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The effect of subclinical mastitis on milk composition in dairy cows

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Summary

Milk samples were collected from quarters of 35 cows with subclinical mastitis (California mastitis test [CMT] positive and somatic cell counts [SCC] >500,000 cells/ml in individual quarter foremilk), as well as from 37 healthy controls. Compared to the levels observed in milk from healthy quarters, milk from quarters with subclinical mastitis showed elevated sodium (91.97 vs 52.93 mg/dl), chloride (>0.14 vs <0.14 g/dl), pH (6.69 vs 6.59), albumin (5.62 vs 2.65 g/dl), lactate dehydrogenase (LDH) activity (1524.04 vs 485.94 IU/L) and immunoglobulins (26.86% vs 7.43%). In contrast, decreased values were found for calcium (90.45 vs 126.29 mg/dl), inorganic phosphorous (24.40 vs 30.59 mg/dl), potassium (151.56 vs 167.74 mg/dl), α-lactalbumin (22.25% vs 28.72%) and β-lactoglobulin (34.21% vs 57.08%). No changes were seen in blood serum LDH activity. Furthermore, an increase in positive response to CMT was found to be accompanied by an almost proportionate increase in immunoglobulin values to 48.20% and decrease of α-lactalbumin levels in milk serum ($P<0.01$). These changes in pH, mineral concentrations, LDH activity and protein fractions in milk of quarters show the presence of tissue damage provoked by SCM. Thus, these parameters can be used in the diagnosis of mastitis.

Key words: Cows, Milk, Composition, Subclinical mastitis

Introduction

In spite of the great progress in genetics, feeding systems, housing and milking conditions, in most of the high-producing herds, there is a significant increase in a group of multi-factorial diseases known as "production diseases". Among these diseases, mastitis is still the most economically important illness leading to drastic milk losses, premature culling of genetically superior cows, drug cost, veterinary cost, increased labour, milk withholding after treatment, reduced genetic improvement (DeGraves and Fetrow, 1993; Leslie and Dingwell, 2000), changes in the hygienic and compositional quality of milk and impairment of the technological properties of milk (Kitchen, 1981; Munro *et al*., 1984; Auldist *et al*., 1995; Wielgosz-Groth and Groth, 2003) and decreased reproductive performance (Cullor, 1991; Barker *et al*., 1998; Schrick *et al*., 2001). Additionally, mastitis can be harmful to suckling newborns.

Mastitis—inflammation of the mammary gland—is caused by several species of common bacteria, fungi, mycoplasmas and algae. Subclinical infections are those for which no visible changes occur in the appearance of milk or the udder, but milk production decreases, somatic cell count increases, pathogens are present in the secretion, and the milk composition is altered. Clinical mastitis is recognized by abnormal milk, varying degrees of mammary gland inflammation (redness, heat, swelling, pain) and with or without illness of the cow. Milk production declines, bacteria are present in the milk and the milk can vary from having a few milk clots to serum with clumps of fibrin in the secretion (Tyler and Cullor, 1990).

Early identification of udder health problems is essential for dairy farmers and veterinarians to ensure not only the animal

well-being but also the milk quality and dairying productivity. Economic aspects interfere with the routine application of bacteriologic examination of quarter milk samples. For this reason, alternative parameters are used to identify trends in the development of the udder health in a dairy herd, although these parameters indicate inflammation.

The present study aimed at assessing the relationship between a set of chemical parameters including pH, mineral concentrations, lactate dehydrogenase (LDH) activity and protein fractions and subclinical mastitis occurred naturally on dairy herds.

Materials and Methods

Animals were selected from three Holstein dairy herds located in Urmia in West Azerbaijan province of Iran. In each herd, cows were housed in free stall barns. Cows were in the second to fifth lactation and were milked twice daily by machine milking. Cows were fed *ad libitum* by a total mixed diet that had been formulated to meet the nutritional requirements of a 650-kg cow, yielding 15–35 kg of milk/d with about 3.5% milk fat and 3.4% protein. All cows were subjected to post-milking teat disinfection, those were dried off approximately two months before expected calving and all quarters of cows were infused with an antibiotic preparation approved for use in non-lactating cows following the last milking of lactation.

Milk samples were sampled from quarters of 35 cows with subclinical mastitis (SCM), as well as from 37 healthy controls just before morning milking. Teats were washed thoroughly and dried with a single-use paper towel. The first three streams of milk from each teat were discarded. The teat end and orifice was sanitized with cotton swabs soaked in 70% ethyl alcohol and approximately 10 ml foremilk sample were collected from each quarter of cow in a sterile tube held horizontally. The experimental material was divided into four groups according to the California mastitis test (CMT) results—0 = negative or trace, 1 = weak positive, 2 = distinct positive and 3 = strong positive—obtained from the test performed directly in the cowshed, using the method described by Schalm *et al*. (1971). Blood specimens were also collected from jugular vein for the LDH assay. Samples were immediately placed in crushed ice and submitted to the laboratory within 2–4 hrs. For diagnosis of SCM, the total somatic cell count of milk was determined, using Breed's smears with Newman's stain and leukocyte count more than 500,000 cells/ml of individual quarter milk was taken as a positive index of mastitis (Harrigan, 1998). In all other cases, the samples were considered uninfected (healthy). All milk and blood samples were tested at mid-lactation and none of the cows were sampled twice in the study.

Milk serum (whey) was prepared at a two-step centrifugation procedure. At first, milk samples were centrifuged at 3000 rpm for 10 min to remove their creams and cells. Samples were then treated with 0.1 M hydrochloric acid at the controlled pH of 4.8 for casein precipitation. Treated samples were recentrifuged and the supernatants (whey) were collected. The pH of milk samples was determined electrometrically (Metrohm 620 pH-meter, Swiss). Total calcium and phosphorous concentrations were determined by colorimetric method, a hand-held spectrophotometer (pharmacia LKB) using commercial kits (Pars Azmun, Karadj, Iran) based on cresolphthalein complexion and phosphomolybdic acid complex formation, at wavelengths of 578 and 340 nm, respectively. Albumin was determined by bromocresol green method, using commercial kit (Pars Azmun, Karadj, Iran) at wavelength of 546 nm; sodium and potassium by flame photometer (Jenway, Clinical, PFP7C, England); chloride based on rapid spot test using K chromate and silver nitrate (observation of yellow colour, >0.14 g/dl and brownish colour less than that amount) (Cole, 1986). LDH activity was measured by spectrophotometer, using commercial kit (Pars Azmun, Karadj, Iran) by the method of Bergmeyer (1974) at wavelength of 340 nm. Protein fractionation of milk was separated according to molecular mass using cellulose acetate membrane electrophoresis (Sebia

preference, France) at 100 V for 15 min and barbital buffer; pH = 8.6. After fractionation, membranes were stained with fixative dye solution (0.3% Ponceau red, 3.5% trichloroacetic acid, 96.5% double distilled water) at 15 min and then decolorized and cleared. After drying, the relative levels of proteins were determined by densitometry at wavelength of 530 nm.

Data were analysed by SPSS (version 10). Student's t-test was carried out to find the differences between the results of mastitic and non-mastitic milk and serum. The changes in the content of protein fractions in milk with different positive CMT scores were assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The results were given as mean ± SEM.

Results

The concentrations of calcium, phosphorous and potassium were significantly lower in the milk of inflamed (SCM) quarters than those in normal milk ($P<0.01$). In contrast, the concentrations of sodium, chloride and albumin were significantly higher in the milk of inflamed quarter than those in normal ones ($P<0.01$). The pH was significantly higher in the subclinical mastitic milk than in the normal ones ($P<0.01$; Table 1).

Table 2 shows the LDH activities of normal and subclinical mastitic milk and blood serum samples. The mean LDH activity was significantly higher in milk from inflamed (SCM) quarters than in normal milk ($P<0.01$); no significant difference was in blood enzyme values.

The percent of protein fractions were significantly different between normal and SCM milk ($P<0.01$). SCM caused increment in the immunoglobulin and albumin content in milk. While, α-lactalbumin, β-lactoglobulin and pre-albumin content in SCM milk decreased (Table 3).

Table 4 shows the contents of protein fractions depending on the CMT progression. Statistically significant ($P<0.01$) influence of high mastitis progression (+++) on the increase in milk immunoglobulin values to 48.20% was detected. Milks obtained from highly infected quarters (+++) contained significantly ($P<0.01$) lower α-lactalbumin, albumin and pre-albumin, but the content of β-lactoglobulin in milk was similar between quarters with various CMT scores.

Table 1: Changes in the minerals, albumin and pH of milk as a result of SCM in quarters

Parameters	Normal milk	SCM milk
Calcium (mg/dl)	126.29 ±0.88* (37)	90.45 ±1.91 (24)
Phosphorous (mg/dl)	30.59 ±0.38* (36)	24.40 ±0.33 (26)
Sodium (mg/dl)	52.93 ±1.20* (24)	91.97 ±5.55 (11)
Potassium (mg/dl)	167.74 ±2.60* (22)	151.56 ±2.44 (11)
Chloride (g/dl)	<0.14* (35)	>0.14 (26)
pH	6.59 ±0.02* (37)	6.69 ±0.08 (35)
Albumin (g/dl)	2.65 ±0.08* (37)	5.62 ±0.12 (26)

*Significant at $p<0.01$. Parenthesis shows the number of samples

Table 3: Changes in the level of protein fractions (%) in milk as a result of SCM in quarters

	Normal milk	SCM milk
Immunoglobulin	7.43 ±0.32* (35)	26.86 ±0.68 (35)
α-Lactalbumin	28.72 ±0.45* (35)	22.25 ±0.98 (35)
β-Lactoglobulin	57.08 ±0.74* (35)	34.21 ±1.12 (35)
Albumin	6.99 ±0.68* (35)	17.21 ±1.47 (35)
Pre-albumin	0.18 ±0.07* (35)	0.09 ±0.04 (35)

*Significant at $p<0.01$. Parenthesis shows the number of samples

Table 2: Changes in the level of LDH in milk and blood serum as a result of SCM in cows

	Normal milk	SCM milk	Normal serum	SCM serum
LDH (IU/L)	485.94±13.66* (35)	1524.04±111.74 (25)	867.16±31.31 (35)	809.32±21.67 (25)

*Significant at $p<0.01$. Parenthesis shows the number of samples

Table 4: Changes in the concentration of protein fractions (%) in milks with different positive CMT scores

	(+)	(++)	(+++)
Immunoglobulin	11.70 ±0.31[a] (10)	20.68 ±0.45[b] (13)	48.20 ±1.30[c] (12)
α-Lactalbumin	29.19 ±0.84[a] (10)	25.21 ±1.10[b] (13)	12.37 ±1.01[c] (12)
β-Lactoglobulin	31.52 ±1.06[a] (10)	36.02 ±1.06[a] (13)	35.11 ±1.24[a] (12)
Albumin	27.40 ±1.43[a] (10)	17.96 ±1.66[b] (13)	6.27 ±1.34[c] (12)
Pre-albumin	0.11 ±0.06[a] (10)	0.07 ±0.35[b] (13)	0.10 0.29[a] (12)

The means with different superscript (a, b and c) in each row are significantly different ($P<0.01$). Parenthesis shows the number of samples

Discussion

Inflammation of the mammary gland leads to a variety of compositional changes in milk either because of local effects or because of serum components entering the milk and the movement of some normal milk components out of the alveolar lumen into the perivascular space (Harmon, 1994). Theoretically, all changes in mammary secretion during inflammation might be used to measure the effects of mastitis, but problems of instrumentation and standardization have hampered farm application of most tests.

The pH of SCM milk was higher than that of normal milk, which is consistent with the results of previous reports (Kitchen, 1981; Sena and Sahmani, 2001; Wielgosz-Groth and Groth, 2003). The indirect pH testing can be considered as a guide to detect the subclinical mastitis as this is economical, easy and rapid. It can be done in the field at the time of milk collection. After determining pH, the positive samples can be tested to isolate the causative organism for further confirmation of SCM. Mastitis also markedly changed the ionic environment. Sodium and chloride are increased. In contrast, potassium, normally the predominant mineral in milk, is declined. These increases in sodium and chloride and decrease in potassium levels have been confirmed by other authors as methods of monitoring udder health (Kitchen, 1981; Fernando *et al*., 1985; Vijayalakshmi *et al*., 2001; Bruckmaier *et al*., 2004). Intramammary infection results in damage to the ductal and secretory epithelium, an opening of the "tight junctions" between secretory cells, and the increased permeability of the blood capillaries. Thus, sodium and chloride (which are high in extracellular fluid) pour into the lumen of the alveolus and, in order to maintain osmolarity, potassium levels decrease proportionately. The levels of calcium and phosphorous is also affected by mastitis. The reduction in calcium and phosphorous levels in the case of intramammary infections have been reported by Bogin and Ziv (1973) and Coulon *et al*. (2002).

Albumin content of milk in subclinical mastitis was significantly increased compared to the healthy ones. The increase of albumin content in milk during mastitis has been reported in cows (Urech *et al*., 1999; Vijayalakshmi *et al*., 2001; Coulon *et al*., 2002), sheep (Leitner *et al*., 2004a) and goats (Leitner *et al*., 2004b). Although, It is generally thought that the main site of albumin synthesis is in the liver, and that the albumin enters the milk by leaking through the epithelial tight junction from the blood stream (De wit, 1998), the extrahepatic synthesis of albumin has been demonstrated in mammary gland epithelial cells, albeit lesser than the liver (Shamay *et al*., 2005). The marked increases of albumin in mastitic animals suggest that a major source of the increase in the content of albumin in milk under inflammatory conditions is the gland itself.

Our findings showed that tissue disturbances of the mammary gland in subclinical mastitis were accompanied by marked increase of LDH activity in the secretions, but without obvious influence on enzyme levels in blood serum. Higher LDH activity in milk serum of inflamed udders has been previously reported in cows (Kovac and Beseda, 1975; Grun *et al*., 1992) and sheep (Nizamliglu and Erganis, 1991; Batavani *et al*., 2003). The higher level of LDH in mastitic milks than blood serum LDH activity shows that blood serum was not the sole source of this enzyme in mastitic milk and it was probably also liberated from disintegrated leukocytes and the parenchymal cells of the udder (Bogin *et al*., 1977; Michel, 1979; Kato *et al*., 1989).

The present study showed that the types of proteins present in all of the milking

fractions from quarters with subclinical mastitis undergo dramatic changes. Quarters with SCM showed higher immunoglobulins and lower lactalbumin than did the corresponding milking fractions taken from healthy ones. The increased proportion of immunoglobulins associated with inflammatory responses of the udder compensated for the significantly lower proportion of lactalbumin. In fact, there is an approximate balance between this decrease and increase. Changes in protein fractions of milk obtained from mastitic cow have been documented in earlier studies (Atiekh, 1979; Anderson *et al*., 1986; Kostyra, 1990; Urech *et al*., 1999; Coulon *et al*., 2002). Immunoglobulins in mammary secretions are serum-derived or produced in the udder and pass into the milk through the mammary epithelium. The concentrations of immunoglobulins in normal milk are low and depend on the degree of vascular permeability of the udder tissues. When this permeability barrier is broken during inflammation, immunoglobulin concentrations increase in secretions from infected glands. The immunoglobulin has several important functions. They are believed to prevent bacterial adherence to epithelial membranes, inhibit multiplication, agglutinate bacteria and neutralize toxins. In addition, a major function of immunoglobulins is opsonization of microorganisms for phagocytosis. The increase in milk immunoglobulins may be effective in reducing severity of mastitis (Nickerson, 1985; Persson, 1992). Specific proteins (e.g., α-lactalbumin and β-lactoglobulin) are largely synthesized in the mammary gland. This decrease in α-lactalbumin associated with SCM could be due to the reduced synthetic activity of mammary gland. Some studies suggest that α-lactalbumin may leak out of the alveolus between epithelial cells; this component has been measured in urine or blood of cows with mastitis (McFadden *et al*., 1988). α-lactalbumin and β-lactoglobulin have physiological properties of whey proteins including immunoenhancing effects. The possible role of α-lactalbumin as an antitumour agent is being investigated (Shah, 2000).

In conclusion, the present study showed that 1) modifications of the foremilk chemical composition are linked to the subclinical mastitis; and that 2) mastitis progression of quarters (CMT scores) influenced protein fractions in milk.

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