

Effect of Calcium bolus at calving on postpartum performance and milk composition in dairy cows



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SUMMARY

During the late stages of gestation, calcium requirements increase to support the development of the calf and the production of colostrum. Low blood calcium after calving can result in various issues such as reduced feed intake, decreased milk production, muscle weakness, and in severe cases, recumbency. The study aimed to investigate the effect of calcium supplementation after calving on performance and milk composition of dairy cows during the first 28 days of lactation and lactational performance (days open, lactation length, cumulative lactation milk yield, and 305-d mature equivalent milk). Holstein cows (parity number: 2.6 ± 0.46) were randomly allocated into 2 groups: (1) oral supplement of Calcium bolus (CaB; $n=9$) containing calcium formate and lithothamnion calcareum (red coral alga) immediately and 12 - 18 h post-calving and (2) Control (CON; $n=11$) that did not receive oral Ca. Milk yield was recorded daily and data of body weights and milk composition were collected weekly from calving to 28 d postpartum. Blood sampling was performed between partum and 72 h from the coccygeal vein after morning milking and before feeding. Ca supplementation at calving tended to increase daily milk yield ($P=0.062$) and increase total milk yield ($P=0.049$) for 28 d postpartum. Also, CaB treatment significantly increased ($P=0.038$) blood total Ca levels (2.15 mmol/L vs 1.87 mmol/L) in the first 72 hours after calving but did not affect magnesium and phosphorus concentrations. However, body weights and milk composition (total solids, fat, protein, fat:protein ratio, lactose, casein and urea-N), and lactational performance (energy and fat corrected milk, fat and protein yields) did not differ between treatments. Also, there was no effect of the treatments on days open, lactation length, 305-d mature equivalent milk, and lactation milk yield. In conclusion, supplementing dairy cows with oral Calcium boluses at parturition improves milk yield during early lactation, but not entire lactation.

KEY WORDS

Calcium bolus; dairy cow; calving; milk yield; milk composition.

INTRODUCTION

Calcium (Ca) is the most plentiful mineral found in the animal body. Nearly 100% of the body Ca is found as a constituent of the skeleton and teeth. Moreover, Ca is an important component of body cells and tissues fluids. It plays roles in nerve impulse transmission, muscle contractile properties, as well as blood coagulation (1).

Calcium is a crucial part of metabolism, immune system, and defence against inflammation because of its roles in muscle contraction and immune function (2). Due to the dramatically increased demand of Ca at the beginning of the lactation, post-calving dairy cows are often hypocalcemic (3, 4). Clinical hypocalcemia or parturient paresis affects less than 5% of postpartum cows. Subclinical hypocalcemia (SCH), however, is less apparent but much more common (5), thus creating herd-level

effects on cow health and production (2). In fact it is a usually accepted risk factor for periparturient diseases (e.g. displaced abomasum, metritis, mastitis, retained placenta, ketosis) and, also, decreased milk production. It was reported that cows with SCH are 3 to 5 times more likely to develop postpartum diseases and 50% more likely to be culled from the herd in early lactation contrast to normocalcemic cows (6).

Practically, the giving of a low DCAD prepartal diet improves postpartum Ca status in multiparous cows with improvements in production and health (7). However, such diets require appropriate balancing, attention to stocking density, and cow movement patterns (6). Even using a negative DCAD diet in prepartum high incidence of SCH still exists. It has been proposed that oral Ca supplementations is a recommended approach for cows which experience hypocalcemia without any symptoms (8). In fact, when supplying substantial amounts of highly soluble Ca forms, a high concentration gradient will occur between the alimentary canal and interstitial fluids, subsequently, leading to the passive absorption of ionized Ca (9). Oral Ca may be provided as gel, liquid, paste or bolus; however, boluses are preferred since it is the best form to minimize the risk of pneumonia aspiration (8, 10).

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Table 1 - Descriptive statistics at the experiment start [mean \pm standard error (min - max)].

	Control (n= 11)	Calcium Bolus (n= 9)
Lactation number	2.5 \pm 0.48 (1 - 5)	2.7 \pm 0.43 (1 - 4)
Body weight at partum (kg)	627.6 \pm 15.40 (551 - 710)	617.8 \pm 22.11 (537 - 780)
Previous lactation length (day)	383 \pm 33.62 (254 - 599)	341 \pm 13.93 (276 - 414)
Previous lactation milk yield (kg)	8736 \pm 409 (6604 - 11940)	8497 \pm 387 (6834 - 10590)

Data are lacking from controlled studies to determine the effects of Ca bolus supplementation at parturition on lactational performance of postpartum first month and long - term duration. Also, currently, no data are available to evaluate whether Ca bolus would change the whole lactation performance. Therefore, the objective of this study was to determine the effect of Ca bolus at parturition on blood total calcium (tCa), magnesium (Mg) and phosphorus concentrations within 72 h, and body weight, milk yield and milk composition during the first 28 d postpartum. Measurements were extended to lactational performance (days open, lactation length, 305-d mature equivalent milk and total milk yield).

MATERIAL AND METHODS

All procedures involving animals were complied with the Institutional Animal Care and Use Committee of Cukurova University (protocol number: 2022 - 8). The herd consisted of mainly Holstein-Friesian cows (100 - 150 lactating cows, 50 - 75 replacement heifers and dry cows, and 100 calves). The average lactation yield for the herd was 7847 \pm 865 kg/cow (mean \pm standard deviation).

Animals, management and diets

Thirty dry cows (Table 1) were moved from a far-off pen to a close-up pen 1 month before the expected parturition date. The cows were moved into maternity pens when they showed signs of impending calving (for example, relaxation of the pelvic ligaments, udder enlargement, and milk let-down). Wheat straw was used as a bedding material in maternity pens. Calves were separated from dams after parturition. Fresh cows were moved from the fresh cow pen to a colostrum pen for calf nutrition during the first 72 - 96 hours twice daily.

After calving, the cows were sheltered in a free stall - barn. During the experiment (prepartum and early lactation period) all cows were fed the same balanced TMRs that prepared to meet the requirements of dry and early lactating cows (11). In briefly, the prepartum TMR consisted of 33.7% corn silage, 15.5% alfalfa hay, 16.1% wheat straw, 11.5% corn ground, and 23.2% concentrate and mineral mix on a DM basis (DM: 53.4%, and on a DM basis CP: 11.1%, NDF: 48.0%, ADF: 28.1%). The early lactating cows TMR consisted of 31.7% corn silage, 10.6% alfalfa hay, 10.9% wheat straw, 46.8% concentrate and mineral - vitamins mixture on a DM basis (DM: 53.7%, and on a DM basis CP: 15.3%, NDF: 38.8%, ADF: 24.8%). Cows received, approximately, 15-17 kg of TMR per cow (on a dry matter basis) in prepartum while 20-23 kg of TMR per cow in early lactation period. The TMRs was delivered twice daily at 08:00 and 14:00 h. The feed was pushed in front of the cows 2 or 3 times a day to ensure maximum feed consumption. Cows had free access to water. Constant management conditions (TMR dis-

tribution, working routine, etc.) were maintained during the study. The herd policy of manipulating DCAD was not enforced.

Experimental groups

During the dry period of the study, cows were randomly allocated into two groups; treatment or control. Cows in the treatment group (CaB, n = 9) received one Ca Bolus immediately after calving and a second bolus approximately 12 - 18 h later. The cows were observed for a while (5 - 10 minutes) following bolus administration to verify they did not throw up the bolus. Cows in the control group (n= 11) were unsupplemented with Ca bolus. Oral supplement of Ca bolus (Calseatec, Vittek Vitamin Teknolojileri İlaç Hayvancılık San. ve Tic. Ltd. Sti, Adana, Türkiye) containing 21 g Ca (from Lithothamnium and Calcium Formate, Table 2) was used.

Blood samples were collected thrice (at the bolus administration, 24 h, and 72 h from the administration of the bolus) from the coccygeal venipuncture after morning milking and before feeding. Plain tubes (10 mL; Aysel, Türkiye) were used for drawing of blood. Serum was separated by centrifugation (2,136 \times g, for 5 min at 4°C) and stored at -20°C until further analyses. Blood total calcium (tCa), magnesium (Mg), and phosphorus (P) were analyzed according to the following colorimetric methodologies: total calcium: Arsenazo III (Mindray CA0102); magnesium: Xylidyl Blue (Mindray MG0102); phosphorus: Fosfomolibdat (Mindray P0102). Before the start of blood analysis, biochemistry analyzer (BS-120 Vet, Mindray, China) was calibrated with control calibrator serum (Mindray Multi Control Sera N and P).

Cows were milked twice daily (04:30 and 16:00 h) and milk yields were recorded at each milking for individual cows. Milk samples were taken on days 7, 14, 21 and 28 postpartum for total solids, protein, casein, urea - N, fat, and lactose analysis (FOSS Milkoscan FT - 120, Denmark). The following equation was used to calculate 4% fat corrected milk (FCM) of each cow (12):

4% FCM (kg) = [(0.4 \times kg milk) + (0.15 \times kg milk \times fat %)]. The yield of energy-corrected milk (ECM) was calculated by the following formula (13):

ECM (kg) = Milk production (kg) \times (383 \times fat% + 242 \times protein% + 783.2)/3140.

Table 2 - Composition of Calcium Bolus used in the experiment.

Component name	1 Bolus / 90 g
Lithothamnium (g)	6.30
Magnesium oxide (g)	3.70
Magnesium stearate (g)	0.70
Vitamin D3 (IU)	25750
Calcium formate (g)	14.70

Table 3 - Effects of Calcium Bolus on lactation performance of postpartum Holstein cows during 28 days.

Items	Treatment ¹		SEM ²	P-value		
	Control	CaB		Trt	Week	Trt×Week
Body weight ³ (kg)	583.8	596.1	11.76	0.470	<0.01	0.440
Milk yield (kg/d)	27.2	29.8	0.89	0.062	<0.01	0.735
Total milk yield (kg)	763.0	833.8	23.47	0.049	-	-
ECM ⁴ (kg/d)	25.7	27.9	1.15	0.202	<0.01	<0.01
FCM ⁵ (kg/d)	26.1	28.5	1.14	0.148	<0.01	<0.01
Fat yield (kg/d)	1.00	1.09	0.05	0.243	<0.01	<0.01
Protein yield (kg/d)	0.85	0.90	0.04	0.393	<0.01	0.512

¹Control= received no Calcium bolus, CaB: Calcium bolus; ²SEM: Standard error of means; ³Body weight at calving; ⁴ECM: Energy corrected milk; ⁵FCM: Fat corrected milk.

All cows were weighed weekly before morning feeding during the study. Days open, lactation length, cumulative lactation milk yield and 305-d mature equivalent milk yield were obtained from the milking system (De Laval, Tumba, Sweden) of the herd.

Statistical analysis

Cows (n = 9) that developed a clinical disease (lameness, mastitis, and metritis) and cow (n = 1) that had twins were excluded from the study. The final data set included 20 cows. All statistical analyses were conducted using SAS (14). Data were tested with PROC UNIVARIATE for normality by the Shapiro-Wilk. In the case of non-Gaussian distributions, parameters were normalized by box-cox transformation. Blood tCa, Mg, P, milk yield and composition were analyzed as repeated measures by using a Mixed model (PROC GLIMMIX (15)). The model included oral Ca bolus (with and without), time and 2 ways interaction as fixed effects and body weights at parturition as a covariate. Cow nested within oral Ca bolus was a random term. Time (hour for blood analysis and week for milk analysis) was included in the model as a repeated variable. Compound symmetric (CS), autoregressive order 1 (AR(1)), unstructured (UN), heterogeneous compound symmetry (CSH), heterogeneous autoregressive 1 (ARH(1)), antedependence 1 (ANTE(1)), spa-

tial power (SP(POW)), autoregressive moving average (ARMA(1)) were used as covariance structures (16). The structure that best fitted the model was chosen based on the smallest values of Akaike information criteria (AIC). Results are reported as least - squares means. Statistical significance was declared at $P \leq 0.05$, and tendencies were considered when $0.05 < P \leq 0.10$.

RESULTS

Postpartum body weight ($P = 0.470$), ECM ($P = 0.202$), FCM ($P = 0.148$), fat yield ($P = 0.243$), and protein yield ($P = 0.393$) were not different between the Control and CaB groups (Table 3). Interactions of treatment and week were observed for ECM ($P < 0.01$), FCM ($P < 0.01$), fat yield ($P < 0.01$). Daily milk yield ($P = 0.062$) and total milk yield ($P = 0.049$) for postpartum 4 wk were higher for the CaB group than those for the Control group (Table 3).

Blood tCa, Mg and P concentrations within 72 h and milk composition during the first 28 days post-calving are reported in Table 4. The CaB administered cows had a higher tCa concentration (2.15 mmol/L vs 1.87 mmol/L) in their blood than

Table 4 - Effects of Calcium Bolus at partum on blood tCa, Mg and P within 72 h and milk composition during 28 days.

Items	Treatment ¹		SEM ²	P-value		
	Control	CaB		Trt	Week ³	Trt×Week ³
Blood ⁴						
tCa (mmol/L)	1.87	2.15	0.18	0.038	0.258	0.647
Mg (mmol/L)	0.84	0.89	0.07	0.546	0.197	0.453
P (mmol/L)	2.38	2.03	0.13	0.789	0.354	0.651
Milk						
Total solids (%)	11.4	11.3	0.18	0.769	<0.01	0.040
Fat (%)	3.71	3.65	0.14	0.764	<0.01	0.014
Protein (%)	3.11	3.03	0.08	0.468	<0.01	0.776
Fat:Protein ratio	1.19	1.22	0.04	0.646	0.112	0.045
Lactose (%)	4.52	4.61	0.10	0.513	0.186	0.670
Casein (%)	2.42	2.37	0.06	0.499	<0.01	0.558
Urea N (mg/dl)	12.1	11.4	0.453	0.296	0.271	0.829

¹Control= received no Calcium bolus, CaB: Calcium bolus; ²SEM: Standard error of means; ³Hour and week were for blood and milk analyses, respectively. ⁴tCa: total calcium, Mg: magnesium, P: phosphorus.

Table 5 - Effects of Calcium Bolus at partum on lactational performance .

Items	Treatment ¹		SEM ²	P-value
	Control	CaB		
Days open (d)	120.7	120.0	19.41	0.962
Lactation length (d)	315	323.1	18.93	0.768
Cumulative lactation milk yield (kg)	7679.5	8914.6	591.20	0.380
305-d mature equivalent milk (kg)	7864.8	8942.7	472.48	0.136

¹Control= received no Calcium bolus, CaB: Calcium bolus; ²SEM: Standard error of means.

Control cows. In contrast to the tCa, the concentrations of Mg (0.84 mmol/L vs 0.89 mmol/L) and P (2.38 mmol/L vs 2.03) were similar between treatments.

No significant differences ($P > 0.05$) were observed in on total solids, fat, protein, fat:protein ratio, lactose, casein and urea-N between Control and CaB groups. There was a Trt by Week interaction on total solids, fat, and fat:protein ratio. Relative to the Control cows, the CaB administered cows increased total solids, fat and fat:protein ratio after week 3 (data not shown). There was no effect of the treatments on days open ($P = 0.962$), lactation length ($P = 0.768$), 305-d mature equivalent milk ($P = 0.136$), and lactation milk yield ($P = 0.380$).

DISCUSSION

The present study was designed to evaluate the effect of oral Ca bolus administration at parturition on blood tCa, Mg, and P concentrations within 72 h, body weight, milk production, milk components during the first 4 weeks of lactation, and lactational performance (days open, lactation length, 305-d mature equivalent milk, and cumulative lactation milk yield). Supplementing oral Ca bolus increased daily and total milk yield for 28 days of lactation, respectively 9.6% and 9.3% compared with unsupplemented cows. However, milk composition and lactational performance were not different between the Ca bolus administered cows and the Control cows. Therefore, the positive effect of Ca bolus is not sustained through the whole lactation.

Normocalcemic cows produced 1.1 to 2.9 kg/d more milk compared with clinical hypocalcemic cows (3, 6, 17). Oral Ca boluses, also, improved milk yield in grazing cows ≥ 3 lactations (18). However, Domino et al. (19) reported that oral or subcutaneous Ca supplementation did not improve average daily milk yield compared with nontreated cows (47.0 kg/d, 47.1 kg/d and 46.7 kg/d, respectively). Reasons for the differences in the results of the experiments were attributed to parity, the threshold applied, Ca sampling time, and the type of Ca salts used (2, 20). The positive effects of Ca boluses may be attributed to their significant role in facilitating smooth muscle contractions and supporting nerve function (8, 21). Therefore, decreased blood Ca probably negatively affects skeletal muscle strength and gastrointestinal motility and is thought to be causal to reduced DMI and milk yield (8, 23).

Because of the role of Ca in muscle contraction, hypocalcemia affects organs including the uterus, forestomach and abomasum (24 - 26). These organs depend on the contraction of smooth muscles to carry out their functions. When the ruminoreticular motility is compromised by hypocalcemia, structural carbohydrate degradation and subsequently acetate pro-

duction can reduce. Therefore, lower milk fat levels may be observed in the Control cows. However, why this effect was seen after 3 weeks is unclear. Valdecabres and Silva-del-Rio (27) found that milk fat and protein concentration during the first 3 monthly tests were not affected by oral Ca supplementation. Similarly, a study administering Ca subcutaneously postpartum observed no effect on milk fat and protein concentration across the first 3-month test (28).

Martinez et al. (29) implied that the Ca supplement affected productivity as gradually and time dependent manner, namely, the oral Ca supplement did not affect milk yield during postpartum 150 DIM while there was a difference in milk production during the early lactation days. In other words, the effect of Ca bolus on milk yield diminished as the lactation advanced.

No positive effect of CaB on days open was detected in the current study which is in line with previous experiments looking into the effect of oral Ca supplementation at parturition on reproductive performance. Similarly, Oetzel and Miller (22) and Valdecabres et al. (17) reported no difference in time to conception or pregnancy to first service between bolus treated and untreated cows. However, management strategies that improve the Ca balance in the transition cows will likely have a positive effect on reproductive outcomes such as days to first heat, time to first service, time to conception (3, 30). Therefore, the magnitude of the effect may change with management, nutrition and parity. Also, observed results vary with the timing of blood sampling, threshold values, and study design. Due to the limited sample size of cow in this study, our results should be taken with caution.

Immediately after parturition, supplementing oral Ca bolus provided a short-term effect on lactation performance without altering milk composition. Future research should work on a higher number of cows or should be done on large-scale dairy farms while considering metabolic profiles.

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Disclosure statement

The authors report there are no competing interests to declare.

References

1. McDonald P, Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A., and Wilkinson R.G. 2010. *Animal Nutrition* 7. Edition, Pearson Education, London, UK.
2. Couto Serrenho R., Devries T.J., Duffield T.F., and LeBlanc J. 2021. What

- do we know about the effects of clinical and subclinical hypocalcemia on health and performance of dairy cows? *J. Dairy Sci.*, 104: 6304 - 6326.
3. Grossi, S., Compiani, R., Baldi, G., Buehler, K., and Rossi, C.A.S. 2022. Effect of the administration of a ruminal bolus containing 1,25-dihydroxyvitamin D3 glycosides from *Solanum Glaucophyllum* on blood calcium levels, hypocalcaemia, production performance, health status and fertility rate in lactating dairy cows. *Large Anim. Rev.*, 28: 273-284.
 4. Zhang X., Glosson K.M., Bascom S.S., Rowson A.D., Wang Z., and Drackley J.K. 2022. Metabolic and blood acid-base responses to prepartum dietary cation-anion difference and calcium content in transition dairy cows. *J. Dairy Sci.*, 105: 1199-1210.
 5. Martinez N., Risco C.A., Lima F.S., Bisinotto R.S., Greco L.F., Ribeiro E.S., Maunsell F., Galvão K., and Santos J.E.P. 2012. Evaluation of periparturient 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.
 6. Venjakob P.L., Pieper L., Heuwieser W., and Borchardt S. 2018. Association of postpartum hypocalcemia with early-lactation milk yield, reproductive performance, and culling in dairy cows. *J. Dairy Sci.*, 101: 9396 - 9405.
 7. Wilkens M.R., Nelson C.D., Hernandez L.L., and McArt J.A.A. 2020. Transition cow calcium homeostasis - Health effects of hypocalcemia and strategies for prevention. *J. Dairy Sci.*, 103: 2909 - 2927.
 8. Oetzel G.R. 2013. Oral calcium supplementation in periparturient dairy cows. *Vet. Clin. North Am.: Food Anim. Pract.*, 29: 447 - 455.
 9. Goff J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. *J. Dairy Sci.*, 101: 2763 - 2813.
 10. Lawlor J., Fahey A., Neville E., Stack A., and Mulligan F. 2019. On-arm safety and efficacy trial of cow start calcium bolus. *Anim. Vet. Sci.*, 7: 121-126.
 11. NRC 2001. Nutrient Requirements of Dairy Cattle. 7th rev. Edition. National Academy Press, Washington, DC.
 12. Gaines W.L. 1928. The energy basis of measuring milk yield in dairy cows. Illinois agricultural experiment station bulletin 308. Urbana: University of Illinois.
 13. Sjaunja L.O., Baevre L., Junkkarinen L., Pedersen J., and Setälä J. 1990. A Nordic proposal for an energy corrected milk (ECM) formula. Paper presented at Proceedings of the 27th Biennial Session of the International Committee for Animal Recording (ICAR); 2-6 July, Paris, France. EAAP publication no. 50.
 14. SAS Institute Inc. 2014. SAS® OnDemand for Academics: User's Guide. Cary, NC: SAS Institute Inc.
 15. Stroup W.W., Milliken G.A., Claassen E.A., and Wolfinger R.D. 2018. SAS® for Mixed Models: Introduction and Basic Applications. Cary, NC: SAS Institute Inc.
 16. Littell R.C., Milliken G.A., Stroup W.W., Wolfinger R.D., and Schabenberger O. 2006. SAS for Mixed Models, Second Edition, Cary, NC: SAS Press.
 17. Valdecabres, A., Branco-Lopes, R., Bernal-Córdoba, C., and Silva-del-Río, N. 2023. Production and reproduction responses for dairy cattle supplemented with oral calcium bolus after calving: Systematic review and meta-analysis. *JDS Communications*, 4(1): 9-13.
 18. 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. *Livest. Prod. Sci.*, 248: 104510.
 19. Domino A.R., Korzec H.C., and McArt J.A.A. 2017. Field trial of 2 calcium supplements on early lactation health and production in multiparous Holstein cows. *J. Dairy Sci.*, 100: 9681 - 9690.
 20. Al-Qaisi M., Kvidera S.K., Horst E.A., McCarthy C.S., Mayorga E.J., Abeyta M.A., Goetz B.M., Upah N.C., McKilligan D.M., Ramirez-Ramirez H.A., Timms L.L., and Baumgard L.H. 2020. Effects of an oral supplement containing calcium and live yeast on postabsorptive metabolism, inflammation and production following intravenous lipopolysaccharide infusion in dairy cows. *Res. Vet. Sci.*, 129: 74 - 81.
 21. Miltenburg C.L., Duffield T.F., Bienzle D., Scholtz E.L., and LeBlanc S.J. 2016. Randomized clinical trial of a calcium supplement for improvement of health in dairy cows in early lactation. *J. Dairy Sci.*, 99: 6550 - 6562.
 22. Oetzel G.R., Miller B.E. 2012. Effect of oral calcium bolus supplementation on early-lactation health and milk yield in commercial dairy herds. *J. Dairy Sci.*, 95: 7051-7065.
 24. Chapinal N., Carson M., Duffield T.F., Capel M., Godden S., Overton M.W., Santos J.E.P., and LeBlanc S.J. 2011. The association of serum metabolites with clinical disease during the transition period. *J. Dairy Sci.*, 94: 4897 - 4903.
 25. Goff J.P. 2015. Minerals, Bones, and Joints. Dukes' physiology of domestic animals. 13th edition/editor: William O. Reece, Wiley Blackwell, Ames, Iowa.
 26. Rodríguez E.M., Aris A., and Bach A. 2017. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. *J. Dairy Sci.*, 100: 7427 - 7434.
 27. Valdecabres A., Silva-del-Río N. 2020. 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.
 28. Amanlou H., Akbari A.P., Farsuni N.E., and Silva-del-Río N. 2016. Effects of subcutaneous calcium administration at calving on mineral status, health, and production of Holstein cows. *J. Dairy Sci.*, 99: 9199 - 9210.
 29. Martinez N., Sinedino L.D.P., Bisinotto R.S., Daetz R., Risco C.A., Galvão K.N., Thatcher W.W., and Santos J.E.P. 2016. Effects of oral calcium supplementation on mineral and acid-base status, energy metabolites, and health of postpartum dairy cows. *J. Dairy Sci.*, 99: 8397 - 8416.
 30. Mahen, P.J., Williams, H.J., Smith, R.F., and Grove-White, D. 2018. Effect of blood ionised calcium concentration at calving on fertility outcomes in dairy cattle. *Vet. Rec.*, 183(8): 263-269.