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Milk composition and health status from mammary gland quarters adjacent to glands affected with naturally occurring clinical mastitis

Marcel G. Paixão,*†[1] Luiz R. Abreu,† Roxann Richert,‡ and Pamela L. Ruegg*
*Department of Dairy Science, University of Wisconsin, Madison 53706
†Departamento de Ciência dos Alimentos, Universidade Federal de Lavras, Minas Gerais, Brazil 37200-000
‡2171 Rd B, Ulysses, NE 68669

ABSTRACT

Mammary gland quarters have usually been considered to be anatomically and physiologically independent, but some recent research has indicated more interdependence than previously reported. The objective of this study was to compare milk composition (fat, total protein, lactose, solids-not-fat, and chloride) and health status (somatic cell count, differential leukocyte count, and lactate dehydrogenase) of milk samples from unaffected mammary glands of an udder with a single clinically inflamed quarter to results of milk samples from healthy mammary glands of healthy cows. The study was designed as a prospective case control study with case and control cows matched by parity and days in milk. Cases were defined as cows (n = 59) experiencing clinical mastitis in a single mammary gland, and controls (n = 59) were defined as cows that had not experienced clinical mastitis during the current lactation. Quarter milk samples were collected from all mammary glands adjacent to clinically affected quarters of cases and from the same mammary glands of controls. Samples were used to assess concentration of chloride and lactate dehydrogenase, fat, total protein, solids-not-fat, somatic cell count, and differential leukocyte count. Microbiological analysis was also performed on milk samples obtained from clinically affected mammary glands (n = 59). Logistic regression models were used to assess possible associations among quarter somatic cell count (≥150,000 cells/mL) and quarter type (adjacent to case or control). Multivariate linear models were used to compare milk composition and health status between quarter types. A total of 170 quarters were enrolled per group. Milk obtained from adjacent quarters of cases contained a lesser concentration of total protein, lactose, and solids-not-fat, but had a greater concentration of fat and chloride. The somatic cell count, total leukocyte count, and absolute numbers of neutrophils, lymphocytes, and macrophages were all increased in milk obtained from adjacent quarters of case cows compared with milk obtained from quarters of control cows. The relative proportion of neutrophils was increased, whereas the proportion of macrophages was decreased in milk obtained from cases. Approximately 30% of milk samples obtained from adjacent quarters of cases had a somatic cell count ≥150,000 cells/mL compared with 12% of milk samples obtained from quarters of control cows. The position of the mammary gland was not associated with any outcomes. In conclusion, our results support previous research that indicates the immune response to intramammary infection in a single mammary gland quarter alters milk composition and health status throughout the udder.
Key words: mastitis, milk composition, somatic cell count, milk quality

INTRODUCTION

The bovine udder is divided into 2 distinct halves separated by suspensory ligaments, and a thin septum of connective tissue divides the front and rear quarters with no direct connections. Each mammary gland quarter is considered anatomically and physiologically independent and has its own vascular and nervous system, and suspensory apparatus (Nickerson and Akers, 2011). The assumption that each quarter is independent has resulted in use of within-udder experiments that include both treated and control quarters of a single cow (Grönlund et al., 2006; Lutzow et al., 2008; Rinaldi et al., 2010).

The theory of independence among mammary gland quarters has been challenged as several studies have demonstrated changes in milk of adjacent healthy quarters when a single quarter is affected with mastitis (Bansal et al., 2005; Hamann et al., 2005; Jensen et al., 2013). Differences in lactose, fat, electrical conductivity, SCC, and *N*-acetyl-β-D-glucosaminidase were observed among milk samples obtained from cows free of subclinical mastitis in all quarters compared with milk obtained from cows that had at least one quarter with subclinical mastitis (Bansal et al., 2005). Hamann et al.

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[1]Corresponding author: marcelgpaixao@gmail.com

(2005) reported differences in the proportion of neutrophils and lymphocytes between milk samples collected from quarters of healthy cows (no subclinical mastitis in any glands) compared with milk samples collected from cows with at least one gland affected with subclinical mastitis. They suggested that inflammation in the infected glands influenced responses in the noninfected glands and proposed that the mammary gland cannot be considered as 4 isolated entities. Similarly, Jensen et al. (2013) studied transcriptional response after experimentally induced IMI. They reported profound changes in the expression of mRNA of healthy quarters adjacent to quarters infected with *Staphylococcus aureus* and *Escherichia coli*. They suggested that mastitis pathogens directly affect host mammary cells but also influence adjacent glands with the purpose of reducing subsequent transmission (Jensen et al., 2013).

Although these studies suggest that composition and quality of milk from mammary glands adjacent to affected quarters is likely altered in response to infection in a single quarter, associations between a single quarter affected with clinical mastitis (visual abnormalities of milk and udder) and milk composition in adjacent quarters have not been previously reported. A better understanding of relationships among the mammary gland will help to clarify immune mechanisms after a clinical IMI.

Milk from quarters with visible changes in milk appearance (clinical mastitis) are immediately discarded and cannot be used by processors. Previous cited authors have investigated the effect of unseen signs of infection (SCC, LDH, leukocytes, and microbiological analyses) in milk of adjacent quarters, but researchers have not investigated the influence of a single quarter-case of clinical mastitis on composition of milk from adjacent quarters. The effect of a clinical case of mastitis on composition of milk of adjacent quarters may be greater than that of cows experiencing subclinical signs as the magnitude of inflammation is greater and the etiological agents typically differ. The objective of this study was to compare composition [fat, total protein (**TP**), lactose, chloride, and SNF] and health status [SCC, differential leukocyte count, and lactate dehydrogenase (**LDH**)] in milk samples from unaffected mammary glands of an udder with a single clinically inflamed quarter to results of milk samples from healthy mammary glands of healthy cows.

MATERIALS AND METHODS

Eligibility and Inclusion

The study was conducted on a commercial Wisconsin dairy farm that contained 3,152 lactating Holstein cows. The herd had a milking routine that included observation of foremilk for detection of mastitis, complete records of clinical mastitis at the quarter level, and monthly SCC testing of individual cows. The enrolled farm had a daily average milk production of 33.6 kg per cow. The average bulk tank milk values of SCC, fat, and TP were 301,000 cells/mL, 3.62%, and 3.13%, respectively. The cows were housed in a freestall barn, with digested manure solids as bedding, and fed a balanced TMR. Cows were milked 3 times per day in a 72-stall rotary parlor.

The study was designed as a prospective, matched case-control study. Primiparous and multiparous cows with 4 functional mammary gland quarters were potentially eligible for participation in the study if clinical mastitis was detected in a single quarter during November 4 to December 30, 2015. Cases were defined as cows with a single quarter that experienced mild (occurrence of abnormal milk only) or moderate (occurrence of abnormal milk and swelling, redness, or pain in the udder) clinical signs of mastitis (Pinzón-Sánchez and Ruegg, 2011). Cows that had experienced previous clinical cases were not eligible until at least 13 d after a previous mastitis event. Control cows were matched by parity and were required to be within 30 DIM of cases, were in the same milking group as cases and had no history of clinical mastitis within the current lactation.

Each week, on the day before researchers visited the farm, a list of enrolled cases was obtained and used to identify eligible control cows. Information about enrolled cows (parity, DIM, milk yield, pregnancy status, mastitis history, severity of mastitis, affected quarter) was obtained from herd management software (Dairy Comp 305, Valley Agricultural Software, Tulare, CA). Formal randomization was not used to select controls, but researchers maintained a list of eligible controls and selected the first eligible control for each case as cows entered the rotary parlor during milking.

This research was approved by the College of Agricultural and Life Sciences Animal Care and Use Committee, protocol number A005251.

Sample Size

Prior to the study, power calculations were performed to estimate sample size needed to provide an excess of 95% confidence and 80% power to detect the following differences: (1) fat of 0.35%, (2) TP of 0.15%, (3) lactose of 0.1%, (4) chloride of 8.0 mg/100 mL, (5) SCC of 50,000 cells/mL of milk, (6) total leukocyte count (**TLC**) of 50,000 cells/mL, and (7) LDH; EC number 1.1.1.27) of 0.9 U/I. Based on the most limiting variable (fat), sample size was estimated to be a minimum of 170 quarters per group.