Influence of Bovine Mastitis on Lipolysis and Proteolysis in Milk

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ABSTRACT

Lipolysis and proteolysis in milk were determined before, during, and after experimentally induced mastitis. *Streptococcus agalactiae* was infused into one quarter of five cows to elicit an infection. Milk protease activity was higher during infection, but milk lipase activity was unchanged. Lipolytic damage to milk fat and proteolytic damage to milk casein occurred in the udder prior to milking during an infection. Lipolysis increased due to increased susceptibility of the milk fat to lipase action during infection. The mechanism of the increased susceptibility of the fat to lipolysis was not determined.

After infections were eliminated, SCC, initial and stored FFA concentrations, and initial tryosine values returned to preinfection levels. However, after infections were eliminated, milk protease activity as determined by an increase in tyrosine values remained elevated as milk SCC returned to preinfection levels. Protease activity returned to preinfections levels within 10 d after SCC returned to preinfection levels.

INTRODUCTION

The effect of mastitis and the resulting immune response on compositional and quality characteristics of bovine milk has been documented (21, 31). Of particular importance for dairy product quality is the influence of mastitis on breakdown of proteins (proteolysis) and development of rancid off-flavors through hydrolysis of fatty acids from triglycerides (lipolysis). Concentrations of FFA in milk at milking and after refrigerated storage are higher in milk from cows with mastitis (15, 17, 24, 26, 29, 37). However, there is disagreement on the effect of mastitis on milk lipase activity. It has been suggested by several researchers that milk lipase activity increases with infection (8, 26, 37) but others have found that it decreases or remains the same (15, 29). Because of the increased FFA levels during cold storage, milk from cows with mastitis has been associated with "spontaneous lipolysis", which by definition is a substantial increase in FFA that occurs simply upon cooling of the milk (11). After elimination of infection it is not clear if milk retains its "spontaneous lipolysis" character.

Earlier work on protein composition of milk from cows with mastitis suggested a change in the synthetic mechanism of milk secretion causing alterations in the casein and whey protein fractions (31). Current research suggests that an increase in proteolytic activity due to the inflammatory response may be responsible for the changes in the milk protein fraction (4, 6, 9, 10, 21, 27). Increased proteolytic activity can be detrimental to dairy product manufacture (2, 18, 25). Extensive proteolysis in milk can result in accumulation of small peptides, which can cause bitterness in certain dairy products (20, 39). Casein proteolysis products can be measured by the determination of tyrosine values (TV), which have been shown to be elevated in milks with high SCC (32).

The objective of this research was to determine the effect of mastitis on milk lipolysis and proteolysis with emphasis on postinfection im-

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provement in milk quality as milk SCC returned to normal concentrations.

MATERIALS AND METHODS

Experimental Design

Cow and Quarter Selection. Five Holstein cows were selected based on the following criteria: 1) second or third lactation, 2) production of >16 kg of milk/d, 3) no mastitis pathogens present in the udder as determined by standard bacteriological procedures (23), 4) current lactation average linear score SCC less than 3.5 (geometric mean SCC <140,000/ml of milk), and 5) no tendency for spontaneous lipolysis defined as a milk FFA less than .350 meq FFA/L after 48 h at 5°C. The left rear quarter of each udder was infused with an inoculum of *Streptococcus agalactiae* to induce mastitis as previously described (28). The right front quarter of each udder served as an untreated control.

Experimental Periods. The experiment was divided into four periods: 1) preinfection: baseline period prior to the infusion of the *Streptococcus agalactiae* inoculum, 2) infection: period corresponding to elevated SCC linear score greater than 5.6 (>600,000 somatic cells per ml of milk) and the presence of *Streptococcus agalactiae* as determined by standard bacteriological procedures (23) in the treatment quarter, 3) postinfection 1: period beginning when the infection was eliminated in treatment quarter and linear score SCC was less than 4.3 (<250,000 SCC/ml of milk), and 4) postinfection 2: period consisting of 7 to 10 d after postinfection 1. There were 3 sampling d within each period. Management of cows and antibiotic treatment of quarters during the infection period was as described previously (28).

Milk Analysis

Somatic Cell Counts. Milk SCC and linear scores were determined as previously described (28).

Free Fatty Acid Determinations. Free fatty acids were determined by a modified copper soap method as described by Shipe et al (33). Milk samples were acidified with .7 N HCl (.5 ml milk to .1 ml HCl) immediately after milking (to prevent further lipolysis) for determination of initial concentrations of FFA. Another unacidified portion of milk was cooled immediately on ice and stored at 5°C for 48 h as an indication of cold storage lipolysis (spontaneous lipolysis). Relative lipase activity was determined by adding .5 ml of cooled raw milk to be tested to 9.5 ml of freshly pasteurized homogenized milk substrate (Cornell Dairy Plant, 3.5% milk fat) followed by incubation at 5°C for 24 h. Relative lipase activity is expressed as the increase in FFA concentration.

Tyrosine Value Determinations. Merthiolate (.01%) was added as a preservative to milks immediately after milking. Tyrosine values were determined by the method of Juffs (19) as modified by Senyk et al (32) on fresh milks and milks after storage at 5°C for 48 h. As an index of relative proteolytic activity, TV were determined on a portion of the milks incubated at 37°C for 24 h. Relative protease activity (RPA) is expressed as the increase in TV (above the fresh milk TV) after 24 h at 37°C.

Statistical Analysis. Indices of lipolysis, proteolysis, and SCC from 3 sampling d within each of the four experimental periods were averaged for treatment and control quarters for each cow. Means were determined by experimental periods for linear score SCC, fresh TV, stored TV, relative protease activity, fresh FFA, stored FFA, and relative lipase activity. Means were tested by analysis of variance (SAS Institute, Cary, NC). The statistical model included terms for cow, period, quarter, and interaction between quarter and period.

RESULTS AND DISCUSSION

Somatic Cell Response

Somatic cell counts of milk for treatment quarters increased within 24 to 36 h after infusion of *Streptococcus agalactiae.* Three cows became clincally infected with the presence of abnormal milk or systemic symptoms. Two cows did not show clinical signs of mastitis; however, these did have elevated milk SCC. Peak linear score SCC for the 5 infected cows ranged from 6.03 (815,000 cells/ml) to >10.5 (19,000,000 cells/ml).

Mean linear scores and corresponding geometric means for SCC during the four experimental periods are summarized in Table 1. Infection period mean linear scores increased ($P<.05$) over preinfection period in the treat-

TABLE 1. Linear score and geometric mean SCC in treatment and control quarter milks.

SCC in Milk	Experimental periods			
	Preinfection	Infection	Postinfection 1	Postinfection 2
	Linear score[1]			
Treatment	2.55[a]	7.88[b]	3.58[a]	3.35[a]
Control	2.47	2.55	2.79	2.75
	Geometric mean ($\times 10^3$)			
Treatment	83	5506	163	152
Control	85	93	111	124

[a,b]Means in the same row with different superscripts differ ($P<.05$); n = 5 cows.

[1] Linear score SCC = $((\log_e (SCC/1000)/.693147) - 3.543856$; SEM = .41.

ment quarter. Mean linear score for the treatment quarter for postinfection 1 and 2 periods were significantly lower than the infection period ($P<.05$) and not significantly different from preinfection SCC ($P>.05$), although they were slightly higher. No differences could be determined for mean linear scores in the control quarters ($P>.05$).

Lipolysis

Initial FFA levels for fresh milks, FFA levels for milks stored for 48 h at 5°C, and relative lipase activity are shown in Table 2. The FFA levels (meq FFA/L) for fresh milk at the time of milking and milks after storage at 5°C for 48 h from the treatment quarters were higher ($P<.05$) during the infection period than the preinfection period. No differences in FFA levels were observed in milks from control quarters. Other investigators have also demonstrated that FFA levels in fresh milk and in milk after cold storage are elevated during mastitis (15, 24, 29, 37).

The FFA levels of fresh and stored milks from postinfection periods 1 and 2 remained

TABLE 2. Initial FFA levels, stored FFA levels, and relative lipase activity in treatment and control quarter milks.

	Experimental periods			
	Preinfection	Infection	Postinfection 1	Postinfection 2
	(meq FFA/L)			
Initial FFA[1]				
Treatment	.106[a]	.153[b]	.120[a]	.122[a]
Control	.109	.113	.115	.121
Stored FFA[2]				
Treatment	.217[a]	.342[b]	.260[a]	.259[a]
Control	.223	.193	.222	.237
Relative lipase activity[3]				
Treatment	.672	.636	.624	.660
Control	.692	.611	.642	.689

[a,b,c]Means in the same row with different superscripts differ ($P<.05$).

[1] Initial FFA concentration in milks immediately after milking; SEM = .006.

[2] Stored FFA = FFA concentration in milks after 5°C for 48 h; SEM = .020.

[3] Relative lipase activity = Increase in FFA concentration in pasteurized homogenized milk (3.5% milk fat) on addition of raw milk sample (.5 ml raw: 9.5 ml pasteurized, homogenized) and incubation at 5°C for 24 h; SEM = .028.

slightly higher than preinfection levels although this was not significant ($P>.05$). In this study, even though milks became "spontaneously lipolytic" during severe mastitic infection, they did not retain this characteristic when the infection was eliminated and SCC returned to low levels.

Relative milk lipase activity was not different for any of the periods tested for milks from both treatment and control quarters. This agrees with others who have also found no effect of mastitis on milk lipase activity (15, 29). Therefore, in mastitic milks, a change in milk lipase activity does not appear to be responsible for increased FFA of the fresh or stored milks. The milk fat substrate apparently becomes more accessible, allowing greater lipolysis to occur as seen from the higher initial FFA and stored FFA during the infection period of this study (Table 2). The mechanism by which this occurs has not been defined, but increased lipolysis has been correlated to an increased association of lipoprotein lipase to the milk fat globule (34, 35). This association apparently occurs at the surface of the milk fat globule membrane (MFGM), which is thought to act as a barrier to lipolysis in normal milk. The MFGM must be altered to allow milk lipoprotein lipase to attack the milk fat substrate. The mechanism by which this occurs in mastitic milk is not clear, although compositional changes in the MFGM (3, 14), influence of bovine blood serum components (5, 36) and somatic cell enzymes (7) have been implicated. Other components of the inflammatory response (e.g., IG), which have not been fully investigated, may also contribute to increased lipolysis of mastitis milk.

Although the mechanisms have not yet been determined, it is clear that a mastitic infection and concurrent inflammatory response will cause an increase in "spontaneous lipolysis" while an infection persists. It is important to note that once an infection is eliminated and milk SCC returns to a preinfection level, the milk returns to the lipolytic character it had before the cow had a mastitic infection.

Proteolysis

Mean TV for five cows are shown in Table 3. During the infection period there was a significant increase ($P<.05$) in initial TV of milks from treatment quarters. This indicates that proteolysis occurred in the mammary gland prior to milking and agrees with observations of casein proteolysis products in fresh milks measured by PAGE (10, 28) and column chromatography elution patterns (9). An increase in premilking proteolysis may be accompanied by an increase in milk relative protease activity. During the infection period the

TABLE 3. Initial milk tyrosine values (TV), stored TV, relative protease activity in treatment and control quarter milks.

	Experimental periods			
	Preinfection	Infection	Postinfection 1	Postinfection 2
	Tyrosine (mg/L)			
Initial TV[1]				
Treatment	31.7[a]	69.5[b]	40.4[a]	39.1[a]
Control	30.7	36.8	37.3	38.0
Stored TV[2]				
Treatment	4.2	7.0	0.5	1.1
Control	4.8	2.2	0.7	0.7
Relative protease activity[3]				
Treatment	12.7[a]	62.5[c]	24.5[b]	19.0[ab]
Control	13.3	18.9	19.1	16.3

[a,b,c]Means in the same row with different superscripts differ ($P<.05$).

[1] Initial TV of milks immediately after milking; SEM = 3.8.

[2] Stored TV = Tyrosine at 5°C for 48 h minus initial TV; SEM = 1.1.

[3] Relative protease activity = TV at 37°C for 24 h minus initial TV; SEM = 3.9.

increase in TV after incubation of merthiolate preserved milks for 24 h at 37°C (RPA) of the treatment quarters was greater ($P<.05$) than the RPA in the preinfection period for treatment quarters. Higher milk protease activity due to infection agrees with results of others (4, 6, 9, 10, 27, 28). Increased protease activity could be at least in part responsible for higher initial TV in the fresh milks during the infection period.

In postinfection periods 1 and 2, the initial TV for fresh milks were lower than during the infection period ($P<.05$) and were not different from preinfection levels ($P>.05$). This suggests that once an infection is eliminated and SCC returns to normal, premilking proteolysis is not detectably different by TV determination from that before mastitis. Similar results were obtained by electrophoresis (28).

The RPA of treatment quarter milks in postinfection 1 was lower than during the infection ($P<.05$) but remained higher than the preinfection RPA. Postinfection 2 RPA was not significantly different from preinfection values or from postinfection 1. This was not entirely in agreement with Saemen et al (28). Under the same experimental conditions they measured RPA by SDS-PAGE expressed as the percent decrease of intact α-casein plus β-casein of treatment and control milks incubated at 37°C for 24 h. Proteolysis of α-casein and β-casein was earlier found to be higher ($P<.05$) than preinfection proteolysis in postinfection periods 1 and 2 (28), but in this study RPA was different only in postinfection 1. Saemen et al (28) attributed proteolysis during the postinfection periods primarily to plasmin activity, whereas nonplasmin proteolysis made a significant contribution during the infection period. Assuming plasmin as the major proteolytic enzyme in milk in the postinfection periods could explain why no significant difference in RPA (between preinfection and postinfection 2) was found as measured by TV in this study even though SDS-PAGE found significantly higher RPA under the same conditions. Plasmin preferentially hydrolyzes α-casein and β-casein to polypeptides with molecular weights of greater than 3000 daltons (1, 13, 30, 39). Tyrosine values are a measure of tyrosine- and tryptophan-containing peptides and amino acids, which are soluble in 10% TCA. Ten percent TCA-soluble peptides have been found to have molecular weights of less than 350 daltons (16). Most of the plasmin proteolysis products would not be soluble in 10% TCA, thus making TV a less sensitive indicator of plasmin hydrolysis of caseins. Verdi et al (39) came to a similar conclusion in a study of farm bulk milk samples where they found that TV and TCA-soluble N determinations were not as sensitive as SDS-PAGE techniques in determining the extent of proteolysis and RPA in fluid milks.

During the infection period, concentrations of nonplasmin proteases, which are assumed to be of somatic cell origin, increase (28). Proteolysis products produced by these enzymes are not as well-defined as plasmin proteolysis products (6, 10, 28). However, a recent study found they are different from plasmin proteolysis products (38). Our results indicate clearly that during an infection these enzymes are capable of hydrolyzing casein into 10% TCA-soluble components as demonstrated by the elevated TV during the infection period (Table 3). This is important for milk quality, because accumulation of these components can lead to bitterness in dairy products (20, 22, 32, 40).

Milks stored at 5°C for 48 h had no increase ($P>.05$) in TV over preinfection levels in any of the other periods studied (Table 3). This suggests that the enzymes responsible for the increased TV in fresh mastitic milks and those incubated at 37°C for 24 h are not active or have very low activity at refrigeration temperatures. Senyk et al (32) found an increase in TV of preserved raw milks with elevated SCC when stored at 6°C for 72 h. The enzyme plasmin has been found to be active at refrigerated temperatures (12, 30). The extent of cold storage proteolysis of casein and the effect on quality and dairy processing of milks produced by cows during and after mastitis needs further investigation.

CONCLUSIONS

Concentrations of FFA in milk initially and after storage of milks at 5°C for 48 h were higher during induced mastitis. After the infection was eliminated and the milk SCC was reduced, the initial and stored FFA concentrations in milk returned to preinfection levels. Total relative milk lipase activity did not change during or after mastitis. Increased lip-

olysis in mastitic milk apparently results from increased susceptibility of the milkfat substrate to lipolysis and not increased lipase activity.

Proteolytic damage to casein in fresh milk and relative protease activity were higher during mastitic infection. After infection, milk protease activity remained elevated longer than milk SCC.

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