

milk production of the cows enrolled in this study. The poor prognosis group was composed of cattle that died within 1 week or were culled due to poor milk production within 30 days after the first medical examination. Thirty-nine healthy, lactating cattle were used as controls. The mean concentrations of trace and major elements in serum were detected by particle induced X-ray emission (PIXE) methods. For normally distributed data, the mean values for each dependent variable were compared among groups, using the Tukey test, after ANOVA with the F test. For non-normally distributed data, the Kruskal-Wallis test was employed for comparison among groups. The ROC curves were used to characterize the sensitivity and specificity of a parameter to poor prognosis. The significance level was set at  $p < 0.05$ .

### Results

Of the 53 dairy cattle with ACM, good and poor prognosis groups were composed in 35 and 18 cattle, respectively. The PIXE method allowed detection of 29 elements. The average concentrations of Fe, Zn, and Br were lower in the ACM than those of the controls ( $p < 0.05$ ), but there were no significant differences in the levels of the remaining eleven elements. The median serum Zn and Br concentrations in control cattle were  $1.32 \mu\text{g/mL}$  and  $26.6 \mu\text{g/mL}$ , respectively. The median Zn ( $0.90 \mu\text{g/mL}$ ,  $p < 0.01$ ) and Br ( $10.4 \mu\text{g/g}$ ,  $p < 0.01$ ) in serum were significantly lower in ACM compared to control.

The ACM were found to have lower amounts of Fe compared to those without mastitis ( $1.46 \mu\text{g/mL}$ ,  $p < 0.01$ ). Serum Fe concentration ( $0.75 \mu\text{g/mL}$ ) was significantly lower in dairy cattle with poor prognosis compared to the cows that had longer survival and good production ( $0.95 \mu\text{g/mL}$ ,  $p < 0.001$ ). The area under the ROC curves for Fe concentrations was  $0.713$  ( $p < 0.05$ ). The proposed diagnostic cutoff points for Fe concentrations in serum for identifying poor prognosis of acute coliform mastitis based on the analysis of the ROC curves were set at  $< 0.82 \mu\text{g/mL}$ . Sensitivity and specificity of proposed diagnostic cutoffs for serum Fe concentration was  $77.8\%$  and  $77.0\%$ , respectively.

### Significance

We found how acute coliform mastitis in dairy cattle is associated with the concentrations of some trace and major elements in serum. The dairy cattle with acute coliform mastitis were found to have lower concentrations of Br, Fe, and Zn in serum compared to those without mastitis. In addition, serum Fe concentration was significantly lower in dairy cattle with poor prognosis compared to good prognosis group. Therefore, the proposed diagnostic cutoffs for serum Fe concentration based on ROC curves analysis in detecting a poor prognosis was set at  $< 0.82 \mu\text{g/mL}$ .

## Detecting endotoxin activity in raw milk using an automated testing system

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### Introduction

Gram-negative bacteria are among the most common environmental pathogens causing mastitis. Endotoxin plays a major role in the pathophysiology of gram-negative bacterial sepsis; therefore, attempts have been made to detect and quantify it, with conflicting findings, in various states of infection. The simple and easy portable test system (PTS) for endotoxin activity offers several advantages over the traditional microplate kinetic limulus amebocyte lysate (LAL)-based test used by diagnostic laboratories. It is small, inexpensive and

portable, requires only small quantities of raw milk, and provides results relatively rapidly in comparison to traditional methods. Thus the portability and ready availability of this cartridge system adds to its attractiveness for use in acute coliform mastitis (ACM). However, since the results obtained using this portable test will often dictate the course of clinical care and hygiene management, it is important that the cartridge system provides accurate information. The aim of the present study was to compare endotoxin activities detected in raw milk samples obtained from healthy dairy cattle without mastitis by a commercially available PTS™ and

traditional microplate LAL-based assay, which determined activities using a kinetic turbidimetric (KT) assay.

### Materials and Methods

Raw milk samples were obtained from 53 dairy cattle without mastitis and 12 cattle with clinical mastitis. Approximately 4 mL of raw milk was collected and diluted 100-, 200-, or 400-fold in endotoxin-free water and agitated in a vortex for 10 seconds. Endotoxin activity in milk was measured using KT assay and PTS system. Friedman test was performed for comparisons between the KT assay and PTS. The Pearson product moment correlation coefficient was also calculated to evaluate associations between any 2 continuous variables. Linear regression model analysis was also performed to obtain the equation associating the results of these 2 assays. The significance level was set at  $p < 0.05$ .

### Results

The endotoxin activities detected in 200- or 400-fold diluted milk samples using PTS were similar to those using the KT assay ( $p = 0.705$  and  $p = 1.000$  by the Friedman test, respectively), whereas a significant difference was observed in endotoxin activity detected in 100-fold diluted milk ( $p < 0.001$ ). The results obtained for 200- ( $r^2 = 0.778$ ,  $p < 0.001$ ) and 400-fold diluted milk samples ( $r^2 = 0.945$ ,  $p < 0.001$ ) using PTS correlated with those using the KT assay, respectively. The medians (range) of endotoxin activities in 100-, 200-, and 400-fold diluted raw milk samples were

15.00 endotoxin units (EU)/mL (range: 0.89 to 83.10 EU/mL), 2.99 EU/mL (range: 0.10 to 43.10 EU/mL), and 2.69 EU/mL (range: 0.10 to 40.80 EU/mL), respectively. PTS for endotoxin activity effectively recovered reference endotoxin from 100-, 200-, and 400-fold diluted raw milk samples. The median milk endotoxin activity in gram-positive clinical mastitis cows was 0.655 EU/mL (range: 0.280 to 450.00 EU/mL). Therefore, the dilution factor was adequate for 200 or 400-fold. On the other hand, a sample dilution was required of more than 160,000-fold to measure of endotoxin activity by PTS because the median of milk in coliform mastitis cow was significantly higher (median; 11,523.49 EU/mL, range: 4,707.38 to 49,035.21 EU/mL).

### Significance

In conclusion, photometric PTS represents a rapid, simple, and accurate technique using the quantitative, kinetic chromogenic LAL method for assessing endotoxin activity in raw milk, and meets all the requirements for endotoxin activity including the percentage of coefficient of variation (CV) and recovery of the positive control. In addition, the results of PTS using 200- and 400-fold diluted milk samples correlated with those obtained by the traditional KT assay. Therefore, the results of the present study confirmed that PTS is practical for simple and easy use to assess endotoxin activity in raw milk.

## Lipidomic biomarkers in colostrum and milk from production-related metabolic disease (PRMD) resistant and susceptible dairy cows

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### Introduction

Although the highest incidence for most production-related metabolic diseases (PRMDs; hypocalcemia, hepatic lipidosis, ketosis, left displaced abomasum-LDA, mastitis, laminitis) occurs within 60-days-in milk (DIM), the disease incidence has not been altered by transition diets, manipulating prepartum dietary cation anion balance, and overconditioning avoidance. PRMDs significantly affect economic returns with altered milk composition or decreased produc-

tion, conception, life expectancy, and cull value. The risk for PRMD in early lactation has been correlated with increased free fatty acids (FFAs), non-esterified fatty acids (NEFAs), triglycerides (TG), and beta-hydroxybutyrate ( $\beta$ HBA) serum concentrations, hepatic TG:glycogen, and fecal stable isotope differences (<sup>13</sup> carbon/<sup>12</sup> carbon ratio,  $\delta^{13}C$ ). These observations of biochemical changes prior to PRMD onset prompted investigation of periparturient colostrum (CS) and milk (MK) lipid profiles at the beginning of lactation. The objectives of this study were to determine postpartum