

DOI 10.24425/pjvs.2020.134691

Short communication

Peripartum changes in serum activities of three major alkaline phosphatase isoenzymes in Holstein dairy cows

A. Chiba^{1,2}, R. Onomi³, K. Hatate⁴, T. Moriyama², A. Goto^{2,4}, N. Yamagishi^{1,3,4}¹United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, Japan²Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8550, Japan³Graduate School of Veterinary Life Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8550, Japan⁴Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8550, Japan

Abstract

This study investigated changes in serum levels of hepatic, bone, and intestinal alkaline phosphatase (ALP) isoenzymes (ALP2, ALP3, and ALP5, respectively) in Holstein cows around parturition. Tartrate-resistant acid phosphatase 5b (TRAP5b) activity and calcium (Ca) concentrations were also measured. We analyzed blood samples from 11 late-pregnant heifers (primiparous group) and 13 multiparous (2–4 lactations; multiparous group) cows at 3 weeks (18–24 days prepartum; -3 weeks), 2 weeks (17–11 days prepartum; -2 weeks), and 1 week (10–4 days prepartum; -1 weeks) before parturition; the day of calving (within 12 h post-calving; day 0); and 5 days postpartum (5 days). ALP3 activity was significantly higher in the primiparous group than in the multiparous group, whereas the activities decreased significantly in both groups after 5 days. ALP2 and ALP5 activities did not change, whereas ALP2 activity was significantly higher in the primiparous group than in the multiparous group. TRAP5b activity was significantly higher in the primiparous group than in the multiparous group and showed a transient significant increase at day 0. Ca concentration significantly decreased at day 0 in both groups; the Ca level at day 0 was significantly higher in the primiparous group than in the multiparous group. These data show that ALP3 activity in serum may indicate a change in osteoblastic bone formation after calving, but further study is needed to determine the clinical application for measuring ALP isoenzymes in bovine medicine.

Key words: agarose gel electrophoresis (AGE), alkaline phosphatase isoenzyme, periparturient cow

Introduction

A commercial agarose gel electrophoresis (AGE) kit can discriminate among three major alkaline phosphatase (ALP) isoenzymes in bovine sera, namely, a hepatic ALP isoenzyme derived from hepatic tissue (ALP2), a bone ALP isoenzyme derived from osteoblasts (ALP3), and an intestinal ALP isoenzyme derived from intestinal tissue (ALP5) (Chiba et al. 2020). We recently reported that the AGE method using both protease- and protease and neuraminidase-treated sera could be used to measure serum activities of these isoenzymes in lactating Holstein cows (Chiba et al. 2020). Dairy cows often suffer from metabolic diseases such as ketosis and hypocalcemia after parturition; these may result from hepatic dysfunction due to mobilized body fat and inhibited bone metabolism (Goff and Horst 1997). Therefore, this study investigated changes in circulating ALP isoenzyme activities in cows around parturition.

Materials and Methods

The experimental design was approved by the Obihiro University of Agriculture and Veterinary Medicine Laboratory Animal Care and Use Committee (approval #19-49). We took blood samples from 11 late-pregnant heifers (primiparous group) and 13 multiparous (2-4 lactations; multiparous group) Holstein cows around parturition. They were given water and hay *ad libitum* with a total mixed ration containing corn and grass silage, concentrated mix, vitamin, and minerals. All diets fell within normal compositional ranges typically fed to transition dairy cows on commercial dairy farms in Japan (e.g., Ca: 0.6% dry matter [DM], Mg: 0.2% DM, dietary cation-anion difference: 7.8-23.3 mEq/100 g DM). All cows underwent normal calving and were declared clinically healthy during blood sampling. Blood samples were obtained via coccygeal venipuncture at 3 weeks (18-24 days prepartum; -3 weeks), 2 weeks (17-11 days prepartum; -2 weeks), and 1 week (10-4 days prepartum; -1 weeks) before parturition; the day of calving (within 12 h post-calving; day 0 days); and 5 days postpartum (5 days). After centrifugation, the sera were stored at -60°C before analyses.

AGE was performed using an automatic electrophoresis system (Epalyzer-2; Helena Laboratories, Saitama, Japan), as described previously (Chiba et al. 2020). Total ALP (t-ALP) activity and calcium (Ca) concentration were measured enzymatically using a biochemical autoanalyzer (TBA-120FR; Toshiba Medical Systems, Tokyo, Japan). A fluorometric method was used to detect tartrate-resistant acid

phosphatase 5b (TRAP5b) activity and was modified from Janckila's method (Matsuo et al. 2014) using a multimode microplate reader (NIVO 5S, PerkinElmer, Waltham, MA, USA).

The statistical analyses were performed using SAS Enterprise Guide version 7.1 (SAS Institute, Cary, NC, USA). All numerical data are presented as mean \pm standard deviation. The data were analyzed using the mixed model (PROC MIXED) and the minimum variance quadratic unbiased estimation method (MIVQUE) for repeated measures. The model was assessed with fixed treatment effects [(primiparous vs. multiparous), day (-3 weeks, -2 weeks, -1 week, day 0, and 5 days), and day \times treatment interaction] and the random effect of individual animals. The ante-dependence covariate structure was utilized, and Tukey's post hoc comparison test was employed to explore differences between the groups. A p -value < 0.05 was considered significant.

Results and Discussion

Figure 1 shows the changes in t-ALP, ALP2, ALP3, ALP5, and TRAP5b activities and Ca concentration around parturition. T-ALP and ALP3 activities were significantly higher in the primiparous group ($p < 0.01$ and 0.001) than in the multiparous group during the entire period. These parameters remained unchanged in both groups from -3 weeks to day 0 but significantly decreased at 5 days ($p < 0.001$). By contrast, TRAP5b activity was significantly higher in the primiparous group ($p < 0.05$, 0.001 , and 0.001) than in the multiparous group from -2 weeks to 5 days and transiently showed a significant increase at day 0 ($p < 0.001$). Ca concentration significantly decreased at day 0 in both groups ($p < 0.001$); the Ca levels at day 0 were significantly higher ($p < 0.01$) in the primiparous group than in the multiparous group. The decrease in ALP3 activity after parturition suggests a diminution of osteoblastic bone formation, whereas the transient increase in TRAP5b activity at day 0 indicates acceleration of osteoclastic bone resorption in response to hypocalcemia at calving (Kim et al. 2010). ALP2 and ALP5 activities did not significantly change during the entire period. However, ALP2 activity in the primiparous group was significantly higher ($p < 0.05$ or 0.001) at -2 weeks and day 0 than in the multiparous group, suggesting that liver function was more active in younger cows.

In conclusion, serum ALP3 activity was useful for evaluating bone formation related to parity in periparturient cows. It remains unclear whether ALP2 and ALP5 activities reflect the functions of liver and intestinal tissues around parturition. A further study is needed to pursue the possibility of clinical applica-

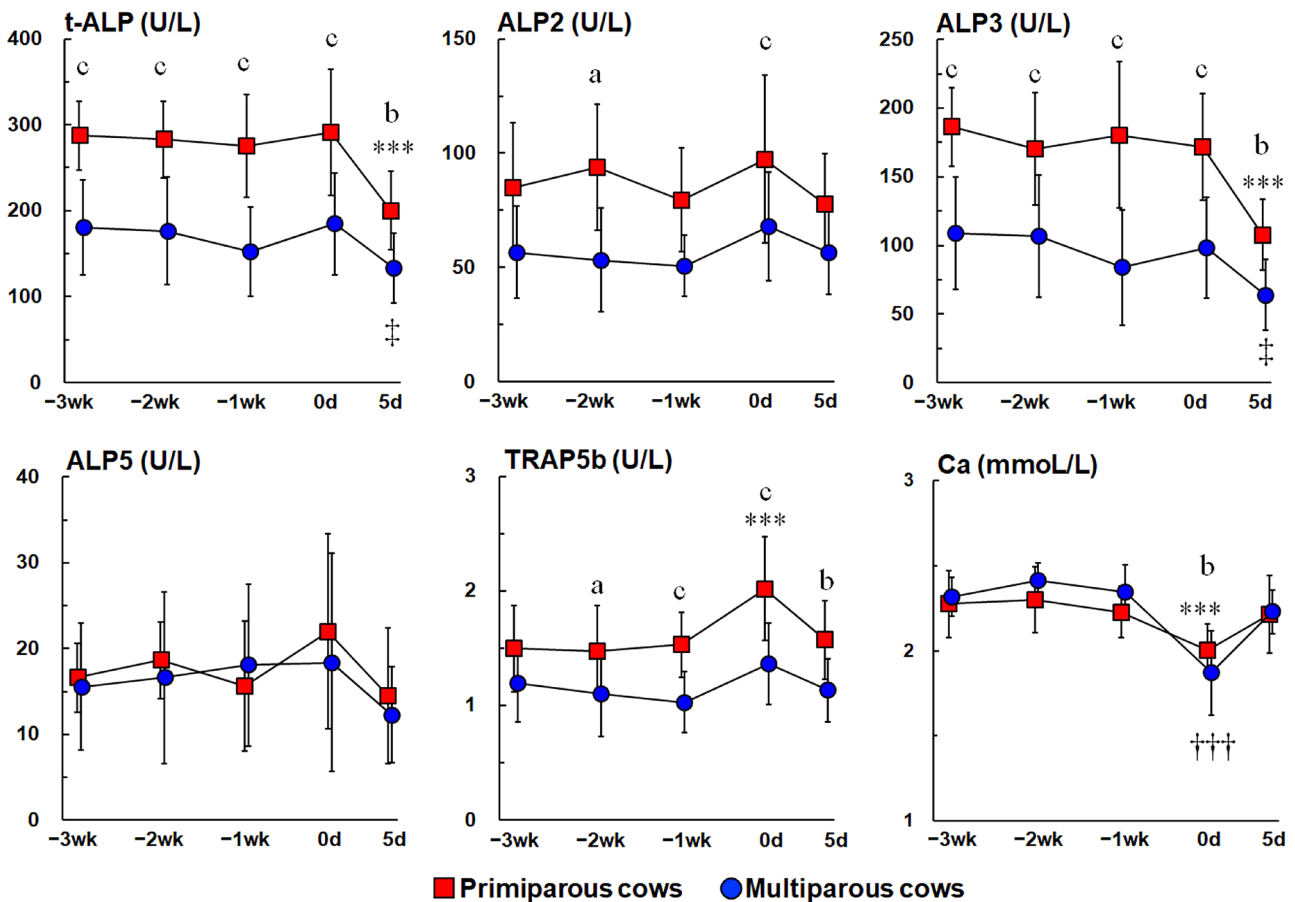


Fig. 1. Changes in t-ALP, ALP2, ALP3, ALP5, and TRAP5b activities and Ca concentrations in serum around parturition; t-ALP: total alkaline phosphatase; ALP2: alkaline phosphatase isoenzyme 2; ALP3: alkaline phosphatase isoenzyme 3; ALP5: alkaline phosphatase isoenzyme 5; TRAP5b: tartrate-resistant acid phosphatase 5b; Ca: calcium. wk: week; d: day. Significant differences between the primiparous and multiparous groups at each time point are shown: ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$. Significant differences compared to the levels at -3 weeks within the group: * $p < 0.001$ in the primiparous and ** $p < 0.001$ in the multiparous groups.

tion of measuring ALP isozymes in bovine medicine by analyzing the blood samples of cows with various diseases.

References

- Chiba A, Hatate K, Onomi R, Moriyama T, Goto A, Yamagishi N (2020) Agarose gel electrophoresis pattern of the serum alkaline phosphatase isoenzymes in Holstein cows during lactation. *Polish J Vet Sci* 23: 317-319.
- Goff JP, Horst RL (1997) Physiological changes at parturition and their relationship to metabolic disorders. *J Dairy Sci* 80: 1260-1268.
- Kim D, Yamagishi N, Ueki A, Miura M, Saito F, Sato S, Furuhashi K (2010) Changes in plasma bone metabolic markers in periparturient dairy cows. *J Vet Med Sci* 72: 773-776.
- Matsuo A, Togashi A, Sasaki S, Devkota B, Hirata T, Yamagishi N (2014) Diurnal variation of plasma bone markers in Japanese Black calves. *J Vet Med Sci* 76: 1029-1032.