Effect of Exogenous Somatotropin on Hematological Variables of Lactating Cows and Their Offspring

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ABSTRACT

Eighty-two lactating Holstein cows in their first, second, or third lactation received either one, three, or five concurrent i.m. injections of a unit dose (0.6 g) of zinc methionyl bovine somatotropin (bST) or five doses of the vehicle. Injections were administered at 14-d intervals from 60 ± 3 d postpartum until the end of lactation or until necropsy. Thirty-eight cows were continued on the treatment for a 2nd yr. Blood samples were collected at wk –2, –1, 3, and 7 relative to the start of treatment and then every 8 wk (yr 1) or 4 wk (yr 2) thereafter. Untreated cows that were included in a survey of the resident herd were bled at wk 7 or 8, wk 10 or 11, and wk 13 or 14 of lactation and every 4 or 8 wk thereafter. Calves were bled within 72 h of birth and at approximately 5 wk of age. Most parameters associated with erythrocytes were decreased mildly in cows that were treated with bST. However, data remained within generally accepted reference ranges, and changes were not of clinical importance. Decreased hematocrit was not associated with increased hemolysis, hemodilution, or clinical anemia. No morphological lesions related to treatment were noted in the bone marrow or spleen; bST did not affect the incidence of immature cell types. Energy and protein balances did not significantly affect the hematological results of the cows. Calves generally were unaffected by bST treatment of the dam, but heavier calves had higher parameters associated with erythrocyte and lymphocyte counts than did calves with lower body weight. Exogenous bST treatment caused predictable changes in hematological parameters of dairy cows.

(**Key words**: hematology, dairy cows, somatotropin, lactation)

Abbreviation key: **EB** = NE_L balance, **HCT** = hematocrit, **HGB** = hemoglobin, **PB** = protein balance, **RBC** = erythrocyte, **WBC** = leukocyte.

INTRODUCTION

Physiological changes in constituents of blood cells and blood biochemistry occur dynamically during growth, pregnancy, and lactation. For example, cyclic changes in the erythron during the lactation cycle are well documented. Measurements associated with erythrocytes (**RBC**), such as hematocrit (**HCT**) and hemoglobin (**HGB**) concentration, normally are highest in nonlactating cows, decrease postpartum until the 3rd or 4th mo of lactation, and then increase again (24, 27). The normal increase in HCT after the 3rd or 4th mo of lactation can be delayed if dietary protein is inadequate (17); low dietary protein can also limit erythropoiesis (27). Cows with higher milk yield tend to have lower HCT than do cows with lower milk yield (27, 31). The explanation for sequential HCT changes in dairy cows with stage of lactation is unknown, although the changes are affected by protein balance (**PB**). Previous reports (23, 26) also have documented decreased HCT in cows treated with exogenous bST.

The objective of this study was to evaluate the effects of very high doses of bST on hematological parameters of dairy cows and their offspring. Data from cows treated with bST also were compared with hematological variables in the normal range for lactating cows maintained under defined management conditions.

MATERIALS AND METHODS

Experimental Design

Eighty-two Holstein cows in first, second, or third lactation were assigned to treatments in a randomized block design in a study on chronic toxicity

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caused by bST. Cows had not received bST treatment previously. Treatments were 0 (control), 0.6, 1.8, or 3.0 g/14 d of zinc methionyl bST (sometribove; Monsanto Co., St. Louis, MO) in an oil-based formulation (7, 10). Intramuscular injections were administered every 2 wk in alternating sites within the left or right gluteus, semitendinosus, or semimembranosus muscles. At each interval, the control treatment or 0.6, 1.8, or 3.0 g of bST were administered as five, one, three, or five independent injections, respectively. The study was conducted according to good laboratory practice guidelines of the FDA (13) and animal care guidelines of the National Institutes of Health (21). The study was approved by the Monsanto Institutional Animal Care and Use Committee.

Cows were housed at the research facility for at least 30 d prior to calving. All cows were screened prior to arrival for bovine paratuberculosis, tuberculosis, anaplasmosis, and brucellosis. Cows were treated from 60 ± 3 d postpartum until the end of lactation or until necropsy. Two cows were removed from treatment early in lactation and were excluded from all analyses. A subset of 38 cows continued through a second lactation with the same treatment regimen. Necropsies were conducted as described previously (7), and sections from 30 or more tissues were included for microscopic evaluation. Routine tissue sections included spleen, bone marrow smears, and sections of the right femur and 10th rib that included marrow. Sixty-nine calves that were born during the study also were evaluated through 5 wk of age.

A hematological survey was conducted in the same facility using the resident, untreated cows. One hundred seventy-six lactating cows (35 primiparous and 141 multiparous cows) were evaluated.

Cows were housed in artificially ventilated tie-stall barns during both the nonlactating and lactating periods. Stalls were bedded with wood shavings over rubber mats. Unless precluded by research procedures or weather conditions, all cows were exercised on a dirt and concrete lot at least 2 h daily. Cows were milked twice daily at approximately 0500 and 1700 h. Calves were housed in individual pens for the first 2 to 3 mo of age and then were transferred to group housing. All pens and stalls received approximately 20 h of light from artificial lighting and 4 h of darkness. All housing facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care (Rockville, MD).

Cows were fed total mixed diets for ad libitum intake. Diets contained corn silage, chopped alfalfa hay, beet pulp, whole cottonseed, and premixed protein, grain, and mineral supplements (Purina Mills, St. Louis, MO) and were formulated to meet or exceed nutritional requirements recommended by the NRC (22). Cows were switched between diets that differed in energy density according to milk yield and body condition as was previously reported (10). Potable water was available for ad libitum consumption. Estimated NE_L balance (**EB**) and PB were calculated as described previously (10). Calves were fed colostrum at birth and a commercial milk replacer (Land O' Lakes, Minneapolis, MN) and calf starter (Purina Mills) thereafter.

The general health status of all cows was evaluated daily throughout the study. Clinical health data included information from daily observations, physical examinations, and reproductive examinations. Body weights were measured once every 2 wk prior to lactation, except during the peripartum period when cows were not weighed. During lactation, milk yield and feed intake were measured daily; BW and milk composition were measured weekly. Calves were weighed weekly through 6 wk of age.

Sample Collection

For the bST study, blood samples were collected from lactating cows at wk –2, –1, 3, and 7 relative to the start of treatment (60 ± 3 d of lactation) and then every 8 wk (yr 1) or 4 wk (yr 2) thereafter. In addition, cows were bled during physical examinations at wk 6 and 26 of lactation and at the end of lactation. Untreated cows included in the survey of the resident herd were bled at wk 7 or 8, wk 10 or 11, and wk 13 or 14 of lactation and every 4 or 8 wk thereafter. Calves were bled within 72 h of birth and at approximately 5 wk of age. All whole blood samples were collected by coccygeal or jugular venipuncture into tubes containing 15% liquid EDTA (Becton Dickinson Vacutainer Systems, Rutherford, NJ). One aliquot of blood from each sample was collected for serum preparation.

Blood Analyses

Complete cell counts were determined, and blood films were prepared within 4 h of collection for differential counts. Leukocyte (**WBC**), RBC, and platelet counts and HGB concentration were determined using a Coulter counter (model S-Plus II; Coulter Electronics, Hialeah, FL). Cellular indices, including HCT, mean cell volume, mean cell HGB, mean cell HGB concentration, red cell distribution width, and mean platelet volume were derived electronically. Minor modifications were made to the WBC and RBC aperture currents to achieve optimal relationships among bovine RBC, platelets, and WBC

(30). The WBC aperture current was lowered slightly from 127 to 119.7 V to reduce the interference by debris on the WBC count and to improve the position of the WBC histogram with respect to the 45-fl threshold. The RBC aperture current was increased from 191 to 210 V to improve the lower threshold. Serum albumin and total bilirubin concentrations were analyzed using a clinical analyzer (ACA IV; DuPont, Wilmington, DE).

Statistical Analyses

Pretreatment data were analyzed using a linear model including effects of treatment, block (based on location in barn), parity (primiparous or multiparous), and all two-way interactions. Analyses of the treatment period used a model that included a pretreatment baseline covariate and the effects of treatment, block, parity, week of treatment, and two-way interactions between treatment and week and treatment and parity. The effect of milk yield (at the time of blood sampling or cumulative up to the time of sampling) was examined by inclusion of yield quartile and the interaction of treatment and quartile in the whole-plot model. Unless otherwise noted in the text, results from the 2nd yr were similar to those from the 1st yr, and data from only the 1st yr are listed or plotted (12).

For the survey of the resident herd, data were adjusted for season, parity, and week of lactation. Covariates of EB and PB (at the time of blood sampling or change in nutrient balance since the last blood sampling) were evaluated.

For calves, the treatment of the dam was coded as control or bST to accommodate unequal conception rates between treatments (7), and data from yr 1 and 2 were analyzed together. The effects of calf birth weight and average daily gain also were examined as covariates. All two-way interactions between sex, season, parity of the dam, and birth weight were examined, but these interactions did not contribute ($P > 0.25$) to the variation in response. Data within each sampling interval were described as least squares means, population ranges, and number of cows sampled, unless otherwise stated. All analyses utilized SAS (25) procedures.

Clinical health data for the bST study were grouped by physiological system (e.g., digestive, mammary gland, respiratory, etc.), subsystem within a system (e.g., udder or teats within mammary gland), and incidence within a subsystem as described previously (12). Incidence rate, except for fetal loss, was calculated on an individual basis as the number of incidents divided by the number of test days. The number of incidents, regardless of test

TABLE 1. Mean and 95% confidence intervals (CI) for the hematological parameters for the resident (untreated) cows and cows on the bST study.[1]

Variable[2]	Resident herd		Cows treated with bST							
			0 g/14 d		0.6 g/14 d		1.8 g/14 d		3.0 g/14 d	
	$\bar{X}$	CI	$\bar{X}$	CI	$\bar{X}$	CI	$\bar{X}$	CI	$\bar{X}$	CI
Cows, no.	176		20		21		18		20	
HCT, %[a]	30.6	25.3–36.0	32.6	27.6–37.5	30.0	25.1–35.0	29.4	24.5–34.4	28.0	23.1–33.0
RBC, $10^6/\mu l$[a]	6.5	5.4–7.6	6.8	5.7–8.0	6.5	5.4–7.6	6.5	5.4–7.6	6.1	5.0–7.3
HGB, g/dl[a]	10.6	8.9–12.4	12.0	10.4–13.7	11.1	9.5–12.7	10.9	9.3–12.6	10.3	8.7–11.9
MCH, pg	16.4	13.7–19.2	17.7	14.9–20.6	17.2	14.3–20.0	17.0	14.1–19.8	16.9	14.1–19.8
MCHC, g/dl	34.9	30.1–39.7	37.3	31.8–42.8	37.3	31.7–42.8	37.5	32.0–43.0	37.1	31.5–42.6
MCV, fl[a]	47.4	40.7–54.1	47.7	41.6–53.7	46.1	40.0–52.1	45.2	39.2–51.3	45.8	39.7–51.8
RDW, %[a]	18.6	15.2–22.0	18.9	16.2–21.6	17.9	15.2–20.6	17.7	15.0–20.4	18.4	15.7–21.1
Platelets, $10^3/\mu l$[a]	455	192–718	416	148–686	474	205–743	624	355–892	576	307–845
MPV, fl	5.9	4.4–7.5	6.8	5.6–8.1	6.9	5.6–8.2	6.7	5.4–8.0	6.6	5.3–7.9
Total WBC, $10^3/\mu l$	8.7	4.0–13.3	8.9	4.8–13.0	9.7	5.6–13.8	10.2	6.0–14.3	9.5	5.4–13.6
Lymphocytes	4.3	0.9–7.7	4.1	2.1–6.2	4.3	2.2–6.3	4.1	2.1–6.2	4.2	2.1–6.1
Segmented neutrophils[a]	3.6	0.9–6.4	3.8	0.5–7.1	4.5	1.2–7.8	5.1	1.8–8.4	4.6	1.3–7.9
Band neutrophils	0	0–0.1	0	0–0.1	0	0–0.1	0	0–0.1	0	0–0.1
Eosinophils	0.4	0–1.1	0.6	0–1.3	0.4	0–1.2	0.4	0–1.1	0.4	0–1.1
Monocytes	0.2	0–0.7	0.3	0–0.8	0.3	0–0.9	0.4	0–0.9	0.3	0–0.9
Basophils	0	0–0.1	0	0–0.4	0.1	0–0.5	0	0–0.4	0	0–0.5

[a]$P < 0.01$.

[1]Somatotropin was administered at 0, 0.6, 1.8, and 3.0 g/14 d to 79 cows beginning at wk 9 of lactation (10). Hematology samples were collected at –2, –1, 3, 7, 15, 23, and 31 wk of treatment. Values from untreated cows in the resident herd are included as references.

[2]HCT = Hematocrit, RBC = erythrocyte, HGB = hemoglobin, MCH = mean cell HGB, MCHC = MCH concentrations, MCV = mean cell volume, RDW = RBC distribution width, MPV = mean platelet volume, and WBC = leukocyte.

TABLE 2. Probability values from repeated measures analysis of hematological parameters of lactating dairy cows treated with bST.[1]

Variable[2]	Treatment	Pretreatment	Block	Parity	Treatment by parity	Week of treatment	Treatment by week
				P			
HCT	<0.0001	0.0007	0.4084	0.8770	0.1519	<0.0001	0.3028
RBC	<0.0001	<0.0001	0.5729	0.2618	0.9343	<0.0001	0.0569
HGB	<0.0001	0.0012	0.1670	0.3748	0.2711	<0.0001	0.6508
MCH	0.0532	<0.0001	0.0123	0.0579	0.2512	<0.0001	0.0631
MCHC	0.7310	0.4572	0.0007	0.9907	0.4205	<0.0001	0.6827
MCV	<0.0001	<0.0001	0.4959	0.6209	0.1139	<0.0001	<0.0001
RDW	0.0080	0.0174	0.5790	0.1016	0.9510	<0.0001	<0.0001
Platelets	0.0001	0.0019	0.4080	0.3530	0.2112	<0.0001	0.3878
MPV	0.4415	0.0001	0.0013	0.5669	0.0083	<0.0001	0.1246
WBC	0.0520	<0.0001	0.2012	0.0008	0.1341	0.3149	0.0025
Lymphocytes	0.7785	0.0003	0.2376	0.0084	0.5828	0.0487	0.1213
Segmented neutrophils	0.0061	0.0037	0.2172	0.0642	0.5699	0.0359	0.0023
Band neutrophils	0.8291	0.1784	0.3999	0.4441	0.7916	0.5115	0.5750
Eosinophils	0.0359	0.0012	0.2860	0.8218	0.8417	0.0317	0.0253
Monocytes	0.0337	0.3344	0.0483	0.3373	0.0670	<0.0001	0.3574
Basophils	0.1267	0.6681	0.5644	0.1726	0.3521	0.0036	0.3998

[1]Bovine somatotropin was administered at 0, 0.6, 1.8, and 3.0 g/14 d to 79 cows beginning at wk 9 of lactation (10). Hematology samples were collected at –2, –1, 3, 7, 15, 23, and 31 wk of treatment.

[2]HCT = Hematocrit, RBC = erythrocyte, HGB = hemoglobin, MCH = mean cell HGB, MCHC = MCH concentrations, MCV = mean cell volume, RDW = RBC distribution width, MPV = mean platelet volume, and WBC = leukocyte.

days, was analyzed for fetal loss. Spearman correlation coefficients were calculated between rank-transformed data for hematological parameters and the incidence rate of diarrhea, fetal loss, high orts, high temperature, lameness, mastitis, and total clinical signs. The effects of bST on these clinical conditions have been reported previously (7). Total clinical disorders included all health-related observations made during the treatment period (12).

RESULTS

Ninety-five percent confidence intervals for the hematological parameters of cows in the resident herd (untreated cows) and of cows on the bST study are presented in Table 1. Somatotropin lowered ($P < 0.05$) parameters associated with RBC (RBC count, HCT, HGB, and RBC distribution width) but did not affect mean cell HGB concentration compared with control cows during each year of study (Tables 1 and 2; Figure 1). Week of bST treatment also affected ($P < 0.05$) most hematological parameters (Table 2). Overall, mean cell HGB also tended to be lower ($P = 0.0532$) in all cows treated with bST than that in control cows. Mean cell volume was decreased in cows treated with bST, but RBC distribution width was inconsistently affected by bST (Tables 1 and 2). Only the cows treated with 1.8 g of bST had lower RDW than did the control cows ($P < 0.05$). Total serum bilirubin concentrations were increased ($P < 0.01$), and albumin concentrations were lower, in cows treated with bST ($P < 0.01$) during each year of study (Figure 1).

Somatotropin increased ($P < 0.05$) platelet count during the study, but mean platelet volume was unaffected. Total WBC tended to increase ($P = 0.0520$) in cows treated with bST (Table 1). Elevations in WBC were due to increased ($P < 0.05$) segmented neutrophil counts during bST treatment (Table 1). Lymphocytes were unaffected by bST. The presence of immature (band) neutrophils, basophils, monocytes, and eosinophils in the blood was not affected by bST (Table 1).

Energy balance, PB, and changes in EB and PB did not affect ($P > 0.10$) hematological results (data not presented). Somatotropin generally did not affect ($P > 0.10$) the correlations between hematological variables and the incidence of diarrhea, fetal loss, high orts, high rectal temperature, lameness, mastitis, or total clinical signs (Table 3). The occasional significant correlation was not consistent among parameters, and no dose pattern was present. All cows and representative calves were necropsied at the end of the study, and no morphological lesions related to treatment were noted in the bone marrow or spleen.

Hematological results for neonatal calves (within 72 h of birth) and for growing calves (5 wk old) are presented in Table 4. Average daily gain did not affect ($P > 0.10$) hematological parameters of the calves from birth through 5 wk of age (Table 4). Similarly,

previous treatment of the dam with bST had limited effects on hematological results of their offspring. Slight decreases in mean cell HCB concentration and RBC distribution width and increased mean cell volume were observed in neonatal calves but not in older calves from dams treated with bST (Table 4). Total WBC counts tended to be increased, primarily because of an increase ($P < 0.05$) in lymphocytes in calves from dams treated with bST. Birth weight affected HCT, RBC counts, HGB, mean cell HGB, mean cell volume, RBC distribution width, and lymphocyte counts of neonatal calves ($P < 0.05$); calves with higher birth weights had higher hematological counts. Several of these trends persisted in 5-wk-old calves (Table 4).

DISCUSSION

The primary hematological differences noted during bST treatment were decreased RBC count, HCT, HGB, and RBC distribution width, which were consistent with previous reports (5, 9, 19). However, in those trials and in the present study, hematological data remained within published reference ranges, and clinical anemia was not present (15, 16, 32). Because the capacity of HGB to carry oxygen greatly exceeds the need, the response to bST did not include anemia or increased reticulocytes, as expected. Responses were similar to normal adjustments to changes in the physiological state. Hematocrit and HGB concentrations are normally decreased postpartum until the 3rd or 4th mo of lactation and then are increased again (24, 27). In the present toxicology study, analytes associated with RBC of cows treated with bST increased at a slower rate relative to control cows. By the pretreatment period of the second lactation (at least 4 mo after the previous bST injection), all hematological parameters were similar among groups (Figure 1). Similar recovery at lower doses of bST

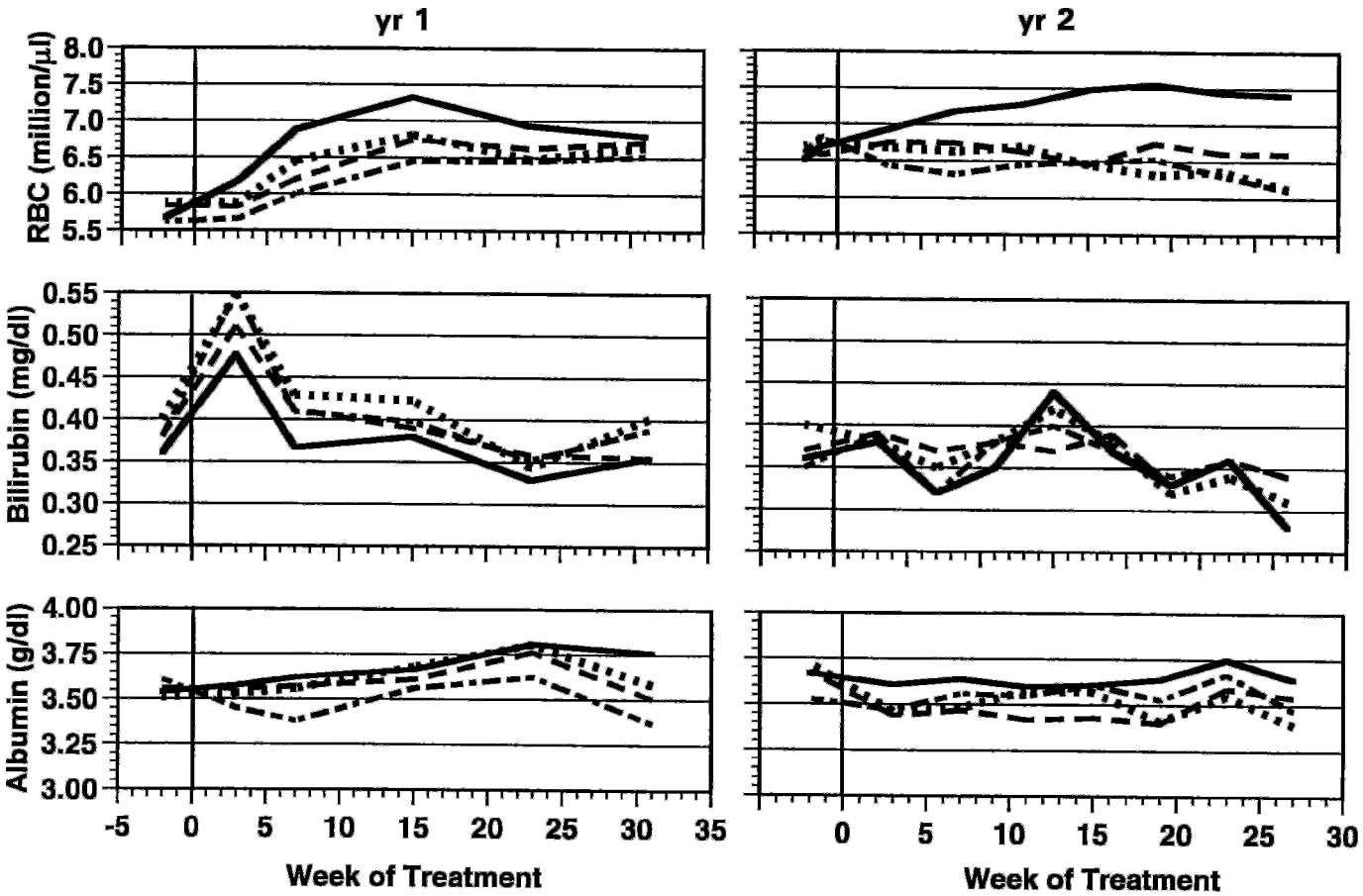

Figure 1. Effect of bST on erythrocyte (RBC) counts and on serum bilirubin and albumin concentrations in lactating dairy cows during two lactations. Pooled standard errors: RBC = 0.208 $10^6/\mu$l, bilirubin = 0.019 mg/dl, and albumin = 0.065 g/dl. Treatments were control (0 g of bST; —), 0.6 g of bST (- - -), 1.8 g of bST (· · ·), and 3.0 g of bST (· – ·).

was described previously (6). During the treatment period, stage of lacation or season within year had a greater effect on RBC than did bST (Figure 1).

Lowered HCT would be expected for cows treated with bST because those cows produced approximately 4 to 10 kg/d more 3.5% FCM than did the control cows (10). Studies of metabolic profiles (27), which utilized more than 1500 Holstein cows in 38 herds, revealed that HCT generally is lower in higher yielding cows. In contrast, inclusion of milk yield in the statistical model as a discrete or continuous variable did not alter the effect of bST on hematological measures in the present study. This lack of effect was probably due to the relatively small population of cows on the test and the unequal distribution within milk yield quartile; for example, no control cows were in the upper quartile. In addition, the milk yield from all control cows and cows treated with bST in this

TABLE 3. Probability values of the correlation coefficients between mean hematocrit (HCT), hemoglobin (HGB), erythrocyte (RBC) count, and platelet volume and incidence rates of diarrhea, fetal loss, high orts, high temperature, lameness, mastitis, and total clinical signs.[1,2]

Variable	bST Treatment	Diarrhea	Fetal[3] loss	High orts	High rectal temperature	Lameness	Mastitis	Total clinical disorders
	(mg)							
HCT	Control	0.87	0.23	0.62	0.09	0.80	0.06	0.67
	0.6	0.29	0.12	0.47	0.97	0.22	0.13	0.43
	1.8	0.14	0.94	0.11	0.63	0.89	0.34	0.66
	3.0	0.25	0.39	0.37	0.01+,[4]	0.72	0.41	<0.01−
HGB	Control	0.42	0.40	0.96	0.13	0.87	0.51	0.98
	0.6	0.86	0.23	0.28	0.85	0.82	0.01+	0.32
	1.8	0.24	0.83	0.38	0.95	0.87	0.25	0.78
	3.0	0.04−	0.67	0.12	0.43	0.36	0.31	0.30
RBC Count	Control	0.20	0.77	0.90	0.11	0.88	0.17	0.45
	0.6	0.22	0.20	0.16	<0.01+	0.95	<0.01+	0.13
	1.8	0.59	0.33	0.82	0.36	0.33	0.87	0.12
	3.0	0.93	0.21	0.56	0.05−	0.66	0.59	0.01−
Mean platelet volume	Control	0.24	0.50	0.68	0.66	0.96	0.80	0.46
	0.6	0.15	0.69	0.24	0.11	0.59	0.31	0.72
	1.8	0.23	0.99	0.44	0.31	0.01−	0.93	0.88
	3.0	0.09	0.69	0.27	0.13	0.15	0.69	0.02−
Segmented neutrophil count	Control	0.19	0.96	0.29	0.24	0.64	0.44	0.35
	0.6	0.11	0.57	0.07	0.88	0.25	0.42	0.19
	1.8	0.99	0.02+	0.19	0.82	0.22	0.92	0.83
	3.0	0.90	0.71	0.12	0.88	0.74	0.59	0.74
Band neutrophil count	Control	0.74	0.48	0.21	0.82	0.10	0.08	0.27
	0.6	0.82	0.58	0.74	0.58	0.58	0.58	0.20
	1.8	0.55	0.04+	0.08	0.17	0.55	0.45	0.64
	3.0	0.05+	0.54	0.73	0.46	0.46	0.18	0.25
Eosinophil count	Control	0.70	0.18	0.49	0.79	0.27	0.05−	0.95
	0.6	0.85	0.25	0.79	0.72	0.73	<0.01+	0.76
	1.8	0.39	0.35	0.90	0.59	0.17	0.71	0.97
	3.0	0.33	0.47	0.14	0.95	0.52	0.79	0.80
Basophil count	Control	0.36	0.84	0.82	0.53	0.56	<0.01+	0.43
	0.6	0.58	0.06	0.42	0.61	0.86	0.06	0.89
	1.8	0.98	0.81	0.78	0.67	0.24	0.23	0.92
	3.0	0.86	0.11	0.70	0.21	0.77	0.92	0.19
Monocyte count	Control	0.25	<0.01+	0.19	0.21	0.76	0.37	0.17
	0.6	0.38	0.14	0.59	0.81	0.61	0.07	0.09
	1.8	0.21	0.80	0.61	0.21	0.52	0.70	0.01−
	3.0	0.07	0.93	0.23	0.44	0.87	0.65	0.55

[1]Total clinical disorders included all health-related observations made during the treatment period (12). Incidence rate was calculated on an individual cow basis as the number of incidences divided by the number of test days.

[2]Somatotropin was administered at 0 (control), 0.6, 1.8, and 3.0 g/14 d to 79 cows beginning at wk 9 of lactation (10). Hematology samples were collected at −2, −1, 3, 7, 15, 23, and 31 wk of treatment.

[3]The number of incidences of fetal loss was analyzed.

[4]For significant correlation coefficients ($P < 0.05$), the nature of the relationship is indicated as positively correlated (+) and negatively correlated (−).

study was greater than the cutoff for the upper production group (18 kg of FCM/d) in the report of Stevens et al. (27).

The causes for the sequential changes in HCT in dairy cows with week of lactation and for the delayed increase in erythroid mass in cows treated with bST are unknown. Clinically depressed HCT typically is associated with one of three general disorders: 1) increased hemolysis or shortened RBC survival, 2) alteration of the fluid phase of circulating volume (hemodilution), or 3) decreased erythropoiesis.

Shortened RBC survival is associated with compensatory hyperplasia of erythroid marrow. Increased polychromasia, reticulocytosis, increased cell volume above the normal range, and an increased proportion of erythroid precursors in the marrow occur in conjunction with moderate to marked hyperplasia (14). However, bST did not affect the appearance of immature RBC, tended to decrease rather than to increase mean cell volume, and only marginally affected serum bilirubin concentration, a marker of heme turnover. The minor change in bilirubin was consistent with other RBC data. In this study, all cows and representative calves were necropsied at the completion of the study. No pathologic effects were noted in hematopoietic organs (7, 11).

Hemodilution has previously been suggested as an explanation of a portion of the bST effect on HCT (1, 23, 29). The results of a 2-wk study by Vicini et al. (29) supported this concept because, in that study, a rapid change in HCT that was compatible with hemodilution was observed in response to the administration of 30 g of bST. However, if hemodilution was the sole explanation for the depression in HCT, a decrease in serum albumin concentrations that was similar to that of HCT would be expected. Albumin concentrations in this study tended to be lower in cows treated with bST [Figure 1; (29)], but the profile did not coincide with period of reduced HCT. The pattern could have been confounded because of increased globulins in cows treated with bST (11, 29). Changes in plasma volume across lactation could explain the differences between control cows and cows treated with bST in two scenarios. If both control cows and cows treated with bST had mild increases in total body RBC mass but the plasma volume of cows treated with bST also increased, the increase in HCT would be masked in cows treated with bST. Alternatively, if the total body RBC mass remained unchanged in both control cows and cows treated with bST and the plasma volume of untreated cows is normally decreased with stage of lactation, then the HCT of control cows would increase relative to that of cows treated with bST. Increased plasma and RBC volumes have been reported in early studies with rats treated with somatotropin and in studies with acromegalic humans (2, 28).

TABLE 4. Mean and 95% confidence intervals for the hematological parameters for the calves conceived by dams treated with bST[1]

Variable[2]	Treatment of the dam of neonates					Treatment of the dam of 5-wk old calves				
	Control		bST		*P*	Control		bST		*P*
	$\bar{X}$	CI	$\bar{X}$	CI		$\bar{X}$	CI	$\bar{X}$	CI	
Calves, no.	28		41			26		39		
HCT, %	32.0	28.1–35.9	35.1	32.8–37.4	0.1499*	32.9	30.3–35.6	35.0	33.4–36.6	0.1568*
RBC, $10^6/\mu l$	7.9	7.1–8.7	8.1	7.7–8.6	0.6291*	8.3	7.7–9.0	8.6	8.3–9.0	0.4010*
HGB, g/dl	9.9	8.7–11.1	10.5	9.8–11.3	0.3572*	10.2	9.3–11.1	10.7	10.2–11.3	0.2883*
MCH, pg	12.7	12.2–13.2	12.9	12.6–13.2	0.4492*	12.4	12.0–12.8	12.4	12.2–12.6	0.8528
MCHC, g/dl	31.4	30.2–32.7	30.1	29.4–30.8	0.0427	31.3	30.5–32.1	30.7	30.3–31.2	0.1662*
MCV, fl	40.4	38.5–42.3	42.9	41.8–44.1	0.0185*	39.5	38.3–40.6	40.4	39.8–41.1	0.1190*
RDW, %	20.2	19.1–21.4	18.7	18.0–19.3	0.0147*	20.5	18.8–22.2	20.4	19.3–21.4	0.8762
Platelets, $10^3/\mu l$	442	333–551	500	430–571	0.3163	510	425–595	582	528–636	0.1122
MPV, fl	5.7	5.2–6.2	5.9	5.6–6.2	0.5399	5.6	5.2–6.0	5.7	5.5–6.0	0.5281
Total WBC, $10^3/\mu l$	9.6	6.7–12.5	12.6	10.9–14.4	0.0578	9.8	7.8–11.8	12.0	10.8–13.2	0.0450
Lymphocytes	2.2	1.5–2.9	3.1	2.6–3.5	0.0192*	2.9	2.7–3.6	3.7	3.3–4.1	0.0310*
Segmented neutrophils	6.4	4.1–8.7	8.0	6.6–9.3	0.2091	5.9	4.4–7.3	6.8	6.0–7.7	0.2066
Band neutrophils	0.5	0–2.6	2.1	0.9–3.3	0.1631	0.5	0–2.2	1.6	0.6–2.6	0.2495
Eosinophils	0.2	0.1–0.3	0.1	0.1–0.2	0.1311	0.2	0–0.4	0.2	0.1–0.3	0.6453*
Monocytes	0.4	0.2–0.7	0.5	0.4–0.7	0.5021	0.5	0.3–0.7	0.7	0.6–0.8	0.0709
Basophils	0	0–0.7	0.3	0–0.8	0.4287	0.1	0–0.3	0.2	0–0.4	0.5170

[1]Dams were treated with 0 (control), 0.6, 1.8, or 3.0 g of bST/14 d beginning at wk 9 of lactation (10). Calves did not receive bST treatment. Hematology samples were collected at ≤3 and 30 to 45 d of age.

[2]HCT = Hematocrit, RBC = erythrocyte, HGB = hemoglobin, MCH = mean cell hemoglobin, MCHC = MCH concentrations, MCV = mean cell volume, RDW = RBC distribution width, MPV = mean platelet volume, and WBC = leukocyte.

*Significant effect of birth weight ($P < 0.05$).

Decreased HCT also may be associated with lowered RBC production caused by disorders such as renal failure, endocrine failures (e.g., hypothyroidism or hypoadrenocorticism), anemia from chronic disease or inflammation, starvation, or injury to bone marrow stem cells. All of these disorders are associated with a decreased proportion of erythroid cells in the marrow, which was not observed (7). Renal failure was not present as was indicated by concentrations of blood urea nitrogen and creatinine and renal histology (11). Stem cell injury, which is associated with a generalized failure to produce RBC, platelets, and WBC, was not observed.

A decrease in RBC mass also may occur because of malnutrition and other causes of negative EB. An attractive explanation for the mild decrease in HCT of dairy cows in early lactation is that negative EB and nitrogen balance result in slightly modified production of RBC. As cows adapt to the metabolic demands of lactation and recover from the demands of late gestation, HCT slowly increases to steady-state values. Minimal modulation in the erythron of cows that were treated with bST could be undetectable by examination of marrow cellularity. In cows treated with bST, protein availability would be lowered because of increased milk protein synthesis and decreased PB (4, 8, 10, 29), which could limit erythropoiesis for a longer period. The delay in the increase of HCT to steady-state values, which can occur if dietary protein is inadequate (24), supported this theory. Low dietary protein has been postulated to impair production of erythropoietin (27). In the present study, however, neither EB nor PB significantly affected hematological values. Even the nadir of estimated EB (approximately –2.5 Mcal/d) or PB (approximately –375 g/d) for the cows that received 3.0 g of bST did not approach the lowest values that occurred during early lactation (–7.0 Mcal/d and –800 g/d, respectively) (10). Moreover, the relative amount of substrate that was required for milk protein synthesis and secretion was much greater than that required for HGB production. Because of the relatively low rate of RBC turnover, production of about 15 g of HGB/d was estimated as necessary to maintain steady-state HCT (15), which is much less than the kilograms of protein required for milk synthesis (22). Only a fraction of the protein requirement would be salvaged by a mild reduction in erythropoiesis.

A more likely alternative to explain the changes in HCT associated with the lactation of dairy cows is a homeorhetic adjustment to divert nutrients from body protein synthesis to milk synthesis, whether milk yield is basal or induced by bST (3, 4). Burton et al. (6) noted that variations in IGF-I concentrations of cows treated with bST did not differentially influence RBC counts apart from the bST effect. Although causality is not proven by that relationship, IGF-I is an integral component of the bST response, which is modulated by the nutritional status of the cow (4, 20). Fine-tuned control of increases in iron, copper, and cobalt secretion in milk during bST treatment (1, 3, 4, 8) could limit HGB synthesis in a manner similar to the limitation that has been reported during deficiency of these HGB precursors (27).

The 0.6-g dosage of bST was most representative of a commercially approved dose (12); therefore, response to that dosage might mimic field conditions. Moreover, an overdose of bST produced predictable results (Table 1). The normal ranges of hematological parameters overlapped for the untreated, resident cows and cows treated with bST and in no instance were indicative of a chronic clinical disorder (Table 3). Thus, any large changes in hematological values of cows that are observed by veterinary services in clinical or farm settings are not likely to be associated with bST treatment. The label insert for POSILAC® (sterile sometribove zinc suspension, prolonged-release recombinant bST product; Monsanto Co., St. Louis, MO) cautions producers and veterinarians that HGB and HCT are reduced during treatment. The present data demonstrate that the reduction of HGB and HCT may be difficult to observe because the cautionary statements are based on statistical differences that were calculated for a defined stage of lactation. In the present study, even with defined sampling intervals, the pattern of parameters associated with RBC was variable from year to year for both control cows and cows treated with bST (Figure 1). For example, during the 1st yr, the RBC of control cows peaked at about 15 wk of treatment and declined thereafter; the RBC of cows treated with bST tended to reach a plateau after 15 wk. In contrast, during the 2nd yr treatment period, the RBC of control cows increased, the mean RBC of cows treated with 0.6 g of bST was stable, and the RBC of cows treated with 1.8 g or 3.0 g of bST tended to decrease. The reason for variation between years is unknown, but the magnitude of difference was judged to be unimportant. Clinical anemia was not present (32).

The marginal changes in parameters for platelets or WBC in this study were not clinically significant and did not reflect a pathologic effect. There was no evidence of a left shift in the neutrophil population of cows treated with bST, i.e., the incidence of immature neutrophil forms, such as bands and earlier precursor

cell types, was generally immeasurable. In contrast, Massart-Leen et al. (18) observed that pretreatment of cows with bST shortly after calving increased circulating immature band neutrophils. It is unclear whether stage of lactation accounted for the differences in bST response between the two studies. In addition, although in this study the cows treated with bST had a higher incidence of diarrhea, fetal loss, high orts, high rectal temperature, lameness, mastitis, and total clinical signs (7), the relationships between these conditions and hematological parameters were not affected by treatment (Table 3). The positive correlation of eosinophil and RBC counts and the incidence of clinical mastitis occurred only in cows in the group treated with 0.6 g of bST. The number of mastitis cases was lowest, and the duration of each case was shortest, for that group during the 1st yr of treatment (7). In summary, acute health disorders might have varied among groups, but cachexia was not present (14), and the observed disorders had no predictable association with treatment.

Average daily gain of the calf and previous treatment of the dam with bST had no effect on the hematological parameters of the calves. Somatotropin had no pathologic effect on fetuses or calves of the dams treated with bST (12) and did not significantly affect the average daily gain of first generation calves (7). However, in larger clinical studies (12), bST was shown to decrease the birth weight of calves from cows treated with bST by approximately 1.5 kg, probably because of a 3-d decrease in gestation of dams treated with bST. In the present study, birth weight affected ($P < 0.05$) several hematological parameters of calves from dams treated with bST; calves with lower birth weights had lower hematological counts. Although the bST treatment of the dam resulted in slightly smaller birth weights, the importance of these changes may be questionable because bST had no impact on calf health or performance (7).

CONCLUSIONS

Exogenous bST treatment caused predictable changes in hematological parameters. Most parameters associated with RBC were decreased marginally in cows treated with bST. However, data remained within generally accepted reference ranges and were not clinically significant. Decreased HCT was not associated with increased hemolysis, hemodilution, or clinical anemia. No morphological lesions that were related to treatment were noted in bone marrow or spleen, and the incidence of immature cell types was not affected by treatment. Erythropoiesis might have been decreased as part of an adjustment to divert nutrients from body protein synthesis to milk synthesis during bST treatment. Energy balance and PB did not affect the hematological parameters of the cows. Calves generally were unaffected by the bST treatment of the dam, but calves with heavier birth weights had significantly higher parameters associated with RBC and lymphocyte counts than did calves with lower birth weights.

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