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PREPARTUM URINE pH AS A PREDICTOR OF LEFT DISPLACEMENT OF ABOMASUM

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ABSTRACT

The aim of this study was to investigate the relationship between prepartum urine pH, blood ionized calcium, and blood pH levels and the incidence of left displacement of abomasum (LDA). Holstein dairy cows (n=115) in their second lactation were selected for the study. Urine and blood samples were collected seven days prior to the expected calving date, and urine pH, blood ionized calcium, and blood pH levels were measured. Of 115 cows, 13 (11%) had LDA at 15 (±7) days postpartum. Urine pH (LDA group: 6.11; healthy control group: 6.65) and blood pH (LDA group: 7.27; healthy control group: 7.37) levels were lower (P<0.05) in the LDA group than in the healthy control group. The sensitivity of urine pH, blood ionized calcium, and blood pH was 46, 62, and 84.6%, respectively, with corresponding specificities of 80.2, 61, and 44.1%. The results revealed that the measurement of urine pH levels in the last days of the dry period represents an inexpensive and fast method for predicting LDA. Future studies should incorporate dry matter intake calculations and larger sample sizes to evaluate the usefulness of urine pH in predicting LDA in practice.

Keywords: Urine pH, LDA, DCAD, overacidification.

INTRODUCTION

Left displacement of abomasum (LDA) is a multifactorial disease with a 20% incidence rate among high-yielding dairy cows (Dawson *et al.*, 1992). The economic impact of reduced milk yield, substantial treatment costs, and herd removal is approximately 250–400 USD per affected animal (Bartlett *et al*., 1995). As a result of increasing milk demand, LDA has become a significant problem in the dairy industry. Severeal factors such as ad libitum feeding during the dry period (Lacasse *et al*., 1993), low quality roughage (Dawson *et al.*, 1992), genetics (Uribe *et al*., 1995), and postpartum negative energy balance (Cameron *et al.*, 1998) play an important role in the etiology of LDA. Aditionally, decreased feed intakes, reduced ruminal fill, and decreased abomasal motility are involved in the ethiopathogenesis of LDA. It has been reported that hypocalcemia increases the incidence of LDA by 4.8×, probably due to the inhibitory effects of low blood calcium levels on abomasal motility, resulting in the accumulation of gases in the abomasum (Massey *et al*., 1993). Therefore, the prevention of hypocalcemia is considered to be one of the main strategies for reducing LDA incidence. Dietary cation anion difference (DCAD) is a widely used procedure for preventing hypocalcemia in dairy herds. High blood pH values interfere with the action of the parathyroid hormone on its target tissues, i.e., bone and kidneys (Goff, 2008). Metabolic alkalosis blunts the response of animals to the parathyroid hormone and increases the risk of subclinical hypocalcemia and milk fever. Metabolic alkalosis is attributed to the intake of diets that are high in cations (especially sodium and potassium) and low in anions (especially chloride and sulfur). Precalving diets, which are high in anions and low in cations or contain anionic salts such as ammonium sulfate, magnesium sulfate, and ammonium chloride, decrease the occurrence of hypocalcemia (Goff, 2008; DeGaris and Lean, 2009). To prevent hypocalcemia, it is necessary to decrease levels of cations (e.g., potassium) and increase levels of anions (e.g., chloride and sulfate) in the feed. By reducing cation and increasing anion levels, the ability of the parathyroid hormone to regulate blood calcium levels is restored (Goff, 2008). Reducing DCAD prepartum significantly decreases the incidence rates of hypocalcemia and milk fever in dairy cows (Block, 1984; Tucker *et al*., 1992; Goff and Horst 1997a; Goff and Horst 1997b; Goff, 2008). The measurement of urine pH represents an inexpensive and fairly accurate assessment method of the appropriate anion supplementation level (Block, 1984; Jardon, 1995; Goff and Horst 1997a; Goff and Horst 1997b; Goff, 2008; DeGaris and Lean, 2009). Urine pH is >8.2 on high-cation diets and ~7.8 on low-cation diets. For the optimal control of hypocalcemia, the average urine pH of Holstein cows should be 6.2–6.8, which essentially requires the addition of anions to the ration (Goff, 2008). Urine pH level>6.8 following the addition of anionic salts are indicative of insufficient DCAD and increased risk of hypocalcemia during the postpartum period. On the other hand, urine pH levels <6.2 are indicative of overacidification associated with DCAD. DCAD-induced overacidification causes a significant reduction in dry matter intake (DMI) of the animals during a period when they are vulnerable to postpartum diseases such as LDA (Cook *et al*., 2006). Reduction of DMI is a major factor in the pathogenesis of LDA, because decreased ruminal fill enables the movement of the distended abomasum within the abdominal cavity. The objective of this study was to evaluate the relationship between prepartum urine pH levels and the incidence of postpartum LDA.

MATERIALS ANDMETHODS

Holstein dairy cows (n=115) in their second lactation with an expected calving date within the subsequent seven days and without any history of LDA in their first lactation, were selected for the study, which was conducted in April and May. All cows were from the same herd and yield group, and had the same management and feeding conditions. Their mean milk production in the last lactation was 9880 ± 68.7 kg (305d) per cow. The cows were fed a diet that had a DCAD value (Na+K- Cl - S, mEq/kg dry matter) of -71 obtained by the supplementation of 160 g/d of magnesium sulfate ($MgSO_4 \cdot 7H_2O$) and 120 g/d of ammonium chloride for 21 d per cow before the expected calving date (Tucker *et al*., 1992). After calving, all cows were fed the same lactation diet. Diets were formulated according to NRC recommendations (2001) and delivered as a total mix ration.

Blood and urine samples were collected from all cows at the same time. Seven days prior to the expected calving date, blood samples were collected by jugular venipuncture from each cow into 10-ml evacuated glass tubes containing lithium heparin for blood ionized calcium and blood pH measurements. All blood samples were transported to the laboratory within two hours and analyzed in same day.

Urine samples were collected via urinary catheter after cleansing the region. Urine pH was measured immediately with an electronic digital pH meter (PT-15, Sartorius, Goettingen, Germany), which was calibrated following each measurement. Blood ionized calcium and blood pH levels were measured in an automatic biochemical analyzer (Stat Profile PHOX, Nova Biomedical, Waltham, MA, USA). The clinical diagnosis of LDA was based on the detection of high-pitched sounds (ping) on the left abdomen over the 9–12th intercostal region. LDA was confirmed by observation of the displaced abomasum at the time of omentopexy. Cows with LDA were assigned to the LDA group, while cows without LDA were assigned to the healthy control (HC) group.

To assess normality in the distribution, the data were first analyzed with the Kolmogorov-Smirnov test. Because the groups were independent, and the data were not normally distributed in all categories, a nonparametric statistical test was required. Therefore, differences between the LDA and HC groups were assessed with the Mann-Whitney U test (Sigma Plot® 12, Systat Software Inc., Chicago, IL, USA). Additionally, in the two groups, variables were evaluated by logistic regression to determine risk factors and odds ratios. Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Receiver operating characteristics (ROC) curves were generated with MedCalc 9.2 (MedCalc Software, Ostend, Belgium). Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were determined using 2×2 tables according to cut-off values established by ROC curves that minimized the sum of false negative and false positive results. This study was approved by the University of Uludag Ethical Committee in accordance with the Animal Welfare Guidelines (2015-08/01).

RESULTS

In this study, cows calved at an average of 8 (±2) d. Of the 115 cows included in this study, 13 (11%) were diagnosed with LDA at 15 (±7) d postpartum. Therefore, the LDA group consisted of 13 cows, while the HC group consisted of 102 animals. The LDA group had significant lower urine and blood pH levels than the HC group; however, there was no significant difference in ionized calcium levels between the two groups (Table 1). Cut-off points of ≤5.76, >1.38 mmol/L, and <7.33 were established by ROC curves for urine pH, blood ionized calcium, and blood pH levels, respectively (Figure 1). The sensitivity of urine pH, blood ionized calcium, and blood pH was 46, 62, and 84.6%, respectively, while the corresponding specificity was 80.2, 61, and 44.1% (Table 2). The predictive values of positive and negative tests, positive and negative likelihood ratios, and areas under the curves for urine pH, blood ionized calcium, and blood pH cut-off points determined by ROC curves are shown in Table 2. The results revealed a negative correlation between urine pH and blood ionized calcium levels (r: -0.285, P = 0.002).

Table-1. Mean (± SE) urine pH, serum ionized Calcium (mmol/L) and blood pH for LDA and healthy groups

	LDA Group	Healthy Group	*P* value
Urine pH	6.11 ± 0.2	6.65± 0.1	$P < 0.05$
Serum iCa^{++}	1.39 ± 0.01	1.36 ± 0.01	Not significant
Blood pH	7.27 ± 0.01	7.32 ± 0.01	$P < 0.05$

Table-2: Sensitivity (Se,%), specificity(Sp,%), predictive values of a positive (pV$^+$,%) and negative (pV$^-$,%) test, positive (LR$^+$) and negative (LR$^-$) likelihood ratios, areas under the ROC curves for urine pH, blood ionized Calcium and blood pH cutoff points determined by ROC curves

Data and cutoff value	Se	Sp	pV$^+$	pV$^-$	LR$^+$	LR$^-$	Area under the ROC curve
Urine pH (≤ 5.76)	46	80.2	23.1	92	2.33	0.67	0.624
Blood Ca (> 1.38mmol/L)	62	61	18.7	94	1.81	0.50	0.645
Blood pH (< 7.33)	84.6	44.1	16.2	95.7	1.51	0.35	0.691

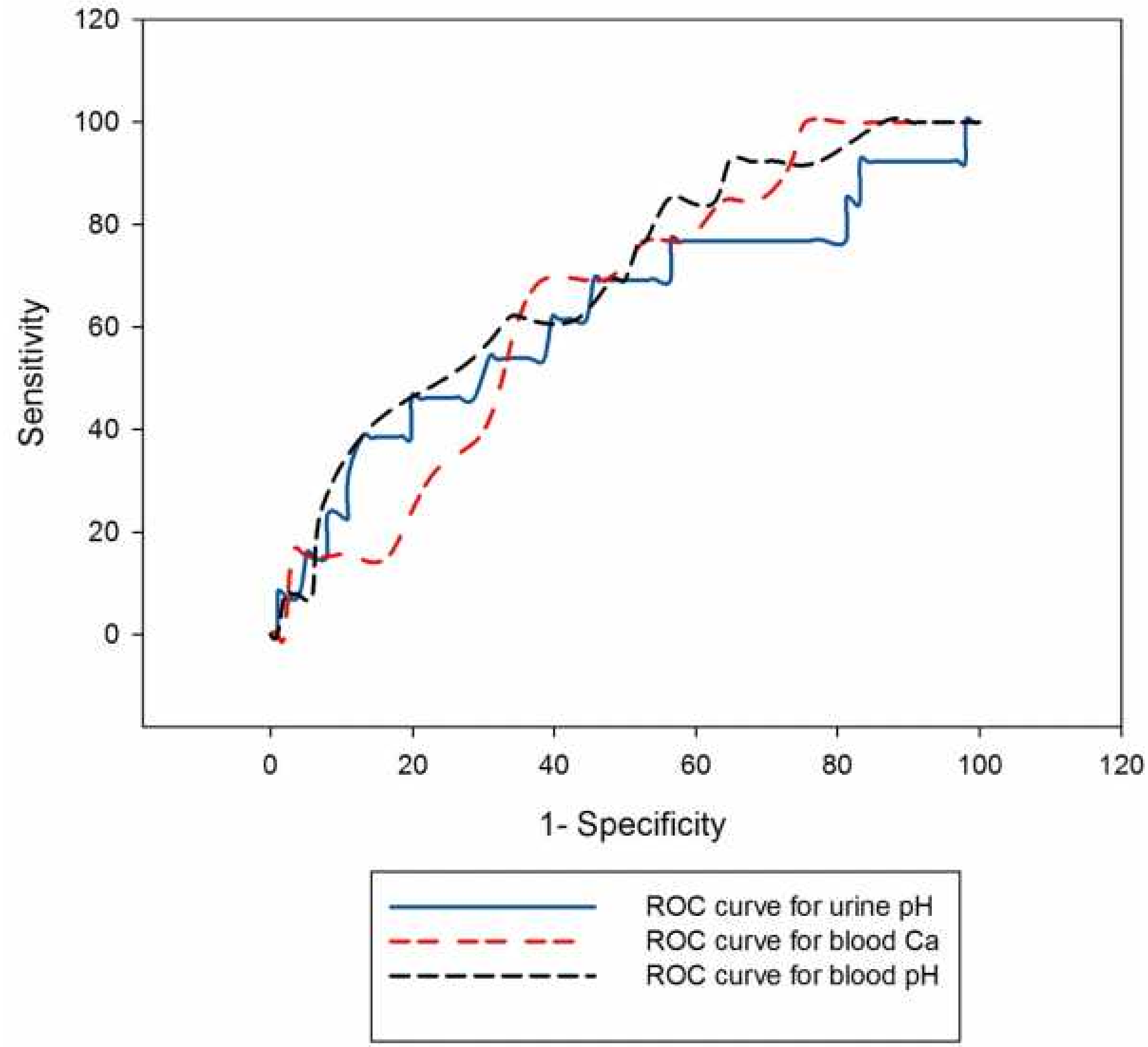

Figure-1: ROC curves for urine pH, serum ionized calcium and blood pH levels

DISCUSSION

LDA, which afflicts dairy cattle during the postpartum stage, causes significant economic losses to the dairy industry. Hypocalcemia is involved in the etiopathogenesis of LDA (Massey, 1993; Van Winden, 2003). Geishauser *et al*. (1998), who reported that blood calcium levels are significantly reduced in cattle prior to abomasum displacement, concluded that low blood calcium levels in the second week of postpartum could be used to predict LDA (Geishauser *et al*., 1998). On the other hand, Madison and Trout (1988) argued that only blood calcium levels as low as those detected in milk fever could cause a reduction in abomasal motility, and that subclinical hypocalcemia could not be an etiological factor for LDA (Madison and Trout, 1988). Massey *et al.* (1993) postulated that mild hypocalcemia could also be involved in the pathogenesis of LDA, because of its inhibitory effects on ruminal motility, resulting in decreased ruminal filling (Massey *et al*., 1993), and Van Winden *et al*. (2003) reported that as calcium is a messenger in parietal cells of abomasum, hypocalcemia could cause displacement of abomasum by reducing abomasal acid secretion.

Regardless of the exact mechanism, the incidence of LDA is higher in hypocalcemic cattle than in non-hypocalcemic cows. DCAD is used to prevent hypocalcemia in dairy cattle. The measurement of urine pH is widely used for monitoring DCAD. There is a strong correlation between high urine pH levels 48 h prior to parturition and increased incidence of hypocalcemia during the postpartum stage (Seifi *et al*., 2004). For the optimum control of hypocalcemia, urine pH levels in Holstein cattle should be 6.2–6.8 following the consumption of anionic salts in the feed (Goff, 2008). On the other hand, pH levels >6.8 are associated with a high incidence of hypocalcemia. In this study, urine pH levels were <6.8 in both groups. Clinical hypocalcemia was not observed in any of the animals. Additionally, there were no differences in blood ionized calcium levels between the two groups. Based on these results, we concluded that LDA was not associated with high DCAD or hypocalcemia.

Urine pH levels <6.2 are indicative of over-acidification (Cook *et al*., 2006). The lower pH levels in the LDA group compared to the HC group (6.11 versus 6.65) could be attributed to overacidification due to the consumption of anionic rations. A slight decrease in the blood pH levels (7.35–7.45) has been reported after reducing DCAD (Constable, 1999). However, in this study, the LDA group had low blood pH levels, probably due to overacidification.

In addition to DCAD, acute or sub-acute ruminal acidosis could contribute to the acidification of urine due to increased renal excretion of H^+. However, urine pH itself is not a good indicator of ruminal acidosis, because of the simultaneous increase in excreted inorganic phosphate levels, which increase urine pH levels (Enemark, 2008).

Overacidification is associated with a reduction in DMI (Seifi *et al*., 2004). Charbonneau *et al*. (2006) reported an 11.3% reduction in DMI after lowering DCAD from +300 to 0 mEq/kg and concluded that acidification of urine pH beyond 7 could further decrease DMI. Comparably, the ration used in the study had a DCAD of -71, which is considerably more acidic than the values provided above. Low feed consumption and low ruminal fill during the transition period are major risk factors for LDA (Shaver, 1997). In this study, LDA was probably caused by reduced feed consumption in overacidified cows.

This was the first study that investigated the relationship between prepartum urine pH levels and postpartum LDA incidence. Theoretically, high urine pH levels would be associated with a higher incidence of LDA due to hypocalcemia. However, in this study, low urine and blood pH levels probably as a result of DCAD-induced overacidification were associated with increased incidence of LDA.

The measurement of urine pH during the last days of the dry period represents an inexpensive, fast, and useful method for predicting LDA. However, caution should be exercised when low urine levels are detected in prepartum dairy cows with DCAD. Future studies should incorporate dry matter intake calculations and larger sample sizes to evaluate the usefulness of urine pH in predicting LDA in practice.

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