Animal and Veterinary Sciences
2024, Vol. 12, No. 6, pp. 154-160
https://doi.org/10.11648/j.avs.20241206.12

Research Article

Can Supplementation with the Cow Start Complete Bolus Result in Elevated Blood Calcium Status in a Group of At-Risk Dairy Cows During the First Four Days of Lactation

John Lawlor[1, *], Enda Neville[1], Alan Fahey[2]

[1]Anchor Life Science Ltd., Cork, Ireland

[2]School of Agriculture and Food Sciences, University College Dublin, Dublin, Ireland

Abstract

The prophylactic supplementation of freshly calved multiparous cows with oral calcium supplements (bolus, drink, drench, paste) has increased in popularity in recent years. Oral calcium supplements are generally only effective for 12 hours, yet the high-risk period for hypocalcaemia in freshly calved multiparous cows is 48 hours post-partum. Therefore, a second application 12 hours post-partum is suggested by manufacturers, yet due to labour shortages on dairy farms, it is very common for the second application to not be carried out. The objectives of this study were to ascertain if a the administration of a single dose of the sustained release bolus (Cow Start Complete, Anchor Life Science, Co. Cork, Ireland) given to the cow at calving, could offer the combined benefits of elevated calcium status over the first 48 hours post-partum from a labour efficient single dose given at calving and also to evaluate if this enhanced calcium status could have a positive effect on daily rumination time and milk production yields in the first three months of lactation. The two groups consisted of an un-supplemented control (CON) group and a Cow Start Complete (CSC) group in which cows were given a single bolus dose at the point of calving. The CON group experienced a clinical milk fever (Blood Ca <1.5mmol/L) incidence rate of 13.3% compared to 0% for the CSC group. Cows given the CSC treatment had significantly ($P<0.0001$) higher total blood calcium levels (2.14 mmol/L) from the point of calving (0h) to 4 days post-partum (96h) compared to CON (1.98mmol/L), with significant increases at 12h ($P<0.01$), 24h ($P<0.001$) and 36h ($P<0.01$) post-partum. Rumination time for CSC cows (471mins/day) was significantly ($P<0.05$) higher during the first 14 days post-partum when compared to CON (434mins/day) group. Milk production was also significantly ($P<0.01$) higher for CSC cows (+1.8kg/day) than in CON cows during the first 90 days of lactation. These findings demonstrate that the convenience of giving a single dose of CSC at calving, can be combined with desired outcomes in a range of key benefits in freshly calved multiparous cows.

Keywords

Sustained Release Calcium, Sub-clinical Hypocalcemia, Transition Cow, Milk Yield, Rumination Status

[*]Corresponding author: John.lawlor@anchorlifescience.com (John Lawlor)

Received: 17 October 2024; **Accepted:** 30 October 2024; **Published:** 26 November 2024

1. Introduction

In an effort to support colostrum and milk production at the onset of calving, cows can experience a sudden and significant increase in the demand for calcium (Ca). The challenge for dairy cows is that, in the days just before parturition dry matter intakes (DMI), and consequently dietary supply of Ca, can be reduced by 30%, yet at calving this coincides with an abrupt 3-fold increase in Ca demand [1]. The cows' natural homeostatic mechanism can take between 24 hours (intestinal Ca delivery) and 48 hours (skeletal Ca delivery) to become fully functional and increase Ca supply to the cow. The combination of a sudden increase in demand, coupled with a time lag in supply of Ca that can result in low blood Ca concentrations or hypocalcaemia in the cow in the first days post-partum [1, 2].

Total blood Ca concentrations of less than 1.5 mmol/L are considered to leave the cow at risk of clinical milk fever and it is reported that a 4 - 10% incidence rate is commonplace for multiparous cows [3-5], with an average cost of $300 per case [1]. The nadir for blood Ca is 12- 24 hours post-partum and this represents the highest risk period for clinical milk fever cases [1, 6, 7].

Blood Ca concentrations of between 1.5 and 2.0 mmol/L are considered to leave the cow at risk of Sub-Clinical Hypocalcaemia (SCH) [1]. Whilst the cost, at $125/case, is less than that of clinical milk fever, the prevalence is much higher, reported at over 50% in multiparous cows, which can mean than 80% of the cost of hypocalcaemia on dairy farms results from SCH [3].

Traditionally blood Ca status on the first day of lactation was viewed as the most appropriate indicator of the freshly calved cow's Ca status, however there is a growing body of research to show that blood Ca status on days two, three and four of lactation may be as important as the first day [8-11]. A research study by Martinez (2012) showed that a case of sub-clinical hypocalcaemia (>2.15 mmol/L) on any of the first four days in lactation resulted in a significant increase in the risk of metritis, an impaired immune response and inferior energy status during the critical first two weeks of lactation. A further study found a statistical trend between cows with SCH at 2 DIM and an increased risk of retained placenta [12]. Another study also found a correlation between increased risk of metritis, displaced abomasum or both, and blood Ca status on day two of lactation in second parity cows (≤1.97mmol/L cutoff) and day four of lactation in third parity or higher cows (≤2.20mmol/L cutoff) [13]. These studies highlight that Ca status beyond the first day in lactation can effect immune function and energy status.

More recent research studies from Cornell University have revealed that not all SCH cases are the same and found that there were three distinct sub-categories. In these studies, it was found that the level to which blood Ca dropped during the first day after calving was not so much a determining factor, but rather, it was her ability to recovery her Ca status from one DIM to four DIM that was found to be a more important indicator of cows' health status and productive performance during lactation. One study found that cows that had recovered their total blood Ca status to Normocalcaemic levels (≥2.2mmol/L) by four DIM when compared to sub-clinical levels (≤2.2mmol/L by four DIM) had fewer metritis cases (5% v. 14.4%), fewer cases of displaced abomasum (1.9% v 9.6%) and lower levels of herd removal (1.2% v. 9.6%) [10, 11]. The recovery in blood Ca levels was linked to higher post-partum dry matter intakes [11].

With such high costs associated with sub-clinical hypocalcaemia, the focus of most modern husbandry protocols has been on trying to better manage Ca status in freshly calved cows, to avoid blood Ca concentrations dropping to clinical or sub-clinical levels. As a means of overcoming the drop in dietary Ca supply, due to depressed dry matter intakes around calving, the practice of orally supplementing Ca to cows; via bolus, paste, drench or drink, has gained acceptance as a means of increasing the cow's dietary Ca supply, independent of the cows' dry matter intakes [14-19].

The challenge for farmers is that whilst cows can benefit from a supplemental supply of Ca for at least the first 24-48 hours post-partum, almost all oral Ca supplement products only elevate Ca for a 12-hour time period [20]. This scenario requires a second dose of oral Ca to be administered to the cow to ensure enhanced Ca status during the critical first 12-36 hours post-partum. It is this requirement for a second dose that is the biggest resource challenge for dairy farmers, who may not have the appropriate facilities or staffing levels for blanket administration of a second oral Ca supplement.

The objectives of this study were to determine if a single dose of Cow Start Complete would be sufficient to reduce the risk of hypocalcaemia in freshly calved, at-risk, multiparous cows by elevating the blood Ca status of the supplemented cows over the first 4 days of lactation. We hypothesised that this elevated blood Ca status would lead to higher levels of rumination and milk production in supplemented cows.

2. Materials and Methods

2.1. Study Population

The study was carried out over a 3-week period on an Irish dairy herd during the spring calving season of 2024. The herd had experienced excessive levels of clinical milk fever early in the current calving season and initiated a blood Ca analysis study to record the profile and incidence rate of hypocalcaemia in their farm. A prophylactic Ca supplementation protocol was also incorporated into the study, to measure the effect and profile of Ca status in orally supplemented cows, when compared to a cohort of un-supplemented cows on the farm. The herd consisted of 280 cows with an average 305d lactation yield of 6,632kg, produced from a predominantly

grass-based diet. Cows were required to have had a full-term pregnancy in their previous lactation with valid on-farm health records. Only second lactation and greater parity cows were eligible for the trail, and any cows that experienced a significant health issue at calving were excluded, such as clinical milk fever or dystocia, as well as lame cows or cows that were deemed to be too thin (<3.00 BCS) or too fat (>3.5 BCS). The cows were blocked by parity and previous lactation yield and randomly assigned to a control group (CON) or a Cow Start Complete (CSC) group.

Table 1. *Cow Age and Production Profile for Control (CON) and Cow Start Complete (CSC) groups.*

Treatment	CON	CSC
n	13	13
Age (average lactation number at calving)	3.2	3.4
Prev. Lact. Length (days)	292	287
Prev. Lact. 305d Yield (Kg)	6,381	6,464

2.2. Study Intervention

26 multiparous cows took part in the study, 13 cows were enrolled in the CON group which received no bolus after calving and 13 cows that were enrolled to the CSC group which received one oral Ca bolus dose on the point of calving. Each CSC dose contained 106g of Ca, 7g of rumen soluble magnesium and 80,000IU of Vitamin D3. 1,600mg of Vitamin E and 2.20mg of selenium are also supplied as an aid to the immune response.

A clinical milk fever case was determined to be any cow that had shown clear clinical signs of milk fever, such as: visibly unsteady on her feet, cold extremities, shivering or unable to rise and stand. There were no cases of clinical milk fever within the CSC group, with 2 cases of clinical milk fever reported in the CON group. These cows were successfully treated with an infusion of Ca borogluconate and subsequently removed from the study.

2.3. Sample Collection and Analysis

Blood Ca records were provided by the farm, from pre-existing farm health records from the Ca supplement intervention study that was carried out. Whole blood samples were taken from the coccygeal vein and immediately refrigerated at 4^{o}C on the farm. Serum was separated by centrifugation for 5 minutes at 12,000 rpm within 48 hours of the sample being taken, then labelled and frozen immediately. Samples were thawed at room temperature and analysed in batches using an IDEXX (IDEXX Laboratories Inc., One IDEXX Drive, Westbrook, Maine 04092, United States) Catalyst chemistry analyser. The samples were taken at 0h (average 15mins post-partum), 12h (average 12 hours), 24h (average 23 hours), 36h (average 35 hours), 48h (average 48 hours) and 96 hours (average 95 hours) relative to the treatment with time 0 denoting the point of calving before the first bolus was administered.

2.4. Daily Rumination Time Analysis

All cows on the trial were fitted with SenseHub collars (Allflex/MSD Animal Health Intelligence, Merck and Co Inc., Rahway, NJ, USA.) which monitor a range of cows movements and activity. The SenseHub collar records the total time per day a cow spends ruminating. These data are sent from the collar to the SenseHub Controller which then transfers this data to the SenseHub farm software programme.

2.5. Production Data Recording

All bolus administration, metabolic diseases and calving events were monitored and recorded by farm personnel. Milk yield was measured by the digital milk meters in the milking parlour for each cow for AM and PM milking and this was recorded by the Afi Milk software system. Daily milk volume data was obtained from this software system for each cow for the first 90 days in milk (DIM).

2.6. Statistical Analysis

The residuals of the data were assessed for normality using the UNIVARIATE procedure of SAS (v9.4 SAS Campus Drive, Cary, North Carolina). Residuals of Ca, rumination and milk production had a normal distribution and did not require a transformation. The data were analysed using the MIXED procedure of SAS (v9.4 SAS Campus Drive, Cary, North Carolina). When analysis the Ca data treatment (CSC or CON), time (0, 12, 24, 36, 48 and 96h post-partum), treatment × time, and lactation (2 or 3) were considered as fixed effects, with previous 305-d lactation and rumination time considered as possible covariates. Cow was considered as a random effect and time was used as a repeated measures with a compound symmetry (co)variance matrix as determined by the lowest Bayesian Information Criterion. Rumination and milk data was analysed in a similar manner except that time was 0 to 14 days post-partum and 5 to 90 days post-partum, respectively.

3. Results

3.1. Blood Calcium Profile 0 DIM-4 DIM

Figure 1 outlines the effect of treatment on total blood Ca concentration from the day of calving to four days post-partum. Over the trial period from 0h to 96h post-partum cows on the CSC treatment had higher blood Ca concentrations of 2.14mmol/L compared to the CON group at 1.98mmol/L (P<0.0001). There was no difference in blood Ca

levels between the CSC and CON groups at 0h post-partum, prior to treatment, (P = 0.72) and also at 48h (P = 0.65) and 96h (P = 0.65) post-partum. However, the CSC group demonstrated a significantly higher total blood Ca level than the CON group at 12 h (+0.22mmol/L, P < 0.01), at 24 h (+0.29mmol/L, P < 0.001) and 36 h post-partum (+0.23mmol/L, P < 0.01).

The CSC group maintained a blood Ca concentration above the normocalcaemic threshold in the first 48 h post-partum. However, by the first 12 h post-partum the blood Ca concentration of the CON group was below the sub-clinical threshold (Ca < 2.0mm/L) and did not recover to normo-calcaemic status until 48 h post-partum.

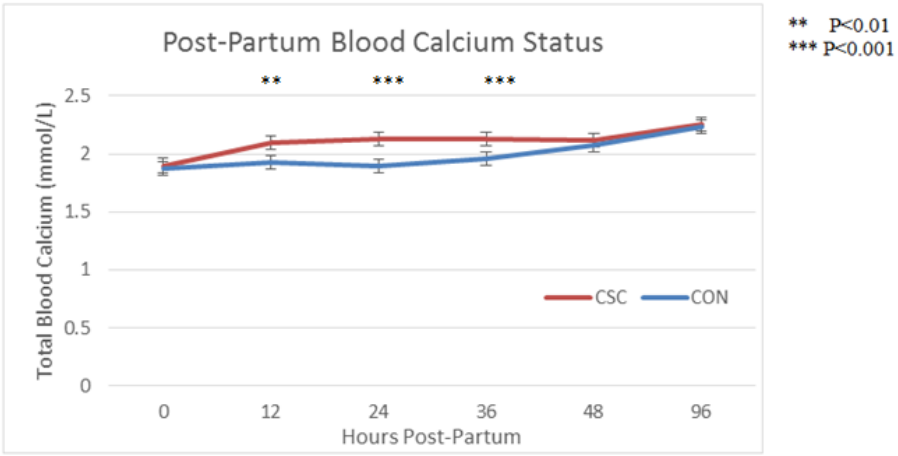

Figure 1. *Blood Calcium Results for Control (CON) and Cow Start Compete (CSC) treatments from 0 to 96 hours post-partum.*

3.2. Rumination Time

The effect of treatment on rumination time is shown in figure 2 which highlights the effect of treatment on rumination time from day of calving to 14 days post-partum. Cows on the CSC group spent a greater amount of time ruminating at 474mins/day compared to the CON group at 434mins/day (P < 0.05).

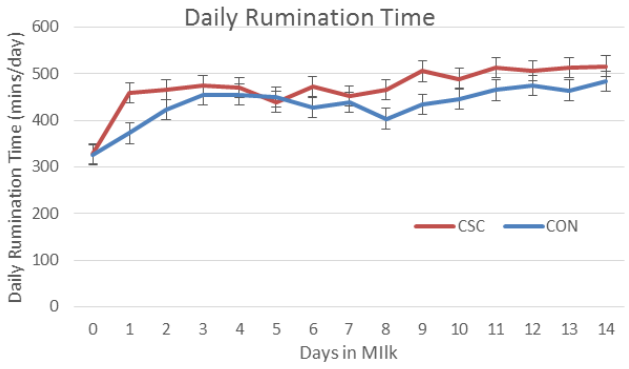

Figure 2. *Daily Rumination Time Results for Control (CON) and Cow Start Complete (CSC) groups from 0 to 14 days in milk.*

3.3. Milk Production

Milk production data was collected from each milking for each cow over the first 12 weeks of lactation. A significant increase in milk production of +1.8kg/day (P < 0.01) was recorded in the CSC group (30.7kg/day) compared to CON (28.9kg/day).

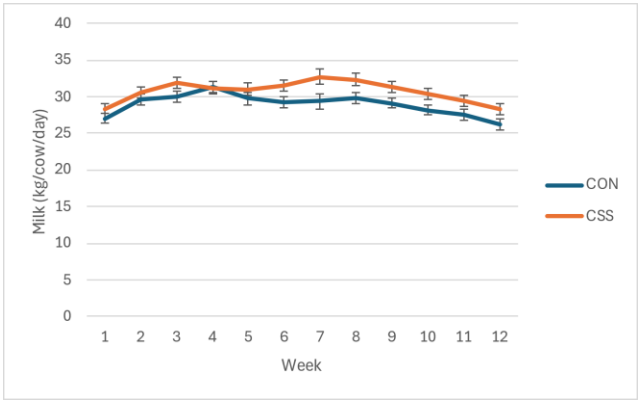

Figure 3. *Milk Production Results for Control (CON) and Cow Start Complete (CSC) groups for the first 12 weeks of lactation.*

4. Discussion

Post-partum oral Ca supplementation of dairy cows has grown in popularity in recent years, as a growing body of research demonstrates the benefit of supporting the Ca status of freshly calved cows. Whilst studies have shown an improved Ca status in orally supplemented freshly calved cows, the effect usually does not last beyond an initial 12-hour period. As cows are at greatest risk of hypo-calcaemia from 12-24 hours post-partum, a

second product application is required in order to prolong the effect of Ca support beyond the initial 12-hour period and is an established protocol recommended by most manufacturers. It is the labour intensive nature of multiple applications of oral Ca products that results in many cows not being supplemented a second time, especially against a backdrop of labour shortages seen on many dairy farms. To the authors knowledge, no studies have yet shown a significantly sustained increase in the Ca status of freshly calved cows for 48 hours post-partum, from a single dose given at calving.

The results of this trial demonstrate that the single application of a dose of Cow Start Complete at calving can successfully elevate the blood Ca status of freshly calved multiparous cows over the critical first 48-hours and 96-hours post-partum. This protocol also resulted in increased rumination time over the first 14 days in lactation and an increase in milk production of 1.8kg/cow/day in the CSC group of cows. These data would suggest that the product offers a practical and effective solution to overcome the challenge of supporting the Ca status of freshly calved cows from a convenient single dose given at calving. This protocol has the potential to help farmers to overcome the conundrum of either short-changing the cow's Ca needs or devoting scarce labour resources to supplement fresh cows sufficiently.

Hypocalcaemia is routinely reported to be a risk factor for transition cow diseases such as dystocia, uterine prolapse, RP, metritis, DA, mastitis and ketosis [21-24]. The benefits of maintaining elevated blood Ca status in the first days of lactation and its effect on reducing the risk of such diseases are well reported and the results of this study may indicate that CSC could play a beneficial role in this regard. The population size of this study was too small to investigate this hypothesis and further larger scale studies would be needed to evaluate fully if there is a link here.

This study also demonstrated that cows supplemented with CSC showed significant increases in rumination time over the first 14 days of lactation. A clear link between Ca status and rumination levels was established in studies where declining rumination levels were monitored in cows and correlated to declining Ca status [25, 26]. Due to the grass-based nature of the production system employed, it was impossible to definitively measure actual kilograms of DMI of these cows and also impractical to accurately measure the dry matter level of the predominantly grazing based diet.

In the absence of DMI data, it was felt that the continuous monitoring of rumination levels might provide an indication of Ca status and rumen activity levels of these cows. The data does suggest that on days 1 and 2 of lactation the CSC group have significantly higher blood Ca levels and also spend significantly more time (+65mins/day) ruminating compared to the CON group. Whilst it is not guaranteed to have also increased DMI in the CSC group, it may indicate that a more active rumen will digest the feed it has taken in in a more comprehensive manner.

Previous research studies on the Cow Start product has shown improved energy status in supplemented cows in early lactation via; significantly reduced milk beta-hydroxy butyrate (BHBA) levels in the milk at weeks 2, 3 and 4 of lactation [17, 18], a numerical improvement in body condition score at 6 weeks in lactation and a numerical reduction in body weight loss in early lactation when compared to un-supplemented cows [18]. This rumination data may support the hypothesis from previous findings that Cow Start supplemented cows in better Ca status have higher levels of DMI and higher rumination levels leading to improvements in early lactation energy status. This theory would need further exploration in an indoor system capable of accurately measuring DMI of freshly calved cows.

The study also found that CSC supplemented cows had higher levels of milk production when compared to un-supplemented CON cows. This finding supports previous research studies on the product that showed an increase of 4.4% in milk production across the first 90 days of lactation, and indeed across the full lactation, under a range of production systems from grass based to intensively feed high production Holstein cows [18, 19, 27]. This finding of increased milk production may be product specific, as numerous studies have not found a consistent link between Ca supplementation and increased milk production [28-30].

5. Conclusions

The results of this trial demonstrate that the prophylactic administration of a convenient single dose of Cow Start Complete at calving, can support the blood Ca status of high-risk multiparous cows for at least 48 hours post-partum. Furthermore, the intervention resulted in significantly increased rumination times during the first 14 days of lactation and demonstrated increased milk production volumes over the first 90 days of lactation, these results were not seen in the control group of cows in this study.

Abbreviations

CON	Control
CSC	Cow Start Complete
Ca	Calcium
DMI	Dry Matter Intakes
SCH	Sub-Clinical Hypocalcemia
DIM	Days in Milk
RP	retained Placenta
DA	Displaced Abomasum

Author Contributions

John Lawlor: Conceptualization, Data curation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing

Enda Neville: Conceptualization, Investigation, Method-

ology, Supervision, Validation, Visualization, Writing – review & editing

Alan Fahey: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing

Acknowledgments

The authors would like to acknowledge the cooperation and efforts of the owners of the participating farm, veterinary practice and analytical laboratory for proving assistance and access to the data and results.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] J. P. Goff, 'The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows', *The Veterinary Journal*, vol. 176, no. 1, pp. 50–57, 2008, https://doi.org/10.1016/j.tvjl.2007.12.020

[2] J. K. Drackley. "ADSA foundation scholar award: Biology of dairy cows during the transition period: The final frontier?" *J. Dairy Sci*. 82: 2259–2273, 1999, https://doi.org/10.3168/jds.S0022 -0302(99)75474-3

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

[4] L. S. Caixeta, P. A. Ospina, M. B. Capel, and D. V. Nydam. "The association of subclinical hypocalcemia, negative energy balance and disease with bodyweight change during the first 30 days post-partum in dairy cows milked with automatic milking systems". *Vet. J*. 204: 150–156, 2015, https://doi.org/10.1016/j.tvjl.2015.01.021

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

[6] D. C. Blood, O. M. Radostits, and J. A. Henderson. "Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs and Horses". 6th ed., 1983, Bailliere Tindall, ed. London.

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

[8] N. Martinez, C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. Galvao, and J. E. P. Santos. "Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease". *J. Dairy Sci*. 95: 7158–7172, 2012, https://doi.org/10.3168/jds.2012-5812

[9] L. S. Caixeta, P. A. Ospina, M. B. Capel, and D. V. Nydam. "Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows". *Theriogenology* 94: 1–7, 2017, https://doi.org/10.1016/j.theriogenology.2017.01.039

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

[11] C. R. Seely, B. M. Leno, A. L. Kerwin, T. R. Overton, and J. A. A. McArt. "Association of subclinical hypocalcemia dynamics with dry matter intake, milk yield, and blood minerals during the periparturient period". *J. Dairy Sci*. 104: 4692–4702, 2021, https://doi.org/10.3168/jds.2020 -19344

[12] R. C. Neves, B. M. Leno, T. Stokol, T. R. Overton, and J. A. A. McArt. "Risk factors associated with postpartum hypocalcaemia in dairy cows". *J. Dairy Sci*. 100: 3796-3804, 2017, https://doi.org/10.3168/jds.2016-11970

[13] R. C. Neves, M. Leno, K. D. Bach, and J. A. A. McArt. "Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: The temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production". *J. Dairy Sci*. 101: 9321–9331, 2018a, https://doi.org/10.3168/jds.2018-14587

[14] G. R. Oetzel. "Oral calcium supplementation in peripartum dairy cows". *Vet. Clin. North Am. Food Anim. Pract.* 29: 447–455, 2013, https://doi.org/10.1016/j.cvfa.2013.03.006

[15] A. Valldacabres, J. A. A. Pires, and N. Silva-del-Rio. "Effect of prophylactic oral calcium supplementation on postpartum mineral status and markers of energy balance of multiparous Jersey cows," *J. Dairy Sci*. vol. 101: 4460–4472, 2018, https://doi.org/10.3168/jds.2017-12917

[16] B. M. Leno, R. C. Neves, I. M. Louge, M. D. Curler, M. J. Thomas, T. R. Overton, and J. A. A. McArt. "Differential effects of a single dose of oral calcium based on postpartum plasma calcium concentration in Holstein cows," *J. Dairy Sci*.vol. 101: 3285–3302, 2018, https://doi.org/10.3168/jds.2017-13164

[17] J. Lawlor, A. Fahey, E. Neville, A. Stack, and F. Mulligan, "On-farm Safety and Efficacy Trial of Cow Start Calcium Bolus," *Anim. Vet. Sci.*, vol. 7, no. 6, pp. 121–126, 2019, https://doi.org/10.11648/j.avs.20190706.11

[18] J. Lawlor, Fahey, A., Neville, E., Stack, A., and Mulligan, F. "Effect of Cow Start Calcium Bolus on Metabolic Status and Milk Production in Early Lactation," *Anim Vet Sci.*, vol. 8, no. 6, pp. 124-132, 2020, https://doi.org/10.11648/j.avs.20200806.12

[19] J. Lawlor, R. Reardon, C. O'Sé, E. Neville, and A. Stack. "Effect of Cow Start Bolus Supplementation on Early Lactation Health and Performance Indicators in a Group of Older, At-Risk Dairy Cows" *Anim Vet Sci.*, vol. 9, no. 5, pp. 134-140, 2021, https://doi.org/10.11648/j.avs.20210905.12

[20] J. Lawlor "Functionality, Effectiveness and Delivery of Oral Calcium Boluses in Elevating Blood Calcium of Freshly Calved Cows" a Review of Commercially Available Products. Anchor Life Science Ltd., 2024.

[21] C. R. Curtis, H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman, and E. J. Pearson. "Association of parturient hypocalcemia with eight peripartu-rient disorders in Holstein cows". *J. Am. Vet. Med. Assoc*. 183: 559–561, 1983.

[22] H. N. Erb, R. D. Smith, P. A. Oltenacu, C. L. Guard, R. B. Hillman, P. A. Powers, M. C. Smith, and M. E. White. "Path model of reproductive disorders and performance, milk fever, mastitis, milk yield, and culling in Holstein cows". *J. Dairy Sci*. 68: 3337–3349, 1985, https://doi.org/10.3168/jds.S0022-0302(85)81244-3

[23] Melendez, P., G. A. Donovan, C. A. Risco, and J. P. Goff. "Plasma mineral and energy metabolite concentrations in dairy cows fed an anionic prepartum diet that did or did not have retained fetal membranes after parturition". *Am. J. Vet. Res*. 65: 1071–1076, 2004, https://doi.org/10.2460/ajvr.2004.65.1071

[24] Kimura, K., J. P. Goff, M. E. Kehrli Jr., and T. A. Reinhardt. "Decreased neutrophil function as a cause of retained placenta in dairy cattle". *J. Dairy Sci*. 85: 544–550, 2002. https://doi.org/10.3168/jds.S0022 -0302(02)74107-6

[25] S. S. Hansen, P. Nørgaard, C. Pedersen, R. J. Jorgensen, L. S. B. Mellua and J. D. Enemark. "The Effect of Subclinical Hypocalcaemia induced by Na_2EDTA on the Feed Intake and Chewing Activity of Dairy Cows". *Veterinary Research Communications* 27: 193-205, 2003, https://doi.org/10.1023/A:1023340506782

[26] R. C. Daniel. "Motility of the rumen and abomasum during hypocalcaemia". *Can. J. Comp. Med*. 47: 276–280, 1983.

[27] J. Lawlor and A. Fahey. "Effect of Cow Start supplementation on cow health, fertility, and milk production performance of high production Holstein cows". Internal White Paper, 2021.

[28] J. A. A. McArt, and G. R. Oetzel, "A stochastic estimate of the economic impact of oral calcium supplementation in post parturient cows," *Journal of Dairy Science* vol. 98: 7408–7418, 2015, https://doi.org/10.3168/jds.2015-9479

[29] G. R. Oetzel, and B. E. Miller. 2012. "Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds," *J. Dairy Sci*. vol. 95: 7051–7065, 2012, https://doi.org/10.3168/jds.2012-5510

[30] A. Valldecabres, R. Branco-Lopes, C. Bernal-Córdoba, and N. Silva-del-Río. "Production and reproduction responses for dairy cattle supplemented with oral calcium bolus after calving: Systemic review and meta-analysis". *JDS Communications*, 2022. https://doi.org/10.3168/jdsc.2022-0235