Effect of prepartum administration of recombinant bovine somatotropin on health and performance of lactating dairy cows

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ABSTRACT

A double-blind, randomized clinical trial was conducted in 5 commercial dairy herds in southern Ontario with 1,362 cows enrolled to evaluate the effect of prepartum administration of recombinant bovine somatotropin (rbST) on health and performance. Cows were randomly assigned to receive either 325 mg of sometribove zinc suspension ($n = 680$) or a placebo injection ($n = 682$; control) subcutaneously every 14 d until calving. Treatments started 28 to 22 d before expected calving, with a maximum of 3 treatments per cow. Serum samples taken at the time of enrollment, 1 wk before calving, and weekly for 3 wk after calving were analyzed for nonesterified fatty acids (NEFA), β -hydroxybutyrate (BHBA), glucose, aspartate aminotransferase, calcium, and haptoglobin. Diseases were recorded by farm staff. Incidences of clinical ketosis, clinical mastitis, displaced abomasum, metritis, retained placenta, milk fever, and lameness were similar between groups. Body condition score was lower for treated than for control cows at 3 wk after calving (3.13 and 3.17, respectively). Serum NEFA tended to be higher for treated than for control cows by 0.01 mmol/L. Overall BHBA was not different between groups, but BHBA for treated cows was higher in wk 1 after calving (750 and 698 μ mol/L, respectively) and tended to be higher in wk 2 after calving (779 and 735 μ mol/L, respectively). Incidence of hyperketonemia was similar between groups. Treated cows had higher serum glucose compared with control cows (2.8 and 2.7 mmol/L, respectively). We detected no differences in serum aspartate aminotransferase, calcium, or haptoglobin between groups. Milk yield was recorded daily for each cow for 63 d, and did not differ between groups (37.1 ± 0.5 kg and 36.7 ± 0.5 kg, respectively) but we detected a tendency for treated cows to produce 0.8 kg/d more milk than control cows in wk 1 after calving.

We observed no difference between groups in the time from calving to first insemination or the probability of pregnancy at the first insemination. Groups did not differ in the proportion of anovular cows at 53 ± 3 d in milk based on serum progesterone measured from a subset of cows (38.0 and 34.3%, respectively, for treated and control groups). We found no difference between groups in dry matter intake from 21 d before calving to 63 d after calving in a subset of cows (17.4 ± 0.4 and 17.5 ± 0.4 kg/d, respectively). Based on results of the current study, biweekly (every 14 d) administration of rbST before calving to prevent disease and enhance performance is not recommended.

Key words: recombinant bovine somatotropin (bST), transition dairy cow, health, performance

INTRODUCTION

Milk production increases gradually until peaking at 7 to 9 wk after calving, whereas DMI does not peak until 10 to 12 wk after calving (Bauman and Currie, 1980), resulting in the inevitable phase of negative energy balance (NEB) that every dairy cow experiences (Herd, 2000). The phase of NEB is also accompanied by a depression in immune function (Goff, 2006) and, as a result, almost 50% of lactating cows are affected with at least one infectious or metabolic disease after calving (LeBlanc, 2010), indicating that these cows failed to adapt to the physiological changes that they underwent during the transition period. As a result, cow health and performance will be suboptimal for affected cows. Therefore, proper transition cow management is emphasized during this critical phase of the lactation cycle.

Administration of bST during the transition period was considered a potential management strategy to enhance cow health and performance due to its positive effects relative to glucose metabolism. Bovine somatotropin results in an increase in gluconeogenesis in the liver, an increase in mobilization of glycogen reserves, a reduction in glucose oxidation, and a decrease in the inhibitory effect of insulin on synthesis of glucose in the

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liver (Peel and Bauman, 1987; Bauman et al., 1989). As a result, readily available glucose will increase. In addition, previous studies found that cows administered with low doses of recombinant bovine somatotropin (**rbST**) in the transition period had an increase in DMI (Gulay et al., 2000, 2004a). The combination of these actions should help in mitigation of NEB and therefore reduce the incidence of metabolic and infectious diseases in the transition period, and in turn enhance performance of lactating dairy cows.

However, previous studies reported a variety of results when **rbST** was administered during the transition period. Some studies showed that administration of a low dose of **rbST** before and after calving reduced ketosis and clinical mastitis but not milk fever, displaced abomasum, retained placenta, or metritis (Gulay et al., 2007). Other studies showed that administration of **rbST** before and after calving increased (Gulay et al., 2003, 2004b), decreased, or did not affect milk production (Eppard et al., 1996; Liboni et al., 2008). When **rbST** was administered only before calving, Simmons et al. (1994) and Vallimont et al. (2001) found no difference in ketosis, displaced abomasum, clinical mastitis, or blood NEFA between treated and control cows, whereas Putnam and coworkers (1999) reported a numerical reduction in cases of clinical mastitis. Milk production was also variable between studies; some reported an increase in cows and heifers (Putnam et al., 1999; Schneider et al., 2012), whereas others showed similar production between groups (Simmons et al., 1994; Vallimont et al., 2001).

Previous studies have used different doses of **rbST**, initiated and discontinued treatments at different times relative to calving, and in general enrolled a small number of cows. As a result, it is difficult to make concrete conclusions about the effect of peripartum **rbST** administration. Therefore, the objective of this study was to evaluate the effect of prepartum administration of 325 mg of **rbST** on health, metabolic indicators, milk production, and early reproductive performance of lactating dairy cattle.

MATERIALS AND METHODS

Study Design

Sample size calculations were based on formulas provided by Dohoo et al. (2009) to compare 2 proportions to provide 80% power to detect a 33% reduction of hyperketonemia at a threshold of 1,400 $\mu\text{mol/L}$ for serum BHBA and 50% reduction in displaced abomasum, and to compare 2 means to detect a 2 ± 10.3 kg/d increase in milk production after administration of **rbST** between the treatment and control groups. The

largest sample size estimate resulted from calculations to detect the reduction in abomasal displacement and was 600 cows per group. Sample size calculation for DMI was estimated based on providing power of 80% to detect an increase of 1 ± 2.12 kg/d in feed intake for cows treated with **rbST** with 95% confidence. This calculation identified that 80 cows per treatment group would be needed.

The study population was a convenience sample of Holstein cows from 5 commercial herds in southern Ontario, Canada. Three of the participating farms milked an average of 520 cows, one farm milked an average of 200 cows, and one farm milked an average of 50 cows. Herds were selected based on the farmer's willingness to participate and ability to record data for trial outcomes. Cows were housed in freestalls in 4 herds and in tiestalls in 1 herd. All cows were fed a TMR. The study was conducted following approval of the Animal Utilization Protocol (AUP: 10R095 and 10R095) by the University of Guelph Animal Care Committee and an experimental study certificate from the Canadian Veterinary Drugs Directorate. Cows in each farm were randomly assigned to receive either an injection of 325 mg of sometribove zinc suspension (Posilac; Elanco Animal Health, A Division of Eli Lilly and Co., Greenfield, IN) or a placebo injection at the time of enrollment (control). Injections were administered subcutaneously at the depression on either side of the tail head. Randomization was based on blocking cows by herd and parity (first-lactation heifers and older cows) using predetermined random number tables that were initially generated in Excel (Microsoft Corp., Redmond, WA). Syringes were identified with a unique number, and the placebo syringe was identical to the sometribove syringe except it did not contain any sometribove zinc. Clinically healthy cows and heifers were enrolled weekly at 28 to 22 d before calving and were treated every other week with a maximum of 3 treatments until calving. Cows were excluded from the study if they were not clinically healthy, had a BCS <2.5 on a 5-point scale at enrollment, were bull-bred, and had no specific calving date recorded, missed a treatment, or if they did not calve within 2 wk after the third treatment. Cows remained in the same group until the end of the study. Body condition score was recorded at enrollment and at 3 wk after calving. Body condition was scored for about 50% of the cows by one individual and, when possible, BCS was measured by 2 experienced individuals who were blinded to the treatment status and then averaged. Researchers involved in administration of treatments, data collection, and analyses, in addition to farm staff, were blinded to the nature of treatments until the end of data collection and analyses.

Data Collection and Analysis

Variables included in any of the regression models used in the current analysis were treatment as the main effect, farm, parity (1, 2, and ≥ 3), BCS at enrollment (categorized to <3.25 , 3.25 to 3.75 , and >3.75 ; Duffield et al., 2009), number of treatments (1, 2, or 3), season (summer, fall, winter, and spring) at enrollment, and days from last treatment to calving (0 to 7 d or 8 to 14 d). The model with main effects was fitted, and nonsignificant variables ($P \geq 0.05$) were removed in a backward stepwise elimination procedure after ensuring that they had no confounding effect on treatment (based on a change of 20% or more in the coefficient for treatment). Interactions between treatment and covariates remaining in the model were assessed one at a time. An interaction term was only kept in the model if significant ($P < 0.05$) or if it was biologically meaningful.

For any of the linear mixed models that included a repeated measures procedure, various correlation structures (unstructured, autoregressive1, autoregressiveH1, Toeplitz, ToeplitzH, and compound symmetry) were tested to account for the repeated measures of the outcome. The correlation structure that provided the smallest Akaike information criterion, indicating the best fit, was chosen to continue the modeling process.

For any of the linear regression models, model fit was assessed graphically by plotting residuals against predicted values to identify outliers and assess the homogeneity of variance. The assumption of normality was assessed by plotting a histogram of the residuals. If required, a log or square root transformation of the dependent variable was performed to achieve a normal distribution.

To ensure efficacy of treatments administered to cows enrolled in the study, growth hormone was measured in serum samples from 30 cows selected randomly from each group (a total of 60 cows). Growth hormone was measured at enrollment, wk 1 after enrollment, and wk 2 after enrollment. All samples were taken before calving. A mixed model with cow as a repeated measure and controlling for treatment effect, week of sampling, and farm was fitted in SAS software (version 9.3., SAS Institute Inc., Cary, NC) using a PROC MIXED statement to measure the difference between groups.

Health. Incidence of the following diseases was recorded by farm staff: clinical ketosis, displaced abomasum, retained placenta, metritis, clinical mastitis, milk fever, and lameness. All participating herds were provided with standardized disease definitions based on previous studies (Kelton et al., 1998; Carson, 2008). Four out of the 5 enrolled herds were recording clinical disease events on farm in herd management software

programs, and information was retrieved by access to backup files collected during farm visits. The fifth herd, which did not record disease events in a herd management software program, was provided with a paper recording form, and disease events were recorded by hand. In the case of multiple disease occurrences, the first disease event was considered for data analysis. The Epi Info software package version 6 (Centers for Disease Control and Prevention, Atlanta, GA) was used for analysis. Two-by-two contingency tables were constructed, and risk ratios (RR) and 95% CI were calculated using a χ^2 test for disease incidences. If any disease incidence had an α significance level <0.2 after screening with 2×2 contingency tables, a Poisson regression model controlling for overdispersion using PROC GENMOD in SAS (version 9.3. SAS Institute Inc.) was fitted to calculate risk ratios after controlling for other variables in the model (Ospina et al., 2012).

Body condition score at enrollment was compared between groups using a Mann-Whitney test. In addition, SAS software (version 9.3, SAS Institute Inc.) was used to analyze the change in BCS between enrollment and wk 3 after calving with a linear regression model using PROC MIXED. Body condition score in wk 3 after calving was used as an outcome and treatment as the main effect.

Metabolic Indicators. Blood samples were collected at enrollment and then weekly until wk 3 after calving. Blood was collected from the coccygeal blood vessels in a vacuum tube without anticoagulant and stored in a cool place. Within 6 h of collection, blood was centrifuged at $2,990 \times g$ to harvest serum, which was given a hemolysis score from 0 to 2, where 0 indicates no hemolysis and 2 indicates severe hemolysis, and then stored at -20°C . A maximum of 5 blood samples for each cow were submitted for analysis depending on how long a cow remained in the trial before being removed (culling or death). Sera submitted for analysis were the sample at enrollment, the week before calving and for wk 1, 2, and 3 after calving. Serum was submitted in batches to the Animal Health Laboratory at the University of Guelph for analysis using a Roche Cobas 6000 c501 automated chemistry analyzer (Roche Canada, Laval, QC, Canada). Serum was analyzed for NEFA, BHBA, glucose, calcium, aspartate aminotransferase (AST), and haptoglobin. Test reagents for glucose, calcium, and AST were supplied by Roche Diagnostics (Indianapolis, IN) and for BHBA and NEFA by Randox Laboratories (Crumlin, Co. Antrim, UK), and were prepared by the Animal Health Laboratory at the University of Guelph to measure haptoglobin. Results for each analyte were entered into a spreadsheet and then imported into SAS (version 9.3, SAS Institute Inc.) for analysis using the MIXED procedure, accounting for repeated measures

within cow. In addition to the above mentioned variables, week of sampling was included as a covariate in each model.

Elevated serum NEFA concentrations between the treatment and control groups before and after calving were compared by calculating risk ratios. Cutoffs indicating high NEFA concentrations before and after calving used were based on previous studies (Chapinal et al., 2011, 2012).

Hyperketonemia. Further analysis was performed using BHBA as an indicator of hyperketonemia. A cutoff point of 1,400 $\mu\text{mol/L}$ was used to determine cows affected with hyperketonemia. A χ^2 test was used to calculate the difference in the prevalence of hyperketonemia between the treatment and control groups for each of the first 3 wk after calving and for the cumulative incidence of hyperketonemia in the first 3 wk after calving. Using the same cutoff point (1,400 $\mu\text{mol/L}$), a Poisson regression model that controlled for overdispersion (Ospina et al., 2012) was fitted in SAS (version 9.3, SAS Institute Inc.) to compare the cumulative incidence of hyperketonemia over the first 3 wk of lactation. Cows with a serum BHBA concentration $\geq 1,400$ $\mu\text{mol/L}$ in one or more weekly samples in the first 3 wk after calving were considered hyperketonemic. The Poisson model was fitted using PROC GENMOD.

Milk Production. Data for milk production were collected for 1,242 cows. Daily milk weights were collected from farm management software for the first 63 d after calving, and SAS (version 9.3, SAS Institute Inc.) was used to analyze the data. Daily milk weights were averaged into weekly weights for analysis. A repeated-measures procedure was used to evaluate the treatment effect on milk yield using a mixed linear regression (PROC MIXED) model. In addition to the above mentioned variables, week of lactation was included as one of the variables in the model.

Reproductive Performance. Pregnancy diagnosis was performed at approximately 35 ± 3 d following insemination and was done by palpation per rectum or by ultrasonography if less than 35 d. Pregnancy status following the first insemination after calving and the date of first insemination after calving were both retrieved from farm software management programs, and SAS (version 9.3, SAS Institute Inc.) was used for analysis.

The probability of being diagnosed pregnant to the first insemination ("conception risk") was modeled with a Poisson regression model that controlled for overdispersion using PROC GENMOD (Ospina et al., 2012). Time to first insemination was modeled with survival analysis using Cox proportional hazards regression (PROC PHREG). Cows that were denoted as "do not breed" by farm managers, that died before calving, or that were sold before calving were not included in the

analysis, whereas cows that died or were sold after calving but before being inseminated contributed the time they remained on the farm and were censored at the date of culling. Assessment of the assumption of proportionality was performed by including a time-dependent covariate (an interaction between the covariate and the time at risk) within the model. If the assumption was violated, the time-dependent variable was kept in the model (Dohoo et al., 2009).

Progesterone was measured in serum after calving as an indication of cyclic activity. Blood samples were collected from the coccygeal vessels from 335 cows; 50 cows from the tiestall herd and 285 cows from 2 of the freestall herds. Cows were sampled 14 d apart, first at 36 to 42 DIM and second at 50 to 56 DIM. Blood samples were collected and stored in a cool place and were centrifuged at $2,990 \times g$ within 6 h after collection. Harvested serum was stored at -20°C until it was submitted to the Animal Health Laboratory at the University of Guelph for analysis. Progesterone was analyzed using Immulite 1000 Progesterone (Siemens Healthcare Diagnostics, Tarrytown, NY), a competitive immunoassay procedure using enzyme-labeled chemiluminescent technology validated against RIA for cattle. Cows were classified as noncyclic if serum progesterone in both samples was <1 ng/mL (Silva et al., 2007). A Poisson regression model that controlled for overdispersion was fitted in SAS (version 9.3, SAS Institute Inc.) using PROC GENMOD to analyze the difference in cyclic activity between the treatment and control groups.

DMI. Dry matter intake was measured for 52 cows. The cows were housed in individual tiestalls during the trial period and calved in individual box stalls so that individual DMI could be measured. Feed intake and refusals were recorded daily for each cow enrolled in the trial. Two feed samples were collected from each cow's manger twice a week at the time of feeding (Tuesday and Friday) from enrollment until 63 DIM and from the freshly mixed TMR batch fed to dry and lactating cows (Monday and Thursday). Samples were frozen at -20°C . All samples were weighed and then dried in a hot air oven at 105°C for 48 h to calculate the DM content of feed. Total DMI was calculated as the difference between DM of offered and refused feed. For data analysis, data for each cow from 3 wk before calving (in case cows did not calve within 3 wk from enrollment) until 62 DIM was used. For data analysis, daily feed intakes for each cow were averaged into weekly intakes. A mixed linear model (PROC MIXED in SAS version 9.3., SAS Institute Inc.) with cow intakes as a repeated measure was used to analyze the data. In addition to the above-mentioned variables, the week of feed intake was included as one of the variables in the model.

Table 1. Descriptive data for cows treated precalving with either 325 mg of recombinant bST (rbST) or placebo

Lactation	rbST		Placebo (control)		<i>P</i> -value ¹
	No.	%	No.	%	
1	154	22.6	155	22.7	0.97
2	213	31.3	209	30.6	0.79
≥3	313	46.0	318	46.6	0.82
Total ²	680	100	682	100	—

¹*P*-value calculated using χ^2 test.²Total number of cows enrolled in each treatment group.

RESULTS

Eighty-three cows were excluded from analysis for the following reasons: not pregnant at enrollment (1 cow), having wrong expected calving dates (48 cows), behavioral reasons (2 cows), no blood samples collected (5 cows), no information on herd management software (2 cows), inadvertent rbST or placebo treatments (7 cows), and missed treatments (18 cows) as they were not found on the farm at the weekly herd visit. Table 1 shows enrollment by parity for cows in the study.

Cows treated with rbST had higher serum somatotropin concentrations than control cows at the time of enrollment (1.5 ng/mL, 95% CI: 1.2–1.8 and 1.0 ng/mL, 95% CI: 0.8–1.2, respectively; $P = 0.01$), wk 1 after enrollment (1.7 ng/mL, 95% CI: 1.6–1.8 and 1.0 ng/mL, 95% CI: 0.9–1.1, respectively; $P < 0.001$), and wk 2 after enrollment (2.3 ng/mL, 95% CI: 1.9–2.8 and 1.0 ng/mL, 95% CI: 0.8–1.3, respectively; $P < 0.001$).

Health

Health data were analyzed from 1,279 cows. Removal (withdrawal from the trial) and culling (dead and sold cows) from enrollment to 63 DIM are shown in Table 2.

Prepartum treatment of cows with rbST had no significant effect on cow removal or death. Table 3 shows the incidence of clinical disease recorded during the duration of the trial for cows treated with rbST or placebo. The *P*-values obtained from simple associations of treatment and disease were 0.08 for abomasal displacement and 0.16 for retained placenta; therefore, fitting a Poisson regression was warranted for both diseases to investigate the effect of treatment after controlling for other variables.

Before fitting the Poisson regression for displaced abomasum and retained placenta, 119 cows (61 cows from the treatment group and 58 cows from the control group) were excluded as they had no outcomes recorded, and 10 cows were excluded because they were sold or died before calving. Therefore, a total of 1,150 cows were included for analysis in the Poisson regression models for displaced abomasum and retained placenta. In the model for displaced abomasum, number of treatments, season at enrollment, and days from last treatment to calving were removed from the model. The risk of developing abomasal displacement tended to be higher for cows treated with rbST than for control cows (RR = 3.6, 95% CI: 0.88–14.69, $P = 0.08$). For the Poisson model for retained placenta, season at enrollment and

Table 2. Disposition of Holstein cows in a randomized clinical trial of 325 mg of recombinant bST (rbST) injected every 14 d from enrollment at 28 to 22 d before the expected calving date until 63 DIM

Item	rbST		Placebo (control)		<i>P</i> -value ¹
	No.	%	No.	%	
Treated	680	49.9	682	50.1	—
Sold	75	11.0	65	9.5	0.30
Died or euthanized	14	2.1	14	2.1	0.96
Withdrawn ²	44	6.5	39	5.7	0.56
Completed ³	547	80.4	564	82.7	0.73
Total ⁴	636	93.5	643	94.3	—

¹*P*-value calculated using χ^2 test.²Withdrawn = cows excluded from analysis due to various reasons such as having wrong calving dates, missed treatments, and inadvertent treatments.³Completed = cows that were followed up in the trial until 63 DIM.⁴Total = total number of cows that were included in the analysis including sold, died/euthanized, and completed cows.

Table 3. Univariable associations of recombinant bST (rbST) with clinical disease incidence from enrollment to 63 d after calving for cows treated prepartum with rbST or placebo

Item	rbST		Placebo (control)		Risk ratio ¹	95% CI ¹	<i>P</i> -value ¹
	No.	%	No.	%			
Clinical ketosis ²	18	2.8	19	3.0	0.96	0.51–1.81	0.89
Displaced abomasum ²	9	1.4	3	0.5	3.6	0.82–11.15	0.08
Milk fever ²	25	3.9	28	4.4	0.90	0.53–1.53	0.70
Retained placenta ³	44	7.0	32	5.1	1.38	0.88–2.14	0.16
Metritis ³	82	13.0	77	12.2	1.06	0.80–1.42	0.67
Mastitis ²	88	14.4	80	12.4	1.11	0.84–1.47	0.46
Lameness ²	26	4.1	30	4.7	0.88	0.52–1.46	0.61

¹Risk ratio, 95% CI, and *P*-value were calculated using a χ^2 test.

²Denominator excluded cows that were withdrawn from the trial for each group.

³Denominator excluded cows that were withdrawn from the trial for each group and 3 cows died/sold before calving for the rbST group, and 11 cows died/sold before calving for the control group.

BCS at enrollment were removed from the model. We observed a tendency for cows treated with rbST to have an increased risk of retained placenta compared with control cows (RR = 1.45, 95% CI: 0.94–2.24, *P* = 0.09).

Median BCS for both treatment groups at enrollment was 3.5 (*P* = 0.51) on a 5-point scale with 0.25-point increments. Twelve percent of cows were thin (BCS <3.25), 81% were fair (BCS 2.25 to 3.75), and 7% were fat (BCS >3.75). To analyze the change in BCS, a log-transformation of the dependent variable was required to achieve a normal distribution. Days from last treatment to calving and the number of treatments were dropped from the model. Cows treated with rbST had lower BCS 3 wk after calving compared with similar cows in the control group (3.13, 95% CI: 3.10–3.15 vs. 3.17, 95% CI: 3.15–3.20, *P* < 0.001).

Metabolic Indicators

Seventeen cows (11 cows from the treatment group and 6 cows from the control group) were excluded from the serology analysis as no serum samples were submitted. Therefore, the final analysis included results from 1,226 cows. Hemolysis score was not considered during analysis as only about 5% of the submitted serum samples from both groups had a hemolysis score of 1 or 2.

NEFA. Results comparing elevated NEFA between the treatment and control groups before and after calving are shown in Table 4. The only variables eliminated from the NEFA model were the number of treatments given before calving and the days from last treatment to calving. A square-root transformation of the dependent variable was required to achieve a data set with normal distribution. None of the interaction terms with treatment were significant and therefore were not included in the final model. However, the interaction term between treatment and week of sampling (*P* = 0.06) indicated

further analysis, which showed that cows treated with rbST had higher NEFA levels than control cows at the week before calving (0.27 mmol/L, 95% CI: 0.25–0.28 and 0.23 mmol/L, 95% CI: 0.22–0.25, respectively, *P* = 0.003). Overall, cows treated with rbST had a tendency to have higher NEFA concentrations compared with control cows (0.47 mmol/L, 95% CI: 0.45–0.49 and 0.46 mmol/L, 95% CI: 0.44–0.48, respectively, *P* = 0.07).

BHBA. Body condition score at enrollment, number of treatments, and days from last treatment to calving were all eliminated from the final model. A log-transformation of the dependent variable was required to achieve a normal distribution. The interaction term between treatment and week of sampling (*P* = 0.06) suggested further analysis, which showed that cows treated with rbST had higher BHBA levels than control cows in wk 1 after calving (750 μ mol/L, 95% CI: 721–781, and 698 μ mol/L, 95% CI: 671–727, respectively, *P* = 0.009) and tended to be higher in wk 2 after calving (779 μ mol/L, 95% CI: 744–815 and 735 μ mol/L, 95% CI: 702–769 respectively, *P* = 0.07).

Hyperketonemia. The herd-level prevalence of hyperketonemia in the study herds in the first 3 wk following calving ranged from 14 to 45%. Results of the χ^2 test comparing the prevalence of hyperketonemia between the treatment and control groups for wk 1, 2, and 3 after calving and for the cumulative incidence of hyperketonemia after the first 3 wk postcalving are shown in Table 5. For the Poisson regression model, number of treatments and days from the last treatment to calving were removed from the model. The incidence of hyperketonemia was not different between treatment and control groups (RR = 1.17, 95% CI: 0.94–1.47, *P* = 0.16).

Glucose. After transforming the dependent variable to the log scale, days from last treatment until calving, BCS at enrollment, and number of treatments were removed from the model. Four observations that were

Table 4. Risk ratios of elevated NEFA comparing cows treated with recombinant bST (rbST) before calving to control cows using NEFA serum concentrations of 0.4 mmol/L as a cutoff point in the week before calving (wk -1) and 1.0 mmol/L as a cutoff point for each of the following weeks following calving (wk 1 to 3)

Week	rbST		Placebo (control)		Risk ratio ¹	95% CI ¹	P-value ¹
	No. ²	%	No.	%			
-1	158	26.2	133	21.9	1.27	0.96–1.66	0.08
1	217	35.6	214	35.4	1.01	0.79–1.29	0.96
2	155	26.4	157	26.4	1.00	0.77–1.33	0.99
3	99	17.5	114	19.6	0.87	0.64–1.18	0.35

¹Risk ratio, 95% CI, and P-value were calculated using a χ^2 test.

²Number of cows equal to or above the thresholds of NEFA serum concentrations of 0.4 mmol/L for wk -1 and 1.0 mmol/L for wk 1 to 3. Denominator included all cows with a NEFA measurement for the respective week.

outliers were removed from the analysis because of errors in serum glucose measurement by the laboratory. Prepartum administration of rbST had a significant effect on serum glucose concentrations. Serum glucose was higher for treated cows than for control cows (2.79 mmol/L, 95% CI: 2.76–2.83 and 2.73 mmol/L, 95% CI: 2.70–2.77, respectively, $P = 0.004$). Although we found no significant interaction between treatment and week of sampling ($P = 0.11$), cows treated with rbST had higher glucose levels than control cows in the week before calving (3.17 mmol/L, 95% CI: 3.11–3.22 and 3.00 mmol/L, 95% CI: 2.99–3.10, respectively, $P < 0.001$), in wk 2 after calving (2.50 mmol/L, 95% CI: 2.44–2.55 and 2.41 mmol/L, 95% CI: 2.35–2.46, respectively, $P = 0.03$), and in wk 3 after calving (2.51 mmol/L, 95% CI: 2.45–2.56 and 2.41 mmol/L, 95% CI: 2.35–2.46, respectively, $P = 0.01$).

AST. Number of treatments, BCS at enrollment, farm, and days from the last treatment to calving were removed from the model. After log-transformation of the AST values, we observed no difference in serum AST concentrations between the treatment and control groups (68.2 U/L, 95% CI: 66.7–69.8 and 69.4 U/L, 95% CI: 67.8–71.0, respectively, $P = 0.29$).

Calcium. Number of treatments, BCS at enrollment, season of enrollment, and days from the last treatment

were removed from the model. We found no significant difference between treated and control cows (2.35 ± 0.01 mmol/L and 2.34 ± 0.01 mmol/L, respectively, $P = 0.46$).

Haptoglobin. After log-transforming the dependent variable, season at enrollment, days from last treatment to calving, and BCS at enrollment were removed from the model. We found no difference between treated and control cows (0.17 g/L, 95% CI: 0.16–0.18 for both groups, $P = 0.73$).

Milk Production

Milk production from 1,242 cows was analyzed. Daily milk weights were collected until 63 DIM or until cows were sold or died, if before 63 DIM. The only variable eliminated from the model was season at the time of enrollment in the study. Milk production per cow and day was not different between treated and control cows (37.1 ± 0.48 kg and 36.7 ± 0.49 kg, respectively, $P = 0.31$). Although the interaction between treatment and week of milk production was not significant ($P = 0.07$), stratifying milk production by each week showed that cows treated with rbST tended to produce 0.8 kg/d more milk than control cows ($P = 0.07$) in wk 1 after calving.

Table 5. Risk ratios of hyperketonemia comparing cows treated with recombinant bST (rbST) before calving to control cows using a BHBA serum concentration of 1,400 μ mol/L as a cutoff point to define hyperketonemia

Week	rbST		Placebo (control)		Risk ratio ¹	95% CI ¹	P-value ¹
	No. ²	%	No.	%			
1	52	8.5	42	7.0	1.23	0.83–1.81	0.30
2	67	11.4	57	9.6	1.19	0.85–1.66	0.30
3	69	12.2	63	10.8	1.12	0.82–1.55	0.47
Overall ³	130	21.2	115	18.7	1.14	0.91–1.42	0.27

¹Risk ratio, 95% CI, and P-value were calculated using a χ^2 test.

²Number of cows equal to or above the cutoff threshold BHBA serum concentration of 1,400 μ mol/L. Denominator included all cows with a BHBA measurement for the respective week.

³Cumulative incidence of hyperketonemia to 3 wk after calving; if a cow was hyperketonemic in any of the 3 wk after calving, she was considered positive.

Reproductive Performance

Reproductive data were analyzed from 1,224 cows. A summary of different reproductive measures is shown in Table 6. Forty-two cows were excluded from the analysis because they were flagged as “do not breed” by farm staff. Administration of rbST during the prepartum period had no significant effect on reproductive parameters measured in this study. For the Poisson regression model, only treatment, farm, and lactation group (parity) were retained in the model. Conception risk was not different between groups (28.5% vs. 29.4%, RR = 0.99, 95% CI: 0.82–1.19, *P* = 0.91). For survival analysis, the only violation in the assumption of proportionality of hazard was for farm, and therefore a time-dependent variable was forced into the model. Treatment, lactation group, farm, and farm × time were the variables retained in the model. Median time to first insemination was not different between groups (61 DIM, hazard ratio = 0.93, 95% CI: 0.74–1.16, *P* = 0.51). For progesterone analysis, treatment was the only variable retained in the model. We detected no difference in the risk of being anovular by 8 wk postpartum between cows treated with rbST before calving and cows treated with placebo (38.0 and 34.3%, respectively, RR = 1.09, 95% CI: 0.82–1.45, *P* = 0.56).

DMI

Data for DMI were normally distributed. Number of treatments and days from last treatment to calving were removed from the model. We detected no difference in DMI between cows treated with rbST before calving and control cows (17.4 ± 0.43 kg and 17.5 ± 0.44 respectively, *P* = 0.80).

DISCUSSION

The dose of rbST used in this trial was an average of doses used in studies previously conducted; evidence of positive outcomes has been reported for rbST administered at a dose between 143 and 500 mg (Putnam et al., 1999; Gulay et al., 2007).

Health

One point to consider when looking at clinical disease incidence is how compliant farm staff is in terms of recording diseases. Disease incidences reported in this study was mostly based on diagnosis by farmers and not veterinarians. However, considering that overall clinical disease incidence in this study was 44% (561/1,279) for both the rbST and control groups, and that 30 to 50% of dairy cows are affected with one disease or another in the transition period (LeBlanc, 2010), under-reporting of diseases by farm managers did not appear to be a major issue. In addition, diseases reported in the current study are within the range reported previously (Kelton et al., 1998). Although disease definitions were discussed with each farm manager at the beginning of the study, the possibility of misclassification bias remains, depending on how adherent the farm personnel were to disease definitions and to what extent their experience and practices influenced diagnosis of diseases. However, because of the randomization process followed in this trial, introduction of bias (if any) is expected to be equal between groups.

In the current study, we observed no difference in clinical diseases associated with treatment, which is in agreement with work by Gulay et al. (2003), which reported no difference in health when cows were treated with 143 mg of rbST every 14 d starting at 3 wk before calving until 42 DIM. Gulay and coworkers (2007) grouped control and treated cows from 3 different studies for analysis and found no difference in the incidence of displaced abomasum, milk fever, retained fetal membranes, or metritis between the treatment and control groups, but the incidences of ketosis, digestive illness, and mastitis were lower for treated than for control cows. Improvement in postparturient health was explained by those authors as a result of the increase in DMI and an improvement in metabolism of carbohydrates, lipids, and proteins, in addition to enhancement of the immune system to resist infectious diseases. The major difference between the 3 studies summarized by Gulay and coworkers (2007) and the current study is that rbST treatments were continued after calving in the former, whereas rbST treatments

Table 6. Summary of reproductive parameters for first breeding after calving for cows treated precalving with 325 mg of recombinant bST (rbST) or placebo

Treatment	Days from calving to first AI		Cows bred, no.	Cows pregnant, no.	Probability of pregnancy to first AI (conception risk), %
	Mean ± SD	Median			
rbST	61.2 ± 12.7	61	530	151	28.5
Placebo	61 ± 11.1	61	534	157	29.4

were only administered before calving in the current study, which might explain the nonsignificant effect of rbST on clinical disease incidence. In another study (Vallimont et al., 2001), cows were started on 500 mg of rbST 28 d before calving every other week with a maximum of 3 treatments until calving. Those authors found that the incidence of ketosis, displaced abomasum, mastitis, milk fever, and retained fetal membranes was similar between the treatment and control groups. They also found that DMI did not differ between the groups, which might explain similar disease incidences in the treatment and control groups.

Although not significant, the risk of developing abomasal displacement was 3.6 times higher in cows treated with rbST before calving compared with cows in the control group. The incidence of displaced abomasum in the current study was 1.4% (9 cows) for the treatment group and 0.5% (3 cows) for the control group. Such a low incidence of displaced abomasum could be a function of conducting the trial in progressive herds with good management practices, including nutritional management of transition cows. One has to consider that although a tendency was found for increased displaced abomasum risk, clinically this result was not important because of the small number of cows affected with displaced abomasum.

Treated cows had a lower BCS than control cows (3.13 and 3.17, respectively). Although the BCS difference between groups was significant ($P < 0.001$), the difference itself was not considered biologically or clinically important as it was only 0.04 of a BCS unit, which corresponds to approximately 3 kg of BW (Schwager-Suter et al., 2001). Therefore, the similar decline in BCS in both groups in the current study could be explained by the typical NEB and reduction in DMI that cows experience in the peripartum period rather than an effect of rbST. This conclusion is supported by similar milk production and DMI for treated and control cows. Previous studies administering rbST in the prepartum period at different doses (Simmons et al., 1994; Vallimont et al., 2001) found no difference in BCS between treated and control groups, but one recent study (Schneider et al., 2012) reported that late pregnant heifers given 500 mg of rbST every 14 d until calving had a higher BCS than heifers that were not treated.

Metabolic Indicators and Hyperketonemia

Overall, serum NEFA concentrations in the current study tended to be higher for treated cows, and serum NEFA was higher for treated than for control cows in the week before calving. However, the difference was neither clinically nor biologically important. Energy

balance will determine the effect of bST on adipose tissue; an increase in blood NEFA and a decrease in subcutaneous fat will occur as a result of rbST-increased fat mobilization and lipolysis in a cow that is in NEB, and NEFA concentrations will not be affected if administered in positive energy balance as lipogenesis is not enhanced (Bauman et al., 1989). Therefore, we can conclude that rbST was administered in a state of positive energy balance, and that cows started the phase of NEB closer to the time of calving. Similar to the current study, other studies found that precalving rbST treatments did not affect NEFA concentrations (Simmons et al., 1994; Putnam et al., 1999). One study found a reduction in NEFA concentrations when rbST was given to late pregnant heifers (Schneider et al., 2012), and another showed a significant increase in NEFA in the postpartum period for cows treated before and after calving with 143 mg of rbST (Gulay et al., 2003).

Although insulin levels were not measured in the current study, insulin resistance in late gestation may result in lower sensitivity to insulin at the level of adipose tissues, which leads to an increase in lipolysis and increased NEFA (Gulay et al., 2003). Bovine somatotropin can exacerbate the state of insulin resistance by decreasing the effect of insulin to stimulate lipogenesis (Bauman et al., 1989), which simply means that insulin (under the effect of bST) will have a reduced ability to lower blood glucose and store the excess in fat depots, resulting in higher blood glucose concentrations. It could be that the short duration of rbST administration was not enough to exacerbate the state of insulin resistance, which was reflected by similar serum NEFA and glucose concentrations.

Although the difference in glucose concentrations was statistically significant in this study, it was neither biologically nor clinically important, and this conclusion was supported in the current study by having a similar incidence of hyperketonemia, DMI, and milk production between treated and control cows. Other studies that investigated the effect of prepartum rbST administration found no difference in blood glucose levels between treated and control cows (Vallimont et al., 2001; Schneider et al., 2012).

Although overall BHBA was not different among groups, a significant difference in BHBA concentrations was observed in wk 1 following calving. The difference could be due to a carryover effect of rbST that was most prominent in wk 1 following calving, a time when cows are in NEB, resulting in an increase in lipolysis that is enhanced by rbST (Bauman et al., 1989) and coinciding with the period of insulin resistance. However, the differences in BHBA between groups were not biologically or clinically important. When cows were treated with rbST before calving, Putnam et al. (1999)

reported a decrease in BHBA concentration only in the precalving period. In contrast, another study reported an increase in BHBA concentrations in Jersey cows treated with 500 mg of rbST before calving (Eppard et al., 1996).

The cutoff point of 1,400 $\mu\text{mol/L}$ for BHBA used in the current study to identify hyperketonemia was previously identified based on the negative effect on production and health (Duffield et al., 2009). In the current study, we observed no difference in the incidence of hyperketonemia between treatment and control groups. This finding could be explained by similar blood NEFA and glucose concentrations and only minor changes in BHBA in treated and control cows. This finding could also be supported by similar DMI and milk production among groups.

Milk Production

Administration of rbST in the prepartum period had no significant effect on overall milk production. Similar milk production between groups was expected because clinical disease incidence, hyperketonemia, and DMI were not different among treated and control cows. The finding of no difference in overall milk production agrees with previous studies (Simmons et al., 1994; Vallimont et al., 2001). In the current study, when milk production was analyzed by the week after calving, treated cows tended to produce more milk than control cows in the first week following calving. Somatotropin mediates its action on the mammary gland through IGF-I (Bauman, 1992) and although the levels of somatotropin are high after calving, the levels of IGF-I are low due to a reduction in growth hormone receptors in the liver around calving (Radcliff et al., 2003). Thus, the effect of rbST administered on the mammary gland in the current trial might be limited to the time before calving and perhaps resulted in the tendency of treated cows to produce more milk in wk 1 following calving but not in greater production for the first 63 DIM for treated cows.

Reproductive Performance

None of the reproductive performance measures evaluated in this study was affected by prepartum administration of rbST. It could be that the time interval from rbST administration before calving to the first insemination following calving was too long to expect a carryover effect of rbST. The incidence of metritis and retained fetal membranes in this study was the same in both treatment groups and it is not surprising from a uterine health perspective that other reproductive parameters were similar between groups.

DMI

The number of cows ($n = 52$) with data on DMI was lower than initially planned. This part of the study was conducted after preliminary results on disease, blood parameters, and production were obtained and analyzed statistically, showing no significant differences between treatment and control groups. Therefore, we decided to limit the number of cows on which to collect DMI data because the purpose of these data was to be a potential explanatory variable for rbST effects on the outcomes above. Given the lack of differences there, detailed feed intake data collection was curtailed. The fact that DMI was similar in both groups in the current study was supported by the lack of difference in milk production between groups. Furthermore, the fact that serum NEFA concentrations and the change in BCS from enrollment to 3 wk after calving were the same in both groups supports similar DMI between groups. Similarly, in other studies, when rbST was only administered before calving, DMI did not differ between treated and control cows (Simmons et al., 1994; Putnam et al., 1999; Vallimont et al., 2001). On the other hand, when rbST treatments were continued before and after calving, one study showed no increase in DMI (Gulay et al., 2004b), whereas another showed an increase in DMI only in the postpartum period (Gulay et al., 2004a).

CONCLUSIONS

We conclude from this study that biweekly (every 14 d) administration of 325 mg of rbST in the prepartum period had no significant effects on health, DMI, reproductive performance, or milk production of dairy cows. Although statistical differences were observed for some of the variables measured in the current trial, such as lower BCS and higher BHBA and glucose for treated cows, these differences were so small as to not be of practical importance. Based on our results, we do not recommend administering rbST only during the prepartum period as a management approach for transition dairy cows. Rather, currently recommended transition period practices, such as proper nutritional management of dry and fresh cows, elimination of undue stress, and proper barn and stall design, are likely to help improve cow performance and welfare.

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