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Relationship between hypocalcemia immediately after calving with metabolic disorders and body condition score in Holstein cows

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Abstract

Background: Hypocalcemia is one of the most common transition period disorders that affects dairy cows and has been divided into clinical and subclinical types.

Aim: This study aimed to investigate the relationship between postpartum serum calcium (Ca) concentrations with metabolic disorders and body condition score (BCS) in Holstein dairy cows.

Methods: Two hundred and seventy-one Holstein cows were blocked from two commercial dairy herds based on parity (primiparous and multiparous) and serum Ca concentrations on calving day, 1 and 2 days postpartum were allocated to 1 of 3 groups: 1) Serum Ca concentration >8.5 mg/dl at the calving day, 1 and 2 days postpartum (normocalcemic); 2) serum Ca concentration ≤8.5 mg/dl on the calving day and 1 or 2 day postpartum (transient subclinical hypocalcemia (TSCH)); and 3) serum Ca concentration ≤8.5 mg/dl on the calving day, 1 and 2 days postpartum (persistent subclinical hypocalcemia (PSCH)).

Results: The results showed that the primiparous and multiparous cows had the highest TSCH and PSCH percentages, respectively. Ca status after calving did not affect the BCS changes, incidence of milk fever, hypomagnesemia and hyperketonemia, and clinical and subclinical endometritis. The incidence of retained placenta, metritis, and subclinical mastitis was affected by Ca status after calving, so PSCH cows experienced 6.28, 6.43, and 5.9 times more retained placenta, metritis, and subclinical mastitis than normocalcemic cows, respectively. The culling rate within the first 60 days in milk for PSCH cows was 4.61 times more than for normocalcemic cows.

Conclusion: Overall, the results of the study showed that cows with PSCH had a higher incidence of retained placenta; uterine infections, subclinical mastitis, and culling rate, but cows with TSCH were similar to healthy cows in terms of metabolic disorders and culling rate.

Keywords: Transient subclinical hypocalcemia, Persistent subclinical hypocalcemia, Mastitis, Retained placenta, Metritis.

Introduction

During the stressful transition period, many metabolic disorders of dairy cows have a significant impact on the farm economy (Atrian *et al*., 2019) and animal welfare. The occurrences of metabolic disorders can lead to economic losses due to treatment and culling costs. In addition, metabolic disorders increase the number of alternatives, impair reproductive efficiency, reduce cows' lives, and have a detrimental effect on the livestock`s welfare (Jakobsen *et al*., 2003; Dhakal *et al*., 2013). Hypocalcemia is one of the most common transition period disorders that affects dairy cows and has been divided into a clinical (i.e., parturient paresis) and a subclinical state, with the latter receiving much recent research attention (Neves *et al*., 2018a,b). Clinical hypocalcemia affects approximately 5% (Goff *et al*., 2008), and subclinical hypocalcemia affects 50% of multiparous cows in periparturient (Reinhardt *et al*., 2011). Recently, numerous studies have been performed on the rapid diagnosis of subclinical hypocalcemia, using its association with reduced blood Ca in the postpartum period (Rodríguez *et al*., 2017; Wilhelm *et al*., 2017). The day after calving chosen to measure Ca is important. Differences in the day of sample collection between studies have led to difficult interpretation of study results. McParland (2012) reported that cows with a minimum Ca concentration of 2.14 mmol/l in one of three days after calving showed detectable metritis. Neves *et al*. (2017) also reported that the cut point for decreased Ca concentration in the

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early postpartum period varies depending on the time of blood sample collection. They suggested that blood sample collection relative to the day of calving is an important factor in measuring blood Ca, which should be considered when examining the association between subclinical hypocalcemia and its consequences. Therefore, since the level of macro minerals, such as calcium, magnesium, and phosphorus, has not been studied until two days after calving in Iran, the present study aimed to measure serum Ca up to two days after calving on metabolic and infectious disorders such as milk fever, retained placenta, hypomagnesemia, metritis, subclinical endometritis, clinical endometritis, hyperketonemia, subclinical mastitis, and culling within the first 60 days in milk (DIM) and body condition score (BCS) changes in two large herds of dairy cows.

Materials and Methods

Two hundred and seventy-one Holstein cows were blocked from two commercial dairy herds based on the parity (primiparous and multiparous) as well as serum Ca concentrations on the calving day, 1 and 2 days postpartum (regarding ≤8.5 mg/dl serum Ca concentration as the cut point of hypocalcemia) and were allocated to 1 of 3 groups: 1) serum Ca concentration >8.5 mg/dl at calving day, 1 and 2 days postpartum (normocalcemic); 2) serum Ca concentration ≤8.5 mg/dl on the calving day and 1 or 2 days postpartum (transient subclinical hypocalcemia (TSCH)); and 3) serum Ca concentration ≤8.5 mg/dl on the calving day, 1 and 2 days postpartum (persistent subclinical hypocalcemia (PSCH)).

Feeding and management of cows

Cows in both herds were kept in the free stall systems and were fed a total mixed ration (TMR) *ad libitum* two times a day during the experimental period. As soon as the cows showed the first signs of calving, they were transferred to the maternity. After calving, cows were kept in a fresh free stall cows to 21 days of lactation. At the end of 21 days, the cows were transferred to the free stall of high-producing cows. Cows in both fresh and high-producing groups were fed a TMR, three times a day and *ad libitum* to remain 5% to 10% of the residue. Prepartum diets contain anionic salts to prevent hypocalcemia. Diets were formulated using NRC (2001). Components and chemical compositions of diets have been presented in Tables 1 and 2. These cows were also milked three times a day.

Sampling and chemical analysis of diets

Samples of diets of fresh and high-producing cows were collected and frozen weekly, and then samples were dried by the oven (AOAC 1990; method 930.15), crude protein was analyzed by the Kjeldahl method (AOAC 1990; method 984.13), ether extract was analyzed by Soxhlet method using diethyl ether (AOAC 1990; method 920.39), and Ash content was determined by ignition at 600°C for 2 hours according to AOAC (1990), method 942.05. Acid detergent fiber (ADF) was analyzed by cetyltrimethylammonium bromide as well as sulfuric acid 1 normal (method 973.18 AOAC 1990). Neutral detergent fiber (NDF) was also determined by heat-resistant amylase and sodium sulfite (Van Soest *et al*., 1991).

Body condition score

On the calving day and day 90 of lactation, cows were scored for BCS using a five-point system (Wildman *et al*., 1982). Two different individuals identified BCS independently and the results were analyzed on average.

Metabolic disorders after calving

The incidence of disorders after calving was recorded for each herd during the first 60 days of lactation and the incidence of these diseases was recorded. Cows that showed symptoms such as lethargy, staggering, nervous symptoms, and degrees of confusion with cold ears at 72 hours postpartum were identified as cows having milk fever (Oetzel, 2013). Retained placenta refers to cows whose embryonic layers in the vulva, vagina, and uterus were visible on vaginal examinations in the first 24 hours after calving, and the placenta was not removed (Kelton *et al*., 1998). Hypomagnesemia at calving and one day after calving was defined as serum magnesium concentrations less than 1.7 mg/dl (Martín-Tereso and Martens, 2014). Metritis cows from calving to 14 days after the calving were identified based on any abnormal signs in color (presence of pus) and smell of vaginal discharge, by rectal touch, rectal temperature over 39.4°C and reduced feed intake were identified (Sheldon *et al*., 2006). Clinical endometritis was defined as the presence of pus or purulent mucus (more than 50%) detectable in vaginal discharge after 21 days of lactation (Sheldon *et al*., 2006), and subclinical endometritis was also diagnosed by uterine vascularity with 0.9% saline solution on 38 ± 3 days. Two hundred cells were counted on the cytological slide, and the proportion of polymorphonuclear cells (PMNL) was determined. Cows with more than 5% PMNL were identified as subclinical endometritis cows (Gilbert, 2016). Subclinical ketosis was detected in cows with a beta hydroxy butyric acid (BHBA) concentration of 1.2≥ BHBA≥ 2.9 mmol/l in the first week after calving (McArt and Nydam, 2012), and clinical ketosis was also identified as cows with BHBA ≥ 2.9 mmol/l in the first week after calving (McArt and Nydam, 2012). Subclinical mastitis was defined as cows with a somatic cell count of more than 200×10^3 per milliliter of milk. Removal or culling percentage was also recorded from the herd until the 60th day of lactation.

Statistical analysis

Data were analyzed in a randomized complete block design, using SAS 9.1 software (2001). Time was entered as a repetitive effect on the model. The model for duplicate data included fixed effects of Ca status after calving, block, time, and their interaction and random effect of herd, and cows in the Ca status and in the block, entered the statistical model as a subject.

Table 1. Ingredient composition of close-up, fresh, and super cows diets (DM basis).

Ingredient (% of DMI)	Herd 1			Herd 2		
	Close-up	Fresh	Super	Close-up	Fresh	Super
Legume hay, mature	0	11.21	6.31	23.50	14.50	12.55
Corn silage, normal	40.25	25.05	24.13	33.57	21.30	24.00
Wheat straw	5.89	2.45	2.58	0	1.58	1.71
Molasses	0	1.65	2.89	0	0	0
Dry sugar beet pulp	0	4.60	6.46	0	3.70	4.82
Barley grain, ground, dry	15.12	14.35	15.02	8.60	13.68	21.27
Corn grain, ground, dry	14.11	14.35	15.04	15.03	19.70	16.59
Soybean meal	2.64	10.39	10.87	8.45	15.40	10.63
Canola meal	14.24	4.38	4.58	3.00	0	0
Full fat soybean	0	5.23	5.48	0	1.50	0
Cottonseed, whole seed with lint	0	0	0	0	1.80	0
Meat meal	0	0	0	2.67	2.10	2.65
Fat powder	0	0.85	0.89	0	0.32	1.50
Wheat bran	2.26	0	0	0	0	0
Nitroza[1]	0	0.42	0.45	0	0	0
Calcium carbonate	0.93	0.84	0.88	1.42	0.98	1.26
Di-Calcium phosphate	0	0.32	0.33	0	0	0
Magnesium oxide	0	0.32	0.33	0.16	0.34	0.31
Salt	0	0.37	0.39	0	0.45	0.40
Sodium bicarbonate	0	1.64	1.72	0	0.80	1.10
Calcium chloride	0.68	0	0	0.54	0	0
Magnesium sulfate	1.16	0	0	0.84	0	0
Ammonium chloride	0	0	0	0.32	0	0
Vitamin premix[2]	0.94	0.63	0.66	0.95	0.68	0.38
mineral premix[3]	1.78	0.95	0.99	0.95	0.68	0.38
Bentonite	0	0	0	0	0.49	0

(1): Slow-release non-protein nitrogen source for ruminants; (2): Herd 1 Vitamin premix per kg includes: 1,300,000 international units of vitamin A, 360,000 international units of vitamin D and 12,000 international units of vitamin E؛ Herd 1 mineral premix per kg includes: 72 mg cobalt, 2,100 mg copper, 75 mg iodine, 400 mg iron, 5,100 mg manganese, 55 mg selenium, 5,350 mg zinc, and was 15,000 mg monensin; (3): Herd 2 vitamin premix per kg include: 1,200,000 international units of vitamin A, 250,000 international units of vitamin D and 10,000 international units of vitamin E, 200 mg of biotin and 3,000 mg of monensin. The mineral premix of herd 2 per kg included: 105 mg of cobalt, 4,200 mg of copper, 190 mg of iodine, 14,500 mg of manganese, 80 mg of selenium, and 15,000 mg of zinc.

The effect of the time constant and its interactions were removed from the model to analyze the changes in body posture score. Milk fever, retained placenta, and hypomagnesemia at the calving day and one day after parturition, metritis, subclinical endometritis, clinical endometritis, hyperketonemia, subclinical mastitis, and culling within 60 days after parturition were analyzed by logistic regression using generalized linear models mixed procedure of statistical software suite (SAS). The logistic regression model for disease data included fixed effects of Ca status, block, and their interactions. Data were reported as the mean minimum squares and mean standard deviation (LSM ± SEM) with statistical differences of $0.05 < p < 0.1$ and a tendency to the significance of $p < 0.05$. Moreover, a comparison of the means was performed using Tukey's test.

Ethical approval

This study was performed based on the Iranian Council of Animal Care (1995). Guide to the Care and Use of Experimental Animals, vol. 1. Isfahan University of Technology, Isfahan, Iran.

Results

Subclinical hypocalcemia

Lactation statistical data (parity), the incidence of subclinical hypocalcemia, and serum Ca concentration

Table 2. Chemical composition of close-up, fresh, and Super cows diets.

Ingredient	Herd 1			Herd 2		
	Close-up	Fresh	Super	Close-up	Fresh	Super
NEL (Mcal/kg DM)	1.66	1.66	1.57	1.61	1.66	1.54
CP (%)	13.10	16.20	16.30	14.30	16.00	15.30
RDP (%)	9.40	11.30	10.80	10.10	10.80	10.20
RUP (%)	3.70	4.80	5.50	4.20	5.20	5.10
NDF (%)	32.50	29.10	27.60	32.20	28.00	28.10
ADF (%)	20.10	18.50	17.00	22.20	18.00	17.40
NFC (%)	46.20	44.20	45.60	43.10	45.90	45.80
Ether extract (%)	3.20	4.1	4.2	2.80	3.5	3.90
Ca (%)	0.90	1.1	1.2	1.60	1.20	1.30
P (%)	0.40	0.40	0.40	0.50	0.40	0.40
Mg (%)	0.35	0.39	0.40	0.40	0.41	0.38
Na (%)	0.03	0.65	0.70	0.04	0.44	0.50
K (%)	1.01	1.28	1.28	1.31	1.22	1.10
Cl (%)	0.53	0.42	0.41	0.73	0.49	0.44
S (%)	0.36	0.22	0.23	0.30	0.20	0.18
DCAD (mEq/kg)	−106	+355	+375	−43	+242	+260

Table 3. Number and percentage of primiparous and multiparous cows based on calcium status in the first 3 days after calving.

Calcium status	Parity	
	Primiparous (n = 122)	Multiparous (n = 149)
Control[1]	28 (22.95)	22 (14/76)
Transient subclinical hypocalcaemia (TSCH)[2]	82 (67.21)	37 (24/83)
Persistent subclinical hypocalcaemia (PSCH)[3]	12 (9.83)	90 (60/40)

(1): Serum calcium concentration >8.5 mg/dl at calving day, 1 and 2 postpartum; (2): Serum calcium concentration ≥8.5 mg/dl on calving day and 1 or 2 postpartum; (3): Serum calcium concentration ≤8.5 mg/dl on calving day, 1 and 2 postpartum.

in the first three days of calving have been reported in Table 1. In primiparous cows, the proportions of cows in normocalcemic, TSCH, and PSCH groups were 22.95%, 67.21%, and 9.83%, respectively (Table 3). Primiparous cows had the highest percentage of transient hypocalcemia (TSCH, 67.1%) and multiparous cows had the highest percentage of persistent hypocalcemia (PSCH, 60.40%).

Body condition score

The body condition score of primiparous and multiparous cows at the beginning of the experiment and its changes between Ca status groups in primiparous and multiparous cows are shown in Table 4. On the calving day, there were no differences in the ratio of cows with BCS ≤ 3.25 and 3.75 ≤ BCS ≥ 3.25 and BCS ≥ 3.75 ($p > 0.05$) and also the mean of BCS ($p = 0.67$). Changes in body condition score from calving to 90 days of lactation were similar among Ca status groups ($p = 0.3$ Table 4).

Health disorders

The percentage of milk fever, retained placenta, hypomagnesemia on the day of calving and the first day after calving, metritis, subclinical and clinical endometritis, hyperketonemia, subclinical mastitis, and culling in the first 60 days after calving, based on serum Ca status in the first three days after calving, separately in primiparous and multiparous cows, have been reported in Tables 5 and 6.

In the present study, no cases of abomasal displacement and clinical ketosis were observed. The incidence of milk fever was not affected by any of the model components ($p > 0.05$). Grouping of cows based on serum Ca status in the first three days after the calving affected the percentage of retained placenta incidence ($p = 0.06$). However, the lactation number (block) and the interaction effect of the Ca status in the block were not affected. Regardless of the effect of the block, cows in the PSCH group showed 6.28 times more retained placenta than cows in the normocalcemic group (p

Table 4. Body condition score of primiparous and multiparous cows at the beginning of the experiment and body condition score changes between experimental treatments.

Item	Primiparous			Multiparous			*p*-value		
	CON[1]	TSCH[2]	PSCH[3]	CON	TSCH	PSCH	Treat	Block	Treat × Block
	(*n* = 2)	(*n* = 82)	(*n* = 12)	(*n* = 22)	(*n* = 37)	(*n* = 90)			
BCS ≤ 3.25	21.43	18.30	16.67	13.63	16.22	23.33	0.93	0.82	0.68
3.25 ≤ BCS ≤3.75	53.57	60.97	66.66	68.18	59.46	47.78	0.90	0.81	0.28
BCS ≥ 3.75	25.00	20.73	16.67	18.19	24.32	28.89	0.98	0.69	0.60
Average of BCS	3.61 ± 0.09	3.54 ± 0.05	3.52 ± 0.13	3.55 ± 0.10	3.58 ± 0.08	3.48 ± 0.05	0.67	0.81	0.78
Changes of BCS	0.61 ± 0.06	0.65 ± 0.04	0.7 ± 0.08	0.58 ± 0.06	0.53 ± 0.07	0.7 ± 0.04	0.3	0.6	0.65

(1): Serum calcium concentration >8.5 mg/dl at calving day, 1 and 2 postpartum; (2): Serum calcium concentration ≤8.5 mg/dl on calving day and 1 or 2 postpartum; (3): Serum calcium concentration ≤8.5 mg/dl on calving day, 1 and 2 postpartum.

Table 5. Percentage of postpartum diseases and culling within 60 days after calving of primiparous and multiparous cows based on serum calcium status in the first 3 days after calving.

Item	Primiparous			Multiparous			p value		
	CON[1]	TSCH[2]	PSCH[3]	CON	TSCH	PSCH	Treat	Block	Treat × Block
	(n = 28)	(n = 82)	(n = 12)	(n = 22)	(n = 37)	(n = 90)			
Milk fever	3.57	2.43	0	0	2.70	3.33	0.80	0.50	0.63
Retained placental	3.57	4.87	16.66	4.54	10.81	16.66	0.06	0.54	0.74
Hypomagnesaemia day of calving	10.71	12.19	8.33	13.63	13.51	13.33	0.92	0.54	0.94
Hypomagnesaemia 1 day after calving	21.42	20.73	25.00	22.72	24.32	24.44	0.94	0.81	0.95
Metritis	7.14	12.19	33.33	9.09	18.91	38.88	0.003	0.44	0.93
Subclinical endometritis	25.92	30.86	36.36	22.22	40.00	50.58	0.21	0.49	0.66
Clinical endometritis	19.23	23.45	36.36	22.22	31.42	35.29	0.31	0.62	0.85
Hyperketonaemia	10.71	15.85	25	9.09	13.51	20	0.31	0.71	0.58
Subclinical mastitis	10.71	10.97	16.66	13.63	16.21	22.22	0.06	0.45	0.98
Culling rate within the first 60 DIM	3.57	4.87	8.33	4.54	8.1	8.88	0.31	0.67	0.95

(1): Serum calcium concentration >8.5 mg/dl at calving day, 1 and 2 postpartum; (2): Serum calcium concentration ≤8.5 mg/dl on calving day and 1 or 2 postpartum; (3): Serum calcium concentration ≤8.5 mg/dl on calving day, 1 and 2 postpartum.

= 0.03, CI = 1.06–06.06, 95%) but the percentage of retained placenta incidence was similar in TSCH and normocalcemic cows ($p = 0.32$, 95% CI = 0.43–11.84). Hypomagnesemia was similar on the day of calving ($p = 0.92$) and the first day after calving ($p = 0.94$) among cows with different serum Ca status in the first three days after calving.

Cows in the PSCH group were 6.43 times more at risk of developing metritis than normocalcemic cows ($p = 0.002$; 95% C = 1.51–21.56); while the incidence of metritis in TSCH cows was not significantly different from normocalcemic cows ($p = 0.22$; 95% CI = 0.56–6.52). Clinical ($p = 0.21$) and subclinical endometritis ($p = 0.31$) were not affected by serum Ca grouping after calving, block, and the interaction of Ca status in the block ($p > 0.05$). Although clinical endometritis was not affected by Ca status ($p = 0.31$); however, PSCH cows showed a higher incidence of clinical endometritis compared to normocalcemic cows (35.41% *vs* 20.83%; $p = 0.1$; CI = 0.79–5.76, 95%; Odds Ratio = 2.14).

Hyperketonemia was not affected by serum Ca grouping in the first three days after calving ($p = 0.3$), block ($p = 0.71$), and their interactions ($p = 0.58$).

Table 6. The effect of experimental treatments on metabolic disorders after calving.

Item Retained placental (n = 271)	Number, %	Estimate	SE	p value	Odds ratio	CI 95%
CON	4 (2/50)	Referent	–	–	–	–
TSCH	6.72 (8/119)	0.82	0.82	0.32	2.28	0.43–11.84
PSCH	16.66 (17/102)	1.83	0.83	0.03	6.28	1.09–36.06
Metritis						
(*n* = 271)						
CON	8 (4/50)	Referent	–	–	–	–
TSCH	14.28(17/109)	0.71	0.58	0.22	2.05	0.64–6.52
PSCH	38.23(39/102)	1.86	0.61	0.002	6.43	1.91–21.56
Clinical endometritis						
(*n* = 260)						
CON	20.83(10/48)	Referent	–	–	–	–
TSCH	25.86(30/116)	0.36	0.43	0.40	1.43	0.60–3.41
PSCH	35.41(34/96)	0.76	0.70	0.13	2.14	0.79–5.76
Subclinical endometritis						
(*n* = 260)						
CON	25.00(12/48)	Referent	–	–	–	–
TSCH	33.62(39/116)	0.51	0.41	0.21	1.68	0.74–3.81
PSCH	48.95(47/96)	0.85	0.48	0.08	2.35	0.9–6.17
Subclinical mastitis						
(*n* = 271)						
CON	12 (6/50)	Referent	–	–	–	–
TSCH	12.60(15/119)	0.80	0.55	0.14	2.24	0.74–6.74
PSCH	21.56(22/102)	1.62	0.68	0.01	5.09	1.31–19.73
Culling rate						
(*n* = 271)						
CON	4 (2/50)	Referent	–	–	–	–
TSCH	5.88 (7/119)	0.94	86.0	0.27	2.57	0.47–14.06
PSCH	8.82 (9/102)	1.53	1.00	0.12	4.61	0.63- 33.40

(1): Serum calcium concentration >8.5 mg/dl at calving day, 1 and 2 postpartum; (2): Serum calcium concentration ≤8.5 mg/dl on calving day and 1 or 2 postpartum; (3): Serum calcium concentration ≤8.5 mg/dl on calving day, 1 and 2 postpartum.

The incidence of subclinical mastitis was affected by the Ca status ($p = 0.06$), but not by the block effect ($P = 0.45$; $P = 0.98$). Cows in PSCH were 5.9 times most at the risk of developing mastitis ($p = 0.01$; 95% CI = 1.31–3.73). In agreement with retained placenta, uterine, and breast infections, culling percentage within 60 days of the first lactation in PSCH cows tended to increase compared to normocalcemic group cows ($p = 0.1$), so PSCH cows were 4.61 times more likely to be eliminated compared to normocalcemic cows ($p = 0.1$; 95% CI = 0.63%–40.40%). At the same time, the percentage of culling up to day 60 of lactation was similar in normocalcemic and TSCH groups ($p = 0.27$).

The present study showed that cows with TSCH had the same ability as normocalcemic cows to adapt to postpartum physiological changes. In contrast, PSCH cows had lower metabolic adaptation for Ca status than normocalcemic and TSCH cows.

Discussion

The results of the current study briefly showed that the primiparous and multiparous cows have the highest TSCH and PSCH percentages, respectively. Calcium status did not affect the BCS changes, the incidence of milk fever, hypomagnesemia and hyperketonemia, and clinical and subclinical endometritis. The incidence of retained placenta, metritis, and subclinical mastitis was affected by Ca status, so PSCH cows experienced 6.28, 6.43, and 5.9 times more retained placenta, metritis, and subclinical mastitis than normocalcemic cows, respectively. The culling rate within the first 60 DIM for PSCH cows was 4.61 times more than for normocalcemic cows. Based on the study results, cows with PSCH had a higher incidence of retained placenta, uterine infections, subclinical mastitis, and culling rate, but cows with TSCH were similar to healthy cows in terms of metabolic disorders and culling rate.

Precise homeostatic regulation of Ca is essential to ensure the survival and production of livestock. The inability to regulate blood Ca concentrations leads to clinical hypocalcemia, which affects less than 5% of postpartum dairy cows (Goff *et al*., 2008). Still, in clinical hypocalcemia, the reduction in blood Ca concentration has no clinical signs, affecting a large proportion of postpartum cows (Reinhardt *et al*., 2011). The results of studies showed that the concentration of Ca in the blood reaches its lowest level in 12 to 24 hours after birth (Goff *et al*., 2008); however, animals prone to metritis have the lowest concentration of Ca between 24 and 48 hours at postpartum (Martinez *et al*., 2012). Thus, the classification of subclinical hypocalcemia may depend on the sampling time relative to the calving time (Neves *et al*., 2017). However, in several studies, despite differences in postpartum sampling, a correlation has been reported between subclinical hypocalcemia and postpartum and even culling diseases (Martinez *et al*., 2012; Rodríguez *et al*., 2017; Venjakob *et al*., 2018). Also, in a study, 78% of subclinical hypocalcemia has been reported to be less than or equal to 2.41 mmol/l within 48 hours after calving with a cut point of serum Ca (Rodríguez *et al*., 2017) and in another study, 47% of multiparous cows experienced subclinical hypocalcemia (Reinhardt *et al*., 2011). These discrepancies may be due to different Ca cut points for subclinical hypocalcemia (Reinhardt 2011; Rodríguez *et al*., 2017).

Besides, McArt and Neves (2020) reported temporary subclinical hypocalcemia in less than 20% of primiparous and multiparous cows. In comparison, the incidence of persistent hypocalcemia in primiparous and multiparous cows was 22.9% and 12.9%, respectively. However, in the present study, the incidence of each of the Ca classifications was higher; especially the highest incidence in the primiparous cows was related to temporary hypocalcemia (67.21%). In multiparous cows, it was related to persistent hypocalcemia (60.40%). These results emphasize the changes in nutritional management and strategies to prevent subclinical hypocalcemia in the postpartum.

Studies on the correlation between hypocalcemia and periparturient diseases such as retained placenta, ketosis, and abomasal displacement are conflicting. For example, Massey *et al*. (1993) identified hypocalcemia as a risk factor for the development of abomasal displacement; but others did not observe any correlations between the two diseases (LeBlanc *et al*. 2005; Chamberlin *et al*., 2013), which was in agreement with the present study on abomasal displacement.

In uterine health, subclinical hypocalcemia is associated with metritis and retained placenta (Curtis *et al*., 1983; Goff and Horst. 1997). Under subclinical hypocalcemia, immune function is compromised and muscle contraction is reduced (Murray *et al*., 2008). Studies have shown that the incidence of metritis and retained placenta in cows with subclinical hypocalcemia is more common and cows with multiple subclinical hypocalcemia experienced 4.85 times more metritis (Martinez *et al*., 2012), which supported the results of the present study on metritis and retained placenta. Saiful Bari *et al*. (2022) reported the prevalence of subclinical mastitis ranging from about 20% to 80%, with an average of 50% caused by nutritional and non-nutritional problems. They have stated that periparturient diseases such as hypocalcemia are among the common factors in the prevalence of subclinical mastitis. Decreasing blood Ca may induce subclinical mastitis.

If subclinical hypocalcemia is used to predict postpartum disease, a different cut point of serum Ca may be needed. Accordingly, the best predictive cut point values for ketosis, abomasal displacement, retained placenta, and postpartum metritis are different (Ca ≤ 1.93, Ca ≤2.05, Ca ≤ 2.05, and Ca ≤1.2 mmol/l, for ketosis, retained placenta, metritis, and abomasal displacement, respectively). Martinez *et al*. (2012) reported a decrease in Ca levels on days 1, 2, 3, 4, 7, and 12 of lactation compared to healthy cows. Also, in other studies, a correlation has been reported between inpatient hypocalcemia and retained placenta on the second day after calving, which is due to a decrease in intracellular Ca levels in the cells of nuclear polymorphism in hypocalcemia and the consequent decrease in phagocytic activity of the body (Kimura *et al*., 2002; Kimura *et al*., 2006; Martinez *et al*., 2012). The results of the present study also showed a higher incidence of anomalies in the PSCH group compared to the normocalcemic and TSCH groups, which was in agreement with McArt and Neves (2020). They have reported that primiparous and multiparous cows adapted well with TSCH to the conditions of early lactation. In contrast, cows with PSCH, regardless of the parity, were at an increased risk for early lactation disorders and culling events. Interestingly, they have found that cows with TSCH produced more milk than healthy cows, but the mechanism of this event is not clear yet.

The findings regarding the association between hypocalcemia and ketosis are also contradictory. Some studies agreed with the present study (Martinez *et al*., 2012; Neves *et al*., 2017) and some (Rodriguez *et al*., 2017) were in the opposite direction of the study. Clinical trials, regardless of lactation, showed 5.5 times more ketosis than healthy cows. This finding agreed with Curtis *et al*. (1983). This correlation may be due to changes in glucose metabolism. For example, Witzel and Littledike (1973) reported that hypocalcemia reduces the efficiency of pig glucose utilization, which appears to be mediated by reduced insulin secretion in low blood Ca. However, this mechanism is still unknown in ruminants.

Conclusion

The results of this study showed that the serum Ca status of cows in the first three days after calving can affect the percentage of metabolic disorders in cows differently. Cows with persistent hypocalcemia showed higher metabolic disorders such as retained placenta, metritis, clinical endometritis, and subclinical mastitis than those with transient hypocalcemia and healthy cows. However, the incidence of these disorders was similar in temporary hypocalcemic and healthy cows. Also, the culling percentage in the first 60 days of lactation was higher in cows with persistent hypocalcemia than in cows with temporary hypocalcemia and healthy cows. Therefore, it seems that TSCH cows are well adapted to the transition from pregnancy to lactation and the improvement of calcium status occurs with the advancement of lactation in these cows.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors contributions

All authors contributed equally to this study. All authors read and approved the final manuscript.

Data availability

All data are provided in the manuscript.

Reference

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