Hepatic mRNA expression for genes related to somatotrophic axis, glucose and lipid metabolisms, and inflammatory response of periparturient dairy cows treated with recombinant bovine somatotropin

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ABSTRACT

Objectives of this experiment were to evaluate the effects of recombinant bovine somatotropin (rbST) treatment of periparturient dairy cows on hepatic mRNA expression for genes related to the somatotrophic axis, insulin, glucose, and lipid metabolism, inflammation, and oxidative stress. Holstein cows were enrolled in the experiment at 253 ± 3 d of gestation and assigned to 1 of 3 treatments: untreated control ($n = 53$), 87.5 mg of rbST ($n = 56$; rbST87.5), and 125 mg of rbST ($n = 57$; rbST125). Cows in the rbST87.5 and rbST125 treatments received weekly injections of rbST from -21 to 28 d relative to calving. A subsample of cows (control = 20, rbST87.5 = 20, rbST125 = 20) was randomly selected for collection of liver samples according to expected calving date, BCS, and previous lactation 305-d mature equivalent milk yield. Only cows that had liver sampled at -21 ± 3 , -7 ± 3 , and 7 ± 3 d relative to calving were used in the current experiment. Blood, sampled weekly from -28 to 21 d relative to calving, was used to determine the concentrations of growth hormone, insulin-like growth factor 1, insulin, cortisol, fatty acids, β -hydroxybutyrate, glucose, haptoglobin, and tumor necrosis factor- α . Liver samples were used to determine hepatic mRNA expression of 50 genes. Treatment with rbST increased growth hormone concentrations during the postpartum period (control = 9.0 ± 0.7 , rbST87.5 = 15.3 ± 1.0 , rbST125 = 18.5 ± 1.3 ng/mL) and increased insulin-like growth factor 1 concentrations during the prepartum period (control = 107.4 ± 7.2 , rbST87.5 = 126.9 ± 6.6 , rbST125 = 139.4 ± 6.9 ng/mL). Control cows had greater postpartum concentrations of β -hydroxybutyrate (control = 776.4 ± 64.0 , rbST87.5 = 628.4 ± 59.7 , rbST125 = 595.4 ± 60.9 μ mol/L) than rbST cows. The rbST87.5 and

rbST125 treatments upregulated the hepatic mRNA expression for somatotrophic axis genes (*GHR*, *GHR1A*, *IGF1*, *IGFBP3*, and *SOCS2*) on d -7 relative to calving and upregulated the mRNA expression for *SOCS2* on d 7. On d -7 , rbST87.5 and rbST125 treatments increased mRNA expression for genes involved in hepatic lipid transport (*ANGPTL4*, *APOA5*, *APOB100*, and *SCARB1*) and downregulated mRNA expression for *PPARD*, which is involved in lipid storage. On d 7, rbST tended to upregulate the mRNA expression for genes involved in gluconeogenesis (*PCK1*) and fatty acid β -oxidation (*ACOX1*), and downregulated the mRNA expression for genes involved in inflammation (*TNFRSF1A*, *ICAM1*, *CXCL1*, *MYD88*, *HIF1A*, *IL1RN*, *NFKBIA*, and *SOCS3*) and oxidative stress (*XBPI*). Administration of rbST during the periparturient period may improve liver function and health by increasing hepatic capacity for gluconeogenesis and lipid transport and by reducing inflammation and oxidative stress.

Key words: periparturient cows, recombinant bovine somatotropin, hepatic gene expression

INTRODUCTION

The liver performs essential functions in the body by uniquely expressing genes encoding proteins involved in glucose, lipid, and protein metabolism, ketogenesis, immune function, detoxification, hormone catabolism, vitamin and mineral metabolism, and a variety of other functions (Donkin, 2012). Postpartum cow health is closely associated with the capacity of the liver to cope with the changes in nutrient supply and shifting metabolic demands that accompany the initiation of lactation (Drackley et al., 2001). In periparturient dairy cows, the liver has an important role in metabolic regulation and control of the somatotrophic axis to successfully adapt to the negative energy balance (NEB; Grummer et al., 2004). During NEB the expression of growth hormone (GH) 1- α receptors (*GHR1A*) by

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the liver is downregulated to decrease IGF-1 synthesis and its inhibitory effects on synthesis and secretion of GH (Bauman, 2000). Therefore, GH concentrations are increased in periods of NEB, such as the periparturient period, to favor nutrient partitioning for lactogenesis (Bell and Bauman, 1997). The pivotal role of GH in nutrient partitioning includes major alterations in metabolism of carbohydrates and lipids (Lupu et al., 2001). In adipose tissue, GH acts to facilitate lipolysis, increase insulin resistance to reduce glucose uptake and oxidation, and to decrease lipid synthesis (Boyd and Bauman, 1989). In the liver, GH increases gluconeogenesis (Cohick et al., 1989) through the regulation of enzymes, such as phosphoenolpyruvate carboxykinase (**PCK**), suppression of insulin's inhibitory effect on gluconeogenesis (Velez and Donkin, 2004), and increased hepatic fatty acid oxidation to CO₂ (Pocius and Hertlein, 1986).

From late gestation to early lactation, insulin resistance and GH-induced lipolysis cause a marked increase in fatty acids concentration and influx into the liver, which experiences a pronounced metabolic stress to maintain homeostasis (Drackley et al., 2001). The liver takes up approximately one-third of the whole body fatty acid flux, and this often exceeds its oxidation capacity, increasing the risk of liver lipidosis and ketosis (Drackley et al., 2001). During the peripartum period the liver is exposed to a variety of inflammatory factors, such as LPS, proinflammatory cytokines, and reactive oxygen species. These inflammatory factors are increased during the periparturient period due to alterations in gastrointestinal physiology, associated with changes in feed intake and composition, and episodes of infectious (e.g., metritis and mastitis) and gastrointestinal (e.g., SARA and displacement of the abomasum) diseases (Plaizier et al., 2008; Vels et al., 2009). Consequently, periparturient cows may present inflammation of the liver, which may induce an acute phase response (Vels et al., 2009), metabolic disorders, and unfold protein response to oxidative stress (Gessner et al., 2014) that collectively result in impaired liver function.

Treatment of lactating dairy cows with recombinant bovine somatotropin (**rbST**) increased liver gluconeogenesis, suppressed insulin's inhibitory effect on gluconeogenesis (Peel and Bauman, 1987), and increased complete oxidation of fatty acids in bovine liver slices (Pocius and Hertlein, 1986), which are expected to improve metabolic parameters. Effects of rbST treatment of periparturient cows on metabolic parameters and health are less clear. Whereas treatment of periparturient cows with 500 mg of rbST every 14 d increased glucose concentration, reduced concentrations of fatty acids and BHB, and increased milk yield according to some (Putnam et al., 1999), it reduced FCM yield ac-

cording to others (Eppard et al., 1996). Reduced doses of rbST (142.8 to 325 mg of rbST every 14 d) given to periparturient cows increased IGF-1, insulin, and glucose concentrations but, again, effects on milk yield and health were diverse (Gulay et al., 2003, 2004, 2007; Gohary et al., 2014). In a recent experiment, periparturient Holstein cows were treated with 0, 87.5, and 125 mg of rbST weekly, and those treated with 125 mg had increased IGF-1 concentrations, improved responses associated with innate and adaptive immunity, and reduced BHB concentrations (Silva et al., 2015). Therefore, treatment of periparturient cows with reduced doses of rbST has the potential to significantly affect liver function.

Enhanced knowledge of the processes that regulate liver function and factors that potentiate the capacity of liver to coordinate shifts in nutrient supply and demand by other tissues may lead to significant improvements in peripartum cow management and health. Therefore, the hypotheses of the current experiment were that weekly administration of rbST to peripartum dairy cows would upregulate hepatic mRNA expression for genes related to the somatotropin axis (e.g., *GHR*, *GHR1A*, *IGF1*, and *INSR*), gluconeogenesis (e.g., *G6PC*, *PC1*, and *PCK1*), lipid oxidation and transport (e.g., *ACOX1*, *APOA5*, *APOB100*, and *SCARB1*), and downregulate the expression of mRNA for genes related to ketogenesis (e.g., *HMGCLL1*), lipid synthesis and accumulation (e.g., *DGAT1* and *PPARD*), inflammation (e.g., *MYD88*, *NFKB1*, *HIF1*, and *CXCL1*), and oxidative stress (e.g., *XBPI*). The objectives of the current experiment were to evaluate the effects of peripartum rbST treatment of dairy cows on hepatic mRNA expression for genes related to the somatotrophic axis, glucose and lipid metabolisms, inflammation, and oxidative stress.

MATERIALS AND METHODS

Animals, Enrollment, and Treatments

All animal procedures conducted during this experiment were approved by the Institutional Animal Care and Use Committee from the University of Minnesota (protocol #1306-30734A). Cows used in the current experiment are a subgroup of cows used in an experiment that evaluated the effects of rbST treatment during the periparturient period on immune parameters (Silva et al., 2015). Detailed information regarding facilities, management, and nutrition has been previously described (Silva et al., 2015).

Multiparous Holstein cows (lactation = 1 to 6) from a commercial freestall herd located in northwest Wisconsin, with BCS 3.75 to 4.75 (Ferguson et al., 1994) and

locomotion score 1 and 2 (Sprecher et al., 1997), were enrolled in the experiment at 253 ± 3 d of gestation. At enrollment, cows were scored for body condition (1 = emaciated and 5 = obese; 0.25-unit increments) and locomotion (1 = normal locomotion and 5 = severely lame), and BW was estimated using a Holstein dairy cow weigh tape (The Coburn Company Inc., White-water, WI). Cows were balanced for lactation number, BCS, BW, previous lactation 305-d mature equivalent milk yield, and previous lactation interval from calving to conception. Cows were assigned randomly to 1 of 3 treatments: untreated control ($n = 53$), 87.5 mg of rbST (**rbST87.5**; $n = 56$), and 125 mg of rbST (**rbST125**; $n = 57$), which were administered every 7 d from -21 to 28 d relative to calving. Treatments doses of rbST were prepared (6 to 12 h before treatment) by dispensing the contents of commercially available syringes of rbST (Posilac, sometribove zinc suspension for injection; Elanco Animal Health, Greenfield, IN) into sterile containers and aspirating the appropriate volume into sterile 1-mL tuberculin syringes to reach 87.5 and 125 mg of rbST. Treatments were administered subcutaneously in the neck area using 16-gauge and 1.6-cm-long hypodermic needles. A subsample of cows (control = 20, rbST87.5 = 20, rbST125 = 20) was selected for collection of liver samples according to expected calving date and after balancing for BCS and previous lactation 305-d mature-equivalent milk yield. Only data from cows that had liver samples collected at -21 ± 3 , -7 ± 3 , and 7 ± 3 d relative to calving are reported herein.

Milk Yield and BCS

Cows were milked thrice daily at both facilities, but milk yield was not measured in the first 21 DIM because the transition cow facility did not have the capability to do so. Daily milk yield was recorded for individual cows (Afimilk Ltd., Kibbutz Afikim, Israel) starting at approximately 22 DIM and recorded on DairyComp305 software (Valley Ag. Software, Tulare, CA). Beginning between 55 to 50 d postpartum, all cows received 500 mg of rbST (Posilac, sometribove zinc suspension; Elanco Animal Health) every 11 d through 220 to 225 d of gestation. Body condition was scored weekly from -28 to 28 d relative to calving.

Hormones, Metabolites, and Indicators of Inflammation Measurements

Cows that had liver samples collected ($n = 32$) also had blood sampled weekly from -28 to 21 d relative to calving to determine concentrations of GH, IGF-1, insulin, cortisol, fatty acids, BHB, glucose, haptoglobin,

and tumor necrosis factor- α (**TNF- α**). During feeding, when cows were restrained in self-locking headlocks, blood samples were collected from the coccygeal vein or artery using 22-gauge and 2.5-cm-long needles into evacuated tubes without anticoagulants and evacuated tubes containing K₂EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were placed in ice until centrifugation ($1,200 \times g$ for 15 min at 4°C) for serum or plasma separation within 8 h after blood samples were collected. Serum and plasma were aliquoted into microcentrifuge tubes and stored at -32°C until analysis.

Serum GH and total IGF-1 concentrations were determined in triplicate using a modified RIA (Lalman et al., 2000); the intra- and interassay coefficients of variation (**CV**) for the GH and IGF-1 assays were lower than 5%. Serum insulin concentrations were determined by a commercial bovine ELISA kit (Mercodia Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden); the intra- and interassay CV were 8 and 5%, respectively. Serum cortisol was measured by a solid phase RIA using a commercially available kit (Coat-A-Count Cortisol; Siemens Medical Solutions Diagnostics, Los Angeles, CA); the intra- and interassay CV for cortisol were 7 and 6%, respectively. Plasma concentrations of fatty acids were determined using a colorimetric assay (Wako Chemicals USA, Richmond, VA; Ballou et al., 2009) and plasma concentrations of BHB were determined enzymatically (Ranbut, Randox Laboratories, Antrim, UK; Ballou et al., 2009). The intra-assay CV were 7 and 11% for the fatty acid and BHB assays, respectively; the interassay CV were 4 and 8% for the fatty acid and BHB assays, respectively. Plasma glucose concentrations were determined by enzymatic reaction (Stanbio Laboratory, Boerne, TX); the intra- and interassay CV were 4 and 3%, respectively. Plasma haptoglobin concentrations were determined by a colorimetric procedure (Hulbert et al., 2011); the intra- and interassay CV were 4 and 3%, respectively. Concentrations of plasma **TNF- α** were determined by ELISA using a protocol described and validated for bovine plasma (Farney et al., 2011); the intra- and interassay CV were 5 and 3%, respectively.

Liver Samples and mRNA Extraction and Quality Assessment

Liver tissue samples were collected -21 ± 3 , -7 ± 3 , and 7 ± 3 d relative to calving to determine mRNA expression of 50 genes. Eight genes were internal control candidates and 42 genes were genes of interest related to the somatotrophic axis, metabolism of glucose and lipids, inflammation, and oxidative stress (Table 1).

Percutaneous needle (Bard Magnum, Covington, GA) technique was performed to obtain the liver samples

Table 1. Genes and accession numbers according to National Center for Biotechnology Information (NCBI)

Gene symbol	Gene description	Accession number
Somatotrophic axis and insulin		
<i>GHR</i>	Total growth hormone receptor	NM_176608.1
<i>GHR1A</i>	Growth hormone receptor 1 α	AY748827.1
<i>IGF1R</i>	Insulin like growth factor 1 receptor	NM_001244612.1
<i>IGF1</i>	Insulin like growth factor 1	NM_001077828.1
<i>IGFBP3</i>	Insulin-like growth factor-binding protein 3	NM_174556.1
<i>INSR</i>	Insulin receptor	XM_002688832.2
<i>INSRB</i>	Insulin receptor β	XM_002688832.2
<i>SOCS2</i>	Suppressor of cytokine signaling 2	NM_177523.2
<i>STAT5B</i>	Signal transducer and activator of transcription 5B	NM_174617.3
Glucose and lipid metabolism		
<i>ACOX1</i>	Peroxisomal acyl-coenzyme A oxidase 1	NM_001035289.2
<i>ANGPTL4</i>	Angiopoietin-like 4	NM_001046043.2
<i>APOA5</i>	Apolipoprotein A-V	NM_001083492.1
<i>APOB100</i>	Apolipoprotein 100 B	AJ399505.1
<i>DGAT1</i>	Diglyceride acyltransferase	NM_174693.2
<i>G6PC</i>	Glucose-6-phosphatase catalytic subunit	NM_001076124.1
<i>HMGCLL1</i>	3-Hydroxymethyl-3-methylglutaryl-CoA lyase-like 1	NM_001191193.1
<i>PC1</i>	Pyruvate carboxylase	NM_177946.3
<i>PCK1</i>	Phosphoenolpyruvate carboxykinase 1	NM_174737.2
<i>PCK2</i>	Phosphoenolpyruvate carboxykinase 2	NM_001205594.1
<i>PPARA</i>	Peroxisome proliferator-activated receptor α	NM_001034036.1
<i>PPARD</i>	Peroxisome proliferator-activated receptor delta	NM_001083636.1
<i>PPARGC1A</i>	PPAR gamma coactivator 1 α	NM_177945.3
<i>SCARB1</i>	Scavenger receptor class B member 1	NM_174597.2
Inflammation and oxidative stress		
<i>CEBPD</i>	CCAAT/enhancer binding protein delta	NM_174267.2
<i>CXCL1</i>	Chemokine (C-X-X motif) ligand 1	NM_175700.1
<i>GCSF</i>	Granulocyte colony stimulating factor	NM_174028.1
<i>HIF1A</i>	Hypoxia-inducible factor 1 α	NM_174339.3
<i>HP</i>	Haptoglobin	NM_001040470.1
<i>ICAM1</i>	Intercellular adhesion molecule 1	NM_174348.2
<i>IL1RN</i>	Interleukin 1 receptor antagonist	NM_174357.2
<i>JUN</i>	Activating protein-1 AP1	NM_001077827.1
<i>MCSF1</i>	Macrophage colony stimulating factor 1	NM_174026.1
<i>MYD88</i>	Myeloid differentiation primary response gene 88	NM_001014382.2
<i>NFKB1</i>	Nuclear factor kappa enhancer in B-cells 1	NM_001076409.1
<i>NFKBIA</i>	NF-kappa B inhibitor α	NM_001045868.1
<i>NR3C1</i>	Nuclear receptor subfamily 3 group C member 1	NM_001206634.1
<i>SOCS3</i>	Suppressor of cytokine signaling 3	NM_174466.2
<i>TLR2</i>	Toll-like receptor 2	NM_174197.2
<i>TNF</i>	Tumor necrosis factor α	NM_173966.2
<i>TNFRSF1A</i>	TNF receptor superfamily member 1A	NM_174674.2
<i>TNFRSF5</i>	TNF receptor superfamily member 5	NM_001105611.1
<i>XBP1</i>	X-box binding protein 1	NM_001034727.1
Candidate reference genes		
<i>EIF3G</i>	Eukaryotic translation initiation factor 3, subunit G	NM_001079623.1
<i>GAK</i>	Cyclin G associated kinase	NM_001046084.1
<i>HNF4A</i>	Hepatocyte nuclear factor 4 α	NM_001015557.1
<i>HPRT</i>	Hypoxanthine phosphoribosyltransferase	NM_001034035.1
<i>SNAPC2</i>	Small nuclear RNA activating complex 2	NM_001034516.1
<i>SPRYD4</i>	SPRY domain containing 4	NM_001076173.1
<i>UBC</i>	Polyubiquitin	NM_001206307.1
<i>UXT</i>	Ubiquitously-expressed transcript	NM_001037471.1

(Sigl et al., 2013). Briefly, an area of 15 $\times$ 15 cm on the right side of the cow was shaved, washed, and degreased with 70% ethanol and subsequently disinfected with an iodine solution (Povidone-Iodine Solution USP 10%, Ricca Chemical Company, Arlington, TX). Local anesthetic (10 mL; lidocaine hydrochloride injection 2%, Vedco Inc., Saint Joseph, MO) was used to desensitize skin, subcutaneous tissue, and intercostal muscle. An

incision (approximately 2 cm long) was made through the skin at the intersection of an imaginary line running from the tuber coxae to the shoulder joint with the 11th and 12th intercostal space. The trocar was inserted through the incision to collect samples from the caudate lobe of the liver. A skin surgical stapler (APPOSE ULC Skin Staplers, New Haven, CT) was used to close the incision. Staples were removed after

2 wk before subsequent liver sample collection when the incision was healed. Approximately 200 mg of liver tissue were obtained from each sample collection and directly transferred into sterile Eppendorf tubes (Sigma-Aldrich, St. Louis, MO) that were immediately frozen in liquid nitrogen and stored at -80°C until mRNA extraction.

The mRNA was extracted from 50 mg of liver tissue using RNeasy mini kit columns (Qiagen, Valencia, CA). Total RNA restrained on the columns after extraction protocol was eluted with 50 μL of sterile RNase-DNase-free water (Qiagen). Quantity and purity of the extracted RNA were estimated using a NanoDrop spectrophotometer (ND-2000, NanoDrop Technologies Inc., Wilmington, DE) at 230, 260, and 280 nm absorbance waves. Purity was accepted when the 280/260 and 260/230 absorbance ratios were ≥ 1.90 and 1.80, respectively. The ratios obtained in the samples ranged from 1.97 to 2.08 and 1.77 to 2.28 for the 280/260 and 260/230 ratios, respectively. A portion of each RNA sample was diluted in RNase-/DNase-free water (Qiagen) to achieve a consistent sample concentration of 100 ng/ μL .

The integrity of RNA was assessed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and the Agilent RNA 6000 Nano Kit (Agilent Technologies) according to the manufacturer's protocol. All samples had an RNA integrity number of >7 .

Gene Expression Analysis, Reference Gene Selection, and Data Normalization

Hepatic mRNA expression was assessed by single-molecule imaging of targeted mRNA molecules hybridized with color-coded probe pairs and molecular barcodes using the NanoString nCounter System (NanoString Technologies, Seattle, WA). The direct digital readout of each mRNA and its relative abundance was measured in 100 ng of total RNA. The nSolver 2.0 Analysis Software (NanoString Technologies) was used to perform automated quality control (QC) metrics to detect flags on imaging, binding density, linearity, and limit detection of control probes. Imaging and binding density QC were set to detect flags when the percentage of fields of view successfully counted by the digital analyzer scan was less than 75 and binding density was outside the 0.05 to 2.25 range. The positive control linearity and limit of detection QC were set to flag lanes when positive control coefficient of determination values were less than 0.95 and when 0.5 fM detection was ≤ 2 standard deviations above the mean of the negative controls. No QC flags were detected in the samples.

Eight candidate reference genes (*EIF3G*, *GAK*, *HNF4A*, *HPRT*, *SNAPC2*, *SPRYD4*, *UBC*, and *UXT*;

Table 1) were evaluated for their ability to fulfill 2 criteria: to be stably expressed in the samples and to provide mRNA counts that span the range of mRNA counts of the genes of interest. NormFinder software was used to determine the most stable genes (Andersen et al., 2004). Four genes, *HNF4A*, *SPRYD4*, *UBC*, and *UXT*, were the most stable and were used in the data normalization process.

To account for slight differences in hybridization and purification efficiency, data were normalized from each sample to its positive controls and selected reference genes using the nSolver 2.0 Analysis Software (NanoString Technologies). The positive hybridization controls are RNA control transcript sequences that are not homologous to any known organism and generate consistent results in gene expression analyses (NanoString Technologies). Positive controls allowed for the correction of sample-to-sample variation due to assay-specific factors such as differences in amount of input material or reagents. The negative correction was used to subtract the background noise from the positively corrected data using counts of 6 negative controls (sequence blank tags) by subtracting the negative controls geometric mean + 2 standard deviations. The normalization procedure consisted of the adjustment of all mRNA counts data to normalization factors generated specifically for each sample based on the geometric means, within and across all samples, of 6 positive controls and selected reference genes counts.

Statistical Analysis

All statistical analyses were conducted using SAS version 9.4 (SAS/STAT, SAS Inst. Inc., Cary, NC). In all models, cows were used as the experimental unit. Only cows that had a complete set of liver biopsies ($n = 32$) were included in the statistical analysis.

The assumption of normality of variables and residuals of continuous data were assessed using the UNIVARIATE procedure of SAS and the residual output function of SAS. A natural log or square root transformation was applied to achieve a normal distribution, when necessary, and data were back transformed to original scale for reporting. Continuous data without repeated measurements (i.e., previous 305-d mature equivalent milk yield, previous interval from calving to conception, lactation number, days in the close-up pens, BCS and BW at enrollment, and milk yield) were analyzed by ANOVA using the MIXED procedure of SAS, and treatment was included in the model as the fixed effect. The MIXED procedure of SAS was used to analyze continuous data with repeated measurements (i.e., GH, IGF-1, insulin, cortisol, fatty acid, BHB, glucose, hemoglobin, TNF- α , and BCS). For analyses of repeated

measurements, the repeated statement was used and treatment, 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 criteria. Cow was included as the random effect and cows were nested within treatment. The prepartum and postpartum periods were analyzed separately because of inherent physiological changes that occur during calving. Dichotomous data (i.e., incidence of postpartum diseases) were analyzed by logistic regression using the LOGISTIC procedure of SAS, and 2 by 2 tables were generated using the FREQ procedure of SAS.

An ANOVA was used to analyze the effects of treatment on hepatic mRNA expression at the different time points relative to calving using the GLM procedure of SAS with treatment as a fixed effect. In the model to determine the effect of treatment on hepatic mRNA expression on d -7 relative to calving, hepatic mRNA expression on d -21 relative to calving was used as a covariate. Hepatic mRNA expression on d 7 relative to calving was analyzed separately because of inherent physiological changes that occur during calving.

When the effect of treatment on dependent variables was $P \leq 0.15$, 2 orthogonal contrasts were used to compare the effects of treatments: control versus rbST87.5 + rbST125 and rbST87.5 versus rbST125. Statistical significance of the contrast analysis was defined as $P \leq 0.05$ and tendency was defined as $0.05 < P \leq 0.10$.

RESULTS

Descriptive Parameters at Enrollment and Calving

Twenty-eight cows (control = 10, rbST87.5 = 8, and rbST125 = 10) had collection of liver samples interrupted and were removed from the experiment for the following reasons: cows calved <3 wk after enrollment ($n = 17$) and cows calved >3 wk after enrollment ($n = 11$). Thirty-two cows remained in the experiment and their data were used in the statistical analysis (control = 10, rbST87.5 = 12, and rbST125 = 10).

At enrollment, average lactation number (control = 1.9 ± 0.3 , rbST87.5 = 2.1 ± 0.3 , rbST125 = 2.1 ± 0.3 ; $P = 0.89$), BCS (control = 4.0 ± 0.1 , rbST87.5 = 4.0 ± 0.1 , rbST125 = 4.1 ± 0.1 ; $P = 0.60$), BW (control = 800.2 ± 21.4 , rbST87.5 = 788.9 ± 19.5 , rbST125 = 796.3 ± 21.4 kg; $P = 0.92$), previous lactation 305-d mature equivalent milk yield (control = $11,545 \pm 640$, rbST87.5 = $12,901 \pm 585$, rbST125 = $12,252 \pm 640$ kg; $P = 0.31$), previous lactation interval from calving to conception (control = 166.8 ± 22.3 , rbST87.5 = 150.2 ± 20.4 , rbST125 = 163.8 ± 22.3 d; $P = 0.84$), and days

of gestation (control = 253.3 ± 0.2 , rbST87.5 = 253.4 ± 0.1 , rbST125 = 253.2 ± 0.2 d; $P = 0.57$) were not different among treatments. Number of days cows stayed in the close-up pen before calving (control = 27.0 ± 0.7 , rbST87.5 = 28.0 ± 0.7 , rbST125 = 27.5 ± 0.7 d; $P = 0.59$) and percentage of cows calving male calves (control = 50.0, rbST87.5 = 41.7, rbST125 = 50.0%; $P = 0.90$) were not different among treatments.

Effects of rbST Treatment on Hepatic Gene Expression

From the 42 genes of interest and 8 internal control genes analyzed (Table 1), only *HMGCLL1* and *GCSF* were not detected. Hepatic mRNA expression on d -21 was not ($P > 0.15$) different among treatments (Table 2; Figures 1 to 3) except for the *SOCS3* gene, which had ($P = 0.02$) greater expression in rbST cows compared with control cows (Figure 3). Twenty-six genes were modulated by treatment on d -7 or 7 relative to calving (Table 2; Figures 1 to 3).

Effects of rbST Treatment on Somatotrophic Axis

Hepatic expression of mRNA for *GHR* and *GHR1A* on d -7 relative to calving tended ($P = 0.07$) to be and was ($P = 0.03$) affected by treatment, respectively. The contrasts demonstrated an upregulation ($P \leq 0.02$) of mRNA expression for *GHR* and *GHR1A* in rbST cows compared with control cows (Figure 1). On d 7 relative to calving, mRNA expression for *GHR1A* tended ($P = 0.08$) to be affected by treatment because rbST treatment tended ($P = 0.06$) to upregulate mRNA expression for *GHR1A* compared with control treatment (Figure 1). Although during the prepartum period serum GH concentration was not ($P = 0.23$) affected by treatment, postpartum serum GH concentrations were ($P = 0.01$) affected by treatment as rbST cows had ($P < 0.01$) greater GH concentrations than control cows (Figure 1).

Hepatic mRNA expression for *SOCS2* tended to be affected by treatment on d -7 ($P = 0.15$) and 7 ($P = 0.06$). On d -7 rbST treatment tended ($P = 0.09$) to upregulate *SOCS2* expression compared with control, and on d 7 rbST treatment upregulated ($P = 0.02$) mRNA expression for *SOCS2* compared with control cows (Figure 1). Although there was a tendency ($P = 0.08$) for treatment to affect mRNA expression for *IGF1R* on d -7 (Table 2), according to the results of the contrasts there were no differences among control and rbST-treated cows ($P = 0.15$) and between rbST87.5 and rbST125 cows ($P = 0.15$) regarding mRNA expression for *IGF1R*. The hepatic mRNA expression for *IGF1R* on d 7 was not ($P = 0.27$) affected

Table 2. Effect of treatment¹ of periparturient cows with recombinant bovine somatotropin (rbST) on hepatic gene expression (mRNA square root counts LSM $\pm$ SEM) of genes related to somatotrophic axis, insulin, metabolism of glucose and lipid, and inflammation

Gene	d -21				d -7				d 7			
	Control		P-value		Control		P-value		Control		P-value	
	rbST87.5	rbST125	rbST87.5	rbST125	rbST87.5	rbST125	rbST87.5	rbST125	rbST87.5	rbST125	rbST87.5	rbST125
Somatotropic axis and insulin												
<i>IGF1R</i>	6.2 $\pm$ 0.5	6.2 $\pm$ 0.4	6.5 $\pm$ 0.5	6.5 $\pm$ 0.5	5.10 $\pm$ 0.4	6.2 $\pm$ 0.3	5.4 $\pm$ 0.4	5.4 $\pm$ 0.4	6.6 $\pm$ 0.3	5.8 $\pm$ 0.3	6.0 $\pm$ 0.3	6.0 $\pm$ 0.3
<i>INSR</i>	16.7 $\pm$ 2	14.5 $\pm$ 1	16.0 $\pm$ 1	16.0 $\pm$ 1	17.1 $\pm$ 1	15.4 $\pm$ 1	14.7 $\pm$ 1	14.7 $\pm$ 1	16.7 $\pm$ 1	14.5 $\pm$ 1	16.1 $\pm$ 1	16.1 $\pm$ 1
Glucose metabolism												
<i>G6PC</i>	61.2 $\pm$ 3	65.3 $\pm$ 2	68.9 $\pm$ 3	68.9 $\pm$ 3	61.6 $\pm$ 3	67.4 $\pm$ 3	64.0 $\pm$ 3	64.0 $\pm$ 3	65.5 $\pm$ 6	71.4 $\pm$ 6	74.8 $\pm$ 6	74.8 $\pm$ 6
<i>PC1</i>	28.7 $\pm$ 2	28.7 $\pm$ 2	27.9 $\pm$ 2	27.9 $\pm$ 2	31.6 $\pm$ 3	30.6 $\pm$ 2	31.0 $\pm$ 3	31.0 $\pm$ 3	43.8 $\pm$ 3	47.8 $\pm$ 3	41.9 $\pm$ 3	41.9 $\pm$ 3
<i>PCK1</i>	84.2 $\pm$ 5	75.6 $\pm$ 4	79.9 $\pm$ 5	79.9 $\pm$ 5	82.7 $\pm$ 6	83.6 $\pm$ 5	84.3 $\pm$ 5	84.3 $\pm$ 5	76.0 ^A $\pm$ 9	98.0 ^B $\pm$ 9	99.9 ^B $\pm$ 9	99.9 ^B $\pm$ 9
<i>PCK2</i>	25.5 $\pm$ 1	23.3 $\pm$ 1	24.1 $\pm$ 1	24.1 $\pm$ 1	28.2 ^A $\pm$ 2	24.0 ^B $\pm$ 1	25.4 ^B $\pm$ 1	25.4 ^B $\pm$ 1	23.9 $\pm$ 2	24.8 $\pm$ 2	28.4 $\pm$ 2	28.4 $\pm$ 2
Lipid metabolism												
<i>DGAT1</i>	18.1 $\pm$ 1	18.2 $\pm$ 1	17.9 $\pm$ 1	17.9 $\pm$ 1	16.5 $\pm$ 1	17.4 $\pm$ 1	17.1 $\pm$ 1	17.1 $\pm$ 1	20.3 $\pm$ 1	19.3 $\pm$ 1	19.3 $\pm$ 1	19.3 $\pm$ 1
<i>PPARA</i>	29.3 ^A $\pm$ 1	27.1 ^B $\pm$ 1	28.1 ^B $\pm$ 1	28.1 ^B $\pm$ 1	28.5 $\pm$ 1	27.8 $\pm$ 1	27.9 $\pm$ 1	27.9 $\pm$ 1	24.6 $\pm$ 1	25.8 $\pm$ 1	27.7 $\pm$ 1	27.7 $\pm$ 1
<i>PPARGC1A</i>	10.6 $\pm$ 2	7.2 $\pm$ 1	8.8 $\pm$ 2	8.8 $\pm$ 2	11.9 ^A $\pm$ 2	7.5 ^B $\pm$ 1	8.7 ^B $\pm$ 2	8.7 ^B $\pm$ 2	10.6 $\pm$ 2	9.5 $\pm$ 2	12.3 $\pm$ 2	12.3 $\pm$ 2
Inflammation												
<i>CEBPD</i>	18.7 $\pm$ 1	19.0 $\pm$ 1	18.4 $\pm$ 1	18.4 $\pm$ 1	20.1 $\pm$ 1	21.1 $\pm$ 1	20.0 $\pm$ 1	20.0 $\pm$ 1	23.1 $\pm$ 2	19.0 $\pm$ 2	18.4 $\pm$ 2	18.4 $\pm$ 2
<i>HP</i>	247 $\pm$ 62	307 $\pm$ 51	274 $\pm$ 59	274 $\pm$ 59	92 $\pm$ 54	214 $\pm$ 38	157 $\pm$ 44	157 $\pm$ 44	272 $\pm$ 63	287 $\pm$ 60	176 $\pm$ 63	176 $\pm$ 63
<i>JUN</i>	13.0 $\pm$ 1	12.9 $\pm$ 1	12.2 $\pm$ 1	12.2 $\pm$ 1	14.0 $\pm$ 1	15.1 $\pm$ 1	13.4 $\pm$ 1	13.4 $\pm$ 1	17.3 $\pm$ 2	13.5 $\pm$ 2	15.5 $\pm$ 2	15.5 $\pm$ 2
<i>MCSF1</i>	10.3 $\pm$ 1	9.9 $\pm$ 1	9.7 $\pm$ 1	9.7 $\pm$ 1	11.3 $\pm$ 0.5	11.1 $\pm$ 0.4	10.6 $\pm$ 0.5	10.6 $\pm$ 0.5	11.6 $\pm$ 1	10.1 $\pm$ 1	10.1 $\pm$ 1	10.1 $\pm$ 1
<i>NFKB1</i>	9.9 $\pm$ 0.3	9.8 $\pm$ 0.3	9.3 $\pm$ 0.3	9.3 $\pm$ 0.3	10.1 $\pm$ 0.5	10.8 $\pm$ 0.4	10.5 $\pm$ 0.4	10.5 $\pm$ 0.4	11.1 $\pm$ 1	9.8 $\pm$ 1	10.4 $\pm$ 1	10.4 $\pm$ 1
<i>NR3C1</i>	21.8 $\pm$ 1	22.3 $\pm$ 1	22.2 $\pm$ 1	22.2 $\pm$ 1	20.4 $\pm$ 1	22.0 $\pm$ 0.5	21.0 $\pm$ 1	21.0 $\pm$ 1	19.1 ^A $\pm$ 1	20.7 ^B $\pm$ 1	21.1 ^B $\pm$ 1	21.1 ^B $\pm$ 1
<i>STAR5B</i>	10.5 $\pm$ 0.4	10.7 $\pm$ 0.3	10.6 $\pm$ 0.3	10.6 $\pm$ 0.3	10.0 $\pm$ 1	10.1 $\pm$ 1	10.2 $\pm$ 1	10.2 $\pm$ 1	10.7 $\pm$ 1	10.1 $\pm$ 1	9.7 $\pm$ 1	9.7 $\pm$ 1
<i>TLR2</i>	6.6 $\pm$ 1	7.5 $\pm$ 1	6.9 $\pm$ 1	6.9 $\pm$ 1	6.8 $\pm$ 1	8.0 $\pm$ 1	7.1 $\pm$ 1	7.1 $\pm$ 1	7.6 $\pm$ 1	7.3 $\pm$ 1	6.4 $\pm$ 1	6.4 $\pm$ 1
<i>TNF</i>	4.6 $\pm$ 0.5	4.4 $\pm$ 0.4	4.4 $\pm$ 0.5	4.4 $\pm$ 0.5	4.5 $\pm$ 0.4	5.5 $\pm$ 0.4	5.0 $\pm$ 0.4	5.0 $\pm$ 0.4	3.9 $\pm$ 0.5	4.6 $\pm$ 0.5	5.2 $\pm$ 0.5	5.2 $\pm$ 0.5
<i>TNFRSF5</i>	4.3 $\pm$ 0.5	5.0 $\pm$ 0.5	4.3 $\pm$ 0.5	4.3 $\pm$ 0.5	5.0 $\pm$ 1	6.0 $\pm$ 1	5.6 $\pm$ 1	5.6 $\pm$ 1	5.6 $\pm$ 1	4.6 $\pm$ 1	5.3 $\pm$ 1	5.3 $\pm$ 1

^{a,b}Within a row, means of contrast control vs. rbST87.5 + rbST125 with different superscripts differed ($P \leq 0.05$).^{c,d}Within a row, means of contrast rbST87.5 vs. rbST125 with different superscripts differed ($P \leq 0.05$).^{A,B}Within a row, means of contrast control vs. rbST87.5 + rbST125 with different superscripts tended to differ ($0.05 < P \leq 0.10$).¹Control (n = 10) = cows received no treatment; rbST87.5 (n = 12) = cows received 87.5 mg of recombinant bovine somatotropin (rbST) every 7 d from -21 to 28 d relative to calving; rbST125 (n = 10) = cows received 125 mg of rbST every 7 d from -21 to 28 d relative to calving.

by treatment (Table 2). The hepatic mRNA expression for *IGF1* on d -7 was ($P = 0.01$) affected by treatment, as mRNA expression for *IGF1* was upregulated ($P < 0.01$) among rbST cows compared with control cows (Figure 1). On d 7, treatment tended ($P = 0.10$) to affect mRNA expression for *IGF1* because rbST87.5

treatment upregulated ($P = 0.03$) mRNA expression for *IGF1* compared with the rbST125 treatment (Figure 1). The mRNA expression for *IGFBP3* on d -7 tended ($P = 0.14$) to be affected by treatment because rbST treatment tended ($P = 0.07$) to upregulate the expression of mRNA for *IGFBP3* compared with control.

Figure 1. Effect of treatment (LSM $\pm$ SEM) of periparturient cows with recombinant bovine somatotropin (rbST) on serum growth hormone (GH) and IGF-1 concentrations and hepatic mRNA expression for genes related to somatotrophic axis. Treatments were control = cows received no treatment; rbST87.5 = cows received 87.5 mg of rbST 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. Prepartum GH: treatment ($P = 0.23$), day ($P = 0.29$), and interaction between treatment and day ($P = 0.33$); postpartum GH: treatment ($P = 0.01$), day ($P = 0.03$), and interaction between treatment and day ($P = 0.52$); prepartum IGF-1: treatment ($P = 0.01$), day ($P = 0.16$), and interaction between treatment and day ($P = 0.11$); postpartum IGF-1: treatment ($P = 0.39$), day ($P < 0.01$), and interaction between treatment and day ($P = 0.15$). An asterisk (*) indicates control vs. rbST87.5 + rbST125 differed ($P \leq 0.05$); a dagger (†) indicates contrasts control vs. rbST87.5 + rbST125 tended to differ ($0.05 < P \leq 0.10$).

On d 7, however, treatment did not ($P = 0.96$) affect mRNA expression for *IGFBP3* (Figure 1). Treatment affected ($P = 0.01$) prepartum serum IGF-1 concentra-

tions (Figure 1) because rbST treated cows had ($P = 0.01$) higher serum IGF-1 concentrations than control cows. Concentrations of IGF-1 during the postpartum

Lipid metabolism

Figure 2. Effect of treatment (LSM $\pm$ SEM) of periparturient cows with recombinant bovine somatotropin (rbST) on serum fatty acids and BHB concentrations and hepatic mRNA expression for genes related to lipid metabolism. Treatments were control = cows received no treatment; rbST87.5 = cows received 87.5 mg of rbST 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. Prepartum fatty acids: treatment ($P = 0.84$), day ($P < 0.01$), and interaction between treatment and day ($P = 0.80$); postpartum fatty acids: treatment ($P = 0.59$), day ($P = 0.02$), and interaction between treatment and day ($P = 0.46$); prepartum BHB: treatment ($P = 0.57$), day ($P < 0.01$), and interaction between treatment and day ($P = 0.60$); postpartum BHB: treatment ($P = 0.11$), day ($P = 0.02$), and interaction between treatment and day ($P = 0.45$). An asterisk (*) indicates contrast control vs. rbST87.5 + rbST125 differed ($P \leq 0.05$); a dagger (†) indicates contrast control vs. rbST87.5 + rbST125 tended to differ ($0.05 < P \leq 0.10$).

Figure 3. Effect of treatment (LSM $\pm$ SEM) of periparturient cows with recombinant bovine somatotropin (rbST) on hepatic mRNA expression of genes related to inflammatory responses and oxidative stress. Treatments were control = cows received no treatment; rbST87.5 = cows received 87.5 mg of rbST 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. An asterisk (*) indicates contrast control vs. rbST87.5 + rbST125 differed ($P \leq 0.05$); a dagger (†) indicates contrast control vs. rbST87.5 + rbST125 tended to differ ($0.05 < P \leq 0.10$); and double dagger (§) indicates contrast rbST87.5 vs. rbST125 differed ($P \leq 0.05$).

period, however, were not ($P = 0.39$) affected by treatment (Figure 1).

There was no effect of treatment on mRNA expression for *INSRB* on d -7 and 7 relative to calving (P

≥ 0.21 ; Table 2). Hepatic mRNA expression for *INSR* was not affected by treatment on d -7 ($P = 0.28$). On d 7, treatment tended ($P = 0.07$) to affect mRNA expression for *INSR*, as rbST treatment upregulated

Table 3. Effect of treatment (Trt) of periparturient cows with recombinant bovine somatotropin (rbST) on concentrations of insulin, glucose, cortisol, haptoglobin, and tumor necrosis factor- α (LSM $\pm$ SEM)

Item	Treatment ¹			P-value		
	Control (n = 10)	rbST87.5 (n = 12)	rbST125 (n = 10)	Trt	Day	Trt $\times$ day
Prepartum						
Insulin, ng/mL	0.95 $\pm$ 0.1	0.98 $\pm$ 0.1	1.03 $\pm$ 0.1	0.89	<0.01	0.38
Glucose, mg/dL	70.4 $\pm$ 3.0	73.5 $\pm$ 2.7	72.7 $\pm$ 2.9	0.73	0.26	0.11
Cortisol, ng/mL	3.0 $\pm$ 0.1	5.4 $\pm$ 0.1	5.2 $\pm$ 0.6	0.20	0.01	0.86
Haptoglobin, OD ² $\times$ 100	2.5 $\pm$ 0.3	2.1 $\pm$ 0.2	2.1 $\pm$ 0.2	0.56	<0.01	0.57
Tumor necrosis factor- α , log	3.0 $\pm$ 0.1	3.2 $\pm$ 0.1	2.8 $\pm$ 0.1	0.21	0.14	0.96
Postpartum						
Insulin, ng/mL	0.21 $\pm$ 0.05	0.27 $\pm$ 0.04	0.22 $\pm$ 0.04	0.52	0.21	0.53
Glucose, mg/dL	63.9 $\pm$ 2.6	67.0 $\pm$ 2.4	63.7 $\pm$ 2.5	0.57	0.91	0.63
Cortisol, ng/mL	2.7 $\pm$ 0.1	3.8 $\pm$ 0.1	2.6 $\pm$ 0.1	0.56	<0.01	0.49
Haptoglobin, OD $\times$ 100	1.8 $\pm$ 0.4	2.5 $\pm$ 0.4	1.3 $\pm$ 0.4	0.19	0.03	0.74
Tumor necrosis factor- α , log	2.8 $\pm$ 0.1	2.9 $\pm$ 0.1	2.7 $\pm$ 0.1	0.36	0.49	0.57

^{a,b}Within a row, means of contrast control vs. rbST87.5 + rbST125 and rbST87.5 vs. rbST125 with different superscripts differed ($P \leq 0.05$) when prepartum and postpartum periods were analyzed separately.

¹Control = cows received no treatment; rbST87.5 = cows received 87.5 mg of rbST 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.

²OD = optical density.

($P = 0.05$) mRNA for *INSR* compared with control (Figure 1). Serum insulin concentrations, however, were not different among treatments during the prepartum or postpartum periods ($P \geq 0.52$; Table 3).

Effects of rbST Treatment on Glucose and Lipid Metabolism

Treatment did not affect hepatic mRNA expression for the genes *G6PC* and *PC1* on d -7 and 7 relative to calving ($P \geq 0.41$; Table 2). Hepatic mRNA expression for *PCK1* was not affected ($P = 0.98$) by treatment on d -7. On d 7, there was a tendency ($P = 0.15$) for treatment to affect mRNA expression for *PCK1* because rbST treatment tended ($P = 0.06$) to upregulate mRNA expression for *PCK1* compared with control (Table 2). Hepatic mRNA expression for *PCK2* tended ($P = 0.15$) to be affected by treatment on d -7 because rbST treatment tended ($P = 0.07$) to downregulate mRNA expression for *PCK2* compared with control, but treatment did not ($P = 0.38$) affect mRNA expression for *PCK2* on d 7 (Table 2; Figure 2). Surprisingly, treatment did not affect prepartum ($P = 0.73$) and postpartum ($P = 0.57$) glucose plasma concentrations (Table 3).

Hepatic mRNA expression for *ACOX1* was not ($P = 0.47$) affected by treatment on d -7. On d 7, treatment tended ($P = 0.12$) to affect mRNA expression for *ACOX1* because rbST treatment tended ($P = 0.06$) to upregulate its expression compared with control (Figure 2). Hepatic mRNA expression for *ANGPTL4*, *APOA5*, *APOB100* ($P \leq 0.05$), and *SCARB1* ($P =$

0.02) were affected by treatment on d -7, as rbST treatment upregulated ($P < 0.03$) mRNA expression for these genes compared with control (Figure 2). On d 7, treatment did not ($P \geq 0.25$) affect mRNA expressions for *ANGPTL4*, *APOB100*, and *SCARB1*, but tended ($P = 0.10$) to affect mRNA expression for *APOA5*. Treatment with rbST upregulated ($P = 0.05$) mRNA expression for *APOA5* compared with control (Figure 2). Hepatic mRNA expression for *PPARD* was not ($P = 0.20$) different among treatments on d -7. Expression of mRNA for *PPARD* on d 7 was ($P = 0.04$) affected by treatment because rbST treatment downregulated ($P = 0.04$) mRNA expression for *PPARD* compared with control (Figure 2). Expression of mRNA for *PPARGC1A* tended ($P = 0.15$) to be affected by treatment on d -7 because rbST treatment tended ($P = 0.06$) to downregulate mRNA expression for *PPARGC1A* compared with control. Expression of mRNA for *PPARGC1A* on d 7, however, was not ($P = 0.43$) affected by treatment (Table 2). Treatment had no effect on mRNA expression for *DGAT1* and *PPARA* on d -7 and 7 relative to calving ($P \geq 0.22$; Table 2). Plasma concentrations of fatty acids were not affected by treatment during the prepartum ($P = 0.84$) and postpartum ($P = 0.59$) periods (Table 3 and Figure 2). Although treatment did not ($P = 0.57$) affect BHB concentration during the prepartum period, there was a tendency ($P = 0.11$) for BHB concentration during the postpartum period to be affected by treatment, as control cows had ($P = 0.04$) higher plasma BHB concentrations than rbST-treated cows (Table 3 and Figure 2).

Effects of rbST Treatment on Inflammation and Oxidative Stress

Hepatic mRNA expression for *TNFRS1A* on d -7 was not ($P = 0.52$) affected by treatment, but it tended ($P = 0.11$) to be affected on d 7. Control cows had ($P = 0.04$) upregulated expression of mRNA for *TNFRS1A* on d 7 compared with rbST cows (Figure 3). Treatment, however, did not affect plasma TNF- α concentrations during the prepartum and postpartum periods ($P \geq 0.20$; Table 3). Hepatic mRNA expression for *ICAM1* on d -7 was ($P = 0.02$) affected by treatment, as mRNA expression for *ICAM1* was ($P = 0.03$) upregulated by the rbST87.5 treatment compared with the rbST125 treatment (Figure 3). Treatment affected ($P = 0.05$) mRNA expression for *ICAM1* on d 7 because control cows had ($P = 0.02$) upregulated expression of mRNA for *ICAM1* compared with rbST cows (Figure 3). The mRNA expression for *CXCL1* on d -7 was not ($P = 0.29$) affected by treatment, but on d 7 treatment tended ($P = 0.06$) to affect mRNA expression for *CXCL1* expression because control cows had ($P = 0.02$) upregulated mRNA expression for *CXCL1* expression compared with rbST cows (Figure 3). There was no ($P = 0.44$) treatment effect on hepatic mRNA expression for *MYD88* on d -7. On d 7, however, treatment affected ($P = 0.02$) mRNA expression for *MYD88*, as control cows had ($P = 0.01$) upregulated mRNA expression for *MYD88* compared with rbST cows (Figure 3). Hepatic mRNA expression for *HIF1A* tended ($P = 0.12$) to be affected by treatment on d -7, as control cows tended ($P = 0.09$) to have upregulated mRNA expression for *HIF1A* compared with rbST cows. On d 7, treatment tended ($P = 0.07$) to affect the expression of mRNA for *HIF1A* as control cows had ($P = 0.02$) upregulated mRNA expression for *HIF1A* compared with rbST cows (Figure 3). Hepatic mRNA expression for *HP* was not affected by treatment on d -7 ($P = 0.20$) and 7 ($P = 0.32$) relative to calving (Table 2). Plasma haptoglobin concentrations were not affected by treatment during the prepartum ($P = 0.56$) and postpartum ($P = 0.19$) periods (Table 3). Treatment did not ($P > 0.15$) affect hepatic mRNA expression for *CEBPD*, *JUN*, *MCSF1*, *NFKB1*, *TLR2*, *TNF*, and *TNFRSF5* on d -7 and 7 relative to calving (Table 2).

Hepatic mRNA expression for *NR3C1* was not ($P = 0.17$) affected by treatment on d -7. On d 7, treatment tended ($P = 0.10$) to be affect mRNA expression for *NR3C1* because rbST cows had ($P = 0.04$) upregulated mRNA expression for *NR3C1* than control cows (Table 2). Hepatic mRNA expression for *IL1RN* on d -7 was not ($P = 0.57$) affected by treatment but, on d 7, treatment affected ($P = 0.05$) the expression of mRNA for *IL1RN*, as control cows had ($P = 0.02$) upregulated

mRNA expression for *IL1RN* than rbST cows (Figure 3). Similarly, treatment did not ($P = 0.78$) affect the mRNA expression for *NFKBIA* on d -7, but it tended ($P = 0.06$) to affect the mRNA expression for *NFKBIA* on d 7. Control cows had ($P = 0.02$) upregulated mRNA expression for *NFKBIA* on d 7 compared with rbST cows (Figure 3). Hepatic mRNA expression for *SOCS3* did not ($P = 0.23$) differ among treatments on d -7, but it differed ($P < 0.01$) on d 7. Expression of mRNA for *SOCS3* on d 7 was ($P < 0.01$) upregulated in control cows compared with rbST-treated cows (Figure 3). Serum cortisol concentrations during the prepartum ($P = 0.20$) and postpartum ($P = 0.56$) periods were not affected by treatment (Table 3). Hepatic mRNA expression for *XPB1* on d -7 tended ($P = 0.07$) to be affected by treatment because mRNA expression for *XPB1* tended ($P = 0.08$) to be upregulated for rbST-treated cows compared with control cows (Figure 3). On d 7, treatment affected ($P = 0.03$) mRNA expression for *XPB1*, as control cows had ($P = 0.01$) upregulated expression of mRNA for *XPB1* compared with rbST-treated cows (Figure 3).

DISCUSSION

Hepatic adaptive responses to NEB during the periparturient period are mediated by the endocrine system through the uncoupling of constituents of the somatotrophic axis, such as GH, GH receptors, IGF-1, IGF-1 binding proteins, and insulin (Bauman, 2000; Lucy, 2008). During the transition from late gestation to early lactation, the increase in GH concentrations enables the shift of nutrients from diet and body stores toward the mammary gland for milk synthesis (Bauman, 2000). A rise in IGF-1 concentration is not observed during this period of elevated GH concentrations because the decreased abundance of hepatic GH receptors, particularly *GHR1A*, reduces IGF-1 synthesis to prevent a negative feedback of IGF-1 on GH secretion (Rhoads et al., 2004; Lucy, 2008). Although in the subsample of cows used in the current experiment GH concentrations prepartum were not affected by rbST treatment, Silva et al. (2015, 2017) demonstrated that weekly treatment of periparturient cows with 125 mg of rbST increased GH concentration prepartum by approximately 30%; this is similar to the numerical increase observed in the current experiment, suggesting that the current experiment may have been underpowered to detect this difference. Nonetheless, prepartum IGF-1 serum concentrations were increased by the rbST125 treatment, in agreement with previous experiments (Silva et al., 2015, 2017). Cows treated with rbST had increased hepatic mRNA expression for *GHR*, *GHR1A*, and *IGF1* on d -7 relative to calving,

suggesting that exogenous GH treatment may have increased IGF-1 synthesis through improved hepatic response to GH by increasing GHR1A abundance. Furthermore, treatment of periparturient cows with rbST increased the hepatic mRNA expression for *IGFBP3* on d -7 relative to calving. The *IGFBP3* forms a complex with the acid-labile α -subunit and IGF-1 that prolongs the half-life of IGF-1 and regulates its interaction with cell surface receptors (Rajaram et al., 1997), which may also have increased IGF-1 concentration. Carriquiry et al. (2009) demonstrated increases in hepatic expression of *GHR*, *IGF1*, and *IGFB3* during early lactation in cows treated with 500 mg of rbST every 10 d from 12 to 70 d postpartum, but, to our knowledge, no data to date reported the effects of rbST on hepatic gene expression during the periparturient period.

The somatotrophic axis can be regulated by *SOCS2*, whose mRNA expression in mice's liver is regulated by GH through the signal transducer and activator of transcription 5B (**STAT5B**; Davey et al., 2001). Suppressor of cytokine signaling proteins act as part of a negative feedback system to regulate cytokine signaling pathways to attenuate their signal (Hilton et al., 1998). Even though *STAT5B* expression was not modulated by rbST treatment in the current experiment, cows treated with rbST had increased hepatic mRNA for *SOCS2* 7 d postpartum, suggesting that increased postpartum serum GH concentrations in rbST-treated cows may have caused GH-STAT5B-induced upregulation of mRNA for *SOCS2* to attenuate excessive GH signaling. Overfed cows challenged intramammarily with LPS between 7 and 14 d postpartum had a marked decrease in hepatic mRNA for *STAT5B* and *SOCS2*, indicative of an exacerbated uncoupling of the somatotrophic axis (Graugnard et al., 2013). Taken together, our findings and that of others suggest that treatment of periparturient cows with rbST may have acted directly, modulating *SOCS2* hepatic expression, or indirectly, improving the immune function and reducing the incidence of infectious diseases (e.g., metritis; Silva et al., 2015, 2017), to reduce the magnitude of the uncoupling of the somatotrophic axis. It is also possible that the rbST treatment of periparturient cows may have attenuated the uncoupling of the somatotrophic axis through the upregulation in hepatic mRNA for *INSR*, which could have led to increased liver responsiveness to insulin. Rhoads et al. (2004) demonstrated that cows subjected to hyperinsulinemic-euglycemic clamps during late pregnancy and early lactation had increased hepatic *GHR* and *GHR1A* mRNA expression and plasma IGF-1 concentration, suggesting that insulin may be involved in the recoupling of the somatotrophic axis (Lucy, 2008).

Hepatocytes are major targets for GH, which, upon binding to its receptors, activates the Ras-Raf-MEK-

MAPK and JAK-STAT signaling pathways, resulting in the translocation of homodimers and heterodimers (e.g., *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5A*, *STAT5B*, and *STAT6*) to the nucleus to bind to target DNA elements and regulate gene transcription (Zhu et al., 1998). Among other things, GH regulates the mRNA expression for hepatocyte nuclear factors through STAT5 signaling (Costa et al., 2003). Hepatocyte nuclear factor 4, for example, is essential for transcription of *PCK1* and *G6PC* genes through the recruitment of the peroxisome proliferator-activated receptor coactivator-1 α protein encoded by the gene *PPARGC1A* (Rhee et al., 2003). It is interesting that mRNA expression for *HNF4* and *PPARGC1A* did not differ among treatments, resulting in no changes in *G6PC* mRNA expression. Hepatic mRNA expression for *PCK1*, a key enzyme in the conversion of oxaloacetate to phosphoenolpyruvate (Velez and Donkin, 2004), was increased in rbST125 cows 7 d postpartum, indicating that rbST treatment improved hepatic gluconeogenesis. Furthermore, rbST125 cows had increased hepatic mRNA expression for *ACOX1* 7 d postpartum, an enzyme involved in the fatty acid β -oxidation that may increase production of ATP to support gluconeogenesis and prevent lipid esterification and accumulation in the liver (Aoyama et al., 1994). Sheep liver slices treated with rbST had increased complete fatty acid oxidation to CO₂ and decreased rates of fatty acid esterification, sparing ATP for increased rates of gluconeogenesis (Emmison et al., 1991). Although the upregulation of mRNA for *PCK1* and *ACOX1* were not associated with increased plasma glucose concentrations, we speculate that this was a consequence of the increase in milk yield among rbST-treated cows (Silva et al., 2015).

Culture of hepatocytes in the presence of GH reduced the accumulation of intracellular TAG (Emmison et al., 1991), and lactating cows treated with rbST were less likely to develop fatty liver in spite of rbST-induced lipolysis and influx of fatty acids to the liver (Pocius and Hertlein, 1986). On d -7 relative to calving, cows treated with rbST had increased hepatic mRNA expression for *SCARB1*, a receptor for high-density lipoprotein, and for *APOA5* and *APOB100*, apolipoproteins involved in the secretion of very low-density lipoproteins (**VLDL**) from the liver. We speculate that the upregulation of *APOA5* and *APOB100* stimulated the synthesis or secretion of VLDL and activated the lipoprotein lipase in peripheral tissues (Kraja et al., 2010), improving transport of triglycerides from the liver, overall lipid metabolism, and delivery of preformed fatty acids to the mammary gland (Emmison et al., 1991). Furthermore, hepatic mRNA expression for *ANGPTL4*, an angiopoietin-like protein that prevents fat storage and stimulates fat mobilization, was upregulated by rbST

treatment on d -7 relative to calving. Upregulation of mRNA expression for *ANGPTL4* in calf hepatocytes has been correlated with culture in media containing fatty acid concentrations >0.6 mmol/L (Wang et al., 2015). In the current experiment, fatty acid concentrations did not differ among treatments, suggesting that rbST may have acted directly to upregulate mRNA for *ANGPTL4* in periparturient cows, as it has in mid-lactation cows (Koltes and Spurlock, 2012). The hepatic expression of mRNA for *PPARD*, a nuclear receptor activated by fatty acids and associated with lipid accumulation, was downregulated on d 7 in rbST cows. The rbST-induced changes in hepatic expression of mRNA for *SCARB1*, *APOA5*, *APOB100*, *ANGPTL4*, and *PPARD* suggests that treatment of periparturient cows with rbST could alter hepatic lipid metabolism to the point of reducing liver TAG accumulation and increasing liver VLDL secretion, which are expected to improve liver health.

In the current experiment, rbST cows had reduced postpartum BHB concentrations compared with control cows. Ketogenesis is associated with the upregulation of mRNA for *HMGCLL1*, which codifies the 3-hydroxy-methyl-3-methylglutaryl-CoA lyase-like 1 enzyme and catalyzes the cleavage of *HMGCS2* into acetyl-CoA and acetoacetate (van Dorland et al., 2009). Although we expected that differences in the expression of mRNA for *HMGCLL1* would help explain possible differences in BHB concentrations among treatments, mRNA for *HMGCLL1* was not detected in the current experiment. Decreased ketogenesis postpartum is associated with reduced hepatic accumulation of TAG (Graugnard et al., 2013). Silva et al. (2015) did not observe differences in hepatic TAG and glycogen contents between rbST-treated and control cows, but this may have been the consequence of insufficient sample size, as hepatic glycogen content of rbST-treated cows was 75% greater than control cows. Liver lipidosis develops when the hepatic uptake of fatty acids exceeds its oxidation and secretion as TAG in VLDL by the liver, leading to impaired liver function and incomplete fatty acid oxidation to ketone bodies and causing ketosis (Grummer et al., 2004). In the current experiment, cows treated with rbST had reduced BHB concentration postpartum compared with control cows, which can be a result of improved hepatic fatty acid complete oxidation and VLDL secretion, as explained previously. It is important to note, however, that the effects of rbST treatment of periparturient cows on BHB concentrations have been mixed, with some researchers reporting a positive effect (Putnam et al., 1999; Gulay et al., 2007; Silva et al., 2015) and some reporting a negative effect (Eppard et al., 1996; Gohary et al., 2014; Silva et al., 2017).

In the current experiment, hepatic mRNA expression for proinflammatory genes were upregulated among con-

trol cows 7 d postpartum compared with rbST cows. It is likely that the upregulation of proinflammatory genes among control cows was a consequence of differences in incidence of ketosis and metritis compared with rbST-treated cows (Silva et al., 2015). A bovine microarray platform used to study hepatic gene networks underlying nutrition-induced ketosis revealed an upregulation of genes and nuclear receptors associated with cytokine signaling and inflammation (Loor et al., 2007). Inflammation can induce lipolysis, glycogenolysis, and hepatic lipid accumulation (Chioléro et al., 1997). Cows injected with recombinant bovine TNF- α had increased liver TAG content and decreased liver mRNA for *G6G* and *PCK1* (Bradford et al., 2009). Although plasma TNF- α concentrations were not affected by treatments in the current experiment, control cows had increased hepatic mRNA expression for *TNFRSF1A* 7 d postpartum. Liver proliferative response to injury can be initiated by the release of TNF- α , as observed by a 70% reduction in hepatocyte DNA replication in *TNFRSF1A* knockout mice (Yamada et al., 1997). Thus, the increased hepatic *TNFRSF1A* expression exhibited by control cows compared with rbST-treated cows is possibly associated with hepatocyte injury. Treatment of mice with induced hepatic injury with a neutralizing antibody against *CXCL16*, a chemokine that promotes immune cell infiltration in the liver, reduced hepatic macrophage accumulation and steatosis (Wehr et al., 2014). Control cows had greater mRNA for *CXCL1* 7 d postpartum, a gene that is translated to a chemokine produced by macrophages and hepatocytes that acts similarly to *CXCL16* (Charo and Ransohoff, 2006). In addition, control cows had greater mRNA expression for *ICAM1* 7 d postpartum, a gene that encodes an intercellular adhesion molecule involved in the transmigration of leukocytes into tissues and is upregulated upon cytokine stimulation. These findings suggest that control cows may have been more prone to liver infiltration of immune cells than rbST-treated cows. The transcription factor hypoxia-inducible factor 1- α encoded by the gene *HIF1A* is involved in the inflammation process increasing angiogenesis, cell proliferation, and tissue regeneration (Cramer et al., 2003). Zhang et al. (2015) demonstrated that drug-induced upregulation of *HIF1A* protein enhances regeneration of lost and damaged tissue in mice after an earhole punch injury. Among control cows, the hepatic mRNA expression for *HIF1A* tended to be and was upregulated -7 and 7 d relative to calving, respectively, and may be a consequence of increased hepatocyte damage. The expression of the *HIF1A* gene is regulated by the nuclear factor kappa enhancer in B-cells (**NF κ B**) encoded by the *NFKB1* gene. Treatment with rbST did not affect the expression of *NFKB1*; however, the mRNA expression

for *NFKBIA*, an inhibitor of NF κ B signaling pathway, was increased in control cows 7 d postpartum. Taken together, these data suggest that the NF κ B signaling cascade was activated in control cows, resulting in greater hepatic *HIF1A* expression; however, an anti-inflammatory response, through the upregulation of *NFKBIA*, was also induced, perhaps to attenuate the hepatic inflammation process.

Liver macrophages, called Kupffer cells, are able to promote fat accumulation in hepatocytes by expressing IL-1 β and by binding to IL-1 receptor type 1 encoded by the gene *IL1R* (Leroux et al., 2012). The mRNA expression for *IL-1 β* and *IL1R* were not evaluated in the current experiment. Nonetheless, the hepatic mRNA expression for *IL1RN*, an inhibitor of the IL-1 receptor type 1, and *SOCS3*, a suppressor of cytokine signaling pathways, were upregulated in control cows 7 d postpartum. The activation of IL-1 receptor type 1 by IL-1 β in response to LPS stimulation dysregulates the hepatic lipid and lipoprotein metabolism and increases lipid peroxidation, resulting in the accumulation of oxidized low-density lipoproteins in large lipid droplets in the Kupffer cells (Leroux et al., 2012). Consequently, Kupffer cells are activated and produce high amounts of proinflammatory cytokines and chemokines, mediating the recruitment of leukocytes to the liver (Leroux et al., 2012). We speculate that the greater incidence of metritis among control cows (Silva et al., 2015) might have sensitized Kupffer cells to produce proinflammatory cytokines and chemokines, which in turn may have stimulated the upregulation of anti-inflammatory genes (e.g., *IL1RN* and *SOCS3*) to contain the proinflammatory signals. It is also possible that the greater metabolic challenge of control cows resulted in upregulation of *SOCS3*, as hepatic expression of mRNA for *SOCS3* was associated with insulin resistance and steatosis in obese mice (Ueki et al., 2005).

The mRNA expression for *MYD88* was greater in control cows than rbST-treated cows 7 d postpartum. Intramammary challenges with LPS 7 d postpartum increased the hepatic expression of mRNA for *TNF*, *MYD88*, and *NFKB1* (Grauagnard et al., 2013). Therefore, we speculate that the increase in incidence of metritis among control cows (Silva et al., 2015) may have resulted in greater stimulation of toll-like receptors and upregulation of mRNA for *MYD88* among control cows. It is important to mention, however, that treatment of periparturient cows with rbST increased PMNL expression of adhesion molecules, phagocytosis, and oxidative burst, increased antibody concentrations, and decreased the incidence of retained placenta and metritis and the concentration of haptoglobin postpartum (Silva et al., 2015, 2017). Thus, rbST treatment may have directly or indirectly modulated the expres-

sion of mRNA for genes associated with inflammation and immune responses.

Metabolic disorders and inflammation are linked to oxidative stress and endoplasmic reticulum (ER) stress in periparturient dairy cows (Ringseis et al., 2015). During early lactation the liver is exposed to metabolic and inflammatory challenges, such as fatty acids, TNF- α , IL-1 β , reactive oxygen species, and LPS, which are known inducers of ER stress (Ringseis et al., 2015). Increased mitochondrial lipid flux and exposure to high concentrations of fatty acids disrupt ER membranes and result in the release of stored calcium that contributes to oxidative stress and greatly increase cytoplasmic reactive oxygen species concentrations, resulting in impaired ER protein folding (Wei et al., 2009). Protein misfolding triggers the unfolded protein response, of which the transcription factor X-box binding protein 1 is a critical effector through the regulation of genes involved in ER homeostasis (Yoshida et al., 2001). In addition, the unfolded protein response signaling cascades and inflammatory signaling pathways are tightly linked (Darling and Cook, 2014). The ER stress-induced unfolded protein response is believed to contribute to pathophysiologic conditions of periparturient cows, such as fatty liver, ketosis, and hepatic inflammation (Ringseis et al., 2015). In the current experiment, hepatic mRNA expression for *XBP1* was upregulated among control cows, which we speculate to be indicative of a state of oxidative stressed due to inflammation and impaired lipid metabolism as a consequence of the upregulation of mRNA expression for genes related to inflammation and the downregulation of mRNA expression of genes related to lipid oxidation and transport. Grauagnard et al. (2013) demonstrated that hepatic mRNA expression for *XBP1* was upregulated during early lactation of overfed cows challenged intramammarily with LPS, supporting the theory of a synergistic effect of impaired lipid metabolism and inflammation on oxidative stress response. The positive effects of rbST treatment on hepatic expression of mRNA for genes related to lipid metabolism, inflammation, and oxidative stress during the phase of greatest metabolic and infectious challenges of cows may present an important alternative to reduce the incidence of metabolic diseases.

CONCLUSIONS

Treatment of periparturient cows with 87.5 and 125 mg of rbST weekly from -21 to 28 d relative to calving may improve liver health and function and attenuate the decoupling of the somatotrophic axis through upregulation of somatotrophic axis genes (e.g., *GHR* and *GHR1A*), resulting in increased hepatic *IGF1*

mRNA and plasma IGF-1 concentration. Furthermore, treatment with rbST may improve glucose and lipid metabolism, through upregulation of transcription of key hepatic enzymes involved in gluconeogenesis and lipid oxidation, and apolipoproteins and lipid receptors responsible for lipid transport and secretion from the liver, resulting in decreased postpartum BHB concentrations among rbST-treated cows. Treatment of periparturient cows with rbST may also prevent postpartum hepatic infiltration of immune cells and hepatocyte injury, demonstrated by the downregulation of mRNA expression for genes associated with inflammation compared with control cows. The results of the current experiment demonstrate that rbST treatment during the periparturient period improves hepatic lipid metabolism and inflammatory status, which may have acted synergistically to decrease hepatic oxidative stress.

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