Effect of Recombinant Bovine Somatotropin on Milk Production and Composition of Cows with *Streptococcus uberis* Mastitis

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ABSTRACT

The protective effect of bovine somatotropin (bST) during experimental *Streptococcus uberis* mastitis in cows was studied. The left quarters of 10 cows were infected with 500 cfu of *S. uberis* O140J. Five cows were subcutaneously treated with 500 mg of recombinant bST 7 d before and after infection, and 5 control cows received the excipient. In the treated cows, total milk production significantly increased after the first and second bST treatments. After infection, milk production decreased 24 and 40% in the infected quarters, 6 and 14% in the uninfected quarters, and 15 and 28% overall for treated and control cows, respectively. In the bST group, milk production was completely restored after 3 wk, but, in the control group, total production and the production of the infected quarters remained lower than preinfection production. The increase in somatic cell count occurred earlier and more rapidly in the control group, and the return to normal values was also more rapid in these cows. The amount of bacteria in milk was higher in the control cows. Changes in milk composition, such as lactose, protein, fat, Na^+, K^+, and Cl^-, were significantly more pronounced in the control cows. Also, clinical symptoms were more prominent in the control cows. Somatotropin protected the mammary gland from excessive production losses and compositional changes during a subsequent episode of experimentally induced *Streptococcus uberis* mastitis and significantly improved the normalization of production and composition, which indicates a beneficial effect on the restoration of the integrity of the blood-milk barrier.

(**Key words**: *Streptococcus uberis* mastitis, recombinant bovine somatotropin, milk production, milk composition)

Abbreviation key: **MP** = milk production.

INTRODUCTION

Mastitis is economically the most important disease in the dairy industry (39) because of the costs of prevention and curative treatment, veterinary services, culling, technical problems in milk processing, increased labor, and large production losses (15). Because the hygiene on well-managed dairy farms has improved, the relative incidence of infectious mastitis, such as *Staphylococcus aureus* mastitis, has decreased, and the relative incidence of environmental mastitis, such as coliform and *Streptococcus uberis* mastitis, has increased (18, 40). Recombinant bST protected the mammary gland from excessive milk production (**MP**) losses during a subsequent experimentally induced *Escherichia coli* mastitis (47) in cows. Normalization of MP and milk composition was accelerated by bST, especially in severely diseased cows (47). Moreover, during cases of *E. coli* mastitis in cows, increased concentrations of bST, insulin, and cortisol have been observed (48). Administration of bST to dairy cows significantly increases MP. Depending on the stage of lactation, production increases of 10 to 15% have been reported (28, 31, 45).

The purpose of the present study was to investigate whether the protective role of pretreatment with recombinant bST during experimentally induced *E. coli* mastitis could also be observed during experimentally induced *S. uberis* mastitis, which has a completely different pathogenesis. Indeed, acute *E. coli* mastitis after calving is accompanied by severe systemic symptoms, especially in the so-called severe responders (47), with, in some cases, even shock and death. These severe systemic signs are not observed during *S. uberis* mastitis, which is characterized by local mastitis symptoms and very few systemic symptoms (16). In addition, neutrophils are of major

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importance in the pathogenesis of *E. coli* mastitis, but contribute little to the pathogenesis of *S. uberis* mastitis (16, 17). Moreover, antibiotic treatment of acute coliform mastitis always comes too late, but is necessary to cure streptococcal mastitis. We studied whether bST was important in the homeostasis of inflammation of the bovine mammary gland and in the subsequent loss of function. Therefore, an inflammatory model of the mammary gland infected with *S. uberis*, which has a different pathogenesis than *E. coli* mastitis, was used.

MATERIALS AND METHODS

Cows

Ten East Flemish Red Pied cows that were in their first lactation, clinically healthy, and free of mastitis were selected. At the start of the experiment, the cows were between 6 and 15 wk postpartum. Before these cows were purchased, the mammary gland was examined clinically, and milk samples were analyzed from each quarter. Only those cows with an SCC less than 2×10^5/ml for each mammary quarter sample that were negative for mastitis pathogens were selected. Mean SCC before infection was $47 \times 10^3 \pm 6 \times 10^3$/ml. During the experiment, the cows were housed in individual tie stalls. Cows were transferred to these stalls 1 wk before the start of the experiment to allow acclimation. Cows were fed a daily ration of approximately 8 kg of concentrate and had ad libitum access to water and hay. Cows were fed twice daily at 0630 and 1630 h, which did not overlap with sampling or treatment. Cows were milked twice daily at 0700 and 1700 h with a device that measures individual mammary gland quarter milk production. Mean MP before the first treatment was 16.8 ± 0.2 kg/d.

Inoculation Procedure

A *S. uberis* strain O140J (James Leigh, Compton, UK), which is a phagocytosis-resistant strain in the presence of casein (25, 26), was maintained in a lyophilization medium at –20°C. For experimental use, the bacteria were subcultured in Todd Hewitt broth (Lab M, Bury, UK) at 37°C for 18 h. After washing, the organisms were resuspended and diluted in pyrogen-free PBS. On d 0, 1 h after the morning milking, the cows were inoculated in the left front and left rear quarters with a suspension containing approximately 500 cfu of *S. uberis* O140J in a total volume of 20 ml of sterile pyrogen-free saline solution per quarter. The bacterial suspension was inoculated into the teat cistern using a sterile teat cannula. Before inoculation, the teat ends were disinfected with 70% ethanol containing 0.5% chlorhexidine. After the inoculation, each quarter was massaged for 30 s to distribute the bacterial suspension in the mammary gland.

EXPERIMENTAL DESIGN

All cows were experimentally infected with 500 cfu of *S. uberis* at d 0 of the experiment. Five cows were injected subcutaneously into the ischiorectal fossa with 500 mg of recombinant bST (Posilac®; Monsanto Co., St. Louis, MO) 7 d before infection (d –7) and 7 d after infection (d +7). Similarly, five control cows were also subcutaneously injected with the excipient on the same days. The experiment consisted of four different periods: an 8-d period before the first injection (d –7), a 7-d period between the first treatment and infection (d 0), a 7-d period after the infection and before the second treatment (d +7), and a 3-wk period after the second treatment. A mixture (Nafpenzal®; Mycofarm Belga, Turnhout, Belgium) of sodium nafcillin (100 mg), sodium penicillin G (300,000 U), and dihydrostreptomycin sulfate (100 mg) was injected once daily into the infected quarters of all cows 48 h after the first appearance of clinical symptoms. This intramammary antibiotic treatment was continued for 3 consecutive d at 24-h intervals.

Milk Sampling Schedule

Milk samples were aseptically collected from each individual mammary quarter for bacteriological examination. Samples also were collected for determination of milk SCC, fat, total protein, lactose, Na^+, K^+, Cl^-, and BSA. These samples were collected once daily at the morning milking on d –8, –4, –1, +6, +7, +8, +9, +14, +21, and +28. On d 0, +1, +2, and +3, milk samples were collected at 0700, 1400, and 2000 h. For bacteriological examination of the milk, the samples taken aseptically were immediately streaked out on a blood agar plate made of sheep red blood cells using an inoculation loop of 10 μl and incubated at 37°C. Scoring of the bacterial growth was completed 24 h after inoculation by simply counting the number of colonies grown on the plate. Separate milk samples were kept at 4°C and sent to the Belgian Society for Milk Quality (Lier) for determination of SCC, fat, protein, and lactose content. Remaining samples were used to prepare skim milk by centrifugation at 1000 × *g* for 15 min. After the fat layer was discarded, the skim milk was divided in aliquots and stored at –20°C

until further analyses. The MP was measured for each individual quarter.

Observations and Analyses

Somatic cell count was determined for each individual quarter by means of the fluoro-opto-electronic cell counting principle (Fossomatic® 360; Foss Electronic, Eden Prairie, MN), which is based on the binding of ethidium bromide to DNA. For statistical analysis, geometric means were used. Lactose, protein, and fat concentrations of quarter milk samples were determined by the infrared spectrophotometric principle (MilkoScan® 4000; Foss Electronic). Sodium, K^+, and Cl^- were analyzed using ion selective electrodes (Ilyte®; Instrumentation Laboratory, Zaventem, Belgium). Bovine serum albumin concentrations in milk were analyzed according to the method of Guzman et al. (14), which is based on a colorimetric method using bromocresol green, NaOH, and lactic acid.

Cows and quarters were also clinically investigated at each sample collection. Rectal temperature, heart rate, feed intake, and forestomach motility were monitored. Observations on experimentally infected and control quarters included pain, firmness, and swelling of quarters and appearance of milk.

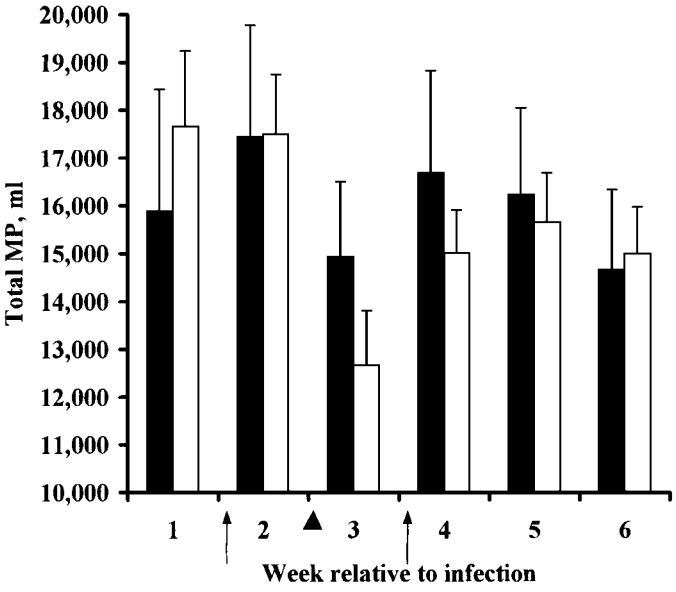

Figure 1. Changes in total milk production (MP) of recombinant bST-treated cows (■) and control cows (□) during experimentally induced (▲) *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Weeks relative to infection: –2 = week before first treatment (arrow), –1 = week after first treatment and before infection, 1 = 1st week after infection and before second treatment (arrow), 2 = 1st wk after second treatment, 3 = 2nd wk after second treatment, and 4 = 3rd wk after second treatment.

Statistical Analyses

Means, standard deviations, and standard errors of the means were calculated using the statistical analysis program package Statistix® (42). Statistical analysis of all data was performed using an ANOVA (mixed linear model) with the following model: $Y = \mu + T + t + C/T + Int1 + Int2 + e$, where Y = dependent variable, μ = overall mean, T = treatment, t = time, C/T = cow nested within treatment, Int1 = interaction between treatment and time ($T \times t$), Int2 = interaction between the cows nested within the treatment and the time ($C/T \times t$), and e = experimental error term. Treatment and time were fixed variables, and individual cows were randomized variables, which were nested within the treatment. To study the effects of treatment, the error term was C/T. To study the effects of time, the Int2 term was the error term. After running the ANOVA, general contrasts were calculated. Significant differences were determined at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS

Total MP

In the bST-treated cows (Figure 1), total MP increased ($P < 0.01$) 10% after the first treatment. During wk +1, total MP decreased 15 and 28% for the treated ($P > 0.05$) and the control groups ($P < 0.01$). At d +2, the maximal decrease was 23 and 36% in the treated and control groups. Total MP of the control cows during wk +1 was much lower ($P < 0.001$) than preinfection MP values. From d +6, MP started to increase ($P < 0.05$) in both groups. During the last week of the experiment (wk +4), total MP decreased ($P < 0.01$) by 11% in the treated cows compared with the MP during the 14 d after the second treatment.

The MP of the control cows during wk +4 was about 15% lower ($P < 0.05$) than the MP at the start of the trial. The MP during wk +4 was lower than the MP during the week before infection in the bST-treated cows ($P < 0.01$) and the control cows ($P < 0.05$). The recovery of total MP 4 wk after infection was complete in the treated cows. Total MP of the treated cows after the second treatment equaled the MP after the first treatment. In contrast, MP of the control cows during the 2nd wk postinfection remained lower ($P < 0.01$) than that before infection. Total MP preinfection was higher (21%; $P < 0.001$) than overall postinfection MP for the control cows. In the treated cows, overall MP after infection slightly increased by 7%. When total MP of the two groups at the different periods was compared, differences ($P < 0.05$) between

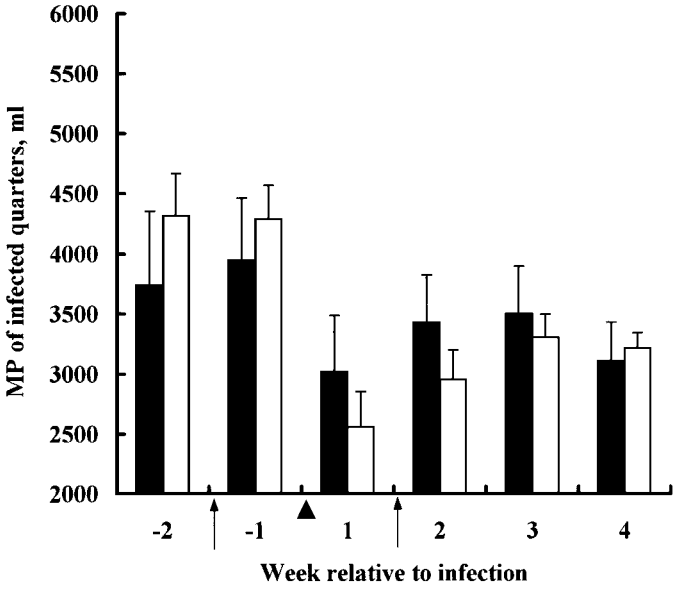

Figure 2. Changes in milk production (MP) of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced (▲) *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Weeks relative to infection: –2 = week before first treatment (arrow), –1 = week after first treatment and before infection, 1 = 1st week after infection and before second treatment (arrow), 2 = 1st wk after second treatment, 3 = 2nd wk after second treatment, and 4 = 3rd wk after second treatment.

groups could be observed at the start of the trial and during wk +1 and +2.

MP of the Infected Quarters

The changes in the MP of infected quarters were similar to those for the total MP, although they were more pronounced (Figure 2). The MP of infected quarters after infection declined by 24 ($P > 0.05$) and 40% ($P < 0.01$) in the treated and control group during wk +1. The maximal decrease was observed on d +2 in both the treated (30%) and control cows (46%). In the control cows, the MP of infected quarters during wk +1 was lower ($P < 0.001$) than the MP before infection. Around d +6, MP started to increase in both groups. In the control group, the MP during wk +3 further increased ($P < 0.001$) compared with the MP during wk +1. The MP of the treated cows increased during 2 wk after the second treatment but decreased ($P < 0.05$) by 11% during the 3rd wk after the second treatment.

During wk +4, MP was 26% ($P < 0.05$) lower than the MP before the first treatment in the control cows and 17% lower for the treated cows ($P > 0.05$). The recovery in the treated cows was already complete during wk +2. The MP during 3 wk after the second bST treatment was lower ($P < 0.001$) than the MP before infection for the control cows. In the treated cows, MP after the first treatment was similar to MP during the 2 wk after the second treatment. The MP during wk +2 and +3 in the control cows was still lower ($P < 0.01$) than the MP before infection. The MP of infected quarters in the control cows during the preinfection period was greater (43%) than that during the total postinfection period ($P < 0.001$). The difference in the treated group was 18% ($P > 0.05$).

MP in the Contralateral Control Quarters

Only minor changes in the MP of control quarters could be observed (Figure 3). After the first bST treatment, MP of contralateral quarters increased ($P < 0.01$) by 12%. After infection, MP of contralateral quarters decreased 6 and 14% in the treated ($P > 0.05$) and control ($P < 0.05$) cows compared with preinfection MP. During the 2nd wk postinfection, MP was increased in both the treated ($P > 0.05$) (9%) and the control groups (17%; $P < 0.05$). During the 4th wk postinfection, the MP of treated cows decreased 11% ($P < 0.001$) compared with a 6% ($P < 0.05$) decrease for control cows.

Recovery of MP in contralateral control quarters to preinfection levels was complete in both groups. The MP by the treated cows after the first and second treatments was higher ($P < 0.01$) in comparison with the MP before the first treatment. Preinfection MP of contralateral quarters did not differ ($P > 0.05$) from the overall postinfection MP for either group.

Milk SCC

In both groups, SCC (geometric means) in the infected quarters started to increase after infection (Figure 4). This increase was more pronounced in the control cows. The SCC peaked at 36 h postinfection in the control group ($P < 0.001$) and between approximately 78 and 96 h postinfection in the bST-treated group ($P < 0.01$). The SCC remained at a very high level from 24 to 9 d after infection in the control cows. During the first 24 h after infection, SCC increased in the treated cows ($P < 0.05$) and in the control cows ($P > 0.05$). During the next 24 h (24 to 48 h postinfection), SCC further increased in the treated group ($P > 0.05$) and the control group ($P < 0.001$). This increase ($P < 0.01$) continued until 96 h after infection in the treated cows. From d +6, SCC started to decrease ($P < 0.01$) in the treated cows. At the end of the experiment, however, SCC was still higher ($P < 0.05$) compared with preinfection value in the treated group. In the control cows, normal SCC was recovered

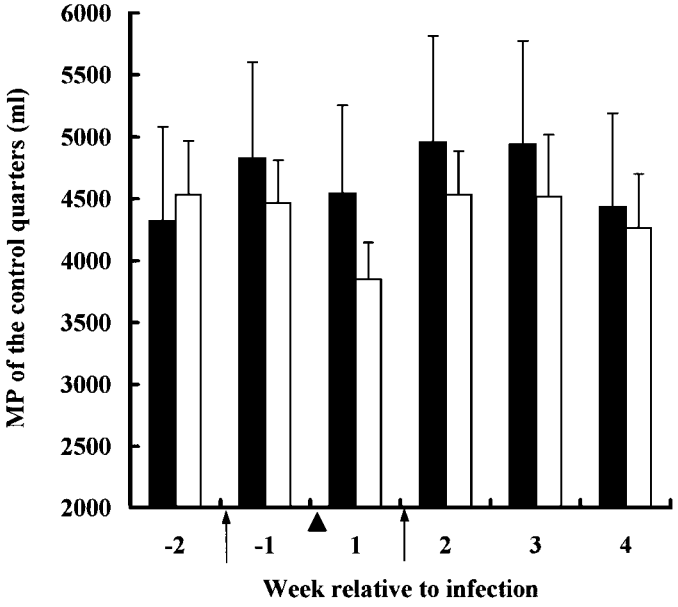

Figure 3. Changes in milk production (MP) of contralateral control quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced (▲) *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Weeks relative to infection: –2 = week before first treatment (arrow), –1 = week after first treatment and before infection, 1 = 1st wk after infection and before second treatment (arrow), 2 = 1st wk after second treatment, 3 = 2nd wk after second treatment, and 4 = 3rd wk after second treatment.

completely. Thus, an increase in SCC after infection occurred earlier and more rapidly in the control group than in the bST-treated group, but the return to normal values at the end of the experiment also was more rapid in the control cows. From d +14, SCC was higher ($P > 0.05$) in the treated cows than in the control cows. Between d +14 and + 28, SCC stabilized in the treated cows but remained higher ($P < 0.05$) than before infection. In the control cows, however, a decrease ($P < 0.01$) was observed between d +14 and + 28 to values that did not differ ($P > 0.05$) from preinfection values.

The SCC in the infected quarters was higher in control cows than in the treated cows on d +2 ($P < 0.001$), +3 ($P < 0.05$), and d +6 to +9 ($P < 0.05$).

Milk Bacterial Counts

Significant differences in the number of bacteria in the milk of the infected quarters were observed only between 48 and 84 h after infection (Figure 5). During this period, the amount of bacteria was higher in the control cows, especially between 78 and 84 h after infection ($P < 0.001$). During the first 24 h after infection, the amount of bacteria in milk increased ($P < 0.001$) for cows in both groups. On d +6, the amount of bacteria increased ($P < 0.01$) in the treated cows. From d +14, the amount of bacteria decreased ($P < 0.01$) again in both groups compared with the counts between d +7 and +14. By the end of the experiment, the bacterial amount did not differ ($P >0.05$) from preinfection numbers.

Milk Lactose

No changes ($P > 0.05$) in the lactose concentration occurred in the contralateral control quarters (Table 1). The two groups only differed at d +2 ($P < 0.05$), which was due to a sudden decrease in milk lactose in the control cows at 36 h after infection.

Based on mammary quarters, numerous changes occurred (Figure 6). Beginning at 24 h after infection, the lactose concentration decreased by 35% in the infected quarters of control cows ($P < 0.01$) but did not change in bST-treated cows. From 48 to 84 h postinfection, lactose concentration continued to decrease ($P < 0.01$) in the control cows; the lowest concentration was reached between 48 and 72 h after infection ($P < 0.001$). Milk lactose concentration from

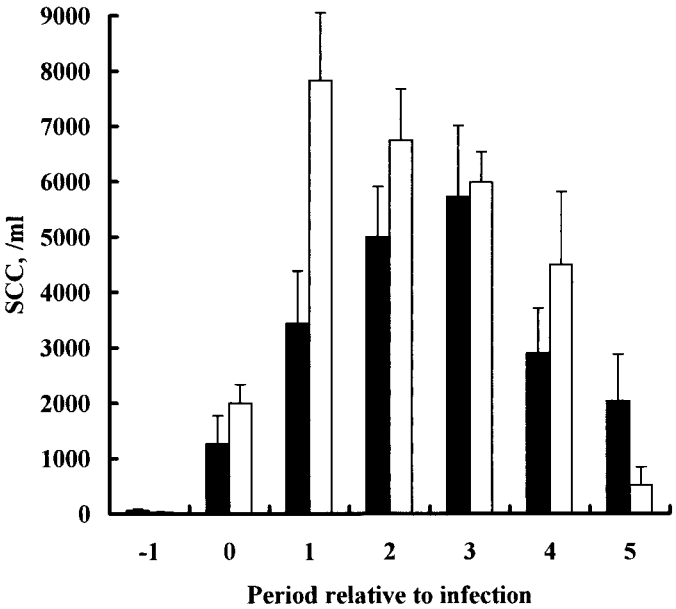

Figure 4. Changes in milk SCC of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Geometric means were calculated. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

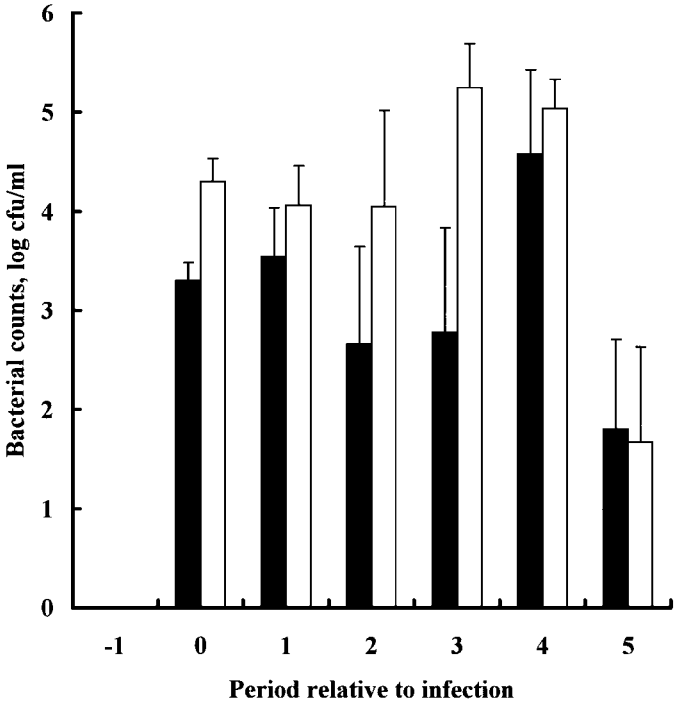

Figure 5. Changes in bacterial count in the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

the treated cows decreased ($P > 0.05$) between 48 and 72 h postinfection. By 72 h postinfection, lactose concentration started to increase again. The concentration between d +6 and +9 was lower ($P < 0.05$) than the preinfection concentration for both groups. A return to normal lactose concentrations was not achieved in either group compared with the preinfection concentration ($P < 0.05$).

For the control cows, the concentration of lactose before infection was higher ($P < 0.001$) than the concentration during the entire postinfection period, but, for the treated cows, no difference ($P > 0.05$) could be observed between these two periods.

The concentration of milk lactose from the two groups of cows differed on d +1 (30 to 48 h; $P < 0.01$), +2 (54 to 72 h; $P < 0.001$), and +3 (78 to 84 h; $P < 0.05$).

Milk Fat

The concentration of milk fat in the infected quarters of the control cows decreased by 25% during the first 24 h after infection ($P > 0.05$). This decrease was followed by an increase ($P < 0.05$) between 30 and 72 h postinfection. By d +6, the fat concentration in the infected quarters of the control cows normalized. No changes ($P > 0.05$) were observed for the treated cows. For the control cows, fat concentration of milk from the control quarters increased ($P < 0.01$), peaking between 54 and 72 h postinfection. This increase was followed by a decrease and a normalization by d +9. No changes ($P > 0.05$) could be observed for the treated cows. Recovery of milk fat in the treated and control quarters was complete for both groups. The fat concentration in the infected and the control quarters before infection did not differ ($P > 0.05$) from the concentration observed during the complete postinfection period for both groups.

Milk Protein

During the first 72 h postinfection, no changes ($P > 0.05$) in milk protein concentration took place in the infected quarters of the treated cows (Figure 7). At 72 h postinfection, total milk protein increased ($P < 0.01$) by 11%. From d +7, the concentration started to decrease but still remained higher ($P < 0.05$) than preinfection concentrations, even on d +28 ($P < 0.01$). The concentration before infection did not differ from the concentration during the entire postinfection period. Treatment with bST on d –7 and +7 had no effect on total protein concentration in the infected quarters.

In the control cows, milk protein in the infected quarters was not altered during the first 24 h after infection. By 30 h postinfection, milk protein had increased ($P < 0.05$) by 29% with a dramatic increase at 48 h postinfection. Although protein concentration decreased after d +3, it still remained higher ($P < 0.01$) than the preinfection concentration until the end of the experiment. Between 54 and 72 h postinfection, protein concentration in milk from the control cows was higher ($P < 0.001$) than that in milk from the treated cows. Overall concentrations in the control quarters of both the treated and the control groups were unaffected during the experiment (Table 1).

BSA

Milk BSA increased ($P < 0.01$) during the first 24 h after infection in both groups (Figure 8). During the next 24 h, the concentration further increased in the control group ($P < 0.01$) and the treated group ($P > 0.05$). Between 48 and 72 h postinfection, BSA increased in the treated group ($P < 0.05$) but not ($P > 0.05$) in the control cows. The greatest increase ($P <$

TABLE 1. Milk composition in the contralateral control quarters of the bST-treated and control cows during the different periods of the experiment.[1]

Milk constituent[2]		Preinfection	Postinfection					
		–192 to 0 h	6 to 24 h	30 to 48 h	54 to 72 h	78 to 84 h	144 to 216 h	336 to 672 h
Lactose (g/L)	bST	50.0 ± 0.7	50.2 ± 0.5	50.5 ± 0.8	51.0 ± 0.4	50.1 ± 1.4	50.3 ± 0.9	49.6 ± 0.8
	Control	50.4 ± 1.2	49.9 ± 1.2	47.9 ± 1.6	50.3 ± 0.8	47.9 ± 2.3	49.6 ± 1.2	49.2 ± 1.0
Fat (g/L)	bST	43.7 ± 2.4	41.5 ± 3.4	51.0 ± 3.1	45.1 ± 3.3	44.5 ± 1.4	40.5 ± 5.9	36.5 ± 4.8
	Control	38.1 ± 4.1	40.9 ± 3.9	50.5 ± 5.0	53.5 ± 5.4	50.7 ± 11.3	43.6 ± 1.8	39.1 ± 3.4
Protein (g/L)	bST	32.3 ± 1.13	32.2 ± 1.20	31.6 ± 1.71	32.1 ± 1.72	32.9 ± 1.68	32.6 ± 1.17	33.6 ± 0.92
	Control	32.2 ± 0.82	32.5 ± 0.89	31.9 ± 0.86	30.7 ± 1.49	30.7 ± 2.12	32.6 ± 0.83	33.4 ± 0.88
BSA (g/L)	bST	0.68 ± 0.27	0.87 ± 0.88	1.09 ± 0.10	1.58 ± 0.16	1.00 ± 0.19	1.51 ± 0.13	1.32 ± 0.19
	Control	0.63 ± 0.13	0.79 ± 0.73	1.09 ± 0.15	1.07 ± 0.14	0.82 ± 0.58	1.41 ± 0.40	1.01 ± 0.73
Na^+ (m*M*)	bST	14.2 ± 0.62	15.5 ± 1.70	13.8 ± 0.99	15.3 ± 1.07	14.9 ± 0.74	15.9 ± 0.96	14.7 ± 1.00
	Control	15.2 ± 0.67	13.8 ± 0.32	20.1 ± 4.51	16.4 ± 0.79	16.5 ± 0.67	19.6 ± 2.12	14.7 ± 0.26
K^+ (m*M*)	bST	40.8 ± 1.08	37.6 ± 0.69	36.5 ± 1.37	40.1 ± 1.32	40.2 ± 1.14	39.4 ± 1.45	40.1 ± 1.20
	Control	41.8 ± 1.65	34.6 ± 1.63	36.7 ± 1.08	40.2 ± 0.95	39.8 ± 1.99	40.0 ± 1.14	41.2 ± 0.72
Cl^- (m*M*)	bST	24.4 ± 1.60	25.4 ± 1.40	24.2 ± 1.42	28.9 ± 1.97	29.5 ± 1.23	31.1 ± 2.72	27.8 ± 2.19
	Control	25.0 ± 1.60	24.8 ± 1.17	26.4 ± 1.39	29.4 ± 0.70	29.6 ± 1.25	28.5 ± 1.10	27.7 ± 0.65

[1]Data are means of five cows ± SEM. Each cow was experimentally infected with 500 cfu *S. uberis* O140J (J. Leigh, Compton, U.K.) into the left quarters. Five bST-treated cows were subcutaneously treated with 500 mg of recombinant bST (Posilac®, Monsanto Co., St. Louis, MO) 7 d before and after infection; 5 control cows received the excipient.

0.001) in the control group was observed between 24 and 72 h after infection. Although BSA began to decrease around 72 h postinfection in the treated group, on d +7, the concentration of BSA in milk was still higher than the preinfection concentration for cows in the treated group ($P < 0.05$) and in the control group ($P < 0.01$). This slow decrease continued until the end of the experiment. In contrast,

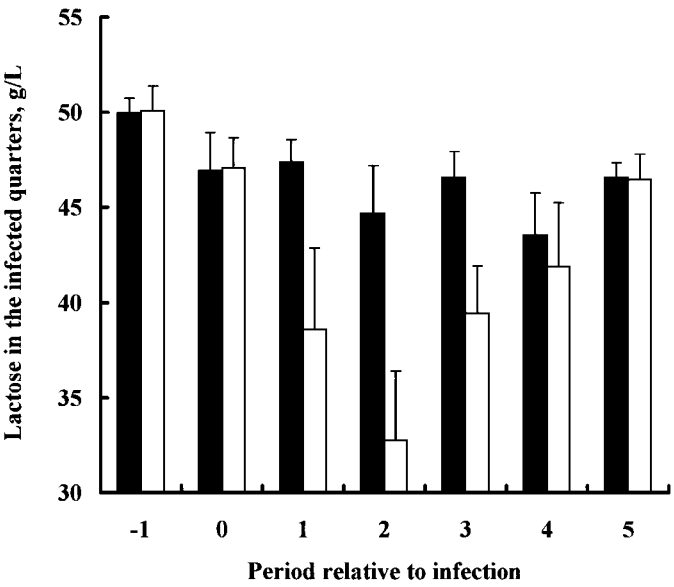

Figure 6. Changes in lactose concentration of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

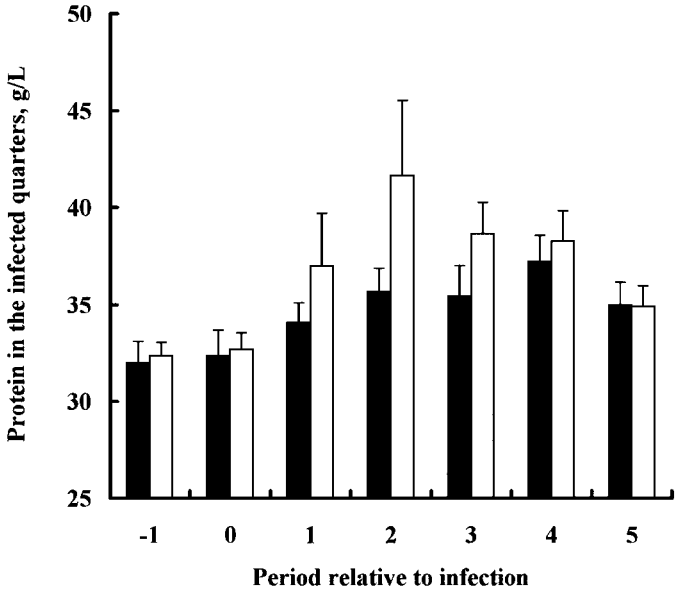

Figure 7. Changes in total protein concentration of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

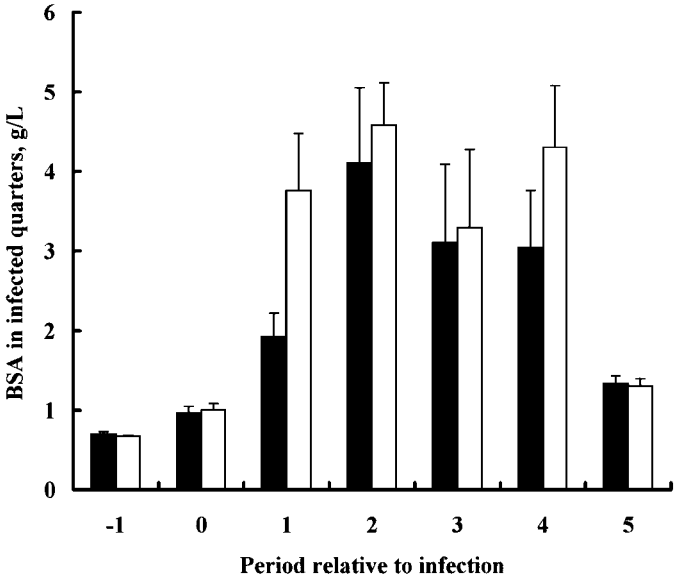

Figure 8. Changes in the concentration of BSA of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

after 72 h postinfection, BSA decreased ($P < 0.05$) in the control group but increased ($P < 0.05$) again between d +6 and +9. After this short episode of increased concentrations, BSA continued to decrease ($P < 0.01$) during the next week. No complete recovery could be accomplished in either group. The concentration on d +28 was still higher ($P < 0.001$) than the preinfection concentration.

During the first 24 h after infection, an increase ($P < 0.05$) in BSA was observed in the contralateral control quarters of both groups, peaking ($P < 0.001$) at 72 h postinfection in the treated group. After this maximum, BSA decreased ($P < 0.01$) in the treated cows. One week after infection, the concentration increased ($P < 0.001$) again toward concentrations observed at 72 h postinfection. During the last 3 wk of the experiment, the concentration decreased ($P < 0.05$) toward a level that was still higher ($P < 0.001$) than the preinfection value. In the control cows, an increase toward a maximum at 48 h was observed ($P < 0.01$) followed by a second increase ($P < 0.001$) between d +6 and +9 postinfection, which was followed by a decrease ($P < 0.001$) during the last 3 wk of the trial toward a concentration that was still higher ($P < 0.05$) than those before infection.

Milk Na^+

During the first 24 h after infection, Na^+ concentration did not alter ($P > 0.05$) in either group (Figure 9), but increased ($P < 0.05$) during the next 24 h in the control group. Between 54 and 72 h postinfection, a three- and fourfold increase was observed in the treated and control cows. After this peak, Na^+ slowly decreased toward values that were still higher than preinfection values in the treated ($P < 0.01$) and the control groups ($P < 0.05$) on d +28.

No changes ($P > 0.05$) could be observed in the contralateral control quarters of treated cows (Table 1).

Milk K^+

During the first 24 h after infection, milk K^+ concentration decreased 24 and 27% in the treated ($P < 0.01$) and control cows ($P < 0.001$) (Figure 10); the lowest values were observed between 30 and 48 h postinfection. In the treated group, K^+ concentrations remained lower ($P < 0.001$) than preinfection concentrations from 6 to 48 h postinfection. Milk K^+ in the control group over the complete postinfection period was lower ($P < 0.05$) than the preinfection concentration. In the control group, recovery by the end of the experiment was not complete ($P < 0.05$).

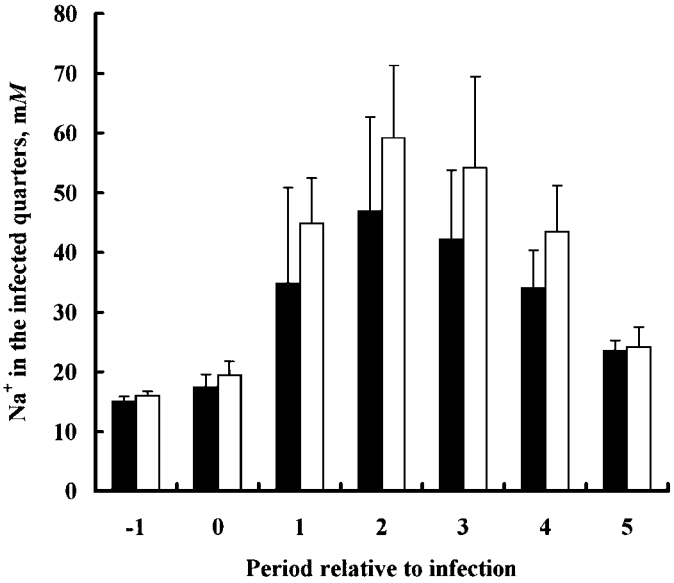

Figure 9. Changes in Na^+ concentration of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

In the contralateral control quarters, K^+ concentration decreased 10 and 17% during the first 24 h postinfection in the treated ($P < 0.05$) and the control groups ($P < 0.001$) (Table 1), remained lower ($P < 0.01$) until 48 h, then increased ($P < 0.001$) in both groups after 54 h. Recovery by the end of the study was complete. In the control group, the overall postinfection concentration was lower ($P < 0.05$) than the preinfection concentration.

Milk Cl^-

Milk Cl^- concentration increased twofold ($P < 0.05$) in the treated group and increased 2.3-fold ($P < 0.01$) in the control group (Figure 11). Peak values occurred between 54 and 84 h after infection. At the end of the trial, the concentration was still higher ($P < 0.001$) than that before infection in both groups, indicating that recovery was not complete. In the control group, the concentration over the entire postinfection period was higher ($P < 0.05$) than the preinfection concentration.

In the contralateral control quarters, Cl^- increased ($P < 0.01$) in both groups between 54 and 72 h after infection (Table 1) and remained elevated until d +9. No difference ($P > 0.05$) could be observed between preinfection and postinfection Cl^- concentrations in either group.

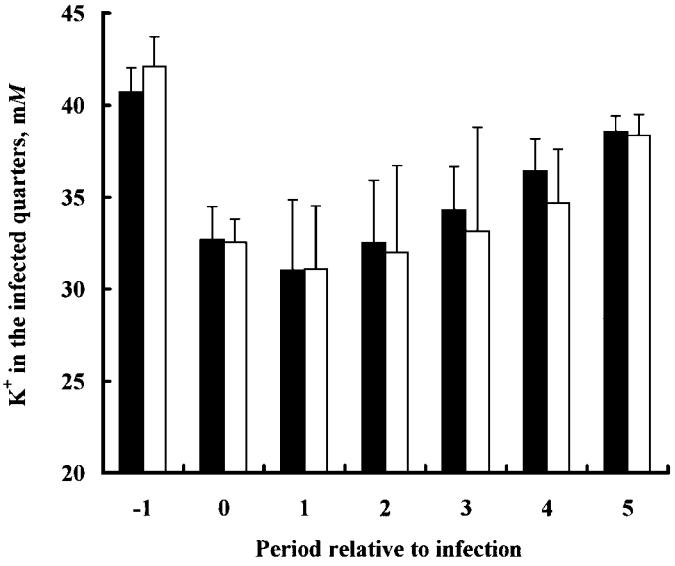

Figure 10. Changes in K^+ concentration of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

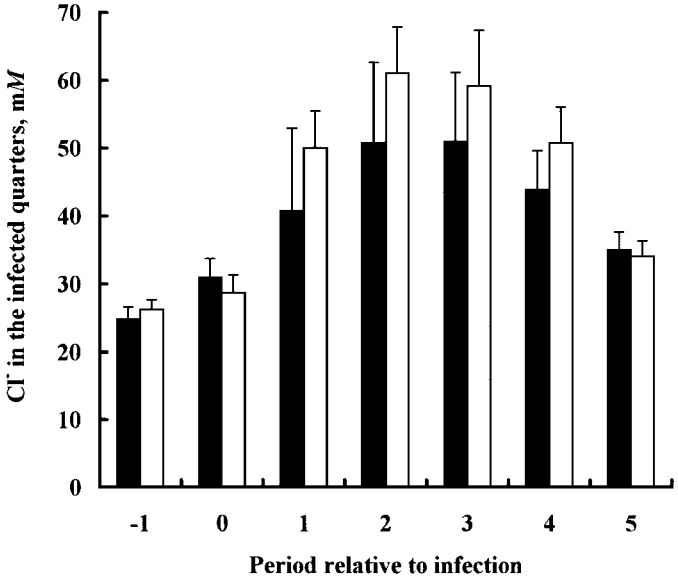

Figure 11. Changes in CL^- concentration of the infected quarters of recombinant bST-treated cows (■) and control cows (□) during experimentally induced *Streptococcus uberis* mastitis. Data are means of five cows, and error bars represent the standard errors of the means. Periods: –1 = d –8 until 0 h, 0 = 6 to 24 h after infection, 1 = 30 to 48 h after infection, 2 = 54 to 72 h after infection, 3 = from 78 to 96 h after infection, 4 = d +6 to +9, 5 = d +14 to +28.

General and Local Clinical Symptoms

The general and local clinical symptoms are discussed more extensively in a companion paper (19). At 24 h after intramammary inoculation of *S. uberis*, the infected quarters were swollen. Between 24 and 48 h postinfection, the firmness of the infected quarters significantly increased, especially in the control cows. Flakes and clots appeared in the milk of the infected quarters from 30 h after infection, especially in the control cows, and disappeared in some cows only after 9 d. In the most severely diseased cows the milk became watery and yellow. Symptoms of pain appeared from about 36 h after infection and disappeared 1 wk after infection. Local symptoms were more pronounced and of longer duration in the control cows. The appearance of local signs in the treated cows occurred later than in the control cows.

Local symptoms were accompanied by general symptoms such as fever, tachycardia, and mild depression. In the treated cows, fever was observed between 6 and 30 h after infection and peaked at 39.4 ± 0.19°C. In the control cows, fever was observed between 1 and 3 d after infection and peaked at 40.3 ± 0.14°C. Rectal temperature showed several fluctuations, especially in the control group. Tachycardia was observed between 24 and 48 h after infection in

the control group and between 48 and 72 h after infection in the treated group. Loss of appetite and rumen motility was not observed ($P > 0.05$).

For ethical reasons, cows had to be treated with antibiotics, but, to minimize interference with the effects of bST, this antibiotic treatment was postponed as long as possible. All cows in the control group were treated for the first time on d +6 for 3 consecutive d. One cow received a second treatment on d +14. In the treated group, 4 cows were treated for the first time on d +6. The 5th cow received her treatment on d +10. One cow was treated for a second time on d +17.

DISCUSSION

The administration of recombinant bST (Posilac®) in healthy cows subsequently infected with experimental *S. uberis* mastitis was associated with a positive effect upon changes in MP and milk composition. Injection of bST in healthy cows induced an increase in MP after 2 d and a plateau around 4 to 5 d after injection, which is comparable with the results of French et al. (11), who observed an increase by 14% and a plateau after 5 to 6 d. Decreases in MP of the contralateral control quarters after experimental infection were only minor compared with the decreases in total MP and MP of the infected quarters. This result might suggest that the systemic impact of *S. uberis* mastitis is very low, which is in contrast to *E. coli* mastitis. Indeed, in the *E. coli* experiment of Vandeputte-Van Messom and Burvenich (47), the decline in MP of the uninfected quarters was much higher (32 and 61% for moderate and severe responders) than the MP losses of the contralateral quarters observed during *S. uberis* mastitis (14%). The decrease in total MP and MP in the infected and contralateral quarters during the 3rd wk after the second treatment indicates that this sustained-release form of recombinant bST exerts its effect during 2 wk. Pretreatment with bST of healthy cows subsequently intramammarily infected with *S. uberis* has a positive effect on the MP losses during mastitis and the recovery of MP after mastitis. Vandeputte-Van Messom and Burvenich (47) reported similar findings during experimental *E. coli* mastitis.

We observed a time interval of 2 to 3 d between the administration of bST in healthy cows and the increase in MP, which is in agreement with the results of others (11, 32, 33). The reduced synthesis of fatty acids in adipose tissues 3 d after bST administration (49) supports the theory of repartitioning of nutrients by bST (11, 30, 51) as a mechanism by which bST increases MP in healthy mammary glands. The inflamed mammary gland appears to be susceptible for the galactopoietic effect of somatotropin, although the mammary epithelial cells and the blood-milk barrier are damaged. The mechanism by which bST is able to increase MP of an inflamed gland is not completely understood. The presence of receptors for IGF-I and somatotropin on mammary tissue might be involved (2, 6, 12).

The increase in SCC after infection and the return to normal values at the end of the trial occurred earlier and faster in the control cows, and recovery was complete in the latter. This result is in contrast to the results of the *E. coli* trial of Vandeputte-Van Messom and Burvenich (47), who observed a faster influx of leukocytes into milk in the bST-treated cows. Recombinant bST appeared to slow down the influx of defense cells during a subsequent experimental *S. uberis* infection. A high neutrophil influx induces severe damage during *S. uberis* mastitis, and the rate of influx of polymorphonuclear leukocytes during *E. coli* mastitis is determinant for the outcome and severity of *E. coli* mastitis (17). Neutrophils are ineffective at controlling infection caused by *S. uberis* (16); however, macrophages are important (46). Treatment with bST had no effect on the recovery of SCC in both the *E. coli* and *S. uberis* mastitis studies. In healthy cows, bST has no effect on SCC (29), except in very high dosages of >960 mg/28 d. In the present experiment, the treated cows received about 1000 mg/28 d, which is indeed higher than the highest dosage in the study of McGlary et al. (29). These dosages may induce an increase in SCC, which might explain why the SCC in the treated cows was higher compared with the control cows during the last week of the experiment. Elvinger et al. (8) could not detect stimulating effects of somatotropin on the migration and chemotaxis of polymorphonuclear leukocytes, although McGlary et al. (29) and Burvenich et al. (4) suggested an increased diapedesis of polymorphonuclear leukocytes from the circulation to the mammary gland by bST.

The effect of bST on bacterial counts in milk could only be estimated during the first 6 d after infection because all cows received antibiotic therapy 48 h after appearance of clinical symptoms. Bovine somatotropin had no bacteriostatic or bactericidal effects. However, treatment of cows with bST induces an increased concentration of IGF-I (5, 19, 36, 44). Fattal et al. (10) showed that IGF-I is involved in the posttranscriptional modulation of the activity of the plasminogen activator inhibitor type 1 by prolonging

the mRNA half-life. As a consequence, the activation of plasminogen to plasmin is decreased. Because of this decrease, fewer amino acids become available for the bacteria, which suppresses their growth. Indeed, *S. uberis* is an auxotrophic agent, and its growth depends on the acquisition of essential amino acids which become available by the proteolytic effect of plasmin (22, 27). Because of this decreased availability of essential amino acids, the growth of *S. uberis* may be inhibited after bST treatment.

Politis et al. (34) demonstrated an inverse relationship between the concentration of plasmin in milk and somatotropin. During mastitis, the concentration of plasmin and plasminogen increases in milk by damage of the epithelial cells with disruption of the tight junctions and leakage of blood plasmin and plasminogen into the milk (paracellular transport) (37, 38). Also, an increase in the SCC is associated with increased plasmin activity in milk (35). However, it is unlikely that plasminogen activators from these cells contribute to the increased plasmin concentration. In the bST-treated cows, this increased plasmin activity may be counteracted by the increase in IGF-I, which might explain the lower amount of bacteria in the treated cows. It is highly unlikely that the intrinsic plasmin activator activity of *S. uberis* (23, 24) is reduced by the stimulating effect of IGF-I on the plasminogen activator inhibitor type 1.

Concerning the milk composition, the most prominent effects were observed in the infected quarters. Only minor changes could be observed in the contralateral quarters. The decrease in lactose concentration in the control cows could be due to leakage toward the circulation, to decreased production by the damaged mammary epithelial cells, and to an increase in consumption by the multiplying *S. uberis* in the mammary gland. In contrast with the control cows, no significant changes could be observed in the fat concentration of the infected and contralateral quarters of the treated cows. Fat concentration was completely normal 4 wk after infection in both groups. The significantly more pronounced changes in protein concentration of the infected quarters of the control cows were mainly due to the influx of BSA into the mammary gland.

However, the increase in total milk protein cannot be completely explained by the increase in BSA, which indicates that other inflammation-related proteins (e.g., acute phase proteins) appear in the milk. The high concentrations at the end of the experiment might be due to increased concentrations of immunoglobulins and other inflammation-related proteins and to an influx of BSA. Although BSA increased in the control quarters, a decrease in total milk protein was observed, which was probably due to a decreased production of milk proteins. The reduction in milk proteins might be due to a decreased absorption of amino acids in the gut and to a redirection of the use of amino acids toward immunoglobulins. Because of the increased proteolytic activity of the milk during mastitis (1, 7, 20, 37), which is mainly due to increased plasmin activity, caseins are hydrolyzed. These changes in milk composition indicate serious damage to the blood-milk barrier with disruption of the tight junctions between mammary epithelial cells and paracellular leakage (43). Administration of recombinant bST appears to protect this barrier from excessive damage because the composition changes in the treated cows were less pronounced. The lack of a complete recovery of the concentration of milk protein, BSA, and lactose indicates that the integrity of the damaged blood-milk barrier was not completely restored 4 wk after infection.

Changes in the concentration of Na^+, K^+, and Cl^- in the infected quarters were more prominent in the control cows. These changes also suggest damage of the blood-milk barrier. At the end of the experiment, Na^+ and Cl^- concentration were still significantly higher than preinfection concentrations in both groups and K^+ only in the control group. Treatment with recombinant bST protected the mammary gland from excessive losses in MP and compositional changes. However, in both groups, 4 wk after infection, the damaged blood-milk barrier was still not completely restored, even in the bST-treated cows. Indeed, the concentrations of lactose, protein, BSA, Na^+, and Cl^- in the infected quarters of both groups were still significantly different from the concentrations observed before infection.

The mechanism by which the integrity of the blood-milk barrier is preserved and restored is not understood. Positive effects of IGF-I on the cytoskeleton of the epithelial cells and on the tight junctions between these cells may be important. Indeed, effects of IGF-I or somatotropin on the cytoskeleton, tubulin mRNA, and cytoskeletal reorganization have been reported in rats (3, 13) and humans (21, 50). Ericson and Nilsson (9) studied the effect of IGF-I on the epithelial barrier in polarized pig thyrocyte monolayers. Indeed, IGF-I receptors (2) and growth hormone receptors (12) have been observed on mammary tissue. Insulin-like growth factor I increased the transepithelial potential difference without significant changes in the transepithelial resistance. Similar

studies could be performed in a cell culture model of the blood-milk barrier (41).

Because neutrophils contribute little to the pathogenesis of *S. uberis* mastitis (17), which is in contrast to *E. coli* mastitis, the possible positive effects of bST on several neutrophil functions, as suggested by Vandeputte-Van Messom and Burvenich (47) but not confirmed by Elvinger et al. (8), are probably not responsible for the observed beneficial effect of recombinant bST during experimental *S. uberis* mastitis.

In conclusion, treatment of healthy cows with bST protects cows from excessive MP losses and excessive milk compositional changes during a subsequent experimental intramammary infection with *S. uberis*. Although not complete, the recovery of MP and milk composition was markedly improved by protective and postinfection treatment with bST. The protective role of bST is not restricted to *E. coli* mastitis only, and bST is important in the homeostasis of inflammation of the mammary gland.

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