ABSTRACT

The aim of this experiment was to determine effects of treating peripartum dairy cows with body condition score ≥3.75 with recombinant bovine somatotropin (rbST) on immune, inflammatory, and metabolic responses. Holstein cows (253 ± 1 d of gestation) were assigned randomly to 1 of 3 treatments: untreated control (n = 53), rbST87.5 (n = 56; 87.5 mg of rbST), and rbST125 (n = 57; 125 mg of rbST). Cows in the rbST87.5 and rbST125 treatments received rbST weekly from −21 to 28 d relative to calving. Growth hormone, insulin-like growth factor 1, haptoglobin, tumor necrosis factor α, nonesterified fatty acids, β-hydroxybutyrate, glucose, and cortisol concentrations were determined weekly from −21 to 28 d relative to calving. Blood sampled weekly from −14 to 21 d relative to calving was used for hemogram and polymorphonuclear leukocyte (PMNL) expression of adhesion molecules, phagocytosis, and oxidative burst. Cows were vaccinated with ovalbumin at −21, −7, and 7 d relative to calving, and blood was collected weekly from −21 to 21 d relative to calving to determine IgG anti-ovalbumin concentrations. A subsample of cows had liver biopsied −21, −7, and 7 d relative to calving to determine total lipids, triglycerides, and glycogen content. Growth hormone concentrations prepartum (control = 11.0 ± 1.2, rbST87.5 = 14.1 ± 1.2, rbST125 = 15.1 ± 1.3 ng/mL) and postpartum (control = 14.4 ± 1.1, rbST87.5 = 17.8 ± 1.2, rbST125 = 21.8 ± 1.1 ng/mL) were highest for rbST125 cows. Cows treated with rbST had higher insulin-like growth factor 1 concentrations than control cows (control = 110.5 ± 4.5, rbST87.5 = 126.2 ± 4.5, rbST125 = 127.2 ± 4.5 ng/mL) only prepartum. Intensity of L-selectin expression was higher for rbST125 than for control and rbST87.5 cows (control = 3,590 ± 270, rbST87.5 = 3,279 ± 271, rbST125 = 4,371 ± 279 geometric mean fluorescence intensity [GMFI]) in the prepartum period. The PMNL intensities of phagocytosis (control = 3,131 ± 130, rbST87.5 = 3,391 ± 133, rbST125 = 3,673 ± 137 GMFI) and oxidative burst (control = 9,588 ± 746, rbST87.5 = 11,238 ± 761, rbST125 = 12,724 ± 781 GMFI) were higher for rbST125 cows than for control cows during the prepartum period. Concentrations of serum IgG anti-ovalbumin tended to be higher for rbST125 cows than for control cows (control = 0.75 ± 0.11, rbST87.5 = 0.94 ± 0.10, rbST125 = 1.11 ± 0.11 optical density) in the prepartum period. Haptoglobin concentration was significantly reduced 7 d postpartum for rbST125 treatment compared with control and rbST87.5 treatments (control = 2.74 ± 0.28, rbST87.5 = 2.81 ± 0.28, rbST125 = 1.87 ± 0.28 optical density). Although treatment tended to affect postpartum β-hydroxybutyrate (control = 747.5 ± 40.2, rbST87.5 = 753.2 ± 40.1, rbST125 = 648.8 ± 39.7 μmol/L), it did not affect liver contents of total lipids, triglycerides, or glycogen. Incidence of metritis among rbST125 cows was reduced compared with that in control cows (control = 23.1, rbST87.5 = 18.0, rbST125 = 7.8%). Treatment of dairy cows with 125 mg of rbST improved innate immune responses and IgG concentration, with limited effects on metabolism.

Key words: dairy cow, somatotropin, immune response

INTRODUCTION

A large proportion of dairy cows are immunosuppressed during the periparturient period. Phagocytosis and oxidative burst of PMNL may decrease starting approximately 2 wk prepartum, reaching a nadir in the first week postpartum (Hoeben et al., 1997; Kehrli et al., 1989a; Moreira da Silva et al., 1998). Furthermore, decreases in lymphocyte blastogenesis and immunoglobulin production by B cells have been observed from 1 wk pre- to 1 wk postpartum (Kehrli et al., 1989b; Nonnecke et al., 2003; Lacetera et al., 2005). It is not surprising, therefore, that a large proportion of dairy
cows present infectious diseases such as metritis and mastitis during the early stages of lactation (Burton et al., 2001; Hammon et al., 2006).

Dairy cows have increased concentrations of growth hormone (GH) and decreased IGF-1 concentrations immediately before parturition and during the postpartum period (Rhoads et al., 2004; Lucy, 2008). Insulin-like growth factor 1 is an important cell growth and differentiation factor associated with regulation of innate and adaptive immune functions (Heemskerk et al., 1999). Culture of granulocytes in the presence of IGF-1 reduces granulocyte apoptosis and increases phagocytosis, oxidative burst, and expression of adhesion molecules (Inoue et al., 1998; Kooijman et al., 2002). Furthermore, IGF-1 inhibits gene expression of mesenchymal stem cells and production of tumor necrosis factor α (TNF-α), IL-1β, and IL-6 (Guo et al., 2014).

Growth hormone-deficient humans treated with recombinant somatotropin had increased concentrations of IGF-1 and granulocyte colony-stimulator factor and blood neutrophil counts (Solmiya et al., 2005). Piglets treated with recombinant somatotropin before weaning and transportation had greater antibody concentrations (Kojima et al., 2008). Therefore, therapeutic strategies that increase circulating IGF-1 concentrations in periparturient dairy cows may improve their innate and adaptive immune responses.

Treatment of periparturient dairy cows with recombinant bovine somatotropin (rbST) increases IGF-1 serum concentrations during the periparturient period (Vicini et al., 1991; Gulay et al., 2004a,b) and has been associated with positive effects on glucose metabolism (Peel and Bauman, 1987). Recombinant bST may improve glucose metabolism by increasing hepatic gluconeogenesis, by decreasing glucose oxidation, and by blocking the inhibitory action of insulin on hepatic gluconeogenesis (Peel and Bauman, 1987; Bauman et al., 1989; Bauman and Vernon, 1993). Furthermore, treatment of periparturient cows with low doses of rbST was shown to increase DMI, decrease the incidence of ketosis and clinical mastitis, and increase milk yield (Putnam et al., 1999; Gulay et al., 2000, 2004b, 2007). Cows with elevated BCS could be ideal candidates for treatment with rbST during the periparturient period because obese cows have reduced postpartum IGF-1 concentrations (Kasimianickam et al., 2013). Thus, obese cows are more likely to be immunosuppressed (Samartín and Chandra, 2001) and are more susceptible to infectious diseases (Fronk et al., 1980; Treacher et al., 1986) during the peripartum period compared with thin cows. Furthermore, obese cows have suppressed DMI peripartum (Hayirli et al., 2002) and increased mobilization of NEFA (Treacher et al., 1986). Consequently, obese cows have impaired hepatic metabolism, are predisposed to fatty liver during early lactation, and are more likely to develop ketosis than thin cows (Mills et al., 1986; Smith et al., 1997).

The hypotheses of the current experiment were that administration of rbST to peripartum dairy cows with BCS ≥3.75 would increase GH and IGF-1 concentrations, improve immune responses (e.g., leukocyte count, phagocytosis, oxidative burst, and expression of adhesion molecules by PMNL, increased IgG concentration), reduce systemic inflammation (e.g., haptoglobin and TNF-α concentrations), and improve metabolism (e.g., reduced NEFA, BHBA, and liver total lipids and triglycerides, and increased glucose and liver glycogen). Therefore, the objectives of the current experiment were to evaluate the effects of peripartum rbST treatment of obese dairy cows on concentrations of GH and IGF-1, immune parameters, inflammatory response, and metabolism.

**MATERIALS AND METHODS**

The procedures conducted during this experiment were approved by the Institutional Animal Care and Use Committee from the University of Minnesota (protocol # 1306-30734A).

**Animals, Housing, and Nutrition**

One hundred sixty-six Holstein cows (≥ first lactation) were enrolled in the experiment at 253 ± 1 d of gestation. Cows with BCS ≥3.75 and locomotion score ≤2 from a commercial freestall dairy herd located in northwest Wisconsin were enrolled in the experiment from September 2012 to April 2013 and were followed until October 2013. From approximately 244 d of gestation to 21 d postpartum, cows were housed in a naturally ventilated freestall barn. As cows demonstrated signs of calving (discomfort, restlessness, tail twitching, and allantoic sac visible through the vulva), they were moved to a box stall. Within 12 h after calving, cows were moved to a postpartum pen (1 to 14 d postpartum) for daily observation and examination for diagnosis of postpartum diseases. Cows free of clinical diseases were then moved to a pen in which they stayed from approximately 14 to 28 d postpartum. At 28 d postpartum, cows were moved to 1 of 2 naturally ventilated freestall dairies (0.5 and 10 miles away) in which they remained until the end of their lactation.

From enrollment to calving, a TMR was fed ad libitum once a day at 0900 h; from calving to 21 d postpartum, a TMR was fed ad libitum once a day at 0400 h. Composition of TMR fed in the prepartum (far-off and close-up) and immediate postpartum (1 to 21 d postpartum) periods are described in Table 1.
Treatments

At enrollment (−28 d relative to calving), each cow’s BW was estimated using a Holstein dairy cow weigh tape (The Coburn Company Inc., Whitewater, WI) and body condition was scored (1 = emaciated and 5 = obese; 0.25-unit increments; Ferguson et al., 1994). Cows with BCS ≥3.75 were balanced for weight, BCS, lactation number, previous lactation 305-d mature-equivalent milk yield, and previous days open. Cows were then assigned randomly to 1 of 3 treatments: untreated control (n = 53), 87.5 mg of rbST (rbST87.5; n = 56), or 125 mg of rbST (rbST125; n = 57). Recombinant bovine somatotropin (Posilac/Elanco, Greenfield, IN) injections were administered every 7 d from −21 to 28 d relative to calving subcutaneously in the neck area using 1-mL tuberculin syringes and hypodermic 16-gauge and 5/8-inch-long needles. Commercially available ready-to-use syringes of rbST were used. To administer the proper dose of rbST to each cow, on the day before treatments, the contents of commercially available syringes of rbST were dispensed into sterile containers and the appropriate volume of rbST was aspirated into sterile syringes that were kept refrigerated until treatment of cows on farm.

Concentrations of GH and IGF-1

Concentrations of GH and IGF-1 were determined weekly from −21 to 21 d relative to calving. Serum GH and IGF-1 concentrations were determined in triplicate using a modified RIA from an ovine GH assay (Lalman et al., 2000). The intra- and interassay coefficients of variation (CV) for the assays were <5%.

Hemogram

Blood samples collected from the coccygeal vein or artery into 3-mL evacuated tubes containing EDTA (Kendall Monoject, Mansfield, MA) were used for the hemogram. Samples were collected −14 ± 1, −7 ± 1, 0 ± 1, 7 ± 1, 14 ± 1, and 21 ± 1 d relative to calving. Blood samples were analyzed within 10 h of sample collection using a Vet Scan HM2 (Abaxis, Union City, CA) hemogram machine.

Innate Immune Response Assays

Blood samples collected into 10-mL heparinized evacuated tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) −14 ± 1, −7 ± 1, 0 ± 1, 7 ± 1, 14 ± 1, and 21 ± 1 d relative to calving were used for determination of ex vivo innate immune responses (Hulbert et al., 2011). Although treatment with rbST started on d −21 relative to calving, a decision was made to start evaluating innate immune responses at −14 d relative to calving because of budgetary issues.

Briefly, indirect immunofluorescence staining was used to determine expression of adhesion molecules L-selectin (also known as CD62L) and β2 integrins (also known as CD18) by peripheral PMNL. The assay consisted of incubating 200 μL of whole blood at 4°C for 30 min with 200 μL (5 μg/mL) of monoclonal antibovine CD62L antibody (BAQ92A; Monoclonal Antibody Center, Washington State University, Pullman, WA) or 200 μL (2.5 μg/mL) of monoclonal anti-bovine CD18 antibody (BAQ30A; Monoclonal Antibody Center, Washington State University). Erythrocytes were lysed with hyperconcentrated PBS to isolate PMNL before a second 30-min incubation of cells with an anti-mouse IgG-fluorescein isothiocyanate (FITC) secondary polyclonal antibody (AbD Serotec, Raleigh, NC) diluted (1:400) in a PBS solution (Sigma-Aldrich, St. Louis, MO). Cells were washed, resuspended in PBS solution, and immediately analyzed by flow cytometry. The hemogram of all cows was performed before innate immune assays, and blood from cows with normal hemogram parameters was used as positive and negative controls in all assays. Negative controls were incubated with 200 μL of PBS solution instead of monoclonal antibodies.

Phagocytic and oxidative burst activities of peripheral PMNL were determined upon challenge with enteropathogenic bacteria (Escherichia coli 0118:H8). Briefly, the assay consisted of incubating 200 μL of whole blood with 100 μM dihydrorhodamine 123 (Molecular Probes/Invitrogen, Eugene, OR), an oxidative-sensitive indicator, and 40 μL of fluorescently labeled bacteria (10^9 cfu/mL) at 38.5°C for 15 min, with surface bacteria fluorescence removed using Trypan dye.
Blue solution (0.4%; Sigma-Aldrich). After washing with MilliQ water (Millipore, Billerica, MA) to remove excess dye, erythrocytes were lysed by the addition of hyperconcentrated PBS solution and resuspended in PBS solution for immediate flow cytometry analyses. Blood from cows with a normal hemogram was used as positive and negative controls. Unlabeled bacteria were used as negative controls for the phagocytosis assay and samples that received no dihydrorhodamine 123 served as negative controls for the oxidative burst assay. Flow cytometry was carried out on a BD FACSCanto II (BD Biosciences, Franklin Lakes, NJ) and analyzed using FlowJo 7.6.4 software (Tree Star Inc., San Carlos, CA). The PMNL population was identified on basis of forward- and side-scattered properties. After strictly gating the PMNL population, forward scatter, side scatter, and log fluorescence data were recorded.

Data reported herein is referent to percentages of PMNL positive for phagocytosis and oxidative burst, and expressing CD18 and CD62L molecules. Furthermore, data referent to intensity of phagocytosis, oxidative burst, and expression of CD18 and CD62L molecules are reported herein as geometric mean fluorescence intensity (GMFI). Intensity of phagocytosis and intensity of expression of adhesion molecules are indirect indicators of the number of bacteria phagocytized by PMNL and the number of adhesion molecules expressed by PMNL, respectively. On the other hand, oxidative burst intensity is an indirect indicator of the amount of reactive oxygen species produced via oxidation of dihydrorhodamine 123.

**Ovalbumin Challenge, Antibody Concentration Assay, and Colostrum IgG Concentration**

Cows were immunized with 0.5 mg of chicken egg ovalbumin (Type VII; Sigma-Aldrich) diluted in 0.5 mL of PBS and emulsified in Quil A adjuvant (0.5 mg of Quil A/0.5 mL of PBS; Accurate Chemical & Scientific Corp., Westbury, NY) by subcutaneous injections in the neck area. Cows were vaccinated with ovalbumin (Type VII; Sigma-Aldrich) diluted in carbonate-bicarbonate buffer (pH 9.4) and then washed with PBS and 0.05% Tween 20 (Sigma-Aldrich) solution (pH 7.4). Plates were blocked with 4% BSA (Santa Cruz Biotechnology Inc., Santa Cruz, CA) solution for 2 h and washed; then, control and diluted test sera (1/200) were added for an additional 2 h. The negative control included a pool of samples of nonimmunized cows’ sera, and the positive control included a pool of samples collected from cows 14 d after the second immunization. After washing the plates, conjugated antibody anti-bovine IgG (KPL Inc., Gaithersburg, MD), diluted (1/2,000) in 10 mM Tris-HCl buffer (Sigma-Aldrich) was added to the plates and incubated for 1 h. Substrate (5 mg/mL) p-nitrophenyl phosphate (Sigma-Aldrich) was added and, after a 30-min incubation, plates were read at 410 and 540 nm using a Eon plate reader (BioTek Instruments Inc., Winooski, VT). The intra- and interassay CV were 4 and 8%, respectively.

Farm personnel were instructed to collect colostrum samples immediately after parturition. Samples were stored at −32°C until analyzed by RIA (University of Saskatchewan, Saskatoon, Canada) for total IgG concentrations. Although farm personnel were instructed to collect samples from all cows enrolled in the experiment, samples from only 15, 6, and 16 cows from the untreated control, rbST87.5, and rbST125 treatments, respectively, were collected.

**Haptoglobin and TNF-α Assays**

Blood sampled weekly from d −21 ± 3 to 21 ± 3 into 10-mL evacuated tubes with EDTA (Becton Dickinson Vacutainer Systems) was used to determine concentrations of haptoglobin and TNF-α. Blood tubes were placed on ice until centrifugation (1,200 × g for 15 min at 4°C) and plasma was stored at −32°C until analysis. Haptoglobin concentrations were determined by a colorimetric procedure (Hulbert et al., 2011) and absorbance was measured using a plate reader (BioTek Instruments Inc.). A pool of plasma samples from cows at 7 d postpartum was used as control. The intra- and interassay CV were 4 and 3%, respectively.

Concentrations of TNF-α were determined by ELISA using a protocol described and validated for bovine plasma (Farney et al., 2011). Eight cows, not enrolled in the experiment, received intravenous infusion of LPS and had blood collected 4 h after infusion. A pool of plasma from the LPS-challenged cows was used as positive control (elevated TNF-α concentration). A pool of plasma samples from non-LPS-infused cows was used as positive control (reduced TNF-α concentration). The intra- and interassay CV were 5 and 3%, respectively.
**Metabolite and Cortisol Concentrations and Liver Biopsies**

Blood samples were collected weekly from −21 to 21 d relative to calving to determine concentrations of NEFA, BHBA, and glucose. Samples were collected from the coccygeal vein or artery into empty evacuated tubes and evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems) immediately after feeding while cows were restrained in self-locking headlocks. Tubes were placed in ice until centrifugation for plasma separation (1,200 × g for 15 min at 4°C). Plasma was aliquoted into microcentrifuge tubes and stored at −32°C until analysis. Concentrations of NEFA were determined using a colorimetric assay (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009), and concentrations of BHBA were determined enzymatically (Ranbut, Randox Laboratories, Antrim, UK; Ballou et al., 2009). Control serum (Randox Laboratories) was used for the NEFA and BHBA assays. The intraassay CV were 7 and 11% for the NEFA and BHBA assays, respectively, and interassay CV were 4 and 8% for the NEFA and BHBA assays, respectively. Glucose concentration was determined by enzymatic reaction (Stanbio Laboratory, Boerne, TX). The intra- and interassay CV were 4 and 3% for the glucose assay, respectively. Serum cortisol was measured by a solid-phase RIA using a commercially kit (Coat-A-Count Cortisol; Siemens Medical Solutions Diagnostics, Los Angeles, CA). The intra- and interassay CV were 7 and 6%, respectively.

A subsample of cows (n = 10/treatment) had liver samples collected at −21, −7, and 7 d relative to calving to determine liver content of total lipids, triglycerides, and glycogen. Liver biopsies were placed in nitrogen immediately after harvesting and later stored at −80°C until analysis. Total lipids and triglycerides extracted from liver tissues were measured by a colorimetric procedure (Fletcher, 1968; Foster and Dunn, 1973; Hara and Radin, 1978). The intra- and interassay CV for the total lipids and triglycerides assay were 2 and 3%, respectively. Glycogen content in the liver was determined by colorimetric assay (Lo et al., 1970). The intra- and interassay CV were 5 and 1%, respectively. Liver total lipids, triglycerides, and glycogen data are reported herein in percentage of wet weight.

**Clinical Examination, Disease Definitions, BCS, and Productive Parameters**

Farm personnel were instructed to evaluate cows within 24 h after calving to determine and record the occurrence of retained fetal membranes. Unfortunately, occurrence of retained fetal membranes was not recorded consistently and this information was not used in the current experiment. Study personnel palpated cows per rectum daily from d 2 to 21 postpartum for the diagnosis of metritis. Metritis was defined as cows having watery, pink/brown, and fetid uterine discharge (LeBlanc, 2010). Ketosis was defined as cows having circulating BHBA concentrations ≥1,400 μmol/L (Duffield et al., 2009). Percentage of cows removed from the herd was recorded for the first 60 d postpartum. Cows’ body condition was scored every 14 d from −28 to 70 d relative to calving and at 90 d postpartum.

Cows were milked thrice daily and started to receive full doses of rbST (500 mg) every 11 d between 55 to 60 d postpartum and remained in the rbST treatment until 220 to 225 d of gestation. Daily milk yield was recorded for individual cows (Afimilk Ltd., Kibbutz Afikim, Israel) starting at approximately 21 d postpartum and recorded on DairyComp305 software (Valley Ag, Software, Tulare, CA). Milk yield was not measured in the first 21 DIM because the transition cow facility did not have the capability to do so. Milk data reported herein are weekly milk yield averages from 21 to 150 DIM.

**Statistical Analysis**

Cows that calved before receiving at least 2 preparum doses of rbST were removed from the experiment and statistical analyses. All statistical analyses were conducted using SAS version 9.3 (SAS/STAT, SAS Inst. Inc., Cary, NC). In all models, cows were used as experimental unit. Binomial data were analyzed by logistic regression using the LOGISTIC procedure and the FREQ procedure of SAS with the Chi-Square and Fisher’s exact tests. Continuous data were analyzed by ANOVA using the MIXED procedure of SAS. For analyses of repeated measurements, the repeated statement was used and treatment (control vs. rbST87.5 vs. rbST125), time, and the interaction between treatment and time were included in the model as fixed effects. The structure of covariance (auto-regressive, unstructured, or compound symmetry) was chosen according to the Bayesian information criterion (BIC). The assumption of normality was assessed using the UNIVARIATE procedure of SAS. A log-transformation of the dependent variables granulocyte:lymphocyte ratio and TNF-α concentration was required to achieve a normal distribution. The prepartum and postpartum periods were analyzed separately because of inherent physiological changes that occur during calving.

Analysis of covariance was used to analyze the effect of treatment on liver contents of total lipids, triglycerides, and glycogen at different time points relative to calving using the GLM procedure of SAS. The model...
incorporated treatment as a fixed effect. In the model to
determine the effect of treatment on liver contents on d
−7 relative to calving, liver content on d −21 relative
to calving was used as a covariate.

Two statistical analyses were conducted to evaluate
the effects of treatment on milk yield. The first analysis
considered the period from 3 to 21 wk postpartum. The
second statistical analysis considered the period from
3 to 7 wk postpartum because in wk 8 of lactation, all
cows started to receive 500 mg of rbST every 11 d.

When the effect of treatment on dependent variables
was \( P \leq 0.15 \), the contrast statement of SAS was used to
test whether rbST dose had a linear or a quadratic effect on
dependent variables using the IML procedure of SAS and the ORPOL function to adjust and obtain
the appropriate contrast coefficients when testing un-
equally spaced dose treatments. Statistical significance
was defined as \( P \leq 0.05 \) and tendency was considered
if \( 0.05 < P \leq 0.15 \). When treatment affected or tended
to affect a dependent variable, the Bonferroni multiple
comparison correction was used and statistical sig-
ificance was defined as \( P \leq 0.015 \) and tendency was
considered if \( 0.015 < P \leq 0.05 \).

RESULTS

Seven cows were removed from the analyses (rbST87.5
= 3, and rbST125 = 4) for the following reasons: not
pregnant at enrollment (1 cow) and calved before rece-
ing at least 2 doses of rbST (6 cows).

At enrollment, lactation number (control = 2.36 ±
0.14, rbST87.5 = 2.34 ± 0.14, rbST125 = 2.37 ± 0.14
lactations; \( P = 0.98 \)), BW (control = 777.6 ± 10.7,
rbST87.5 = 777.8 ± 10.7, rbST125 = 777.8 ± 10.7 kg;
\( P = 0.99 \)), and BCS (control = 4.03 ± 0.03, rbST87.5 =
3.98 ± 0.03, rbST125 = 3.99 ± 0.03; \( P = 0.49 \)) were not
different among treatments. Treatments did not differ
regarding previous lactation 305-d mature-equivalent
milk yield (control = 12,224 ± 331, rbST87.5 = 12,986
± 331, rbST125 = 12,409 ± 331 kg; \( P = 0.24 \)) and
previous lactation interval from calving to pregnancy
(control = 152.2 ± 11.2, rbST87.5 = 143.7 ± 11.2,
rbST125 = 147.6 ± 11.2 d; \( P = 0.87 \)).

Average number of days that cows stayed in the
close-up pen did not differ among treatments (control
= 25.4 ± 0.7, rbST87.5 = 26.8 ± 0.7, rbST125 = 26.2
± 0.7 d; \( P = 0.41 \)). The percentages of cows calving
male calves tended (\( P = 0.14 \)) to be different among
treatments, as rbST125 cows tended to have higher (\( P
= 0.05 \)) incidence of male calves than control cows. No
differences were observed in incidence of male calves
between control and rbST87.5 (\( P = 0.17 \)) or between
rbST87.5 and rbST125 (\( P = 0.56 \)) treatments (Table
2). The percentage of cows calving twins was not (\( P
= 0.39 \)) different among treatments (Table 2).

**GH and IGF-1 Concentrations**

Treatment tended (\( P = 0.06 \)) to affect prepartum GH
concentrations (quadratic effect: \( P = 0.05 \); Figure 1A).
The contrasts among treatments demonstrated that
rbST125 cows tended (\( P = 0.02 \)) to have higher prepartum
GH concentrations than control cows. No differ-
ences were observed in prepartum GH concentrations
between control and rbST87.5 (\( P = 0.08 \)) or between
rbST87.5 and rbST125 (\( P = 0.60 \); Figure 1A). During
the postpartum period, treatment affected (\( P < 0.01 \))
GH concentrations (quadratic effect: \( P < 0.01 \); Figure
1A). The rbST125 cows had (\( P < 0.01 \)) and tended
to have (\( P = 0.02 \)) greater postpartum GH concen-
trations than control and rbST87.5 cows, respectively,
and rbST87.5 tended (\( P = 0.04 \)) to have higher GH
concentrations than control (Figure 1A). Prepartum
IGF-1 concentrations were affected by treatment (\( P
= 0.01 \); quadratic effect: \( P = 0.04 \); Figure 1B). Insulin-like
growth factor 1 concentration was greater for rbST87.5
(\( P = 0.01 \)) and rbST125 (\( P = 0.01 \)) cows compared
with control cows but no difference (\( P = 0.88 \)) was
observed between rbST87.5 and rbST125 cows. Treat-

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>rbST87.5</th>
<th>rbST125</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male calf, %</td>
<td>41.5\textsuperscript{A}</td>
<td>54.7\textsuperscript{AB}</td>
<td>60.4\textsuperscript{B}</td>
<td>0.14</td>
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<tr>
<td>Twins, %</td>
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<td>3.8</td>
<td>1.9</td>
<td>0.39</td>
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<tr>
<td>Metritis, %</td>
<td>23.1\textsuperscript{A}</td>
<td>18.6\textsuperscript{AB}</td>
<td>7.8\textsuperscript{B}</td>
<td>0.12</td>
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<tr>
<td>Ketosis, %</td>
<td>13.2</td>
<td>18.9</td>
<td>11.3</td>
<td>0.52</td>
</tr>
<tr>
<td>Removed from the herd, %</td>
<td>9.4</td>
<td>20.8</td>
<td>18.9</td>
<td>0.26</td>
</tr>
</tbody>
</table>

\textsuperscript{A,B}Within a row, means with different superscripts tended to differ (0.015 < \( P \) < 0.05).

\textsuperscript{1}Control = cows received no treatment; rbST87.5 = cows received 87.5 mg of recombinant (r)bST every 7 d
from −21 to 28 d relative to calving; rbST125 = cows received 125 mg of rbST every 7 d from −21 to 28 d
relative to calving.
ment did not affect postpartum IGF-1 concentrations ($P = 0.38$; Figure 1B).

**Hemogram Parameters**

Prepartum ($P = 0.57$) and postpartum ($P = 0.25$) percentages of leukocytes classified as granulocytes were not affected by treatment (Table 3). Treatment did not affect percentages of leukocytes classified as lymphocytes during the prepartum ($P = 0.49$) or postpartum periods ($P = 0.34$; Table 3). Prepartum ($P = 0.52$) and postpartum ($P = 0.25$) granulocyte:lymphocyte ratios were not affected by treatment (Table 3).

**Innate Immune Parameters**

Although treatment ($P = 0.16$) did not affect percentage of PMNL expressing CD62L (L-selectin) during the prepartum period, treatment tended ($P = 0.15$) to affect CD62L expression during the postpartum period (quadratic effect: $P = 0.05$; Table 3). Percentage of PMNL expressing CD18 during the prepartum period tended ($P = 0.08$) to be affected by treatment (linear effect: $P = 0.03$; Table 3). Cows in the rbST87.5 treatment tended ($P = 0.04$) to have a lower percentage of PMNL expressing CD18 during the prepartum period compared with control cows. We detected no differences in percentage of PMNL expressing CD18 during the prepartum period between control and rbST125 ($P = 0.87$) or between rbST87.5 and rbST125 ($P = 0.07$) cows. No differences among treatments ($P = 0.59$) were observed for the percentage of PMNL expressing CD18 during the postpartum period (Table 3). Treatment affected ($P = 0.02$) intensity of PMNL expression of CD62L during the prepartum period (quadratic effect: $P = 0.01$; Figure 2A). Contrasts revealed that rbST125 cows had ($P < 0.01$) and tended to have ($P = 0.05$) greater intensity of CD62L expression by PMNL during the prepartum period than rbST87.5 and control cows, respectively. Intensity of CD62L expression by PMNL during the prepartum period did not ($P = 0.42$) differ between control and rbST87.5 cows. No differences in intensity of CD62L expression by PMNL among treatments ($P = 0.77$) were observed in the postpartum period (Figure 2A). During the prepartum ($P = 0.43$) and postpartum ($P = 0.96$) periods, treatment did not affect intensity of expression of CD18 by PMNL (Figure 2B).

Treatment did not ($P = 0.21$) affect the percentage of PMNL positive for phagocytosis and oxidative burst during the prepartum period (control = 66.8 ± 1.8, rbST87.5 = 69.1 ± 1.8, rbST125 = 71.5 ± 1.9%). Similarly, treatment had no ($P = 0.88$) effect on the percentage of PMNL positive for phagocytosis and oxidative burst in the postpartum period (control = 64.9 ± 2.0, rbST87.5 = 64.7 ± 2.0, rbST125 = 66.0 ± 2.0%). Treatment affected ($P = 0.02$) PMNL phagocytosis intensity during the prepartum period (quadratic effect: $P < 0.01$; Figure 3A). According to the contrast analyses, during the prepartum period, intensity of phagocytosis by PMNL from rbST125 cows was greater ($P < 0.01$) than that from control cows but no differences were observed between control and rbST87.5 ($P = 0.17$) or between rbST87.5 and rbST125 ($P = 0.14$).
During the postpartum period, treatment did not \((P = 0.35)\) affect PMNL phagocytosis intensity (Figure 3A). Treatment affected \((P = 0.02)\) PMNL oxidative burst intensity during the prepartum period (quadratic effect: \(P < 0.01\); Figure 3B). Intensity of oxidative burst by PMNL from rbST125 cows during the prepartum period was higher \((P < 0.01)\) compared with the control treatment. We found no differences, however, in PMNL oxidative burst intensity during the prepartum period between control and rbST87.5 \((P = 0.13)\) or between rbST87.5 and rbST125 \((P = 0.18)\) treatments. No differences were observed among treatments in PMNL intensity of oxidative burst during the postpartum period \((P = 0.43; \text{Figure 3B})\).

**Adaptive Immune Parameters**

Prepartum concentrations of serum IgG anti-ovalbumin tended \((P = 0.09)\) to be affected by treatment (quadratic effect: \(P = 0.04\); Figure 4). According to the contrast analyses, during the prepartum period, concentrations of IgG tended \((P = 0.03)\) to be greater for rbST125 cows compared with control cows. No differences were observed in IgG concentration among treatments in the postpartum period \((P = 0.43; \text{Figure 4})\).

\(A,B\)Within a row, means with different superscripts tended to differ \((0.015 < P \leq 0.05)\).

\(^1\)Control = cows received no treatment; rbST87.5 = cows received 87.5 mg of recombinant (r)BST every 7 d from \(-21\) to 28 d relative to calving; rbST125 = cows received 125 mg of rbST every 7 d from \(-21\) to 28 d relative to calving.

**Haptoglobin and TNF-α Concentrations**

Haptoglobin concentrations during the prepartum \((P = 0.08)\) and postpartum \((P = 0.03)\) tended, however, for the interaction between treatment and day to affect haptoglobin concentrations during the postpartum period because rbST125 cows had reduced haptoglobin concentration on d 7 postpartum compared with control \((P = 0.03)\) and rbST87.5 \((P = 0.02)\) cows (Figure 5A). Treatments did not affect prepartum \((P = 0.56)\) and postpartum \((P = 0.27)\) TNF-α concentrations (Figure 5B).

**NEFA, BHBA, Glucose, and Cortisol Concentrations**

Treatment did not affect prepartum \((P = 0.85)\) or postpartum \((P = 0.43)\) NEFA concentrations (Table 4). Concentration of BHBA was not affected by treatment during the prepartum period \((P = 0.84)\) but tended \((P = 0.12; \text{quadratic effect: } P = 0.05)\) to be affected during the postpartum period (Table 4). Treatment did not affect prepartum \((P = 0.99)\) or postpartum \((P = 0.58)\) glucose concentrations (Table 4). Similarly, treatment did not affect prepartum \((P = 0.66)\) or postpartum \((P = 0.71)\) cortisol concentrations (Table 4).

**Liver Total Lipids, Triglycerides, and Glycogen Contents**

Percentage of total lipids in the liver was not different among treatments (Figure 6A). Percentage of liver triglycerides tended to be different among treatments on d \(-21\) \((P = 0.45)\), d \(-7\) \((P = 0.33)\), or d 7 \((P = 0.85)\) relative to calving (Figure 6B). Finally, treatment did not affect percentage of glycogen in the liver on d \(-21\) \((P = 0.97)\), d \(-7\) \((P = 0.31)\), or d 7 \((P = 0.48)\); Figure 6C).
BCS, Incidence of Metritis, Ketosis, Removal from the Herd Within 60 DIM, and Milk Yield

Treatment did not affect prepartum BCS (P = 0.83) but tended (P = 0.10; linear effect: P = 0.08) to affect postpartum BCS, as control cows had slightly higher BCS on d 42 postpartum than rbST87.5 and rbST125 cows (Figure 7). Treatment tended (P = 0.12; quadratic effect: P = 0.04) to affect the incidence of metritis (Table 2). According to the contrast analyses, cows in the rbST125 treatment tended (P = 0.04) to be less likely to be diagnosed with metritis than control cows, but no differences were observed between control and rbST87.5 cows (P = 0.53) or between rbST87.5 and rbST125 cows (P = 0.14). Treatment did not affect incidence of ketosis (P = 0.52; Table 2). Percentage of cows removed from the herd within 60 d postpartum was not (P = 0.26) different among treatments (Table 2).

Milk yield from 3 to 7 wk postpartum was not (P = 0.33) affected by treatment (control = 43.4 ± 1.4, rbST87.5 = 44.9 ± 1.5, rbST125 = 46.4 ± 1.4 kg/d). Milk yield from 3 to 21 wk postpartum was not af-
fected \((P = 0.77)\) by treatment (control = 45.9 ± 1.25, rbST87.5 = 45.8 ± 1.35, rbST125 = 47.0 ± 1.33 kg/d; Figure 8). The interaction between treatment and week, however, affected \((P = 0.02)\) milk yield because there was a tendency for rbST125 cows to produce more milk than control cows in wk 3 \((P = 0.09)\) and wk 5 \((P = 0.06)\).

**DISCUSSION**

Dairy cows undergo uncoupling of the somatotropic axis, characterized by reduced IGF-1 concentrations despite increasing GH concentrations, during the periparturient period (Lucy, 2008). Obese cows have reduced

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>(P)-value</th>
<th>(P)-value</th>
<th>(P)-value</th>
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</thead>
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<tr>
<td></td>
<td>Control</td>
<td>rbST87.5</td>
<td>rbST125</td>
<td>Trt</td>
</tr>
<tr>
<td>Prepartum</td>
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<tr>
<td>NEFA, (\mu\text{mol/L})</td>
<td>313.7 ± 24.5</td>
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<td>BHBA, (\mu\text{mol/L})</td>
<td>380.7 ± 13.7</td>
<td>377.1 ± 13.4</td>
<td>369.7 ± 13.5</td>
<td>0.84</td>
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<tr>
<td>Glucose, mg/dL</td>
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<td>75.5 ± 1.1</td>
<td>75.5 ± 1.2</td>
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<tr>
<td>Cortisol, ng/mL</td>
<td>6.6 ± 0.6</td>
<td>7.3 ± 0.6</td>
<td>7.4 ± 0.6</td>
<td>0.66</td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA, (\mu\text{mol/L})</td>
<td>527.5 ± 36.8</td>
<td>592.9 ± 36.8</td>
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<tr>
<td>BHBA, (\mu\text{mol/L})</td>
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<td>753.2 ± 40.1</td>
<td>648.8 ± 39.7</td>
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<tr>
<td>Glucose, mg/dL</td>
<td>68.0 ± 1.1</td>
<td>67.2 ± 1.1</td>
<td>68.8 ± 1.1</td>
<td>0.58</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>4.4 ± 0.5</td>
<td>4.1 ± 0.5</td>
<td>3.9 ± 0.5</td>
<td>0.71</td>
</tr>
</tbody>
</table>

\(^1\)Control = cows received no treatment; rbST87.5 = cows received 87.5 mg of recombinant (r)bST every 7 d from −21 to 28 d relative to calving; rbST125 = cows received 125 mg of rbST every 7 d from −21 to 28 d relative to calving.

**Figure 4.** Immunoglobulin G anti-ovalbumin optical density (OD) according to treatment. Ovalbumin immunizations were performed on d −21, −7, and 7 relative to calving. Treatments: control = cows received no treatment; rbST87.5 = cows received 87.5 mg of recombinant (r)bST every 7 d from −21 to 28 d relative to calving; rbST125 = cows received 125 mg of rbST every 7 d from −21 to 28 d relative to calving.

**Figure 5.** (A) Haptoglobin optical density (OD), and (B) tumor necrosis factor-α (TNF-α) concentration (pg/mL) according to treatment. Treatments: control = cows received no treatment; rbST87.5 = cows received 87.5 mg of recombinant (r)bST every 7 d from −21 to 28 d relative to calving; and rbST125 = cows received 125 mg of rbST every 7 d from −21 to 28 d relative to calving. (A) Prepartum period—treatment \((P = 0.74)\), day \((P < 0.01)\), and interaction between treatment and day \((P = 0.21)\). Postpartum period—treatment \((P = 0.45)\), day \((P < 0.01)\), and interaction between treatment and day \((P = 0.08)\). Contrasts on d 7 postpartum: control vs. rbST87.5: \(P = 0.87\), control vs. rbST125: \(P = 0.03\), and rbST87.5 vs. rbST125: \(P = 0.28\). (B) Prepartum period—treatment \((P = 0.56)\), day \((P < 0.01)\), and interaction between treatment and day \((P = 0.38)\). Postpartum period—treatment \((P = 0.27)\), day \((P < 0.01)\), and interaction between treatment and day \((P = 0.17)\). Results are reported as LSM ± SEM.
feed intake (Hayirli et al., 2002) and are more likely to lose BCS and BW (Fronk et al., 1980; Treacher et al., 1986) during the periparturient period than thinner cows. Increased BCS 1 wk prepartum was associated with more dramatic reductions in IGF-1 concentrations compared with 1 wk postpartum (Kasimanickam et al., 2013). In the current experiment, concentrations of GH and IGF-1 were increased during the prepartum period in rbST-treated cows. In contrast, during the postpartum period, only GH concentration was increased by rbST treatment. Gulay et al. (2004a) observed a slight increase in IGF-1 concentration during the postpartum period when peripartum cows received 142.8 mg of rbST every 14 d from −21 to 42 d relative to calving. Visual inspection of Figure 1B indicates that the pattern of IGF-1 concentration appears to differ between
control and rbST125 cows starting on d 14 postpartum. Therefore, we speculate that no differences in IGF-1 concentrations were observed in the current experiment during the postpartum period because treatment ended 28 d postpartum.

In an observational experiment, Kasimanickam et al. (2013) demonstrated that concentrations of IGF-1 were reduced from 1 wk prepartum to 1 wk postpartum among cows diagnosed with metritis. Insulin-like growth factor 1 stimulates growth and differentiation of cells of the immune system (Heemskerk et al., 1999). Therefore, it is likely that one of the mechanisms associated with more dramatic immunosuppression among obese cows during the periparturient period (Samartin and Chandra, 2001; Sauwein et al., 2014) is their reduced IGF-1 concentration.

Percentages of granulocyte in blood were not different among treatments. Treatment of GH-deficient adult humans with rbST increased IGF-1 concentration and neutrophil count (Solmiya et al., 2005). Insulin-like growth factor 1 modulates hematopoiesis directly through induction of cell proliferation and antiapoptotic signaling (Heemskerk et al., 1999). Frostad et al. (1998) demonstrated that IGF-1 increased production of neutrophils from cultures of CD34+ cells from umbilical cord in a dose-dependent manner. This dose-dependent effect of IGF-1 on neutrophil count leads us to speculate that the doses of rbST administered in the current experiment did not increase IGF-1 concentrations sufficiently to modulate hematopoiesis. Although in vitro and in vivo experiments have demonstrated that GH stimulates lymphocyte proliferation and stimulates function of human and mice thymic cells (Bozzola et al., 1988–1989; Savino, 2003), percentages of lymphocytes in blood did not differ among treatments in the current experiment. It is possible that the concentrations of IGF-1 and GH reached through the treatments applied herein were insufficient to affect lymphocyte count.

Neutrophil adhesion and migration to the inflammatory site are important initial steps during the inflammatory process. Although no differences in percentages of PMNL expressing L-selectin (CD62L) among treatments were observed, rbST treatment tended to increase intensity of L-selectin expression by PMNL. Growth hormone treatment increased number of CD62L+ T cells and expression of CD62L in mice lymph nodes (Smaniotto et al., 2004) and enhanced adhesion of human neutrophils (Ryu et al., 2000). Unexpectedly, percentage of PMNL expressing CD18 during the prepartum period tended to be lower for rbST87.5 cows compared with control cows. It is not clear why treatment did not improve expression of CD18. Culture of human PMNL with GH did not affect expression of CD11b, despite the fact that culture of human PMNL with IGF-1 increased expression intensity of CD11b (Inoue et al., 1998).

Growth hormone can exert direct effects on all major immune cell types (Kelley, 1990) and participates in the development and maintenance of cell-mediated and humoral responses (Meazza et al., 2004). Similarly, IGF-1 plays an important role in the development of immune responses such as T-cell proliferation, chemotaxis, T-cell activation, apoptosis, and natural killer cell cytotoxicity (Weigent, 2013). In the current experiment, intensities of PMNL phagocytosis and oxidative burst were increased during the prepartum period in rbST125 cows compared with control cows. Phagocytosis and oxidative burst were increased when PMNL were incubated with GH or IGF-1 and challenged with E. coli (Bjerknes and Aarskog, 1995; Inoue et al., 1998). Furthermore, treatment of malnourished human hemodialysis patients with GH increased PMNL phagocytosis (Kotzmann et al., 2003), and GH replacement therapy of GH-deficient humans increased release of superoxide anion by neutrophils (Reinisch et al., 1996). In the current experiment, however, differences in GH concentrations between treatments were greatest during the postpartum period but parameters associated with PMNL function were unaltered during the postpartum period. Therefore, it is likely that the main factor affecting prepartum PMNL function was IGF-1.

Cows in the rbST125 treatment had greater IgG anti-ovalbumin concentrations during the prepartum period than control cows. Interestingly, within 7 d of ovalbumin vaccination and start of the rbST treatment, we observed a significant increase in IgG concentration among rbST125 and rbST87.5 cows. On the other hand, there is a significant increase in IgG concentration among control cows only 14 d after ovalbumin vaccination. Piglets treated with somatotropin before weaning and transportation had increased IgM concentrations (Kojima et al., 2008) and mice treated with somatotropin had increased IGF-1 and IgG, IgM, and IgA concentrations (Solmiya et al., 2005). Hattori et al. (2001) demonstrated that expression of GH receptor mRNA is greater in human B cells than in other immune cells types, such as T cells and neutrophils. The rapid increase in IgG concentration within 7 d of the first rbST treatments suggests that the effects of GH, IGF-1, or both, on immune cells are fast.

The improvements in innate and adaptive immune parameters described herein are of great importance for dairy cows during the periparturient period. Several experiments have demonstrated that activity of PMNL from cows diagnosed with metritis is reduced during the prepartum period (Cai et al., 1994; Hammon et al., 2006). In the current experiment, the incidence of
metritis among rbST125 cows tended to be reduced compared with that of control cows. Treatment of cows with 142.8 mg of rbST every 14 d from −21 to 42 d relative to calving did not decrease the incidence of metritis but reduced the overall incidence of periparturient diseases (Gulay et al., 2007). More recently, Gohary et al. (2014) did not observe differences in incidence of postpartum diseases when prepartum cows were treated once to thrice with rbST (325 mg every 14 d). Treatment of cows with rbST at 14-d intervals is known to result in more oscillation of IGF-1 concentration (Gulay et al., 2004a; Ribeiro et al., 2014) compared with shorter intervals (Rivera et al., 2010). Therefore, in the study by Gohary et al. (2014), concentrations of IGF-1 at the time of calving may not have been significantly different between rbST-treated and untreated cows. The half-life of PMNL is approximately 8.9 h (Paape et al., 2003). Therefore, a 7-d interval between rbST treatments was adopted to maintain IGF-1 concentrations constantly higher among rbST treated cows until at least the day of parturition. This is probably a critical factor influencing the maintenance of elevated activity of PMNL from rbST125 cows through the calving period. Another important difference between the current experiment and that described by Gohary et al. (2014) is the dose of rbST. Elevated doses of rbST during the periparturient period may result in greater BCS loss and accentuate negative energy balance (Moallem et al., 1997, 2000), which may result in immunosuppression. Furthermore, treatment of GH-deficient humans with 13 IU of GH/m² per day (10 to 20 times the normal GH replacement dose) resulted in no improvements in release of proinflammatory cytokines by peripheral immune cells compared with placebo (Zarkesh-Esfahani et al., 2000). Furthermore, signal transducer and activator of transcription 5 (Stat5) response by peripheral blood mononuclear cells was greatest when GH concentration was between 1 and 1,000 ng/mL, whereas GH doses >1,000 ng/mL suppressed the Stat5 response (Zarkesh-Esfahani et al., 2000). Lymphocyte proliferation after phytohemagglutinin stimulation was increased when peripheral blood mononuclear cells were cultured in the presence of GH <850 ng/mL, whereas culture in the presence of GH >850 ng/mL reduced lymphoproliferation (Bozzola et al., 1988–1989). Growth hormone concentration of dairy cows starts to increase approximately 1 wk prepartum, as energy balance starts to decrease (Lucy, 2008). Based on our findings and those of others (Putnam et al., 1999), the decision to use 125 mg of rbST every 7 d in periparturient dairy cows seems correct based on improvements in immune function and minimal effects on metabolism.

Although no differences in concentrations of haptoglobin were observed among treatments, rbST125 cows had reduced haptoglobin concentrations 7 d postpartum. Furthermore, among cows with metritis, haptoglobin concentrations 7 d postpartum tended to be lower for rbST125 cows compared with control and rbST87.5 cows (data not shown). The reduced haptoglobin concentration 7 d postpartum among rbST125 cows may have been a consequence of fewer rbST125 cows having metritis or reduced severity of infection or inflammation among rbST125 cows due to greater GH concentrations. Severely burned human patients receiving GH therapy had decreased haptoglobin concentrations (Jeschke et al., 2000). Growth hormone is likely to modulate the acute phase response by increasing IGF-1 and decreasing IL-1 expression, which leads to decreased type 1 acute phase proteins and increased constitutive hepatic proteins (Jarrar et al., 1997; Jeschke et al., 2000). Although rbST treatment did not affect TNF-α concentration in the current experiment, metritic cows treated with rbST had TNF-α concentrations reduced by approximately 17% (data not shown). Pro-monocytic cells engineered by gene transfer to produce human GH secreted less TNF-α in response to challenges with LPS, and GH treatment inhibited TNF-α secretion by human monocytes and macrophages (Haefner et al., 1997). The effects of treatment of peripartum cows with rbST on severity of inflammatory response and secretion of proinflammatory cytokines should be evaluated because prolonged and uncontrolled inflammatory response might be detrimental to the individual and result in increased morbidity and mortality (Moshage, 1997).

In the current experiment, rbST87.5 and rbST125 cows had reduced BCS compared with control cows starting at approximately 14 d postpartum. Concentrations of NEFA, BHBA, and glucose, however, were not different among treatments. Effects of rbST treatment during the periparturient period on BCS and metabolites are inconclusive and are likely dependent on dose of rbST, frequency of treatment, genetics, DMI, and milk yield. Although Putnam et al. (1999) did not observe effects of rbST treatment on BCS during the periparturient period, cows treated with 142.8 mg of rbST every 14 d had reduced NEFA and BHBA concentrations and increased glucose concentrations around the time of calving (Putnam et al., 1999). On the other hand, Gohary et al. (2014) observed reduced BCS in wk 3 postpartum, increased NEFA in wk 1 prepartum, increased BHBA in wk 1 postpartum, and increased peripartum glucose concentrations in cows treated with 325 mg of rbST every 14 d during the prepartum period. Although in the current experiment and Gohary et al. (2014), DMI and energy balance were not determined, it is likely that the reduced BCS during the postpartum among rbST-treated cows represents
greater fat mobilization to sustain greater milk yield early postpartum. Putnam et al. (1999) also reported greater milk yield among cows treated with rbST but rbST treatment also increased DMI, which may have reduced the extent of negative energy balance in response to greater milk yield. Bovine somatotropin can increase basal lipolysis in cows in negative energy balance and consequently increase NEFA concentrations (Bauman, 1992). Increased adipose tissue mobilization in response to rbST treatment, however, may or may not result in changes in plasma NEFA concentrations because rbST also modulates the utilization of NEFA by several tissues (Bauman, 1992). The uptake and oxidation of NEFA by peripheral tissue can be increased by rbST while glucose uptake is decreased, causing a reduction in NEFA supply to the liver and increased oxidation to ketones (Bauman, 1992; Putnam et al., 1999). Furthermore, rbST regulates gluconeogenesis through the regulation of enzymes such as phosphoenolpyruvate carboxykinase, a key enzyme in the conversion of oxaloacetate to phosphoenolpyruvate (Velez and Donkin, 2004). Although in the current experiment glucose was not increased in rbST-treated cows, this may be a consequence of greater milk yield among rbST-treated cows. Interestingly, on d −7 relative to calving, hepatic content of glycogen was numerically higher (43% higher) in rbST125 cows than in control cows. Treatment of lactating cows and cows under positive energy balance with rbST resulted in decreased liver glycogen content (Pocius and Hertlein, 1986; Knapp et al., 1992). Glycogen degradation is believed to be one of the glucose-sparing mechanisms exerted by rbST to divert nutrients for milk synthesis (Bauman and Vernon, 1993). The role of rbST on glycogen synthesis or breakdown in periparturient cows, however, is not well understood. The numerical increase in hepatic glycogen leads us to speculate that rbST treatment of periparturient cows may be optimizing energy through different pathways than those observed in cows under positive energy balance. In agreement with others (Gallo and Block, 1990; Putnam et al., 1999), rbST treatment during the peripartum period did not affect hepatic content of total lipids and triglycerides.

CONCLUSIONS

Treatment of peripartum dairy cows with 125 mg of rbST at 7-d intervals improved PMNL expression of L-selectin, PMNL phagocytosis and oxidative burst, and antibody concentrations, and decreased postpartum acute phase protein and incidence of metritis in obese dairy cows. Administration of somatotropin in transition cows could be used as a prepartum strategy to increase IGF-1 concentrations, improve immune function, and decrease postpartum disorders. Although we observed numerical separation on milk yield among treatments, additional research is warranted to evaluate how peripartum rbST treatment may improve milk yield. Further investigations with larger sample sizes and cows of varying BCS should be conducted to confirm the positive effects of rbST treatment on immune function and incidence of diseases.

ACKNOWLEDGMENTS

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