J. Dairy Sci. 101:1–4
https://doi.org/10.3168/jds.2017-13767

Short communication: Test of the relationship between prepartum milk leakage and blood calcium concentration in Holstein cows—An observational study

Ehsan Mahjoubi,*[1] Davood Zahmatkesh,* Mehdi Hossein-Yazdi,† Mohammad Hadi Khabbazan,* and Mohammad Reza Samadian‡
*Department of Animal Science, University of Zanjan, Zanjan, Iran 45371-38791
†Department of Animal Science, Arak University, Arak, Iran 38156-88349
‡Qiam Dairy Complex, Isfahan, Iran 83145-46611

ABSTRACT

Prepartum milk leakage happens in some pregnant dairy cows close to calving. It has been hypothesized that low blood Ca is a cause of this event. To investigate the possible reason(s) of milk leakage, 137 multiparous pregnant Holstein cows were enrolled in the experiment and categorized by the presence (72 heads; leak group) or lack (65 heads; control group) of milk leakage before calving. The concentrations of Ca and P and the length of the teat were measured for all cows. Data showed that Ca concentration was not different between cows in the leak group (7.90 mg/dL) and those in the control group (7.99 mg/dL). Moreover, neither P concentration (4.62 vs. 4.54 mg/dL) nor teat length (4.28 vs 4.10 cm) differed between leak and control groups. Milk yield was greater for the leak group (53.6 kg/d) compared with the control group (50.1 kg/d) through 4 mo in milk. The leakage did not affect the odds of postpartum disorders such as retained placenta, metritis, mastitis, displaced abomasum, or lameness occurrence. The current results show that hypocalcemia is not a reason for observed prepartum leakage and that cows in the leak group produced more milk in the subsequent lactation period.
Key words: mammary gland, milk leakage, prepartum calcium level

Short Communication

Milk leakage (**ML**) or milk dripping is defined as a condition in which milk loss happens through the teat end not related to milking. Milk leakage is important as it has been linked to mastitis in several studies (Schukken et al., 1993; Elbers et al., 1998; Waage et al., 2001). Persson Waller et al. (2003) found no relationship between ML and milk production, parity, stage of lactation, or estrus status. Klaas et al. (2005) indicated that high peak milk flow rate, short teats, teat canal protrusion, inverted teat ends, and early lactation increased the risk of ML in multiparous cows. To our knowledge, there is no information about ML just before calving. Waage et al. (2001) reported ML in heifers around the time of parturition as a risk factor of mastitis, but the cause of this ML was not determined. Because ML in dairy cows is a symptom of impaired function of the teat sphincter (Klaas et al., 2005), it is believed that Ca deficiency can affect this disorder; smooth muscle contraction, including teat sphincter smooth muscle, is dependent in part on the Ca concentration in the cytosol (Constable et al., 2012; Goff, 2014). Therefore, the objective of this observational study was to investigate the possible relationship of prepartum ML with Ca and P concentrations and its effect on the subsequent milk yield and disorders such as hypocalcemia. We hypothesized that a low plasma Ca can lead cows to have more leakage during the prepartum period.

The study was carried out from January to March 2017 in the Qiam Dairy Complex (Qiam Animal Husbandry and Agriculture Co., Isfahan, Iran) that houses 4,400 dairy cows of the Holstein breed with an average daily production of 42 kg/lactating cow. A total of 137 multiparous Holstein cows were enrolled in this study.

When milk leaked (any ML from teats before calving) from a dry cow (leak group; 72/137 cows), a blood sample was taken and teat length was measured. Visual inspection of close-up cows during the routine daily monitoring, every 2 to 3 h, was used to detect any ML from the lying cows with the milk dropping from the teat on the floor. Blood was taken from the coccygeal vein using an evacuated tube without anticoagulant (Vacumed no additive; FL Medical, Torreglia, Italy). Serum samples were taken following centrifugation at 2,500 × g for 10 min and were stored at –20°C for

Received August 30, 2017.
Accepted December 13, 2017.
[1]Corresponding author: e_mahjoubi@znu.ac.ir

later analysis. After thawing, the serum was analyzed for concentrations of Ca (colorimetric cresolphthalein complexone method; Darman Faraz Kave, Tehran, Iran) and P (colorimetric molybdate method; Ziest-Chem Diagnostics, Tehran, Iran). At the same time, a similar cow was selected based on the expected calving time and the symptom of calving without ML (control group), and the same measurements were carried out. After parturition, ketosis was diagnosed by blood sampling daily throughout the 10 DIM using the blood β-ketone strips (FreeStyle; Abbott Diabetes Care, Doncaster, Australia). Milk yield was recorded monthly by the regular program of the dairy farm so that the milk yield at each shift at 0700, 1500, and 2300 h was recorded and total milk yield was calculated.

Disorders were defined as follows: retained placenta as placenta not expelled by 24 h; ketosis as the blood ketone bodies ≥1.2 mmol/dL; displaced abomasum as the presence of a pinging noise that sounds like a tap dripping into a steel bucket by listening over the abdomen with a stethoscope; mastitis as the presence of coagulant during the predipping process in the milking parlor; metritis as the presence of fetid red-brown watery uterine discharge at 5 to 20 DIM; lameness as cows with a locomotion score of ≥4 on a 5-point scale (Sprecher et al., 1997); and milk fever as cows in head-down recumbency with paresis after parturition that responded to intravenous Ca.

All statistical analyses were carried out with SAS (version 9.4; SAS Institute Inc., Cary, NC). Serum mineral concentrations and teat length were analyzed with cow as the subject using PROC GLM of SAS (Littell et al., 2006). Milk yield was analyzed as repeated measures using PROC MIXED of SAS (Littell et al., 2006). Data from diseases (retained placenta, displaced abomasum, metritis, mastitis, milk fever, and ketosis) were analyzed with PROC GENMOD with binomial distributions. For each binary variable, ML status at precalving (leak vs. control) was offered as a covariate. A logistic regression model for ML was calculated using the GENMOD procedure with generalized estimating equations (Liang and Zeger, 1986) with compound of symmetry covariance structure (Dohoo et al., 2003) using binomial distribution. The logistic regression model included the fixed effects of treatment.

Data are reported as least squares means. Statistical significance was declared at $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a trend toward significance using Tukey's multiple comparison test.

The prepartum serum concentrations of Ca and P, teat length, and milk yield are presented in Table 1. The average day when ML was diagnosed and blood samples were obtained was 1.4 ± 0.9 d before calving (mean ± SD). In contrast with our hypothesis, there was no difference in Ca concentration ($P = 0.70$) between leak and control cows. According to Oetzel and Eastridge (2013), who defined postpartum hypocalcemia as serum Ca concentration ≤8.5 mg/dL, all cows (leak and control groups) were hypocalcemic during the prepartum period. It should be kept in mind that Oetzel and Eastridge (2013) defined this cut point for postcalving cows (not precalving cows). Although it is believed that hypocalcemia reduces the contraction of the teat sphincter muscle responsible for closure of the teat orifice (Goff, 2014), the current data show that precalving Ca concentration is not a causative factor for ML. We are not aware of any data in which prepartum Ca is <8.5 mg/dL and to what extent this low Ca concentration can affect cow performance. More recently, Neves et al. (2017) showed that multiparous cows with Ca concentrations ≤9.6 mg/dL (too far from the achieved values in the current study) in the prepartum period and cows in third or greater parity had a higher risk of being categorized as subclinical hypocalcemic at calving. Neves et al. (2017) defined this value as a cut point for prepartum animals that are more prone to be hypocalcemic after calving. Because the average days to calving in 2 groups was 1.4 ± 0.9, low Ca concentration was partly anticipated as reported by Moore et al. (2000), who showed that Ca level is slightly lesser when a cow is close to parturition. Acidogenic diets are hypothesized to increase serum Ca by increasing Ca mobilization from bone, as indicated by elevated serum hydroxyproline (Goff et al., 1991). Despite the fact that the cows enrolled in the current study were fed an anionic diet, Ca level was below the defined threshold for hypocalcemia. Green et al. (1981) also reported a prepartum Ca level of close to 8 mg/dL at −1 d to calving for cows fed a high-Ca diet. Moreover, P concentration did not differ across treatments ($P = 0.62$). Serum P is closely associated with serum Ca concentration because both make up the hydroxyapatite lattice structure of bone. Because serum Ca did not differ with treatment, serum P was expected to be similar.

Table 1. Average daily milk yield (first 4 mo of lactation), serum Ca and P concentrations at the time milk leakage was diagnosed, and teat length in cows without milk leakage (Control) or with milk leakage (Leak)

Item	Treatment		SEM	P-value
	Control	Leak		
Milk, kg	50.15	53.63	1.24	0.04
Calcium, mg/dL	7.99	7.91	0.15	0.70
Phosphorus, mg/dL	4.54	4.62	0.12	0.62
Teat length, cm	4.30	4.280	0.14	0.93