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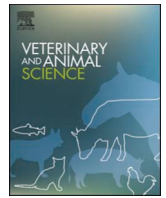
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# Plasma ionized calcium and magnesium concentrations and prevalence of subclinical hypocalcemia and hypomagnesemia in postpartum grazing Holstein cows from southern Chile

Pedro Melendez <sup>a,\*</sup>, Francisca Lopez <sup>b</sup>, Jorge Lama <sup>c</sup>, Bernardita Leon <sup>c</sup>, Pablo Pinedo <sup>d</sup>

<sup>a</sup> School of Veterinary Medicine, Texas Tech University, Amarillo, TX, United States

<sup>b</sup> Universidad Austral de Chile, Valdivia, Chile

<sup>c</sup> Cooprinsem, Osorno, Chile

<sup>d</sup> Colorado State University, Fort Collins, Colorado, United States

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## ABSTRACT

The objective was to determine the plasma concentrations of ionized Ca (iCa) and Mg (iMg) and to establish the prevalence of subclinical hypocalcemia (SCHC) and hypomagnesemia (SCHM) in dairy cows at calving (within 6 hours of parturition) and at 7 days postpartum (pp) in Chilean grazing herds with spring parturitions. Plasma iCa and iMg were assessed using a clinical analyzer. A total of 113 and 175 cows in 18 herds selected at random were sampled at calving and at 7 days pp, respectively. From these 18 herds, 11 herds provided reliable records of lactation number and 8 cows per herd were scored for body condition and sampled at calving and then at 7 days pp. Ionized Ca concentrations for the 18 herds were  $0.99 \pm 0.16$  mmol/L (calving) and  $1.01 \pm 0.13$  mmol/L [7 d pp ( $P > 0.05$ )]. Ionized Mg concentrations were  $0.58 \pm 0.12$  mmol/L and  $0.51 \pm 0.09$  mmol/L ( $P \leq 0.05$ ). For the 11 herds, iCa concentrations at calving were 1.06 mmol/L (lactation 1), 1.02 mmol/L (lactation 2) and 0.89 mmol/L (lactation  $\geq 3$ ), while iMg concentrations were 0.63 mmol/L, 0.60 mmol/L, and 0.61 mmol/L, respectively. Herd prevalence for SCHC (iCa  $< 1.0$  mmol/L) at calving was 64.8%. Prevalence by parity was 40%, 54.5% and 86.7% for lactations 1, 2 and  $\geq 3$ , respectively. Herd prevalence of SCHC on day 7 pp was 30.1%. For SCHM (iMg  $< 0.52$  mmol/L) prevalence was 21.6% and 48.9% at calving and at 7 days pp, respectively.

## 1. Introduction

Hypocalcemia is a metabolic disease of dairy cattle that occurs around parturition with both a clinical (milk fever) and subclinical presentation. Milk fever is characterized by muscle weakness, paresis and sternal recumbency that occurs mainly during the first 48 hours postpartum (pp) (Goff, 2014). Subclinical hypocalcemia (SCHC) occurs when total plasma calcium (tCa) concentration falls below 2.15 mmol/L (8.6 mg/dl), which is also considered a risk factor for other periparturient diseases, decreased milk yield, impaired fertility, and increased culling (Goff, 2014; Melendez and Risco, 2016; Neves et al., 2017).

Ionized Ca (iCa) is the biologically active form of the mineral, with concentrations tightly regulated ( $\sim 1$  mM) by hormones such as PTH and vitamin D3, receptors, and enzymes that detect the fluctuations of iCa instead of total Ca (tCa) and control physiological functions, such as

muscle contraction (Papadopoulou et al., 2021). Consequently, the evaluation of iCa is considered a better indicator of calcemia in animals and humans. Moreover, changes in blood pH, concentration of other minerals and plasma proteins alter the ionized state of Ca, without affecting tCa concentrations (Ott et al., 2021; Papadopoulou et al., 2021; Yu and Sharma 2022). Nevertheless, most studies dealing with hypocalcemia in dairy cattle have evaluated tCa concentrations, due to the complexity of blood sample processing and higher cost of laboratory analysis for iCa.

Although the incidence of milk fever has been reduced over time (5.9% in 1996 to 2.5% in 2014) (USDA, 2014), SCHC is still a concern, as 50% to 80% of dairy cows may be affected at parturition (Reinhardt et al., 2011; Caixeta et al., 2015; Tsiamadis et al., 2016). In addition, new evidence has shown that SCHC still can be frequent beyond 2 days pp (McArt and Neves 2020), with a prevalence of 18.6% at 7 to 8 days pp in confined dairy cattle (Tsiamadis et al., 2021).

\* Corresponding author at: Texas Tech University, 4703 Sussex Drive, Columbia, MO 65203, United States.

E-mail address: [pedro.melendez@ttu.edu](mailto:pedro.melendez@ttu.edu) (P. Melendez).

Chilean milk production systems are characterized by grazing herds located in the southern part of the country (Chilean Dairy Consortium, 2021). Many herds have introduced US, European, and New Zealand Holstein genetics with productions ranging from 5,500 to 8,500 kg per lactation. In this scenario, metabolic diseases originated from energy and mineral imbalances associated with pasture-based systems become a real concern (Grace and Knowles, 2012). Indeed, hypomagnesemia, in particular its subclinical form (SCHM), is another important metabolic disease affecting grazing cattle; however, little is known about ionized Mg (iMg) concentrations in blood which might be also considered a better predictor of Mg status in dairy cows (Goff, 2014). Unfortunately, the determination of iCa and iMg is not a routine strategy and has been barely reported in south American grazing dairy herds. Therefore, assessing prevalence of SCHC and SCHM at calving and 1-week pp by measuring iCa and iMg plasma concentrations would be a more accurate and useful information to establish prevention strategies for these two very important metabolic diseases affecting grazing dairy cattle. In consequence, the objective of this study was to determine the concentrations of iCa and iMg in plasma at calving and at 7 days pp and the prevalence of SCHC and SCHM in grazing Holstein cows with spring parturition in southern Chile.

## 2. Materials and methods

### 2.1. Farms and geographical description

The study was carried out in southern Chile, covering an area of 20,544 km<sup>2</sup> (214 km long, 96 km wide; -39.82 S, -73.23 W; -40.12 S, -72.38 W; -41.39 S, -73.46 W; -41.43 S, -72.94 W). The average temperatures during the autumn and spring are 7°C and 10°C, respectively. The average precipitation during autumn-winter (March to August) is 850 mm, and in spring-summer (September to February) is 150 mm (Chilean Weather Center, 2022).

This region has a dairy cow population of 250,000 heads with 1,000 herds with more than 100 lactating cows, which represents > 75% of the total milk production of southern Chile. The predominant breed is Black Friesian, Holstein, and the cross between these 2 breeds (Chilean Dairy Consortium, 2021).

Farms milk twice a day and manage the grazing cows on ryegrass pastures (*Lolium perenne*) offering a variable amount of dry matter (DM) depending on the time of the year. Ninety percent of the dairies have bi-seasonal parturitions, with 35% and 65 % of the cows calving during the fall and spring, respectively (Chilean Dairy Consortium, 2021). In autumn-winter, grazing provided about 5 kg DM, while during spring the pasture offers between 12 to 18 kg of DM. Therefore, the difference is complemented as partial mixed ration with grass silage, grass hay, corn silage and concentrate (Demagnet et al., 2022).

The reproductive season extends for 12 weeks, starting at 60 days in milk (DIM). At 7 months of gestation cows are dried off and moved to a prepartum group 21 to 28 days before expected parturition. During this period, cows have minimum access to pasture and fed a total mixed ration with anionic salts or low calcium content to prevent clinical hypocalcemia. Cows deliver in the prepartum lot or in a maternity pen. The calf is immediately separated from the dam and the cow is monitored for the first 24 hours for milk fever and retained fetal membranes which are treated according to the protocols established by the farm veterinarian (Chilean Dairy Consortium, 2021).

### 2.2. Study design

The study was completed under the animal welfare protocol established by the Chilean Dairy Consortium (Chilean Dairy Consortium, 2021).

Only dairies with more than 100 milking cows were eligible for the study, as they account for more than 70% of the southern dairy cattle population and have more homogeneous and accurate record keeping

systems and management. To determine the required sample size, two-step process calculations were carried out using the software SAS 9.4 (2017). The first step consisted of randomly selecting a representative number of farms from the total of 1,000 dairies. The assumptions were 95% confidence, 80% power, and 99% of the farms (with a 6% margin of error) having at least one case of SCHC at calving based on the epidemiological survey entitled "Dairy 2014 Health and Management Practices on U.S. Dairy Operations, 2014" (USDA, 2014), using a cut-off concentration value of iCa  $\leq$  1.0 mmol/L (Chamberlin et al., 2013; Yilmaz and Karapinar, 2019). A sample size of 11 dairies was calculated. The total of 1,000 dairies were listed numerically in an excel spreadsheet. Using the RAND, INDEX, and RANK.EQ functions, 20 farms out of the 1,000 farms, without duplicates were randomly selected. Farms were contacted by phone to confirm their participation in the study. From the random selection of 20 farms, 18 agreed to participate in the study and 11 farms out of the 18 were able to provide accurate information about lactation number and allowed for collection of a second sample at 7 days pp in the same cows that were sampled at calving.

A second step for sample size calculation was completed to obtain the number of cows to be sampled within herds. For this purpose, the assumptions were 95% of confidence, 80% of power and an estimation of 65% (range 50-80%) of the cows at calving experiencing SCHC based on the studies of Reinhardt et al. (2011), Caixeta et al. (2015), and Tsiamadis et al. (2016). As a result, a sample size of 88 cows from an estimated total of 126,750 dairy cows with spring parturitions was calculated. A sample size calculation for SCHC at 7 days pp was not conducted.

#### 2.2.1. Sampling and laboratory analysis

Blood samples were collected between July and November 2019 (middle of winter to late spring) by one of the authors. The protocol considered 18 herds with 113 and 175 cows sampled at calving and at 7 days pp, respectively, to calculate raw herd-level and raw cow-level prevalence. Then, 11 out of the 18 herds that notified the occurrence of calving and recorded consistently and provided the information about parity number were analyzed as a cluster. This cluster agreed to collect a second sample at 7 days pp on the same cows sampled at parturition. Body condition score at calving was obtained by the investigator collecting the blood samples.

Cows were first sampled within the first 6 hours post calving. Blood samples were taken from the blood plexus of the tail in a lithium heparin tube for subsequent plasma collection. For the prevention of clinical hypocalcemia, 9 herds out of the 18 farms fed anionic diets during the prepartum period. The rest fed a low calcium diet (0.4% DM).

Immediately after collection, blood samples were placed in a plastic cooler with ice packs and transported within 2 hours of collection to an institutional laboratory (Cooprinsem, Chile), processed, and analyzed to determine the concentration of iCa and iMg. For this purpose, the Stat Profile® pHOx® Ultra clinical analyzer (Nova Biomedical Corporation, Waltham, MA 02454-9141 USA) was used.

### 2.3. Statistical analysis

The statistical analysis from the total 18 herds consisted of a descriptive evaluation of the data, reporting the mean concentrations, SEM, and the range of iCa and iMg. In addition, the percentage of animals presenting SCHC (iCa  $\leq$  1.0 mmol/L) and SCHM (iMg  $\leq$  0.52 mmol/L) at calving and 7 days pp were calculated to obtain the raw herd-level prevalence and raw cow-level prevalence. From the cluster of 11 herds, an ANOVA was carried out to test the differences in iCa and iMg concentrations among different lactations (1, 2,  $\geq$ 3) within days (at calving and 7 days pp) and between days (calving vs. 7 days pp), considering also in the model BCS at parturition, herd, and feeding preventive strategy for milk fever (anionic or low calcium diets). Logistic regression analysis was used to compare the herd-level prevalence and cow-level prevalence of SCHC and SCHM (correcting for lactation

number, BCS at calving, herd and feeding preventive strategy for milk fever) within and between days (at calving and 7 days pp). The analyses were performed using the SAS 9.4 statistical package (SAS 2017). Significant differences were considered for  $P \leq 0.05$ . Trends were reported when the P values were between 0.05 and 0.1.

### 3. Results

A sampling protocol considering 18 herds and 113 cows at calving and 175 cows at 7 days pp was conducted. From this total, 88 cows from 11 herds had samples both at parturition and at 7 days pp with reliable information about BCS at calving and parity number. From these, 34.1% (n=30), 19.3% (n=17), and 46.6% (n=41) were from lactation 1, 2, and  $\geq 3$ , respectively.

Descriptive statistics for plasma iCa and iMg concentration from the 18 farms and the cluster of 11 farms with lactation number information is shown in Table 1. The concentrations of iCa were similar at calving between cows of lactation 1 and 2 ( $P > 0.05$ ), but lower in cows  $\geq 3$  lactations ( $P \leq 0.05$ ) while the concentrations of iCa at day 7 pp were similar among all lactations ( $P > 0.05$ ). When comparing the two sampling points, the concentration of iCa were similar in lactation 1 and 2 ( $P > 0.05$ ), but higher at day 7 pp in cows  $\geq 3$  lactations ( $P \leq 0.05$ ). Concentration of iMg, were lower at day 7 pp compared to calving in all lactations ( $P \leq 0.05$ ). Concentrations did not vary among lactations on the day of calving ( $P > 0.05$ ), but on day 7 pp cows  $\geq 3$  lactations had the lowest values ( $P \leq 0.05$ ). Feeding strategy to prevent milk fever (anionic compounds or low Ca diets) and herd effect were not significant variables in the model ( $P > 0.05$ ).

Herd and cow-level prevalence of SCHC (iCa  $\leq 1.0$  mmol/L) and SCHM (iMg  $\leq 0.52$  mmol/L) at calving and at 7 days pp are shown in Figs. 1 and 2. At calving, first lactation cows had a lower prevalence of SCHC than second lactation cows ( $P \leq 0.05$ ), and second lactation cows in turn had a lower prevalence of SCHC than cows  $\geq 3$  lactations ( $P \leq 0.05$ ). At day 7 pp 21.7%, 23.5% and 42.3% of the cows of first, second, and  $\geq 3$  lactations, respectively, still presented SCHC. In the case of SCHM (iMg  $\leq 0.52$  mmol/L), 21.6% of the cows were affected at calving

**Table 1**  
Ionized calcium and magnesium plasma concentrations (mmol/L) at calving and at 7 d postpartum (pp), total and by lactation, in grazing Holstein cows with spring parturitions in southern Chile.

Item	Ionized Ca (mmol/L)		Ionized Mg (mmol/L)	
	At calving	7 d pp	At calving	7 d pp
Total (18 herds, n=113 at calving; n=175 at 7 d pp)				
Mean	0.99	1.01	0.58 <sup>a</sup>	0.51 <sup>b</sup>
SEM	0.16	0.13	0.12	0.09
Range	0.44-1.24	0.54-1.27	0.19-1.11	0.25-0.84
By lactation (11 herds, n=88 at calving and at 7 d pp)				
1 (n=30)				
Mean	1.064 <sup>*</sup>	1.05	0.63 <sup>a</sup>	0.54 <sup>b, *</sup>
SEM	0.13	0.12	0.06	0.10
Range	0.77-1.19	0.71-1.20	0.55-0.73	0.39-0.84
2 (n=17)				
Mean	1.024 <sup>*</sup>	1.04	0.60 <sup>a</sup>	0.53 <sup>b, *</sup>
SEM	0.13	0.10	0.07	0.09
Range	0.81-1.24	0.84-1.22	0.51-0.74	0.38-0.69
3+ (n=41)				
Mean	0.89 <sup>a, **</sup>	1.01 <sup>b</sup>	0.61 <sup>a</sup>	0.50 <sup>b, **</sup>
SEM	0.17	0.16	0.12	0.10
Range	0.54-1.18	0.54-1.27	0.32-0.89	0.30-0.75

a, b: statistical differences ( $P \leq 0.05$ ) between days.  
\*, \*\*: statistical differences ( $P \leq 0.05$ ) within day among lactations.

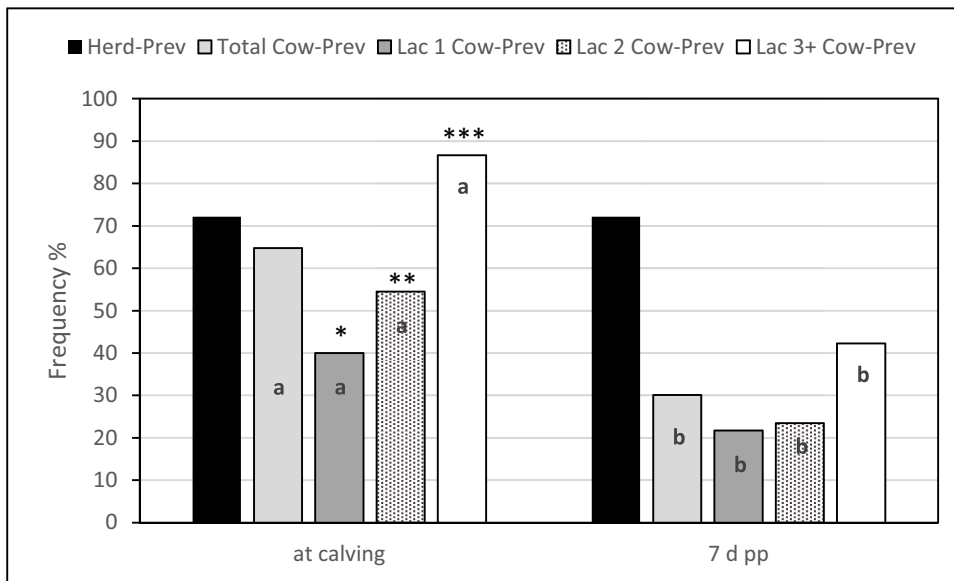
and this percentage increased to 48.9% at 7 days pp ( $P \leq 0.05$ ). In turn, SCHM was higher in cows  $\geq 3$  lactations ( $P \leq 0.05$ ), both on the day of calving and at 7 days pp. Overall, 72.2% of the 18 farms and 81.8% of the cluster farms (n=11) presented at least one cow with SCHC at parturition. In addition, from the cluster farms, 64.8% of the cows presented SCHC at calving, and 30.1% continued with SCHC at 7 days pp; therefore 34.7% of cows improved their iCa concentration from calving to 7 days pp.

### 4. Discussion

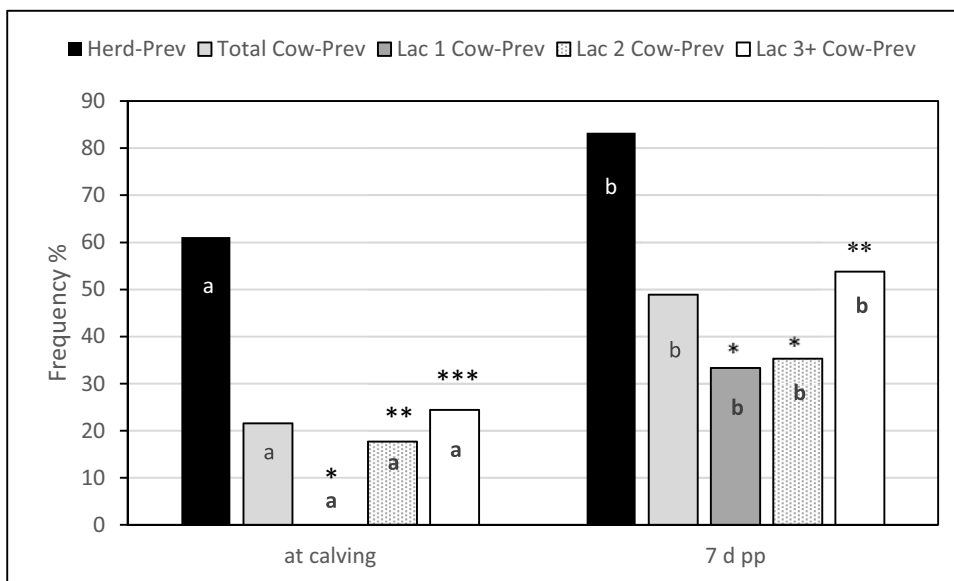
The objective of this study was to determine the concentration of iCa and iMg in grazing dairy cattle from southern Chile with spring parturitions, and to establish the herd-level and cow-level prevalence of SCHC and SCHM at parturition and at 7 days pp, based on the assessment of the ionized form of these two minerals. The use of iCa to characterize the Ca status of dairy cows is based on strong research evidence indicating that iCa is the biologically active form of the mineral, and fluctuations in iCa rather than tCa are detected by hormones, cell receptors, and enzymes that are involved in the tight regulation of calcemia (Papadopoulou et al., 2021). Consequently, the evaluation of iCa might be considered a better indicator of calcium status in dairy cattle. In fact, in a recent study (Silva et al., 2022) the supplementation of vitamin D<sub>3</sub> in prepartum dairy cows increased the iCa concentration during the postpartum period when compared with a control group, without affecting the tCa concentration. Furthermore, Ott et al. (2021) determined that the association between tCa and iCa was moderate ( $r^2 < 0.40$ ), and that the proportion of iCa over tCa varied according to days in lactation, being 48% on the day of parturition and 43% at 7 days pp. Therefore, these authors recommend the evaluation of iCa in plasma as a more accurate predictor of blood Ca status in dairy cattle. In addition, in other species such as dogs (Lebastard et al., 2021) and humans (Papadopoulou et al., 2021; Yu and Sharma 2022), the evaluation of calcemia to establish proper treatment strategies in patients under hospital settings is consistently centered on iCa instead of tCa. Nevertheless, most studies dealing with hypocalcemia in dairy cattle report tCa concentrations, possibly due to the complexity of blood sample processing and the higher cost of laboratory analysis for iCa. For example, one of the factors that may alter the ionized concentration of Ca and Mg is the proper handling of samples. Stored samples up to 8 hours after collection did not affect plasma concentrations of iCa and iMg; therefore, collection of blood samples in lithium heparinized tubes and stored between 1 and 4°C for up to 8 hours after sampling is an acceptable method to obtain a diagnostic value of both minerals in their ionized form (Menta et al., 2020). The current study submitted the samples to the lab within 2 hours of collection.

Previous studies have measured iCa in experimental conditions and in confined dairy cattle (Szenci et al., 1994; Chamberlin et al., 2013), but few studies have reported iCa in dairy cattle under grazing conditions. Consequently, comparing our results with epidemiological studies using tCa instead of iCa is challenging. This is one of the drawbacks of the present investigation; however, the characterization of the calcemia in dairy cows using iCa is required to understand if the association between hypocalcemia and productive variables is comparable to that of tCa. Unfortunately, the lack of reliable records related to milk production, fertility, and incidence of other periparturient diseases from the dairies evaluated in the current investigation made unfeasible to conduct a receiving operating characteristic curve analysis to establish a cut-off value for iCa to categorize cows as SCHC as has been done in other studies with tCa.

When compared with other studies reporting the epidemiology of SCHC using tCa concentrations in plasma (Reinhardt et al., 2011; Neves et al., 2017), our study, using iCa as a biological marker, shows a similar pattern for SCHC at calving, where younger cows had a lower prevalence than older cows ( $\geq 3$  lactations). In the study of Neves et al. (2017), the prevalence of SCHC was 2%, 40%, and 66% for cows in their first,



**Fig. 1.** Herd-level and Cow-Level prevalence (%) total and by lactation of subclinical hypocalcaemia ( $iCa \leq 1.0$  mmol/L) at calving and 7 d postpartum (pp) in grazing Holstein cows with spring parturitions in southern Chile. a, b: statistical differences ( $P \leq 0.05$ ) between days within lactation. \*, \*\*, \*\*\*: statistical differences ( $P \leq 0.05$ ) within day among lactations.



**Fig. 2.** Herd-level and Cow-Level prevalence (%) total and by lactation of subclinical hypomagnesemia ( $iMg \leq 0.52$  mmol/L) at calving and 7 d postpartum (pp) in grazing Holstein cows with spring parturitions in southern Chile. a, b: statistical differences ( $P \leq 0.05$ ) between days within lactation. \*, \*\*, \*\*\*: statistical differences ( $P \leq 0.05$ ) within day among lactations.

second, and third or more lactations, respectively whereas in the study by Reinhardt et al. (2011), the prevalence of SCHC was 25%, 41%, and 50% for cows in the first, second, and third or more lactations, respectively. In the present study, the prevalence of SCHC was 40%, 54.5%, and 86.7% for cows in the first, second, and third or more lactations, respectively. Undoubtedly, the age dynamics are similar among the studies, but with a higher cow-level prevalence of SCHC in cows under grazing conditions using iCa as biomarker.

In another study carried out in New Zealand, also with grazing dairy cattle and spring parturitions (Roberts and McDougall, 2019), it was observed that the cow-level prevalence of SCHC was 52.3%, using a cut-off plasma tCa concentrations  $\leq 2.15$  mmol/L. In our study, the cow level prevalence was 64.8%, which is higher than that reported by the New Zealand study. In other New Zealand study (Roche et al., 2005), it was observed that tCa concentrations were not affected by the different degrees of DCAD, but iCa concentrations did. In this case, iCa concentrations ranged from 1.28 mmol/L in diets with a DCAD of +230 mEq/kg DM, to 1.19 mmol/L in diets with a DCAD of + 880 mEq/kg DM, using the formula  $[K+Na] - [Cl+S]$  (Goff, 2014). These concentrations

were 49.6% and 47.4% of the tCa concentrations, showing that, at similar tCa concentrations the proportion of iCa over tCa decreases while dietary DCAD increases.

In a study with grazing dairy cattle in Argentina (Umaña Sedó et al., 2018), tCa concentrations did not vary between the day of calving ( $2.40 \pm 0.05$  mmol/L) and 7 days pp ( $2.41 \pm 0.05$  mmol/L), as in our study, except in cows  $\geq 3$  lactations. It is noticed that tCa concentrations in the Argentine study were different between cows that calved in the fall ( $2.48 \pm 0.05$  mmol/L) and those that calved in the spring ( $2.24 \pm 0.05$  mmol/L). Furthermore, corroborating our findings, in a study also conducted in southern Chile, it was observed that plasma concentrations of tCa did not differ in second lactation cows between the day of calving and 7 days pp, but it increased significantly from calving to 7 days pp in cows  $\geq 3$  lactations. It was also reported that tCa concentrations were lower as the lactation number increased (Melendez et al., 2021).

At the herd level, our assumption was that 99% of grazing farms in southern Chile would have at least one cow with SCHC at parturition. This assumption was based on the epidemiological survey conducted by the National Animal Health Monitoring System (USDA, 2014) in which

at least 97% of surveyed farms had a case of clinical hypocalcemia. Our results showed that 72.2% (13/18) had at least one case of SCHC, which is slightly lower than our assumption. However, we must extrapolate this finding with caution, since it is valid only for cows calving in spring in dairy herds with more than 100 milking cows. The dynamic of hypocalcemia can be completely different in smaller herds, or in cows calving in fall, because the type of feed and pasture offered to cows delivering in fall is completely different than cows calving in spring (Wagemann et al., 2014).

Regarding iMg, it is important to mention that no studies have determined a cut-off value for iMg to categorize cattle as animals with subclinical or clinical hypomagnesemia. Consequently, our discussion is based on the assumption that plasma concentrations of iMg in dairy cows is 65% of their total form (Goff, 2014). Assuming this percentage, normal concentrations of iMg should be in the range of 0.52 to 0.65 mmol/L. In the case of clinical hypomagnesemia or grass tetany, tMg concentrations are < 1.2 mg/dL (0.50 mmol/L, equivalent to 0.33 mmol/L of iMg). Nevertheless, the lack of a cut-off value for iMg (ROC analysis) to define animals as SCHM, is one of the weaknesses of our study. Unfortunately, due to inconsistency among records from herds considered in the present investigation, running a ROC analysis was not feasible. Therefore, our discussion assumes that the cut-off concentration for iMg in plasma is 0.52 mmol/L (Goff, 2014) to categorize a cow as SCHM.

In our study, iMg concentrations did not differ among lactations on the day of calving but were lower at 7 days pp compared to calving within each parity number and were also lower in cows  $\geq 3$  lactations than lactation 1 and 2. In this sense, the percentage of cows with SCHM (iMg  $\leq 0.52$  mmol/L) was lower at calving than at 7 days pp and raised as the parity number increased. Although the genetic component of metabolic disorders associated with Ca and Mg should not be underestimated (Tsiamadis et al., 2016), the environmental component is the most important factor affecting these minerals. When Mg is low in feeds, or when absorption is impaired in the rumen, especially in the case of lush pastures, Mg concentrations decrease rapidly in blood (Goff, 2014; Goff, 2018; Kumssa et al., 2020). In our study, it is evident that cows subjected to intensive grazing after calving lowered the concentrations of iMg in the blood. This may have been due to the high potassium and nitrogen content of ryegrasses (*Lolium perenne*) during early spring in southern Chile (Sepulveda et al. 2011). In addition, the lush phenological state of spring pastures, resulting in high digestibility, may accelerate ruminal passage further reducing Mg absorption. This can also be exacerbated by the excessive application of K as a fertilizer, when recycling dairy manure, as the excess of potassium reduces the absorption of Mg at the level of the roots of the plant (Goff, 2014; Kumssa et al., 2020; Demanet et al., 2022). In southern Chile, an early estimation of SCHM in 1987 was around 12% (Wittwer et al., 1987). In another study conducted in southern Chile, it was observed that dairy cows managed on grazing had plasmatic concentrations of tMg of  $0.83 \pm 0.03$  mmol/L, equivalent to 0.53 mmol/L iMg, with a range of 0.47 to 0.60 mmol/L (Sepulveda et al., 2015). Assuming a normal distribution of serum concentrations of iMg, it is estimated that the percentage of cows with a concentration  $\leq 0.52$  mmol/L (SCHM) would be around 16%, which is slightly higher than the study of Wittwer et al. (1987). In our study, the percentage of SCHM was 21.6% at parturition, and close to 50% at 7 days pp. Undoubtedly, genetic merit of cows in our study is different than the cows from 20-30 years ago, and the management of pastures is much more intensive today, with varieties of "ryegrasses" much lower in fiber, much more digestible and much richer in potassium and nitrogen than 20-30 years ago (Dairy Chilean Consortium, 2021; Demanet et al., 2022).

## Conclusions

Using iCa and iMg as biological markers, the cow prevalence of SCHC in Holstein herds with more than 100 dairy cows managed under grazing

conditions with spring parturition in southern Chile was higher (64.8%) than expected and to those reported in cows managed in confinement. As in other studies, the prevalence was higher as the lactation number of the cows increased. The prevalence decreased at 7 days pp, presenting 30% of the cows with SCHC, which indicates that this metabolic disorder is still prevalent at 7 days pp. Subclinical hypomagnesemia was 21.6% in animals at calving, but increased to 48.9% at 7 days pp, with a higher presentation in cows with 3 or more lactations. These data suggest that both metabolic disorders are prevalent in dairy cattle in southern Chile and therefore preventive strategies should be reevaluated to reduce their presentation in cows with spring parturitions.

## Supplementary material

There is no supplementary data associated with this article.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests

Pedro Melendez reports was provided by Texas Tech University. Pedro Melendez reports a relationship with Texas Tech University that includes: employment

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