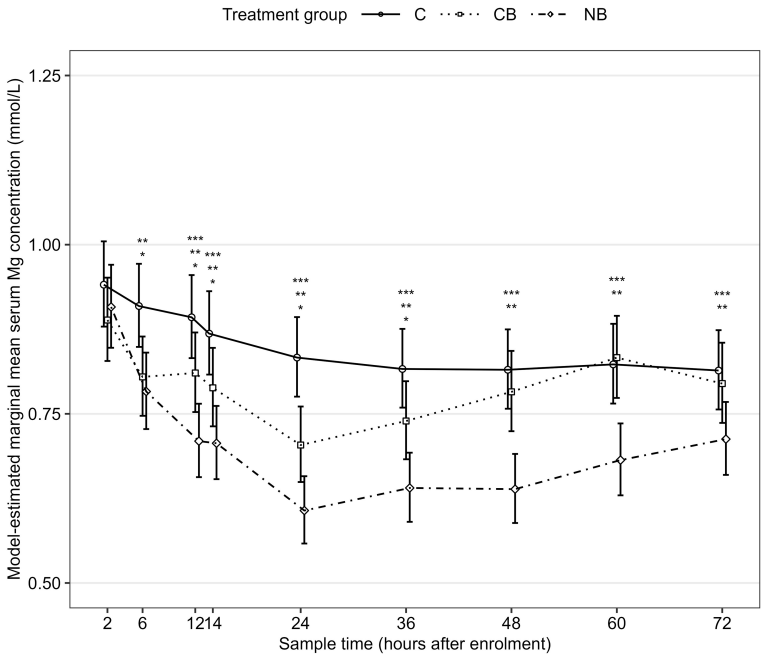

FIGURE 3
Model-estimated marginal mean serum magnesium concentrations for cows in each treatment group, margined across farm and age, in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms. Error bars represent 95% CIs. * indicates that for that timepoint lower limit of the CI for mean difference between CP and C was >0, lower limit of the CI for mean difference between NB and C was >0, * lower limit of the CI for mean difference between NB and CP was >0. Note: At all timepoints without a specific asterisk our data were also compatible with the mean difference being the two groups being >0. (CB = commercially available bolus, NB = New Bolus, C = Control). Baseline (0-hour) Mg concentrations are not shown because they were included as covariates in the model.

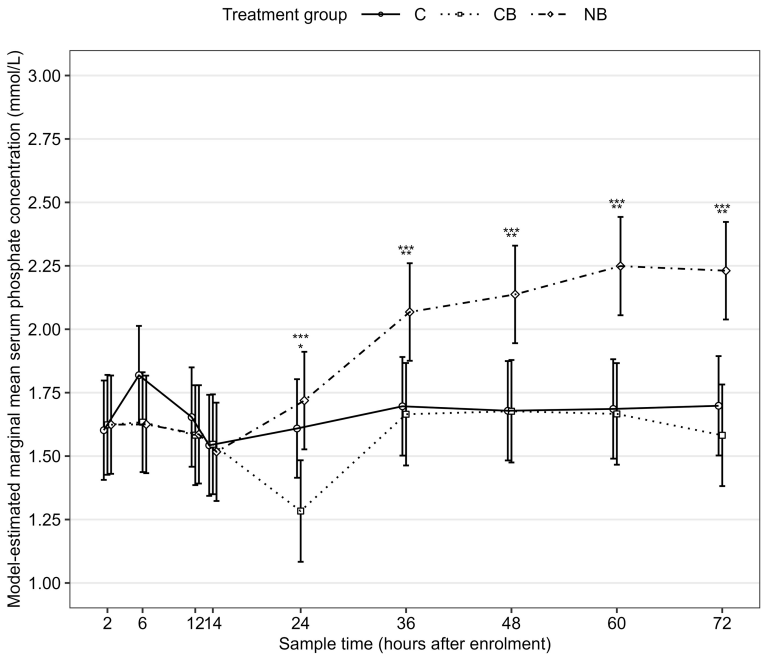

FIGURE 4
Model-estimated marginal mean serum phosphorus concentrations for cows in each treatment group, margined across farm and age, in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms. Error bars represent 95% CIs. * indicates that for that timepoint lower limit of the CI for mean difference between CP and C was >0, lower limit of the CI for mean difference between NB and C was >0, * lower limit of the CI for mean difference between NB and CP was >0. Note: At all timepoints without a specific asterisk our data were also compatible with the mean difference being the two groups being >0. (CB = commercially available bolus, NB = New Bolus, C = Control). Baseline (0-hour) Ph concentrations are not shown because they were included as covariates in the model.

the pattern of change in mean serum phosphorus concentration over time depended on treatment group (see Figure 4). Up to and including the sample taken 14 h post treatment, there was no clear difference between treatment groups in mean serum phosphorus concentrations (see Figure 4), with the 0.22 (95% CI 0.00 to 0.43) mmol/L increase in serum phosphorus seen in the C group between 2 and 6 h still resulting in our data being compatible with no effect of treatment group at 6 h. Between 14 and 36 h the mean serum phosphorus concentrations of the cows in the NB group increased by 0.55 (95% CI 0.34 to 0.76) mmol/L, while that of the cows in the C group increased marginally (mean difference 0.15, 95% CI -0.07 to 0.37 mmol/L), and that of the cows in the CB group decreased and then increased again leading to an increase of 0.12 (95% CI -0.10 to 0.34) mmol/L over that time period. Between 36 and 72 h mean serum phosphorus concentrations were relatively stable in the C and CB groups at ~ 1.6 mmol/L, while the mean serum phosphorus concentration in NB increased by 0.16 (95% CI -0.05 to 0.37) mmol/L over the same time period (see Figure 4).

4 Discussion

Administration of both boluses resulted in biologically important increases in serum tCa concentration compared to untreated cows in the first 14 h after treatment (see Figure 2). The effect of a single bolus of CB on serum tCa concentration in the first 12 h after treatment seen in this study is consistent with the effect reported in pasture-based cattle by Roberts et al. (2019), who reported that mean serum tCa concentrations in cattle treated with a single bolus of CB were higher than in untreated cows in the first 12 h after treatment. Over the first 14 h after treatment, mean serum tCa concentration was higher in NB-treated cows than in CB-treated cows; however, except at 2 h, our data were compatible with no biologically important difference between the tCa concentrations in the 2 bolus-treated groups. Thus, our data are compatible with both boluses increasing serum tCa in the first 12–14 h after treatment, and although supportive of the serum tCa response to NB being greater than that to CB in the first 12 h, they are also consistent with the response to both boluses being similar. Further data are thus required to identify whether the initial response in serum tCa concentration to treatment is better in cattle treated with NB than in cattle treated with CB.

In contrast, from 24 to 72 h, the effect of treatment with NB on serum tCa concentration was clearly superior to the effect of the second CB bolus. Throughout this period, the mean serum concentration in cattle treated with NB was ≥2.2 mol/L and remained higher than that of cattle treated with a second CB bolus and that of the C cattle. These data thus support the hypothesis that combining glycosides of 1,25-$(OH)_2D_3$ with calcium salts can lead to prolonged increases in serum tCa concentrations. One caveat to this suggestion is that differences in the calcium salts in the two boluses (calcium chloride, calcium acetate and calcium lactate for NB and calcium chloride and calcium sulphate for CB) could be responsible for the prolonged increase in serum calcium concentrations seen in NB cows. However, as for CB, the recommendation for most commercial boluses, including those containing calcium lactate or acetate, is that treatment be repeated 12 h later (Verhoef et al., 2021)

so there is no evidence that these salts, on their own, will result in effects on serum calcium concentrations that persist for over 72 h. Furthermore, CB contains calcium sulphate, which has the slowest absorption and thus maintains calcemia for the longest period of any soluble calcium salts (Mann et al., 2019), so on calcium salt constituents alone, CB cows would be expected to have greater persistence in calcium response than NB cows.

Another potential cause of the greater persistence in tCa concentrations after treatment with NB is that all NB cows were initially given 2 boluses containing a total of 80g of calcium, while CB cows were given only 1 bolus (43 g of calcium), which was then repeated 12 h later. Treatment simultaneously with two calcium chloride boluses has been shown to increase the persistence of the elevation in tCa concentration to at least 24 h (Verhoef et al., 2021). However, the data presented by Verhoef et al. (2021) suggest that there was a relatively consistent decrease in Ca concentration between 1 h and 24 h post treatment in double bolus-treated cows, so that at 24 h, double bolus-treated cows had a lower serum tCa concentration than at 1 h (estimated mean difference 0.13 (95% CI -0.26 to 0.018) mmol/L). In contrast, in this study, the serum tCa concentration of NB cows at 24 h was similar to that at 1 h and continued to increase thereafter (see Figure 2). We thus believe that consistent with the results from solanum glycoside-only boluses (e.g. Meyer-Binzegger et al., 2022) that treatment with NB does lead to prolonged increases in serum calcium conc compared to tmt with conventional calcium boluses.

Our data also suggest that, in pasture-based cattle, the response to NB may be better than the response to the recommended two boluses of CB, with the second given 12 h after the first. In both this study and in Roberts et al. (2019), 24 h after the first bolus, serum tCa concentration in CB-treated cows was similar to that in C cows, whereas in this study, serum tCa in NB-treated cattle 24 h after treatment was clearly higher than in C cattle and CB-treated cows.

The impact of the two treatments on serum phosphorus concentration mirrored their effect on serum tCa concentration as in the first 12–14 h after treatment, our data were compatible with no difference in serum P concentration between NB and CB cows, while from 24–72 h serum P concentrations were consistently higher in NB cows and our data were not compatible with the two treatments being equivalent in regards to serum P concentration. These data support the suggestion that NB has significant benefits on Ca/P metabolism beyond simply increasing tCa supply, as we found no evidence that treatment with CB resulted in a meaningful effect on serum phosphorus concentration, a finding which is consistent with the lack of impact on serum phosphorus concentration of treatment with two boluses containing 45 g of calcium (from a mix of calcium chloride, propionate and formate) 24 h apart (Jahani-Moghadam et al., 2018), despite the treatment clearly increasing serum tCa concentration.

Both bolus treatments had a clear negative impact on serum magnesium concentration, with CB cows having lower mean serum magnesium than C cows for 6 to 36 h after treatment, and NB cows having lower mean serum magnesium than C cows for 6 to 72 h after treatment (Figure 3). This effect was unexpected as previous studies of postpartum treatment with calcium boluses have reported no effect of treatment on serum magnesium [e.g. Jahani-Moghadam et al. (2018)] and Melendez et al. (2021), even when they have been combined with prepartum cholecalciferol supplementation (Hajikolaei et al., 2021). Furthermore, the effect was most marked in the NB cows, even though Shock et al. (2019) found that adding cholecalciferol to a bolus containing calcium salts did not influence serum magnesium concentration, and Meyer-Binzegger et al. (2022) found no effect of administering a bolus containing *S. glycophyllum* extract three to four days on subsequent serum magnesium concentration either pre-or postpartum. Further research is required to establish whether the change in serum magnesium identified in this study is a clinically important effect or an unimportant consequence of improving calcium metabolism.

Breed composition was described at the herd level, but we did not record individual cow breed at enrolment or block treatment group allocation by breed. Both herds were predominantly Friesian or Friesian-cross Jersey with few Jersey cows. This study was thus not designed to assess the effect of breed on the response to the new bolus. Subsequent studies should address the effect of breed on the response.

Hypocalcemia is important because of its impact on cow health and on subsequent productivity. This study evaluated the impact of treatment with a new bolus on the mineral status, particularly serum tCa, of postpartum pasture-based cattle. As such, it was not designed to determine the impact of the new bolus on clinical or subclinical hypocalcemia. Nevertheless, we believe that the increase in serum tCa seen after treatment with NB in this study is a potentially biologically relevant increase. Our mean tCa in all three groups prior to bolusing was equivalent to those included by Valldecabres et al. (2018) as "subclinically hypocalcemic", and the response over time of tCa in the C and the conventional bolus in the current study was similar to the responses reported by Valldecabres et al. (2018). However, despite starting out as "subclinically hypocalcemic", the response over time to NB more closely mirrored the change in tCa reported by Valldecabres et al. (2018) in untreated cows that were normocalcemic at calving (mean tCa of 2.3 mmol/L), i.e. treatment of hypocalcemic cows with NB resulted in tCa concentrations that were equivalent to normocalcemic cows that did not need calcium supplementation. Nonetheless, this does not necessarily mean that treatment with NB will necessarily reduce the impact of hypocalcemia (especially subclinical) on cow health and productivity, as the effect of postpartum oral calcium supplementation is conditional on cow-level factors (Valldecabres and Silva-del-Río, 2021). This caveat applies, especially because recent research has demonstrated that calcium concentrations lower than standard thresholds (in the first two days after calving) for determining hypocalcemia can actually be associated with increased productivity (Hernandez and McArt, 2023). This has led to the concept of dyscalcemia, where cows are identified as being subclinically hypocalcaemic based on having a low blood calcium concentration at 4 days in milk, irrespective of their previous calcium concentration. It is thus plausible that administration of exogenous calcium, although beneficial for dyscalcemic cows, might impair the health and production of transiently hypocalcemic cows (Hernandez and McArt, 2023). Thus, further research is required to establish the effectiveness of this new bolus as a means of preventing clinical hypocalcemia and dyscalcemia. Nevertheless, this study has shown that this new bolus is at least as effective at rapidly increasing serum tCa concentration after treatment as a commercial bolus, which is authorized in New Zealand for the prevention and treatment of subclinical hypocalcemia and then maintains elevated tCa for a much longer period.

5 Conclusion

Compared to the C group and to cattle treated with a standard calcium bolus, cattle treated with a new bolus containing glycosides of 1,25-$(OH)_2D_3$ had elevated serum tCa and phosphorus concentrations. These effects were still present 72 h after treatment. Neither calcium bolus produced a biologically important change in serum magnesium over the first 72 h after administration, although both were associated with a transient decrease in magnesium concentration in the first 24 h. As such, this new bolus is likely to be beneficial for pasture-based farms where hypocalcemia (either clinical or subclinical) is an ongoing problem.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by AgResearch Ethics Committee New Zealand. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EC: Investigation, Writing – original draft, Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Project administration. GC: Data curation, Writing – original draft, Validation, Conceptualization, Formal Analysis, Writing – review & editing, Investigation, Methodology, Visualization.

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Conflict of interest

EC and GC were employed by company EpiVets Ltd.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2026.1799456/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Number of cows enrolled on each day in a study of the effect of calcium boluses on dairy cows (n=129) on two commercial New Zealand dairy farms. CB = commercially available bolus, NB = New Bolus, C = Control).

SUPPLEMENTARY TABLE 1
Reasons for missing blood samples in a study of the effect of calcium boluses administered within 12 hours of parturition on serum calcium, magnesium, and phosphate concentrations of dairy cows for 72 hours after administration (n=129) on two commercial New Zealand dairy farms. C, Control; CB, commercially available bolus; NB, New Bolus.

References

Cuttance, E. L., Mason, W. A., Laven, R. A., Denholm, K. S., and Yang, D. (2018). 'Calf and colostrum management practices on New Zealand dairy farms and their associations with concentrations of total protein in calf serum'. *New Z. Veterinary J.* 66, 126–131. doi: 10.1080/00480169.2018.1431159

DeGaris, P. J., and Lean, I. J. (2008). 'Milk fever in dairy cows: A review of pathophysiology and control principles'. *Veterinary J.* 176, 58–69. doi: 10.1016/j.tvjl.2007.12.029

Glosson, K. M., Zhang, X., Zanzalari, K. P., Bascom, S. S., Rowson, A. D., Wang, Z., et al. (2023). 'Negative dietary cation-anion difference and amount of calcium in prepartum diets: Effects on urine and serum minerals'. *JDS Commun.* 4, 449–453. doi: 10.3168/jdsc.2023-0391

Goff, J. P. (2008). 'The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows'. *Veterinary J.* 176, 50–57. doi: 10.1016/j.tvjl.2007.12.020

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

Hajikolaei, M. R. H., Nouri, M., Amirabadi, S. H., Shariari, A., and Constable, P. D. (2021). 'Effect of antepartum vitamin D3 (cholecalciferol) and postpartum oral calcium administration on serum total calcium concentration in Holstein cows fed an acidogenic diet in late gestation'. *Res. veterinary Sci.* 136, 239–246. doi: 10.1016/j.rvsc.2021.02.017

Hernandez, L. L., and McArt, J. A. A. (2023). 'Perspective: Transient postparturient hypocalcemia—A lactation-induced phenomenon of high-producing dairy cows'. *J. Dairy Sci.* 106, 8177–8180. doi: 10.3168/jds.2023-23355

Horan, L., Patton, J., McAloon, C. G., García-Muñoz, Á, Regan, Á, Mee, J. F., et al. (2024). 'Transition cow health and management in pasture-based dairy herds: A farmers' survey'. *PloS One* 19, e0314987. doi: 10.1371/journal.pone.0314987

Horst, R. L., Goff, J. P., Gill, S., Dallorso, M. E., and Pawlak, E. (2003). 'Using Solanum glaucophyllum as a source of 1, 25-dihydroxyvitamin D to prevent hypocalcemia in dairy cows'. *Acta veterinaria Scandinavica* 44, P67. doi: 10.1186/1751-0147-44-S1-P67

Husband, J. (2020). 'An update on hypocalcaemia control'. *Livestock* 25, 125–128. doi: 10.12968/live.2020.25.3.125

Jahani-Moghadam, M., Chashnidel, Y., Teimouri-Yansari, A., Mahjoubi, E., and Dirandeh, E. (2018). 'Effect of oral calcium bolus administration on milk production, concentrations of minerals and metabolites in serum, early-lactation health status, and reproductive performance of Holstein dairy cows'. *New Z. Veterinary J.* 66, 132–137. doi: 10.1080/00480169.2018.1432427

Kerwin, A. L., Ryan, C. M., Leno, B. M., Jakobsen, M., Theilgaard, P., Barbano, D. M., et al. (2019). 'Effects of feeding synthetic zeolite A during the prepartum period on serum mineral concentration, oxidant status, and performance of multiparous Holstein cows'. *J. Dairy Sci.* 102, 5191–5207. doi: 10.3168/jds.2019-16272

Lenth, R. V. (2024). Estimated Marginal Means, aka Least-Squares Means. (R package version 1.10.4). Available online at: https://CRAN.R-project.org/package=emmeans

Liang, D., Arnold, L. M., Stowe, C. J., Harmon, R. J., and Bewley, J. M. (2017). 'Estimating US dairy clinical disease costs with a stochastic simulation model'. *J. Dairy Sci.* 100, 1472–1486. doi: 10.3168/jds.2016-11565

Mann, S., McArt, J., and Abuelo, A. (2019). Production-related metabolic disorders of cattle: ketosis, milk fever and grass staggers. *In Pract.* 41, 205–219. doi: 10.1136/inp.l3041

McArt, J. A. A., and Oetzel, G. R. (2023). 'Considerations in the diagnosis and treatment of early lactation calcium disturbances'. *Veterinary Clinics: Food Anim. Pract.* 39, 241–259. doi: 10.1016/j.cvfa.2023.02.009

McNeill, D. M., Roche, J. R., McLachlan, B. P., and Stockdale, C. R. (2002). Nutritional strategies for the prevention of hypocalcaemia at calving for cows in pasture-based systems. *Aus. J. Agri. Res.* 53, 755–770. doi: 10.1071/AR01100

Mekonnen, S. A., Alelgn, Z., Saudik, S., Molla, W., Fentie, T., and Jemberu, W. T. (2022). Reduced milk production, economic losses, and risk factors associated to subclinical hypocalcemia in holstein friesian × Zebu crossbreed cows in North-West Ethiopia. *Front. Veterinary Sci.* 9. doi: 10.3389/fvets.2022.771889

Melendez, P., Roeschmann, C., Arevalo, A., and Moller, J. (2021). 'The effect of oral calcium boluses at parturition on blood metabolites and milk yield in grazing Holstein cattle'. *Livestock Sci.* 248, 104510. doi: 10.1016/j.livsci.2021.104510

Meyer-Binzegger, M., Ollagnier, C., Eggerschwiler, L., Bühler, K., Meylan, M., and Schlegel, P. (2022). 'Potential of a rumen bolus containing 1, 25-dihydroxyvitamin D3 glycosides for the prevention of hypocalcaemia in primiparous and multiparous dairy cows'. *Animal* 16, 100414. doi: 10.1016/j.animal.2021.100414

Parkinson, T. J., Vermunt, J. J., and Malmo, J. (2010). *Diseases of Cattle in Australasia* (Wellington, New Zealand: New Zealand Veterinary Association Foundation for Continuing Education).

Pinheiro, J. C., and Bates, D. M. (2000). Mixed-effect models in S and S-plus. (New York, NY: Springer New York). doi: 10.1007/0-387-22747-4_8

R Core Team. (2023). R: A Language and Environment for Statistical Computing. 4.3.2 ed. Vienna, Austria: R Foundation for Statistical Computing.

Roberts, K. I., Bennison, J., and McDougall, S. (2019). 'Effect of treatment with oral Ca boluses following calving on concentrations of Ca in serum in pasture-based dairy cows'. *New Z. Veterinary J.* 67, 20–26. doi: 10.1080/00480169.2018.1520654

Roberts, K. I., and McDougall, S. (2019). 'Risk factors for subclinical hypocalcaemia, and associations between subclinical hypocalcaemia and reproductive performance, in pasture-based dairy herds in New Zealand'. *New Z. Veterinary J.* 67, 12–19. doi: 10.1080/00480169.2018.1527732

Roche, J. (2023). 'Transition management in grazing systems: Pragmatism before precision'. *Veterinary Clinics: Food Anim. Pract.* 39, 325–336. doi: 10.1016/j.cvfa.2023.02.005

Roche, J. R., Dillon, P. G., Stockdale, C. R., Baumgard, L. H., and VanBaale, M. J. (2004). 'Relationships among international body condition scoring systems'. *J. Dairy Sci.* 87, 3076–3079. doi: 10.3168/jds.S0022-0302(04)73441-4

Sampson, J. D., Spain, J. N., Jones, C., and Carstensen, L. (2009). 'Effects of calcium chloride and calcium sulfate in an oral bolus given as a supplement to postpartum dairy cows'. *Veterinary therapeutics: Res. Appl. veterinary Med.* 10, 131–139. Available online at: https://europepmc.org/article/med/20037967

Serrenho, R. C., Bruinjé, T. C., Morrison, E. I., DeVries, T. J., Duffield, T. F., and LeBlanc, S. J. (2021). 'Controlled trial of the effect of negative dietary cation-anion difference on postpartum health of dairy cows'. *J. Dairy Sci.* 104, 6929–6943. doi: 10.3168/jds.2020-19389

Shock, D. A., Roche, S. M., Genore, R., and Olson, M. E. (2019). A pilot study to evaluate the effect of a novel calcium and vitamin D-containing oral bolus on serum calcium levels in Holstein dairy cows following parturition. *Veterinary Medicine: Res. Rep.* 10, 151–158. doi: 10.2147/VMRR.S219740

Valldecabres, A., Pires, J. A. A., and Silva-Del-Río, N. (2018). 'Effect of prophylactic oral calcium supplementation on postpartum mineral status and markers of energy balance of multiparous Jersey cows'. *J. Dairy Sci.* 101, 4460–4472. doi: 10.3168/jds.2017-12917

Valldecabres, A., and Silva-del-Río, N. (2021). 'Effects of postpartum oral calcium supplementation on milk yield, milk composition, and reproduction in multiparous Jersey and Jersey× Holstein crossbreed cows'. *J. Dairy Sci.* 104, 795–805. doi: 10.3168/jds.2020-19079

Verhoef, W., Zuidhof, S., Ralston, B., Ross, J. A., and Olson, M. (2021). Dissolution rates of calcium boluses and their effects on serum calcium in dairy cattle. *Veterinary Medicine: Res. Rep.* 12, 23–32. doi: 10.2147/VMRR.S293128

Vieira-Neto, A., Lean, I. J., and Santos, J. E. P. (2024). 'Periparturient mineral metabolism: implications to health and productivity'. *Animals* 14, 1232. doi: 10.3390/ani14081232