Effect of Somatotropin on Changes in Milk Production and Composition During Coliform Mastitis in Periparturient Cows

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ABSTRACT

The potential protective and therapeutic effects of bST during coliform mastitis in periparturient cows were evaluated. In a first study, 19 cows, classified as moderate or severe responders based on the respiratory burst activity of blood neutrophils, were treated with recombinant bST or vehicle 48 h after intramammary inoculation of *Escherichia coli*. Clinical status and changes in milk production and composition were compared in the four groups. In a second study, 8 cows received bST or vehicle 7 d before bacterial challenge.

During mastitis, losses in milk production and compositional changes were most pronounced in infected glands and in severe responders. Milk production of bST cows recovered better than that of placebo cows. Recovery of milk components was accelerated in severe responders treated with bST, but not in moderate responders. Pretreatment of severe responders with bST enhanced milk production before infection, protected the mammary glands from excessive loss of milk during the subsequently induced coliform mastitis, and accelerated normalization of milk composition.

In conclusion, the beneficial effects of bST upon normalization of milk production and composition in periparturient cows suffering from coliform mastitis seem to be restricted to the severe responders. In severe responders that had been treated with bST, changes observed during mastitis resembled those in moderate responders treated with the placebo.

(**Key words**: somatotropin, milk, mastitis, periparturient cows)

Abbreviation key: **IMM** = intramammary, **MP** = milk production.

INTRODUCTION

The great variability in illness and the wide range of pathological responses observed during experimentally induced *Escherichia coli* mastitis in periparturient cows (28, 29, 31) led us to distinguish between moderate and severe responders. As described earlier (29), classification was based upon the capacity of blood neutrophils of cows to generate reactive oxygen species (13) and upon differences in blood and milk components prior to infection. Both groups differed in the course and the intensity of clinical responses and in the changes in milk production (**MP**) and composition after induction of *E. coli* mastitis. Effects were most pronounced in infected glands and in severe responders for which recovery was slow and incomplete (29).

Administration of pituitary or recombinant bST to healthy dairy cows increases MP; increases of 15 to 40% were reported during short-term or long-term treatment studies in early, mid, or late lactation [review, (20)]. The magnitude of the MP responses to bST largely varies with the lactation period. In early lactation, these responses may be absent (2, 19) or small (8, 22). However, recent reports indicate that exogenous bST also is effective at stimulating MP even when treatment began within the first 3 wk after parturition (24, 26). In healthy cows, the action of bST seems to consist in repartitioning of nutrients to the mammary gland in order to support the requirements of milk synthesis (1). In goats,

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pretreatment with recombinant bST increased MP above that for pretreatment controls and stimulated the recovery of MP suppressed during endotoxin mastitis (4). Increasingly, indications are obtained that bST has a role in immunoregulation. Somatotropin has been reported to be synthesized and released by lymphocytes (33). Exogenous bST can increase the proliferative responsiveness of peripheral blood lymphocytes stimulated by mitogen in culture (3), augments granulopoiesis in human bone marrow, and is potent in priming macrophages for the production of $O_2\cdot$ (15). Recently evidence was also obtained that indicates that bST influences the maturation and function of the neutrophils in the cow. Pretreatment of cows with bST shortly after calving increased total leukocyte number, increased the number of circulating immature band cells, and enhanced the burst capacity of the neutrophils induced by phorbol myristate acetate [(16); C. Burvenich et al., 1993, unpublished data]. However, Elvinger et al. (10) did not observe any substantial effect of bST on bovine polymorphonuclear leukocytes in vivo or in vitro. Nevertheless, all these observations suggest that, by stimulating the cow's defense mechanisms, bST may help to protect the mammary gland against invading bacteria. It can be hypothesized that bST plays an important role in the outcome of mastitis and the inflammatory process of the mammary gland. This hypothesis is supported by the observation that somatotropin is released after i.v. injection of endotoxin in goats (5, 30) and during *E. coli* mastitis in cows (31).

In order to obtain more information about a possible protective and therapeutic role for bST, the present study was designed to investigate 1) whether short-term treatment with recombinant bST stimulates the recovery of MP and composition in cows suffering from coliform mastitis; 2) whether these effects differ in moderate and severe responders; 3) whether pretreatment of cows with bST protects the mammary gland against the negative aspects of *E. coli* mastitis; and 4) whether an eventually beneficial effect is comparable with that observed in healthy cows under physiological circumstances, or that, on the contrary, specific mechanisms concerning neutrophil function may be involved. Therefore, experiments were performed on second lactation cows shortly after calving. Changes in MP and composition after induction of mastitis were compared in moderate and severe responders treated with vehicle or bST.

MATERIALS AND METHODS

Cows

Twenty-seven cows of the East Flemish Red Pied breed and mean (± SEM) body weight of 554.4 ± 21.5 kg were used. Cows were in their second lactation, healthy, and free of udder infection. At the commencement of the study, cows were 23.6 ± 2.7 d postpartum. Before the cows were purchased, the udder was examined clinically, and quarter foremilk samples were obtained for bacteriological examination and SCC. Only cows with quarter SCC below 250×10^3 cells/ml and negative for staphylococci and Gram-negative bacteria were transferred to our laboratory, where they were housed in individual box stalls in a climate-controlled stable. Cows were permitted to adjust to the housing facilities and to the milking procedures for at least 5 d. Cows were given a daily ration of 8 kg of concentrates and had free access to hay and water. Feeding was at 0730 and 1600 h and did not overlap sampling or injection. Cows were milked twice a day at 0800 and 1900 h using a quarter milker. Quarter MP were recorded separately. Mean MP was 15.8 ± .7 kg/d.

Inoculation Procedures

An *E. coli* strain (P4: serotype 032) recovered from a cow with a clinical case of bovine mastitis was maintained in stock on nutrient agar CM3 (Oxoid Ltd., Basingstoke, England) at 4°C. For experimental use the organisms were subcultured in brain-heart infusion broth (CM 225; Oxoid) at 37°C over 3 successive d. After washing three times, the culture was diluted in pyrogen-free saline immediately prior to each experiment. One hour after the morning milking, cows were inoculated in both quarters of the left udder half with a suspension containing 10^4 cfu of *E. coli*/ml. Inoculations were made into the teat cistern using a sterile teat cannula after aseptic preparation of teat ends. Following inoculation each gland was massaged for 30 s to distribute the solution.

Experimental Design

In a first study, 19 cows suffering from experimentally induced *E. coli* mastitis were treated with recombinant bST (40 mg/d) or vehicle by daily injection. Each experiment comprised a 5-d preinfection period, a 2-d mastitis period, a 10-d treatment period, and a 10-d posttreatment period. Two kinds of responders were distinguished based upon the respiratory burst activity of blood neutrophils and different blood and milk components during the preinfection phase of the experiment (29). Nine cows, which had blood neutrophils that showed a high capacity to generate reactive oxygen species, were classified as moderate responders. The other 10 cows, with a low capacity to generate reactive oxygen species, were classified as severe responders. Each group was randomly subdivided into a placebo and a bST group. The four experimental groups thus obtained in the first study were 1) moderate placebo group (inoculated, receiving vehicle, moderate responders; n = 4); 2) moderate bST group (inoculated, receiving bST, moderate responders; n = 5); 3) severe placebo group (inoculated, receiving vehicle, severe responders; n = 5); and 4) severe bST group (inoculated, receiving bST, severe responders; n = 5). After the preinfection control period, all cows in the four groups received intramammary (IMM) inoculations of the *E. coli* suspension (d 0). On the inoculation day, placebo cows were 34.3 ± 5.4 d postpartum; bST cows were 27.3 ± 1.8 d postpartum. On d +1, all cows received systemic (after 24 h) and local (after 32 h) antibiotics. A mixture of trimethoprim (1 g) and sulfadoxin (5 g) was i.v. injected twice a day (Duoprim®; Wellcome, Aalst, Belgium). Pomesul® (500,000 IU of polymixin B; Pfizer, Brussels, Belgium) was injected into the infected quarters. Antibiotic treatment was continued for at least 7 d until disappearance of general (e.g., fever) and local (e.g., swelling and firmness of mammary glands) clinical symptoms. On the morning of d +2, cows of the bST group were injected subcutaneously (4 ml) with 40 mg of recombinant bST (sometribove; Monsanto Co., St. Louis, MO). Cows of the placebo group received vehicle only. The bST or vehicle was injected in the area around the shoulder after thorough disinfection of the skin. Cows were treated at 0900 h each day. Treatments were maintained for 10 d. The recombinant bST, prepared as a dry powder, was solubilized in $NaHCO_3$ solution (75 m*M*) and maintained at 4°C until used. Stock solutions were made every 2nd d.

In a second study, cows were pretreated with long-acting recombinant bST (500 mg, bST pretreatment group; n = 5) or vehicle (placebo pretreatment group; n = 3) 7 d before IMM inoculation of *E. coli* organisms. Each experiment comprised a 5-d pre-bST period, a 7-d post-bST but preinfection period, and a 3-wk postinfection period. Pretreatment was by subcutaneous injection (1.3 ml) of a single dose of either a 14-d prolonged-release system of sometribove (Monsanto Co.) or a sterile vehicle.

Milk Sampling Schedule

Milk was sampled for determination of different milk components and for bacteriological examination. During the preinoculation control period and during treatment and posttreatment periods, for each quarter, daily milk samples were taken from evening and from morning bulk milk. Evening and morning samples were pooled per quarter according to MP to give one representative milk sample per day. On the day of experimental mastitis, six samples (every 3 h) were taken, and, on the day thereafter, three samples (every 4 h) were taken. For bacteriological examination of milk, each sample was immediately streaked on a plate of blood agar using an inoculating loop designed to deliver .01 ml of milk. Part of each sample was kept at 4°C for immediate analysis of fresh whole milk. Part was used for skim milk preparation by centrifugation at 3000 rpm for 20 min. After the fat layer was discarded, the skim milk was stored at –20°C until further analyses.

Observations and Analyses

Milk cells were measured in whole milk with a Coulter Counter (ZF; Coulter Electronics Ltd., Luton, England). Skim milk samples were used for analysis of content of casein, lactose, serum albumin, and α-lactalbumin according to methods described earlier (29). Milk chloride was measured using a chloridemeter (EEL 920; Corning, Halstead, England), and sodium and potassium were analyzed by flame

photometry (IL 243; Instrumentation Laboratory, Milan, Italy). Milk fat (cream) was measured using a hematocrit reader (27).

After induction of mastitis, the cows were clinically observed. Cow and quarter observations were made at each sample collection as well as hourly on d 0 and every 4 h on d +1. Rectal temperature, heart rate, respiratory rate, attitude, and appetite were evaluated. Quarter milk appearance and quarter size, swelling, and pain were evaluated. Measurements of body weights, MP, and feed intake were made.

Definitions and Statistical Analyses

All definitions used in this paper in relation to mastitis are in accordance to the definitions proposed by the International Dairy Federation (14).

Daily production per quarter was calculated for MP, cream, lactose, casein, and N. Means per period were taken for infected and uninfected quarters for each cow. Over the entire course of the experiments, results were pooled into four 5-d periods (period −1, +1, +2, +3) and one 2-d period (period 0). The latter included the inoculation day (d 0) and the day after inoculation day (d +1).

Means, standard deviations, standard errors of the mean, and Student's *t* test were computed according to Snedecor and Cochran (25). Differences between groups were calculated according to Student's *t* test. Treatment differences within mastitis groups were tested for statistical differences by analysis of variance with the preinfection period (period −1) as covariate.

RESULTS

Study 1: Effects of bST Treatment of Mastitic Cows

The IMM *E. coli* infections in cows shortly after calving induced clinical signs of quarter inflammation, such as swelling, warmth, and firmness of infected glands. These local symptoms were accompanied by systemic disturbances, including fever, tachycardia, mild depression, loss of appetite, and general illness of the cows. Peak effects were observed between 10 and 14 h. Maximal fever, amounting to 41.7 ± .2°C in the moderate and to 42.0 ± .2°C in the severe responders, was accompanied by tachycardia (92 and 96 beats/min, respectively). For detailed data, see the study by Vandeputte-Van Messom et al. (29). In the moderate responders, most local and systemic symptoms disappeared within 48 h. In the severe responders, effects were delayed and remained significantly above preinfection levels for several days. The contralateral uninfected glands did not show any local signs of inflammation. From all infected quarters, *E. coli* organisms were isolated within 5 to 6 h after inoculation. Multiplication rate in the milk was maximally 1.3×10^5 cfu/ml of milk in the moderate responders and 4.5×10^5 cfu/ml in the severe responders between 18 and 24 h postinfection. The rate of appearance of SCC in the milk was larger in moderate responders than in severe responders. Maximal leukocytosis occurred at 32 (14.7×10^6) and 48 h postinfection (13.7×10^6), respectively, and did not differ significantly between groups. The MP of infected and uninfected quarters was depressed. Maximal losses in MP amounted to 56.2 ± 7.1 and 84.7 ± 4.3% in the infected glands and to 31.6 ± 3.3 and 61.2 ± 6.5% in the uninfected glands of moderate and severe responders, respectively. Milk K^+, fat, α-lactalbumin, casein, and lactose decreased; Na^+, Cl^-, noncasein N, and serum albumin concentrations increased. Changes were most pronounced in infected glands and in severe responders. In order to evaluate the effects of bST treatment on the changes in MP and composition during experimentally induced *E. coli* mastitis, results from infected and uninfected glands were discussed separately. In each series of results, comparison was made between moderate and severe responders and between placebo and bST cows within each group.

For all cows within a group, daily MP values from all quarters were averaged to give one mean value per quarter and per day. Mean daily MP in the infected quarters of the four groups is shown in Figure 1. During mastitis (period 0), the loss in MP compared with that at preinfection is comparable between the placebo and the bST cows in both moderate and severe responders. In not one of the four groups did MP completely return to the preinfection level. Taking into account the initial suppression of MP during period 0, comparison of the MP recovery values in both placebo and bST cows suggested a slight but nonsig-

nificant gain in MP because of bST treatment: 5% (period +2) in the moderate and 10% (period +3) in the severe responders. Concentrations of different milk components before and during mastitis and during and after bST or vehicle treatment in moderate and severe responders are shown in Table 1. During the mastitis period, changes in milk components were comparable in bST and placebo cows. Differences were observed in the course of normalization of milk components between the different groups. In both moderate placebo and bST groups, milk composition gradually returned to normal after 32 h postinfection. Preinfection levels were obtained after 2 d (for K^+ and fat), 7 d (for SCC, lactose, and serum albumin), and 12 d (for Na^+ and Cl^-) postinfection. In the severe placebo cows, the recovery of milk composition was limited and at the end of the experiment amounted maximally to 46.3%. In the severe bST cows, normalization started after 72 h postinfection and was maximally 87.6%. The bST effect was most pronounced during the second half of treatment period (period +2) and during the 5 following d (period +3), resulting in marked and significant differences in milk Na^+ ($P < .01$), Cl^- and lactose ($P < .001$), and α-lactalbumin ($P < .02$) between placebo and bST cows. Treatment with bST had no effect upon the rate of normalization of the number of cells in the milk. Yield patterns of different milk components in infected quarters paralleled that of MP. In moderate responders, recovery of yields of lactose and fat was 5 and 11% better, respectively, in bST-treated cows. Recovery of yields of α-lactalbumin and casein was 13 and 20% better, respectively, in bST cows. In the severe responders, losses in yields of lactose, fat, α-lactalbumin, and casein during period +3 were reduced by 16, 9, 47, and 37%, respectively, from those of placebo cows. Although mean results showed a recovery for all milk components, their statistical level was, except for that of casein ($P < .02$), nearly significant ($P = .07$).

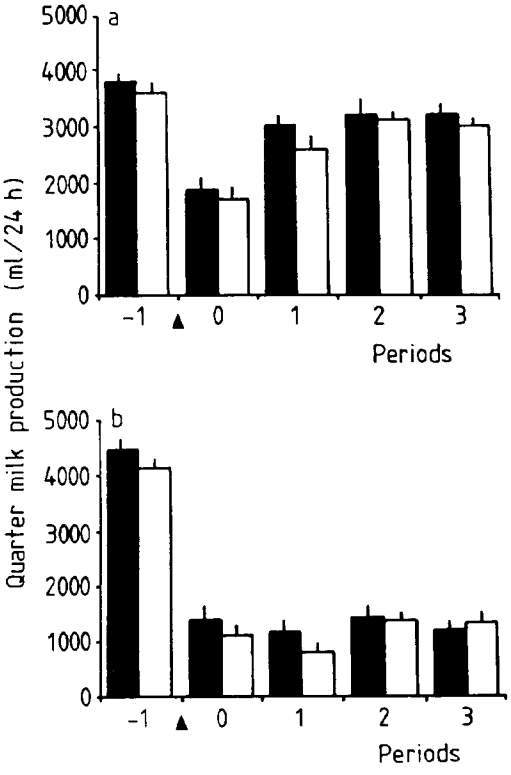

Figure 1. Effect of placebo (■) or bST treatment (□) during periods +1 and +2 on milk production in infected quarters of periparturient moderate (a) and severe (b) responders during experimentally induced (↑) *Escherichia coli* mastitis. Values are means per quarter and per 24 h for a given period. Each period lasted 5 d except period 0, which included d 0 and d +1 after induction of mastitis. Vertical bars represent standard errors of the mean.

Mean daily MP in the uninfected glands of the four groups is shown in Figure 2. Suppression of MP during the mastitis period is comparable in the placebo and bST cows of both moderate and severe responders. In placebo cows, MP recovered maximally by 95 and 69% in the moderate and severe responders, respectively. In the bST cows, however, MP increased above preinfection levels by 7 and 1% in moderate and severe responders, respectively, a gain in MP in the uninfected glands from bST treatment of 12% in moderate and 32% in severe responders. Recovery of MP in the moderate responders (Figure 2a) was statistically significant ($P = .029$). The changes in milk composition in the uninfected mammary glands (Table 2) followed the same pattern as in the infected quarters. Except for α-lactalbumin ($P < .01$), changes during mastitis were often negligible in moderate responders. During and after bST treatment, concentrations of different milk components did not differ from those in the placebo cows. In the severe responders, significant changes in Na^+ ($P < .01$), Cl^- ($P < .001$), and lactose ($P < .01$) were

TABLE 1. Changes in the composition[1] of milk from infected quarters in moderate and severe responders during experimentally induced *Escherichia coli* mastitis and the influence of recombinant bST or vehicle (placebo) treatment upon these changes.

Component and treatment	Pretreatment: Preinfection Period −1		Pretreatment: Mastitis Period 0		Treatment: Period +1		Treatment: Period +2		Posttreatment Period +3	
	Moderate responders									
	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
Lactose, %										
Placebo[2]	5.02	.05	2.72	.17	4.14	.10	4.73	.07	4.78	.07
bST[3]	4.89	.03	2.51	.19	4.01	.15	4.74	.06	4.74	.07
α-Lactalbumin, mg/ml										
Placebo	1.15	.09	.56	.06	1.12	.14	1.06	.09	1.10	.12
bST	1.19	.08	.50	.06	1.28	.08	1.17	.06	1.06	.08
Casein, %										
Placebo	1.82	.12	1.57	.19	1.92	.18	1.77	.12	1.80	.08
bST	2.14	.03	1.75	.06	1.97	.08	2.17	.12	2.05	.05
Fat, %										
Placebo	4.80	.20	4.76	.67	5.20	.35	4.73	.19	4.80	.21
bST	5.22	.27	4.16	.38	4.87	.30	4.91	.21	4.72	.23
Noncasein N, mg/100 ml										
Placebo	107.8	8.4	161.2	20.9	141.6	30.5	120.5	21.1	111.5	10.5
bST	109.6	4.2	214.4	21.1	152.2	24.6	123.2	16.3	116.2	12.4
Na, m*M*										
Placebo	17.8	.4	52.7	7.4	28.9	2.5	24.9	.9	21.5	.9
bST	18.1	.6	78.6	3.1	31.1	2.4	24.3	.6	21.1	.6
Cl, m*M*										
Placebo	27.3	.7	55.8	2.8	42.8	1.3	37.8	1.0	36.1	1.3
bST	28.0	.7	61.4	2.4	44.6	1.5	34.7	1.0	33.3	1.1
K, m*M*										
Placebo	43.4	.8	33.9	1.3	44.7	1.0	42.6	1.0	39.9	1.3
bST	41.8	.9	31.4	1.3	41.2	1.1	40.1	1.0	39.4	1.1
Serum albumin, mg/ml										
Placebo	1.01	.03	8.40	1.28	2.86	.60	1.55	.15	1.32	.18
bST	1.11	.05	9.91	1.68	2.95	.64	1.73	.27	1.57	.17
SCC, log 10										
Placebo	4.950		6.922		6.463		5.451		5.701	
bST	5.132		6.762		6.463		5.771		5.460	

	Severe responders									
Lactose, %										
Placebo[4]	4.41	.08	1.78	.24	1.65	.30	2.10	.37	2.02	.32
bST[5]	4.80	.05	.82	.15	1.93	.22	3.67	.14	4.20	.07
α-Lactalbumin, mg/ml										
Placebo	1.45	.12	.40	.09	.14	.05	.38	.10	.42	.10
bST	1.35	.13	.47	.07	.87	.96	1.39	.19	1.28	.09
Casein, %										
Placebo	1.80	.01	.70	.26	.48	.02	.80	.35	.92	.38
bST	1.93	.23	1.29	.4	1.12	.53	1.35	.35	1.40	.40
Fat, %										
Placebo	5.82	.45	2.21	.25	3.24	.47	3.09	.38	3.75	.72
bST	5.52	.38	2.57	.38	3.48	.41	4.16	.49	5.56	.48
Noncasein N, mg/100 ml										
Placebo	110.5	3.5	315.2	94.9	592.6	25.9	603.9	5.8	535.3	15.8
bST	111.5	9.9	226.3	66.5	305.7	95.8	336.4	85.1	328.5	33.3
Na, m*M*										
Placebo	20.5	.9	67.1	4.3	84	5.9	75.9	6.9	78.9	6.6
bST	16.1	.6	73.4	4.7	63.2	4.6	40.7	3.7	31.6	1.9
Cl, m*M*										
Placebo	32.1	.9	65.2	3.1	78.9	3.9	72.4	5.5	80.4	5.5
bST	25.9	.7	66.9	3.2	62.4	3.8	42.9	3.4	35.5	1.1
K, m*M*										
Placebo	47.7	1.3	29.4	1.9	21.8	2.4	25.1	2.5	24.6	2.2
bST	42.7	.6	23.1	1.6	25.9	1.8	33.1	1.5	37.5	.6
Serum albumin, mg/ml										
Placebo	.75	.02	11.5	1.2	12.1	1.7	7.31	2.11	6.42	1.84
bST	.83	.02	13.0	1.2	11.5	1.9	3.69	1.20	1.94	.30
SCC, log 10										
Placebo	4.965		6.655		6.661		6.412		6.440	
bST	4.952		6.711		6.925		6.473		6.442	

[1]Means per quarter and per 24 h during each period.

[2]n = 4.

[3]n = 5.

[4]n = 5.

[5]n = 5.

TABLE 2. Changes in the composition[1] of milk from uninfected quarters in moderate and severe responders during experimentally induced *Escherichia coli* mastitis and the influence of recombinant bST or vehicle (placebo) treatment upon these changes.

Component and treatment	Pretreatment				Treatment				Posttreatment	
	Preinfection Period −1		Mastitis Period 0		Period +1		Period +2		Period +3	
	Moderate responders									
	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
Lactose, %										
Placebo[2]	4.85	.07	4.46	.06	4.88	.06	4.96	.06	4.85	.05
bST[3]	4.91	.03	4.51	.05	5.01	.06	5.04	.04	4.98	.04
α-Lactalbumin, mg/ml										
Placebo	1.31	.16	.88	.15	1.25	.20	1.05	.12	1.18	.15
bST	1.20	.08	.80	.06	1.39	.08	1.18	.06	1.01	.07
Casein, %										
Placebo	1.69	.18	1.62	.18	1.70	.15	1.65	.14	1.83	.29
bST	2.04	.1	1.88	.12	2.10	.09	2.08	.08	2.01	.12
Fat, %										
Placebo	5.22	.28	7.90	.60	5.07	.21	4.93	.22	5.16	.22
bST	5.27	.2	7.44	.47	5.65	.29	4.99	.22	4.82	.23
Noncasein N, mg/100 ml										
Placebo	101.8	7.6	147.8	9.9	124.2	16.5	114.2	13.5	99.8	4.6
bST	110.7	6.3	108.3	5.9	110.3	11.3	104.9	8.5	107.9	8.8
Na, m*M*										
Placebo	19.1	.6	21.4	.5	19.4	.4	18.6	.3	17.1	.5
bST	17.1	.9	19.4	.4	18.4	.2	17.3	.4	17.3	.5
Cl, m*M*										
Placebo	28.0	.6	32.9	.6	33.6	.8	32.4	.7	33.1	.7
bST	28.7	.7	33.8	.5	30.6	.7	28.6	.7	28.5	.9
K, m*M*										
Placebo	44.6	.8	45.3	.7	45.8	.7	45.4	.5	42.2	1.8
bST	41.1	.9	43.4	.9	43.9	.9	42.5	1.0	40.8	1.1
Serum albumin, mg/ml										
Placebo	.74	.03	.89	.0	.82	.06	.74	.06	.73	.08
bST	.85	.06	1.12	.1	.92	.06	.87	.04	.87	.06
SCC, log 10										
Placebo	5.025		5.221		5.021		4.953		5.016	
bST	5.082		5.232		5.115		5.041		5.001	

	Severe responders									
Lactose, %										
Placebo[4]	4.28	.08	3.98	.08	3.88	.21	3.71	.19	3.95	.21
bST[5]	4.68	.06	3.91	.07	3.96	.08	4.45	.06	4.70	.08
α-Lactalbumin, mg/ml										
Placebo	1.48	.12	.83	.08	.69	.07	1.01	.0	1.52	.17
bST	1.27	.11	.79	.04	1.15	.12	1.12	.06	1.21	.15
Casein, %										
Placebo	1.82	.06	1.74	.07	2.55	1.05	2.96	1.21	2.71	.86
bST	2.04	.19	1.61	.39	1.98	.46	2.03	.30	2.18	.54
Fat, %										
Placebo	5.32	.61	5.09	.51	5.47	.44	4.80	.49	4.80	.62
bST	5.60	.41	6.62	.73	6.82	.63	6.29	.50	5.64	.29
Noncasein N, mg/100 ml										
Placebo	115.1	9.5	132.2	8.6	166.5	46.2	156.2	25.2	180.2	39.5
bST	101.8	6.6	235.6	44.6	218.2	50.8	176.6	20.1	193.7	20.3
Na, m*M*										
Placebo	19.9	.7	22.6	.9	30.2	3.3	31.7	2.6	35.7	3.1
bST	19.1	.9	20.1	.7	20.5	1.1	17.4	.7	17.2	.6
Cl, m*M*										
Placebo	31.3	.9	35.7	.9	40.9	2.4	42.6	2.2	47.2	2.8
bST	28.1	.7	33.8	.7	31.1	1.2	26.6	.7	28.9	.8
K, m*M*										
Placebo	47.5	1.1	48.8	.9	43.1	1.8	40.1	1.9	40.4	2.3
bST	44.1	.8	44.7	.6	43.2	.8	41.8	.5	42.6	.6
Serum albumin, mg/ml										
Placebo	.61	.04	1.17	.17	1.59	.71	1.66	.80	1.46	.35
bST	.61	.05	1.28	.20	1.08	.20	.91	.18	.86	.18
SCC, log 10										
Placebo	5.043		5.169		6.243		6.433		6.364	
bST	5.056		5.293		5.666		5.894		5.866	

[1]Means per quarter and per 24 h during each period.

[2]n = 4.

[3]n = 5.

[4]n = 5.

[5]n = 5.

observed the day after induction of mastitis. In the bST cows, milk composition returned to normal within 4 to 5 d after induction of mastitis. In the placebo cows, recovery was complete only after 12 to 15 d. However, during the entire course of the experiment, milk Cl^- and Na^+ remained above, and lactose remained below, preinfection level. Only for α-lactalbumin ($P < .02$), Na^+ ($P < .01$), and the Cl^- concentration ($P < .001$) in the milk was a significant difference between placebo and bST cows observed during periods +2 and +3. For both bST-treated moderate and severe responders, daily quarter yields of different milk components returned to or increased above preinfection yields during periods +1 and +2. For the moderate bST cows, this change resulted in a gain of 11, 10, 35, and 31% in the daily quarter yield of lactose, fat, α-lactalbumin, and casein, respectively. For the severe bST cows, only fat and casein yields increased with 2 and 17%, respectively, above those preinfection yields. The gain of daily yields amounted to 23, 11, 38, and 51%, respectively, from bST treatment. Only for casein yield was a difference significant ($P < .05$) between both groups.

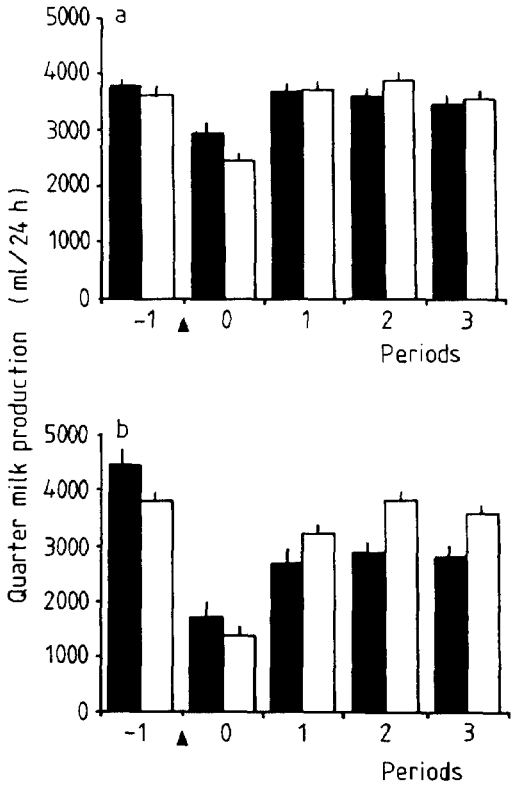

Figure 2. Effect of placebo (■) or bST treatment (□) during periods +1 and +2 on milk production in the uninfected quarters of periparturient moderate (a) and severe (b) responders during *Escherichia coli* mastitis experimentally induced (↑) in the contralateral glands. Values are means per quarter and per 24 h for a given period. Each period lasted 5 d except period 0, which included d 0 and d +1 after induction of mastitis. Vertical bars represent standard errors of the mean.

Study 2: Effects of bST Pretreatment of Mastitic Cows

Based upon the capacity of blood neutrophils to generate reactive oxygen species (with a mean oxygen burst of 1854.6 ± 182.9 mmol of H_2O_2/min and number of neutrophils) measured before the bST treatment period, cows in the second study were classified as potentially severe responders. Those cows also showed characteristics of severe responders for some blood (glucose and packaged cell volume) and milk (serum albumin and lactose) components prior to infection. For all these parameters, data (Table 3) were similar to those observed for severe responders in the first study and differed significantly ($P < .05$ to .001) from those measured for moderate responders [see Vandeputte-Van Messom et al. (29) for detailed data]. Pretreatment of periparturient cows with a single dose of long-acting bST stimulated daily MP. Over the entire post-bST treatment, preinfection period, the mean increase in MP was 16.7 ± 2.9 kg per cow ($P < .005$; range, 11 to 27.3 kg). The increase was observed for all cows and was most obvious between d 3 and 5 after treatment. During the same period, MP of placebo-pretreated cows was not altered.

Local inflammation symptoms during *E. coli* mastitis in cows pretreated with bST (n = 5) were mild and characteristic of a moderate form of acute coliform mastitis. Peak fever of 40.5 ± .5°C and tachycardia (96.4 ± 4.2 beats/min) were observed around 10 h postinfection. In the placebo pretreatment cows, peak effects (41.1 ± .1°C) were delayed by 2 to 3 h compared with those of bST pretreatment cows. In the infected glands, the number of *E. coli* bacteria rapidly multiplied to a maximum of

TABLE 3. Blood and milk components in moderate and severe responders prior to infection.[1]

Parameter	Responders			
	Severe		Moderate	
	$\bar{X}$	SEM	$\bar{X}$	SEM
Glucose, g/100 g	49.3	3.2**	59.06	1.98
Packed cell volume, %	29.1	.9*	31.21	.55
Burst capacity, nmol H_2O_2 × neutrophils	1854.6	182.4***	3812.4	322.3
Lactose, g/100 g	4.41	.21*	4.78	.14
Serum albumin, mg/ml	.66	.08**	.84	.04
SCC, × 10^3/ml	138.2	17.3	132.7	11.6
Fat production, g/24 h	216.6	16.6	174.9	11.3

[1]Severe responders (n = 8) significantly different from moderate responders [n = 9; for details see Vandeputte-Van Messom et al. (29)]: *$P < .05$, **$P < .01$, ***$P < .001$.

8.2×10^4 cfu/ml of milk 12 h postinfection in the bST pretreatment cows and to 6.2 ± 10^4 cfu/ml of milk in the placebo cows. In the bST cows, early bacterial multiplication was rapidly followed by leukocytosis into the infected glands, which increased maximally to 1.1×10^7 cells/ml between 15 and 27 h after inoculation. In the placebo cows, maximal leukocytosis amounting to 1.0×10^7 cells occurred between the 27th and the 48th h postinfection. The pattern of leukocytosis in the bST pretreatment cows was very similar to that in moderate responders in the first study (Figure 3). As expected, based on the pretreatment data, the effects in placebo cows resembled those observed in severe responders.

During mastitis, MP was suppressed in both infected and uninfected glands of all cows; inhibition was maximal on d +1 and d 0, respectively. In the uninfected glands of bST pretreatment cows, maximal loss in MP amounted to 0 and 17.5 ± 5.1% ($P < .01$) compared with pre- and post-bST treatment values, respectively. In the placebo cows, losses in MP were maximally 35.3 ± 3.1% below preinfection level. In the infected glands of bST pretreatment cows, the maximal loss ($P < .01$) in MP amounted to 40.1 ± 9.5 and to 46.1 ± 8.9% compared with pre- and post-bST MP, respectively. In the placebo cows, MP loss was 56.5 ± 12.5% greater than preinfection values. In all cows, MP returned to normal values within 5 to 10 d. Depression of MP in the infected glands was, as expected, accompanied by a significant ($P < .001$) decrease in lactose, milk fat, and K^+ and by an increase ($P < .001$) in Na^+, Cl^-, serum albumin, and SCC in both groups. In bST cows, the pattern of changes in milk composition resembled those in moderate responders. In the placebo cows, changes resembled those in the severe responders. Peak effects were delayed approximately 8 h, and complete normalization was delayed for several days compared with effects in the bST pretreatment cows.

DISCUSSION

In periparturient cows suffering from *E. coli* mastitis, recombinant bST seems to have a beneficial effect upon the recovery of MP and composition. In general, these recovery-stimulating effects of bST are more pronounced in severe responders than in moderate responders and more pronounced in the uninfected than in the infected quarters within a cow. Because of severe inflammation in infected quarters of both moderate and severe responders, MP did not completely recover. The effects of bST upon this recovery were very limited; recovery of MP in bST cows was maximally only 5% higher than that in the placebo cows. In the uninfected contralateral quarters, MP completely recovered and even increased above preinfection MP in bST-treated moderate and in severe responders. Because of bST treatment, recovery of MP in moderate and severe responders was 12 and 32% higher, respectively, than in the placebo cows. Effects were most obvious during the second half of the bST treatment period, which is in agreement with observations on healthy cows during early lactation (21, 26). On aver-

age, although experimental circumstances are completely different, the stimulating effects of bST on MP are in line with those observed in normal lactating cows. It seems that bST can influence milk secretion by way of different pathways. Several investigators (1, 18, 20) suggested that, in healthy cows, bST produces its effect on MP indirectly by partitioning more nutrients to the mammary gland and by enhancing the mammary gland's ability to synthesize milk. Recent studies provided evidence that alveolar epithelial cells may synthesize growth hormone receptors (11). Those findings raise the possibility of direct action of growth hormone on mammary cells. Both the indirect actions on milk production and the direct actions on the mammary alveolar epithelium are probably partially mediated through insulin-like growth factors (9, 12). Also, some of the changes may be due to direct action of growth hormone-dependent mediators, such as somatomedins, on the mammary gland secretory tissue (20).

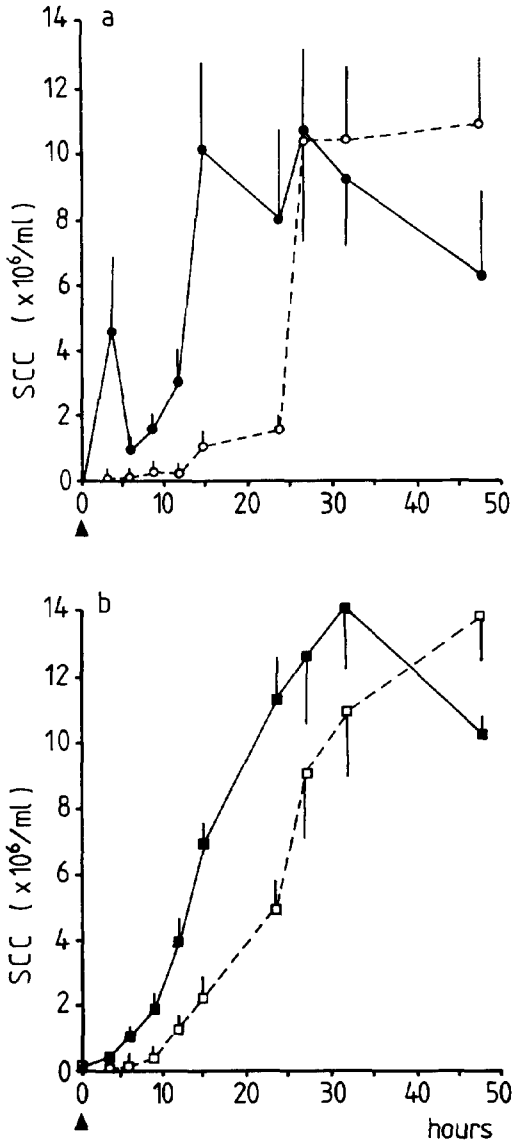

Figure 3. Patterns of leukocyte influx into milk during experimentally induced (↑) *Escherichia coli* mastitis shortly after calving (a) in cows pretreated with bST (●) or the vehicle (○) (data from the second study) and (b) in moderate (■) and severe (□) responders treated with the vehicle (data from the first study). Vertical bars represent standard errors of the mean.

The beneficial effects of bST upon normalization of milk composition are limited to the severe responders. Only for severe responders receiving bST did all milk constituents in the infected and uninfected glands return to preinfection level. For the severe cows receiving the placebo, most milk constituents remained 60 to 70% below or above preinfection level for the entire experiment. In the infected glands of moderate responders, recovery was complete whether cows received bST or not. Treatment with bST had no effect upon recovery of SCC in milk. The differences observed in the concentration of several milk components between moderate and severe responders during the preinfection period were not significant and could probably be explained by differences in MP and days postpartum between groups. Severe responders might be in a stronger negative energy balance. Changes in milk composition when healthy cows are treated with bST usually are minimal and reflect the nutritional status of the cow (20). Daily quarter yields of most milk components paralleled that of the MP. However, unlike the effects in healthy cows, mean MP and yield of milk constituents did not increase above the initial preinfection level. Only in the uninfected glands was this level reached or slightly exceeded.

Normalization of milk composition in mastitic cows suggests restoration of the integrity of the blood-milk barrier. From the present study, bST apparently helps to restore the integrity of the blood-milk barrier damaged during IMM infection only in the severe responders. The different effects of bST on

normalization of MP and composition in mastitic moderate and severe responders can be connected to differences in the role of endotoxins and leukocytes in both groups. Effects induced by *E. coli* mastitis in severe responders resembled those induced by intravenous challenge with endotoxin (6). The delayed leukocyte influx in this group seems to be responsible for the massive release of endotoxin, which seriously affects the integrity of the blood-milk barrier. After absorption into circulation, endotoxin has a serious impact upon the blood profile of the cows and probably affects the synthesis of different milk constituents. These cows have difficulties in restoring their milk secretion spontaneously, and, consequently, the integrity of the blood-milk barrier remains weak. The effects are therefore severe and of long duration. In these cows, leukocytes may play an important role in detoxification of endotoxins in the damaged glands. Endotoxins are known to be detoxified by the lysosomal enzyme acyloxyacyl hydroxylase in blood and milk neutrophils (17). After bST administration in normal healthy subjects, lysosomal enzyme activities of polymorphonuclear leukocytes increased, suggesting that this substance may influence the functional activity of polymorphonuclear leukocytes (23). In the cow, bST seems to influence the maturation and function of the neutrophils [(16); C. Burvenich et al., 1993, unpublished data]. Thus, in severe responders, stimulation of leukocyte function by bST may contribute to the cow's defense system. In moderate responders, effects resembled those during *E. coli* endotoxin (lipopolysaccharide) mastitis in ruminants (7). In such experimental circumstances, absorption of endotoxin into circulation is, although not entirely excluded, very unlikely. The effects observed may rather be ascribed to formation of endopyrogens in inflamed glands and their subsequent release into circulation (32). In the moderate responders, most of the endotoxins are probably detoxified in the mammary gland as a result of the fast influx of blood neutrophils into the milk. In those cows, the effects of coliform mastitis are therefore of short duration without serious impact upon milk secretion. Consequently, the spontaneous restoration of the milk secretion process promotes the integrity of the blood-milk barrier. Under these circumstances, stimulation of leukocyte function by bST treatment is apparently not of any advantage to the cow's defense system.

The results from the first study, suggesting that the role of leukocytes in the outcome and development of mastitis and the effect of bST on leukocyte function are more important in severe than in moderate responders and that bST interferes with the leukocyte detoxification of endotoxin in the mammary gland, are supported by observations in the second study. In addition to a stimulating effect on MP in healthy quarters, pretreatment with bST seems to protect the mammary gland from excessive loss in MP during the subsequently induced coliform mastitis. These observations are in line with those observed in the goat during endotoxin mastitis (4). The present study shows that pretreatment of cows with bST could not prevent the suppression of MP during the subsequently induced *E. coli* mastitis. However, losses in MP and compositional changes of milk were limited, and bST accelerated the recovery of mastitic cows. Thus, it seems that newly calved cows, which show all characteristics of severe responders, after pretreatment with bST will react as moderate responders to IMM *E. coli* infection. Earlier C. Burvenich et al. (1993, unpublished data); and Massart-Leën et al. (16) observed that pretreatment of cows with bST shortly after calving increased the number of circulating immature band cells. These observations and the present data indicate that the beneficial effects of bST on the recovery of newly calved cows during *E. coli* mastitis could probably be ascribed to enhancement of the rate of diapedesis of the neutrophils into the milk during early infection; to mobilization of mature and immature neutrophils from bone marrow into the circulating pool, thereby increasing the neutrophil number; and to stimulation of their reactive oxygen species producing activity.

Clinical mastitis in dairy cows is usually treated by IMM antibiotics, which function by destroying life-maintaining processes of bacterial cells. However, the role that antibiotics play in the therapy of coliform mastitis is doubtful. Many coliform mastitis cases are self-limiting and cure without antibiotic treatment; in contrast, some cows die despite an early and intense antibiotic treatment. In the present experiments, cows received a local and

systemic antibiotic treatment. No other drugs were administered. The observed effects must therefore be considered to be the result of the combination of bST and antibiotics. The substantial contribution of antibiotic therapy to the faster and better recovery observed in bST-treated cows during experimentally induced *E. coli* mastitis is not clear from the present experiments because polymixin drastically reduced the number of growing bacteria in infected glands of placebo as well as of bST-treated cows. However, recovery of milk quantity and quality was only markedly improved in bST-treated cows.

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