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Short communication

Agarose gel electrophoresis pattern of serum alkaline phosphatase isoenzymes in Holstein cows during lactation

A. Chiba[1,2], K. Hatate[1,3], R. Onomi[4], T. Moriyama[2], A. Goto[2], N. Yamagishi[1,3,4]

[1] United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, Japan
[2] Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8550, Japan
[3] Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8550, Japan
[4] Graduate School of Veterinary Life Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8550, Japan

Abstract

A recent study found that an agarose gel electrophoresis (AGE) method yielded two distinct major bands corresponding to the hepatic and bone ALP isoenzymes (ALP2 and ALP3, respectively) in bovine serum treated with protease and neuraminidase (PN-treatment), although there were concerns that the intestinal ALP isoenzyme (ALP5) often overlapped with ALP3 in human serum treated with neuraminidase. Because ALP5 was separated from ALP3 in bovine serum treated with protease alone (P-treatment), we used a modified method employing both P- and PN-treated bovine sera to measure the activities of the three ALP isoenzymes in 53 lactating Holstein cows: 24 primiparous and 29 multiparous. Upon electrophoresis, 51 of 53 samples (96.2%) subjected to P-treatment yielded a distinct fraction corresponding to ALP5, as did the control serum. All PN-treated sera yielded a definite ALP2 fraction. The ALP3 fraction was calculated as the remainder after excluding ALP2 and ALP5. The activities of total ALP (t-ALP) and ALP3 in primiparous cows were higher than those in multiparous cows ($p < 0.001$) at early-to-peak [10–110 days in milk (DIM)] and mid (111–220 DIM) lactation. In the multiparous cows, the ALP3 activity at late lactation (221−477 DIM) was significantly higher than that at early-to-peak lactation. Thus, the modified AGE method described here is able to discriminate three fractions of ALP isoenzymes in the sera of lactating cows. The AGE pattern of circulating ALP isoenzymes will contribute to the understanding of the physiological bone metabolism status in lactating cows.

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

Correspondence to: N. Yamagishi, e-mail: yamagishi@obihiro.ac.jp, tel.: +81 155 49 5541

Introduction

A method using a commercial agarose gel electrophoresis (AGE) kit yielded two distinct major bands corresponding to the hepatic ALP isoenzyme (ALP2) and ALP3 in bovine plasma treated with protease and neuraminidase (PN-treatment) (Onomi et al. 2019); however, there were concerns that the intestinal ALP isoenzyme (ALP5) often overlaps with ALP3 following neuraminidase treatment (Van Hoof et al. 1988). Because ALP5 can be separated in human serum treated with protease alone (P-treatment) (Ooi et al. 2007), a modified AGE method that used both P- and PN-treated sera simultaneously was explored to better resolve the ALP isoenzymes in bovine serum. We used this modified AGE method to measure the serum ALP2, ALP3 and ALP5 levels in lactating Holstein cows. The aim of this study was to evaluate the distribution of the serum activity of each ALP isoenzyme in primiparous and multiparous cows at different lactation stages.

Materials and Methods

We assayed 53 frozen (-20°C) serum samples collected from 53 lactating Holstein cows [age 1.9-8.4 years; 24 primiparous and 29 multiparous (2^{nd} - 6^{th} parity)] kept at our university farm. The samples had been used previously for a metabolic profile test (MPT). All cows were declared healthy based on the MPT results.

AGE was performed using an electrophoresis system purchased from Helena Laboratories (Saitama, Japan), as shown previously (Onomi et al. 2019). Control serum (5139; Helena Laboratories) containing extract of bovine liver or intestinal tissue was used as a reference for ALP2 and ALP5, respectively. Each serum sample (60 µL) was subjected to two treatments before electrophoresis; one half (30 µL) was mixed with a 300 U/mL protease cocktail (4 µL) and distilled water (2 µL) (P-treatment), and the remainder (30 µL) was mixed with the protease cocktail (4 µL) and a separator solution containing neuraminidase (2 µL) (PN-treatment). After electrophoresis (23 min at 230 V and 15°C), the gels were stained and scanned as densitometric images. The P-treated serum showed a distinct ALP5 fraction emerging on the cathode side and a fraction containing poorly separated ALP2 and ALP3 on the anode side (Ooi et al. 2007), whereas the PN-treated serum showed a definite ALP2 fraction on the anode side and a poorly resolved fraction of overlapping ALP3 and ALP5 on the cathode side (Fig. 1A). The relative percentages of the ALP2 and ALP5 fractions were determined by the optical absorbance of the bands. The percentage of the ALP3 fraction was assessed by subtracting the percentage of the ALP5 fraction from the percentage of the overlapping ALP3 and ALP5 fraction in the PN-treated serum. The absolute activity (U/L) of each isoenzyme was calculated from the t-ALP activity measured spectrophotometrically.

All statistical analyses were performed with EZR software (Kanda 2013), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). The cows were divided into three lactation periods: the early-to-peak [10−110 days in milk (DIM)], middle (111−220 DIM), and late (221−477 DIM) periods. All numerical data were expressed as medians, with the minimum and maximum values and an interquartile range (IQR). The data were analyzed using the Kruskal-Wallis test with the Steel-Dwass test to evaluate the differences among the lactation periods within primiparous or multiparous cows. The Mann-Whitney *U*-test was used to compare the values between primiparous and multiparous cows at the same lactation period. The level of significance was set at $p<0.05$.

Results and Discussion

After the P-treatment, 51 of 53 serum samples (96.2%) yielded a fraction corresponding to ALP5 on the cathode side and a poorly resolved fraction of ALP2 and ALP3 on the anode side (Fig. 1B). After the PN-treatment, all samples (100%) yielded a definite ALP2 fraction on the anode side and a poorly resolved ALP3 and ALP5 fraction on the cathode side. These observations suggest that this AGE method can discriminate three ALP isoenzymes using P- and PN-treated sera of cows simultaneously.

Fig. 1C shows the distributions of the serum activities of t-ALP and each ALP isoenzyme in primiparous and multiparous cows at various lactation periods. The primiparous cows had significantly higher t-ALP and ALP3 activities than the multiparous cows ($p<0.001$) in early-to-peak and mid lactation. In the multiparous cows, the ALP3 activity in late lactation was significantly higher than that in early-to-peak lactation. There were no statistical differences in the ALP2 and ALP5 activities. Primiparous cows are still growing, and bone growth and remodeling are active throughout lactation; therefore, the higher serum t-ALP and ALP3 activities in the primiparous cows are indicative of skeletal growth. The increase in serum ALP3 activity at late lactation in the multiparous cows may show the activation of bone formation due to the decreased milk yield.

In conclusion, the modified AGE method described here was able to discriminate three ALP isoenzymes in the sera of lactating cows. The AGE pattern of circu-

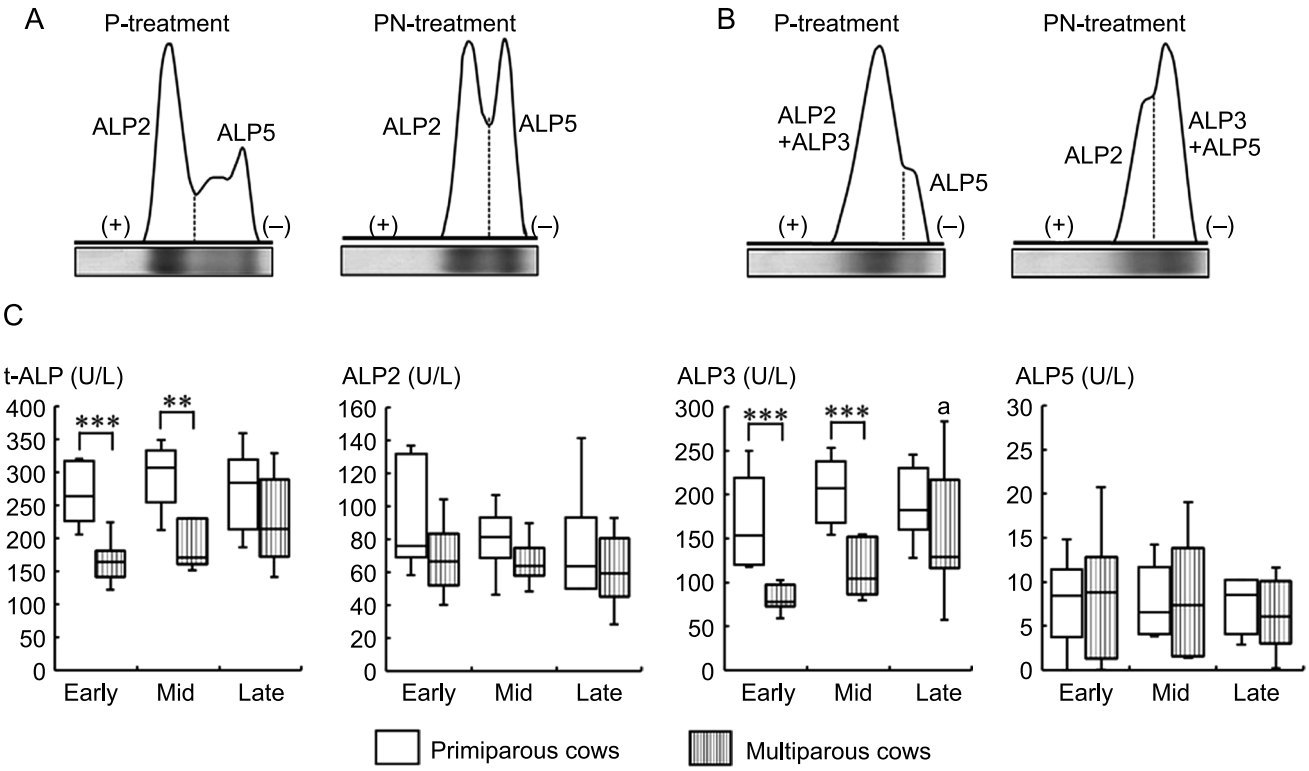

Fig. 1. Representative densitometric images (electrophoretograms) of (A) control serum and (B) a serum sample from a 2.1-year-old primiparous cow at 264 days of lactation and (C) box-and-whisker plots (medians, interquartile ranges, maxima, and minima) showing the distributions of the serum activities of total ALP (t-ALP) and each ALP isoenzyme in primiparous and multiparous cows in the early-to-peak (n = 9 and 13, respectively), middle (n=8 and 7, respectively), and late (n = 7 and 9, respectively) lactation periods. ALP2, hepatic ALP isoenzyme; ALP3, bone ALP isoenzyme; ALP5, intestinal ALP isoenzyme; ALP2+ALP3, the overlapping ALP2 and ALP3 fraction; ALP3+ALP5, the overlapping ALP3 and ALP5 fraction; Early, early-to-peak lactation; Late, late lactation; Mid, mid lactation; P-treatment, protease treatment; PN-treatment, protease and neuraminidase treatment. Significant differences between the primiparous and multiparous cows at each time point are shown: $^{**}p<0.01$, $^{***}p<0.001$. The letter means a significant difference from the values at the early to middle in multiparous cows: $^{a}p<0.05$.

lating ALP isoenzymes will contribute to the understanding of physiological bone metabolism status in lactating cows, although further studies are needed to examine its clinical implications.

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