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Activity and milk compositional changes following experimentally induced *Streptococcus uberis* bovine mastitis

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ABSTRACT

Milk constituents and physical activity of cows experimentally infected with *Streptococcus uberis* mastitis were compared with those of uninfected cows. Twelve late-lactation Holsteins cows were paired based on milk production and parity. One cow in each pair was experimentally infected in the right front mammary gland with *Strep. uberis*. The remaining cow in each pair served as an uninfected control. Real-time analyses of milk constituents provided fat, protein, and lactose percentages at each milking. Pedometers were placed on the left front leg of all cows and activity was measured. Intramammary infections with *Strep. uberis* reduced milk yield in experimental cows by approximately 1.6 kg/d in the first week after challenge compared with control cows. Lactose percentage in milk was reduced on d 3, 4, 5, and 6 after challenge in treatment cows compared with controls. Percentages of fat and protein in milk did not differ between infected and uninfected cows the week after infections were induced. Total steps per day were reduced and minutes resting per day were increased the week after experimental challenge in infected cows compared with control cows. The number of resting bouts did not differ between infected and uninfected cows. Changes in percentage of lactose in milk and animal activity caused by experimentally induced *Strep. uberis* mastitis were detected by the automated milk analyzer and pedometer systems.
Key words: mastitis, lactose, behavior

INTRODUCTION

Mastitis causes changes in composition of milk and physical activities of infected cows (Kitchen, 1981; Fogsgaard et al., 2012). Automated monitoring systems allowing for determination of major milk components and measurements of activity offer a potential for diagnosing changes in mammary health. However, the predictive value of changes in specific milk components during mastitis depends on the degree of inflammation and causative agents (Kitchen, 1981; Leitner et al., 2006). Changes in physical activity such as time spent lying and number of steps per day are similarly influenced by housing conditions and the inflammatory agent (Cyples et al., 2012; Fogsgaard et al., 2012; Medrano-Galarza et al., 2012). Many previous trials have concentrated on the effects of experimentally induced IMI caused by *Escherichia coli* or intramammary challenge with endotoxin to determine changes in milk composition and deviations in behavior (Zimov et al., 2011; Cyples et al., 2012; Yeiser at al., 2012). Leitner et al. (2006) reported that naturally occurring, chronic IMI caused by *Streptococcus dysgalactiae* resulted in significantly reduced lactose concentrations in milk of infected cows. However, the ability of automated monitoring systems to detect concurrent changes in activity and milk components in cows with IMI caused by other gram-positive bacteria is not as well documented. Therefore, the purpose of the current trial was to evaluate the ability of an automated milk analyses and pedometer system to detect daily changes following experimental intramammary challenge with *Streptococcus uberis*.

MATERIALS AND METHODS

Animals

Twelve late-lactation Holsteins, averaging 200 DIM (range = 159 to 239 DIM), in the Ohio Agricultural Research and Development Krauss Dairy were paired by parity and milk production. Experimental animals were 4 pairs of multiparous and 2 pairs of primiparous cows. Average daily milk production for all cows during the 7 d before initiation of the experiment was 34.1 ± 6.0 kg (mean ± SD). All cows were housed in the same freestall pen, fed a TMR once daily, and milked twice daily as a single group. One cow in each pair was experimentally infected in the right front mammary gland with *Strep. uberis*. The remaining cow in each pair was the uninfused control.

Treatment and husbandry of cows were conducted in a manner to avoid unnecessary discomfort of animals by use of proper management and experimental

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procedures as approved by The Ohio State University Institutional Animal Care and Use Committee Protocol Number 2011A00000041.

Intramammary Challenge

Each experimental cow infused with *Strep. uberis* was challenged on the same day, 3 h after morning milking with bacteria prepared from the same challenge inoculum. The bacterial strain used to experimentally induce mastitis was *Strep. uberis* 0140J, originally isolated from clinical mastitis (Finch et al., 1997). *Streptococcus uberis* 0140J was cultured in trypticase soy broth (Becton Dickinson and Co., Sparks, MD) at 37°C for 18 h at 100 rpm in a shaking incubator. Bacteria were pelleted by centrifugation and diluted in sterile PBS to final challenge concentration. Challenge inoculum was 2,300 cfu in 1 mL of sterile PBS infused into the mammary quarter using a sterile 1-mL syringe fitted with a sterile teat cannula (Jorgensen Laboratories, Loveland, CO). The challenge inoculum was determined by plating 1.0 and 0.1 mL in trypticase soy agar (Becton Dickinson and Company) plus 0.05% ferric ammonium citrate (Fisher Scientific, Fair Lawn, NJ) and 0.1% esculin (Sigma-Aldrich, St Louis, MO) for incubation aerobically at 37°C for 24 h. Only uninfected mammary glands were infused. The decision was made a priori, per The Ohio State University Institutional Animal Care and Use Committee Protocol Number 2011A00000041, to treat challenged quarters by intramammary infusion with 125 mg of ceftiofur hydrochloride (Spectramast LC, Florham Park, NJ) once daily on d 4, 5, and 6 after challenge if quarter milk SCC exceeded 6.0 $\log_{10}$ SCC/mL or mammary glands showed clinical signs of mastitis by d 4 after challenge. Consequently, each experimentally challenged mammary gland received the antibiotic therapy as described above.

Quarter Foremilk Samples

Quarter foremilk samples also were collected 7, 5, and 3 d before bacterial challenge to determine eligible, uninfected mammary glands. Sample collection and microbiological procedures were as previously described (Hogan et al., 1995). Quarters were classified as uninfected and eligible for experimental challenge if each of the 3 samples collected before challenge were bacteriologically negative and the milk and mammary gland displayed no clinical signs of mastitis. Quarter foremilk from challenged quarters was collected for enumeration of streptococci on d 1, 2, 3, and 7 after challenge. Total counts (cfu) of streptococci were calculated by serial dilution of milk samples plated in or on the surface of trypticase soy agar plus 0.05% ferric ammonium citrate and 0.1% esculin. Plates were incubated (37°C, 18 h) aerobically before count determination. The SCC per milliliter of milk were determined by Bentley Somatocount 150 milk somatic cell counter (Bentley Instruments Inc., Chaska, MN). Samples from clinical quarters were diluted 1:10 and 1:50 (milk:PBS, vol/vol) for counting. Data were expressed as $\log_{10}$ colony-forming units per milliliter of milk and $\log_{10}$ SCC per milliliter of milk.

Clinical score of all quarters was recorded at the time quarter foremilk samples were obtained. Clinical score was recorded on a 5-point scale: 1 = normal milk and normal quarter, 2 = normal quarter but milk was questionable, 3 = normal quarter but abnormal milk, 4 = a swollen quarter and abnormal milk, and 5 = swollen quarter, abnormal milk, and systemic signs of infection (Hogan et al., 1995).

Milk Composition and Cow Activity

Milk production and percentages of milk fat, lactose, and protein were measured at each milking (AfiLab, SAE Afikim, Kibbutz Afikim, Israel) as described by Kaniyamattam and De Vries (2014). Milk compositional data were analyzed as weighted daily averages for the 7 d after challenge. Pedometers (Afi PedometerPlus, SAE Afikim) were placed on the left front leg of all cows. Pedometers measured daily totals of number of steps taken, bouts of rest, and amount of time resting. Activity data were recorded daily totals.

Statistical Analysis

Differences in daily milk weights, milk compositional data, and activity data were measured by least squares ANOVA (SAS Institute, 2003). Main effects tested were treatment and pair.

RESULTS

Intramammary Challenge

Milk SCC (mean ± SD) in challenged quarters was 4.6 ± 0.4 $\log_{10}$/mL immediately before infusion. Each challenged mammary gland of cows infused with *Strep. uberis* had a SCC of >6.0 $\log_{10}$/mL by d 3 after challenge. Five of the 6 cows challenged by intramammary infusion with *Strep. uberis* had clinical scores of mastitis ≥3. *Streptococcus uberis* counts in milk from challenged mammary glands increased daily after infusion with a peak counts (mean ± SD) of 4.1 ± 2.3 cfu/mL on d 3 after challenge. Rectal temperatures did not differ between challenged and control cows during the experimental period ($P > 0.05$). Mammary health