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 production, conception, life expectancy, and cull value. The risk for PRMD in early lactation has been correlated with increased free fatty acids (FFAs), non-esterfied fatty acids (NEFAs), triglycerides (TG), and beta-hydroxybutyrate (βHBA) serum concentrations, hepatic TG:glycogen, and fecal stable isotope differences (13 carbon/12 carbon ratio, δ13C). 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

day 1 CS and day 4 MK lipid profiles in PRMD resistant and susceptible cows, and to determine if lipids in this CS and/or MK secreted after the transition period can be used as biomarkers to predict risk for development of PRMD.

Materials and Methods

A prospective study was conducted with randomly chosen, age, lactation, parity matched (primiparous (n=101), multiparous (n=108)) Holstein cows (Brigham Creek Dairy, Elberta, UT). Colostrum (CS, 0 to 12 h postpartum) and milk (MK, day 4 postpartum) were obtained and health records maintained. Samples were submerged in crushed ice, flash frozen with liquid nitrogen, and stored at -80 C until analysis. Samples were thawed, centrifuged to isolate the lipid fraction, extracted with chloroform:isopropanol (2:1.25), the bottom organic phase removed and diluted 500x with chloroform:methanol:isopropanol (2:1:1.25) with 15mM ammonium acetate. Archeol 6nM was used as an external standard. Lipidomic analyses of CS and MK from matched PRMD resistant and susceptible cows were performed with 6230 time-of-flight mass spectrometry (MS) via electrospray ionization to detect + and - charged lipids. Student t-test identified significant lipid molecular weights in CS and MK. Lipids significant to health score (HSC) ranking: 0 (healthy) or 1 (PRMD treated/culled/died within 60 DIM) were determined via step-wise discriminant analysis. PROC GLM, independent variable HSC, determined if CS or MK measures were different for PRMD cows (p<0.05 for all tests). LIPID MAPS, QSTAR Pulsar I quadrupole orthogonal time-of-flight MS through an IonSpray Source, 6530 accurate-mass quadrapole/time-of-flight MS and MS/MS fragmentation were used for lipid identification.

Results

Differences existed between lipid profiles in CS and MK from cows that remained healthy (n=22) or later developed PRMD (n=20). Lipidomic analyses of 42 CS and 36 MK samples revealed 3 lipids in CS that were significant predictors of PRMD risk with 90% sensitivity, 88% specificity, a positive (PV+) predictive value for PRMD of 87% and negative result (PV-) of 92%, and 2 lipids in MK with 89% sensitivity, 90% specificity, PV+ of 89%, and PV- of 90%. Lipid biomarkers classified as diacylglcerols and triacylglcerols were found to be significant predictors of PRMD HSC and were identified in CS: 1) C37H62O4, 2) C35H68O5+NH4, and C55H98O6+H, and MK: 1) C39H76O5+NH4, **2) C57H108O6**. The presence or absence of each lipid had an estimated effect on HSC. A positive number raised the health score, predicting increased risk for metabolic disease (cow was at increased risk for PRMD), while a negative number lowered it (cow was likely to remain healthy).

Significance

Beginning lactation colostral and milk lipidomics in periparturient cows may provide biomarkers indicative of resistance or susceptibility to PRMD. Testing for the presence of specific lipids in these substrates may be a management tool that can be used during the peripartum period to provide an economical method to identify cows resistant or susceptible to PRMDs, for retention, breeding, early treatment intervention, or culling decisions to increase profit margins.

Practitioner survey: can a new obstetrical instrument really make the difference?

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Introduction

Dystocia in cattle affects up to 50% of primiparous and 30% of adult cows[1]. Obstetrics are crucial in order to reduce stillbirth rates. GYNstick[2,3,4], a new tool for treating dystocia, has been available for practitioners for almost 3 years. Nearly 400 vets in Germany and Austria are currently working with this obstetrical instrument in their daily practice, and approximately 2000 vets are using it worldwide. A survey was conducted among 49 German and 5 Austrian practitioners to obtain feedback about their experience with this instrument and their recommendations for its use.

Materials and Methods

Fifty-four veterinarians responded to an online questionnaire initiated by the Faculty for Veterinary Medicine of the University of Giessen. Veterinarians were chosen based