Research Article

Slow Release Rbst Administration During the Transition Period on Performance and Blood Cell Counts of Dairy Cows

Jefferson R. Gandra[1*], Tiago A. Dell Vale[3], Caio S. Takiya[4], Erika R. S. Gandra[5], Euclides R. Oliveira[2], Rafael H. T. B. Goes[2], Isabelle Z. Noia[2], Cibeli A. Pedrini[2], Giovana S. Urio[2], Jamille D. O. Batista[2], Giovani Antônio[2], Anderson P. Acosta[6]

[1]Instituto de Estudo em Desenvolvimento Agrário e Regional. Universidade Federal do Sul e Sudeste do Pará, Marabá PA, Brazil; [2]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Rodovia Dourados-Itahum, Dourados, MS, Brazil 79804-970; [3]Faculdade de Agronomia, Universidade Federal do Pampa, campus Itaqui RS, Brazil 97650-000; [4]Department of Animal Sciences and Industry, Kansas State University, Manhattan, United States. 66506; [5]Instituto de Estudo do Trópico Umido. Universidade Federal do Sul e Sudeste do Pará, Xinguara PA, Brazil; [6]Faculdade de Medicina Veterinária. Universidade Estadual de Maringá, Umuarama PR, Brazil.

Abstract | This study aimed to evaluate recombinant bovine somatotropin (rbST) supply on feed intake, performance, serum metabolites, and blood cells count of transition cows. Thirty pregnant Holstein cows (607 ± 28.6 kg of body weight) were randomly allocated to one of the following treatments: 1) Control (CON), without rbST supply; and 2) rbST: 250 mg of slow release rbST (Lactotropin, Elanco Animal Health, USA) supplied every 14 d from 28 d of the expected calving date until 28 d of lactation. rbST group had increased dry matter intake during the post partum period and higher body condition score in both pre- and post-partum periods compared to CON. rbST treatment increased milk yield and milk fat content. Although no effects were observed on serum metabolites before parturition, rbST increased post-partum serum concentrations of glucose, cholesterol, and ionized calcium, and decreased serum concentration of triacyglyceride. rbST had minor effects on complete blood cell count, which included reduced neutrophil counts. In conclusion, this study supports that rbST supply during transition period improves performance of dairy cows that is partially related to improvements in metabolism.

Keywords | leukocyte, milk yield, post-partum, rbST, serum metabolites.

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***Correspondence** | Jefferson R Gandra, Instituto de Estudo em Desenvolvimento Agrário e Regional. Universidade Federal do Sul e Sudeste do Pará, Marabá PA, Brazil; **Email:** jeffersongandra@unifesspa.edu.br
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INTRODUCTION

The transition period of dairy cows is characterized by a sharp decline in dry matter intake (DMI) and negative consequences in animal's health and productivity after parturition (Grummer, 1995). Improved milk production increases energy requirements and results in negative energy balance (NEB), in which cows mobilize energy reserves (Barletta et al., 2017). Besides high growth hormone (GH) concentration, animals in NEB shows insulin resistance and reduced glucose uptake by peripheral tissues (Bell and Bauman, 1997). Incomplete oxidation of mobilized fatty acids could comprise liver function and predispose animals to ketosis (Grummer et al., 2004). On the other hand, animals that drastically lose body score condition (BCS) during transition period shows are more susceptible to health problems (Gandra et al., 2017; Barletta et al., 2017), probably in consequence of an altered structure and function of immune cells.

Recombinant bovine somatotropin (rbST) is a synthetically-derived formulation of somatotropin, that has been traditionally used to increase milk production efficiency in dairy cows: rbST increases lactation milk yield by altering the lactation curve (de Morais et al., 2016). Recombinant bovine somatotropin reduces insulin negative effects on gluconeogenesis (Peel and Bauman, 1987) and increases fatty acids oxidation (Pocius and Herbein, 1986). In addition, rbST increases glucose availability for lactogenesis and immunological response. Putnam et al. (1999) found improved DMI, serum glucose concentration, and milk yield of cows treated with 500 mg of rbST every 14 d, in the peripartum. Gohary et al. (2014) evaluated 325 mg of rbST supply every 14 d, and reported increased blood glucose concentration during pre-partum, without major effects on DMI, post-partum diseases incidence, and reproductive performance. Insulin-like factor 1 (IGF-1) stimulates growth, differentiation and functionality of immune cells (Heemskerk et al., 1999). Silva et al. (2017) reported that 125 mg of rbST, every 7 d, from -21 to 28 d relative to calving increased IGF-1 concentration, intensity of phagocytosis, and oxidative burst of polymorphonuclear leukocytes.

Therefore, the hypothesis of the current study was that rbST supply to transition cows would improve DMI, productive performance, serum glucose concentration, and blood leukocyte counts. This study aimed to evaluate the effects rbST supply on milk yield and composition, serum metabolites, and complete blood cell count of transition Holstein cows.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of the Federal University of Grande Dourados. This experiment was carried out in a commercial dairy farm located at Mato Grosso do Sul State – Brazil, between January and August 2016.

Management, Animal, and Treatment

Thirty multiparous and pregnant Holstein cows (607 ± 28.6 kg of body weight - BW) were housed in individual pens (8 × 4 m), 28 d before expected calving date. Then, animals were randomly allocated to one of following treatments: 1) CON, without rbST supply; and 2) rbST, with 250 mg of rbST (Lactotropin®, Elanco Animal Health, USA), supplied every 14 d until 28 d of lactation. The rBST dose was administered after fractionation of the original 500 mg packaging in half with the aid of sterile syringes. Pre- and post-partum diets were formulated according to NRC (2001) and animals of different experimental groups received the same diets (Table 1). Evaluations were performed weekly until 28 d post-partum. Amounts of feed and orts of each cow were weighed daily and orts were adjusted to 5% to 10% of feed intake as-fed basis.

Table 1: Ingredients and chemical composition of experimental diets

Item	Diets	
	Pre-partum	Post-partum
Ingredients, g/kg DM		
Corn silage	700	450
Ground corn	171	290
Soybean meal	92.0	220
Mineral premix[1]	27.0	36.5
Urea	10.0	3.50
Chemical composition (g/kg DM)		
Dry matter, *g/kg as fed*	450	591
Organic matter	914	914
Neutral detergente fiber	414	313
Non-fiber carbohydrate	385	422
Acid detergente fiber	221	163
Crude Protein	139	176
Lignin	36.1	27.9
Ether extract	28.2	28.4
Net energy of lactation (Mcal/kg)[2]	1.56	1.76
DCAD (meq/kg)[2,3]	-170	-

[1]Mineral premix: pre-partum, each kg contained: 131 g Cl, 130 g Ca; 90 g S; 31 g Na; 30 g P; 20 g Mg; 2,400 mg Zn, 1,600 mg Mn, 600 mg Fe, 600 mg Cu; 80 mg biotin, 60 mg I, 30 mg Cr, 16 mg Se, 12 mg Co, 480,000 U vit. A; 200,000 U vit. D, 12,000 U vit. E, and 500 mg monensin.
Post-partum, each kg contained: 123 g Na, 110 g Ca, 42 g P, 20 g Mg, 18 g S, 2800 mg Zn, 2,000 mg Mn, 1,050 mg Fe, 600 mg Cu, 600 mg monensin, 80 mg biotin, 28 mg I, 20 mg Cr, 18 mg Se, 14 mg Co, 240,000 U vit. A, 100,000 U vit. D, 100,000 U vit. E;
[2]Calculated according to NRC (2001);
[3]Dietary cation and anion difference.

Sample Collection and Chemical Analyses

Samples of corn silage were taken weekly (n = 8) and concentrate ingredients were sampled during the preparation of the mixture (n = 8) for chemical analysis. Orts samples (10% of total daily orts) were collected daily from each cow and were combined into a composite sample per week. Feed intake was recorded daily as the difference between feed offered and orts. Samples of corn silage, concentrate, and orts were analyzed for dry matter content (method 930.15, AOAC 2000). Diet ingredients were analyzed for neutral detergent fiber (NDF), acid detergent fiber, and lignin, using heat-stable α-amylase (Van Soest et al., 1991), ash (method 942.05), ether extract (EE; method 920.39), and crude protein (CP; method 984.13) according to AOAC (2000). Non-fiber carbohydrate (NFC) content was estim-

Table 2: Feed intake, body weight, and body condition score of dairy cows treated with 250 mg of rbST every 14 d, during transition period

Item	Treatments[1]		SEM[2]	Probabilities[3]		
	CON	rbST		Treat.	Week	Treat×Week
Dry matter intake (kg/d)						
Pre-Partum	9.51	10.1	0.13	0.166	<0.001	0.115
Post-Partum	13.3	15.8	0.22	0.008	<0.001	0.181
Body weight (kg)						
Pre-Partum	560	593	6.0	0.179	0.008	0.365
Post-Partum	518	532	7.3	0.559	0.004	0.865
Body score condition						
Pre-Partum	2.63	3.17	0.042	0.002	0.001	0.177
Post-Partum	2.38	2.81	0.051	0.003	0.088	0.248

[1]Treatments: CON, without rbST supply; and 2) rbST, with 250 mg of rbST (Lactotropin, Elanco Animal Health, USA), supplied every 14 d; [2]Standard error of mean; [3]Probabilities: Treatment effect (CON vs rbST); week effect; and treatment by week interaction effect.

Table 3: Milk yield and composition of dairy cows treated with 250 mg of rbST every 14 d, during transition period

Item	Treatments[1]		SEM[2]	Probabilities[3]		
	CON	rbST		Treat.	Week	Treat×Week
Production (kg/d)						
Milk yield	22.5	24.9	0.70	0.026	0.008	0.850
3.5% FCM	22.2	26.0	0.74	0.013	0.321	0.349
Lactose	1.03	1.07	0.038	0.515	0.001	0.721
Fat	0.820	0.941	0.037	0.037	0.795	0.254
Protein	0.808	0.839	0.022	0.460	0.001	0.814
Milk composition (g/kg)						
Lactose	42.8	43.0	0.41	0.206	0.895	0.588
Fat	34.1	37.8	1.01	0.045	0.045	0.233
Protein	33.4	33.7	0.42	0.571	0.792	0.628

[1]Treatments: CON, without rbST supply; and 2) rbST, with 250 mg of rbST (Lactotropin, Elanco Animal Health, USA), supplied every 14 d; [2]Standard error of mean; [3]Probabilities: Treatment effect (CON vs rbST); week effect; and treatment by week interaction effect.

Table 4: Serum metabolites of dairy cows treated with 250 mg of rbST every 14 d, during transition period

Item	Treatments[1]		SEM[2]	Probabilities[3]		
	CON	rbST		Treat.	Week	Treat×Week
Glucose (mg/dL)						
Pre-Partum	112	118	3.05	0.345	0.180	0.608
Post-Partum	97.9	116	4.26	0.044	0.422	0.445
Total cholesterol (mg/dL)						
Pre-Partum	79.1	72.9	7.18	0.776	0.157	0.349
Post-Partum	78.3	90.0	8.56	0.024	0.394	0.959
Triglycerides (mg/dL)						
Pre-Partum	44.5	47.6	3.33	0.766	0.314	0.311
Post-Partum	33.5	29.5	3.19	0.022	0.561	0.084
Total protein (g/dL)						
Pre-Partum	10.8	10.8	0.29	0.967	0.016	0.148

Post-Partum	11.1	11.0	0.40	0.936	0.025	0.239
Albumin (mg/dL)						
Pre-Partum	3.68	3.29	0.155	0.429	0.155	0.464
Post-Partum	3.84	3.38	0.155	0.367	0.704	0.147
Total calcium (mg/dL)						
Pre-Partum	10.4	10.6	0.47	0.855	0.659	0.617
Post-Partum	9.42	9.90	0.373	0.697	0.574	0.032
Ionized calcium (mg/dL)						
Pre-Partum	5.22	5.49	0.235	0.721	0.373	0.612
Post-Partum	4.60	5.07	0.203	0.018	0.743	0.015
Urea (mg/dL)						
Pre-Partum	37.7	40.8	2.63	0.726	0.770	0.920
Post-Partum	45.1	45.3	3.03	0.984	0.775	0.089

[1]Treatments: CON, without rbST supply; and 2) rbST, with 250 mg of rbST (Lactotropin, Elanco Animal Health, USA), supplied every 14 d; [2]Standard error of mean; [3]Probabilities: Treatment effect (CON vs rbST); week effect; and treatment by week interaction effect.

Table 5: Blood cells count of dairy cows treated with 250 mg of rbST every 14 d, during transition period

Item	**Treatments[1]**		**SEM[2]**	**Probabilities[3]**		
	CON	**rbST**		**Treat.**	**Week**	**Treat×Week**
Erythrocytes (μL^{-6})						
Pre-Partum	6.01	6.53	8.582	0.136	0.183	0.136
Post-Partum	5.74	6.22	11.59	0.178	0.591	0.981
Haemoglobin (g/dL)						
Pre-Partum	10.3	11.0	0.13	0.206	0.419	0.630
Post-Partum	9.63	10.2	0.18	0.280	0.343	0.811
Haematocrit (%)						
Pre-Partum	29.4	30.6	0.42	0.320	0.383	0.459
Post-Partum	27.7	29.7	0.60	0.222	0.931	0.269
Leucocytes (μL^{-1})						
Pre-Partum	18,889	17,605	8.4	0.727	0.030	0.690
Post-Partum	18,245	16,485	14.0	0.677	0.040	0.227
Lymphocytes (μL^{-1})						
Pre-Partum	10,391	10,683	8.0	0.935	0.077	0.938
Post-Partum	10,479	8,898	10.7	0.621	0.825	0.024
Neutrophils (μL^{-1})						
Pre-Partum	7,993	6,309	2.8	0.005	0.051	0.912
Post-Partum	8,064	6,052	6.8	0.003	0.102	0.035
Lymphocytes (%)						
Pre-Partum	50.2	56.6	1.63	0.298	0.004	0.292
Post-Partum	54.1	52.6	2.42	0.831	0.390	0.049
Neutrophils (%)						
Pre-Partum	47.0	40.3	1.54	0.245	0.008	0.833
Post-Partum	44.7	44.5	2.24	0.966	0.470	0.016
Neutrophils: lymphocytes ratio						
Pre-Partum	1.06	0.87	0.077	0.442	0.025	0.772

Post-Partum	1.09	1.12	0.142	0.936	0.422	0.081
Platelets (μL^{-5})						
Pre-Partum	2.61	2.27	12.09	0.340	0.588	0.699
Post-Partum	3.07	3.34	21.52	0.651	0.351	0.527

[1]Treatments: CON, without rbST supply; and 2) rbST, with 250 mg of rbST (Lactotropin, Elanco Animal Health, USA), supplied every 14 d; [2]Standard error of mean; [3]Probabilities: Treatment effect (CON vs rbST); week effect; and treatment by week interaction effect.

ated as follows: NFC = 1,000 – [(CP – CP from urea + urea) + EE + ash + NDF], with all values expressed in g/kg DM. Net energy content and dietary cation and anion difference (DCAD) were estimated according to NRC (2001) model.

Body Condition Score and Body Weight

Body weights were measured weekly after milking and before the morning feeding, using a livestock scale for large animals (Brete ME 2.80; Coimma®, Dracena, Brazil). Body condition score was measured weekly using a five-point system (1 = emaciated to 5 = obese) according to Wildman et al. (1982).

Milk Yield and Composition

Cows were mechanically milked twice daily (0500 and 1600 h). Milk samples were automatically collected (Alpro®) from two consecutive milkings every week. Milk samples were analyzed fresh for fat, protein, and lactose by infrared method (Lactoscan®, Entelbra, Sao Paulo, Brazil). Milk production was corrected to 3.5% of fat (FCM) according to Sklan et al. (1994), in which:

FCM (kg/d) = (0.432 + 0.1625 × milk fat %) × milk yield (kg/d).

Plasma Metabolites and Red and White Cells Counts

Blood samples for plasma metabolites and complete blood cell count were collected weekly, before the morning feeding, by puncture of coccygeal vessels. Blood samples were also collected during the first 24 h after parturition. Samples were collected in 10-mL vacuum tubes for glucose, total cholesterol, triglycerides, total protein, albumin, total calcium, and urea concentrations. After clot formation, samples were centrifuged at 2,000 × *g* for 15 min, and serum was frozen until analysis, the samples were centrifuged and frozen while still on the farm. Concentrations of plasma metabolites were analyzed using colorimetric kits (Bioclin, Belo Horizonte, Brazil) and readings were performed in a spectrophotometer (Bioplus 2000 IL, Barueri, Brazil). Ionized calcium (IC) was calculated according to (Oetzel, 2004) as:

Complete blood cell count was performed within 2 h after blood sampling using a hematologic analyzer (Vet Scan BC-2800®, Bio Brasil, São Paulo, Brazil).

Statistical Analysis

Data were analyzed using PROC MIXED of SAS (version 9.4, version 9.4, SAS Institute Inc., Cary, NC), according to the following model:

$Y_{ijk} = \mu + T_i + a_{j:i} + W_k + T{\times}W_{ik} + + e_{ijk}$,

with $a_{j:i}$ and e_{ijk}; where Y_{ijk} = dependent variable; μ = overall mean; T_i = fixed effect of treatment (i = 1 and 2); $a_{j:i}$ = random effect of animal j within i treatment (j = 1 to 30); W_j = fixed effect of week (k = 1 to 4); $T{\times}W_{ik}$ = fixed effect of interaction between treatment and week of evaluation; e_{ijk} = residual error; = Gaussian distribution; = variance associated with animals random effect; = multivariate normal distribution; R = matrix of variance and covariance due to repeated measures. Akaike's method was used to choose R for each variable. Significance was defined at $P \leq 0.05$.

RESULTS

There was no treatment and time interaction effect ($P \geq 0.115$) on those variables associated with animal's performance (Tables 2 and 3). Animals treated with rbST showed higher ($P \leq 0.008$) DMI and BCS than those animals of CON group, in the post-partum (Table 2). Treatment had no effect ($P \geq 0.179$) on BW, and increased ($P = 0.002$) pre-partum BCS. Administration of rbST improved ($P \leq 0.045$) milk yield and milk fat content (Table 3). However, there is no treatment effect ($P \geq 0.206$) on protein and lactose milk content and daily production.

There was no rbST effect ($P \geq 0.345$) on serum metabolites during pre-partum (Table 4). However, during postpartum, rbST increased ($P \leq 0.044$) serum concentrations of glucose, total cholesterol, and ionized calcium; decreased ($P = 0.022$) triglycerides, without affect ($P \geq 0.367$) total protein, albumin and urea serum content. rbST had no effect ($P \geq 0.136$) on red blood cells, hemoglobin, hematocrit, total leucocytes count, and lymphocyte counts and percentage (Table 5). However, rbST-treatment decreased ($P \leq 0.005$) neutrophils count, without affect ($P \geq 0.245$) neutrophils proportion, neutrophils and lymphocytes ratio, and platelets count in pre- and post-partum periods.

DISCUSSION

In the present study, slow release rbST increased serum glucose and cholesterol of cows during the post-partum period. Sanchez et al. (2014) reported positive effects of rbST application on serum glucose and cholesterol. The latter authors suggested that higher glucose concentration was likely associated with improved gluconeogenesis and DMI. In addition, Sanchez et al. (2014) reported improved milk yield of animals treated with low doses of rbST during transition period, due to higher efficiency of convert propionate into glucose. Increased serum concentration of glucose and insulin in animals treated with rbST have been observed when samples are obtained during peripartum period (Gohary et al., 2014; Gandra et al.2016).

Besides the positive effects on cholesterol, rbST reduced triglycerides concentration. Fat is mainly stored as triglycerides and transported as free fatty acids bound to albumin. Animals treated with rbST showed higher BSC in both evaluated phases, suggesting a less intense fat mobilization. As observed by Pocius and Herbein (1986), rbST improves fatty acids oxidation and reduces free triglycerides serum concentration, as observed in the present study.

The reduction in triglycerides for cows supplemented with rBST is associated with better BCS, showing less mobilization of body reserves, providing better metabolism of glucose and total cholesterol by the liver, thus increasing milk yield, as well as milk fat content.

rbST increased serum ionized calcium concentration. Eppard et al. (1996) supposed that rbST may have increased bone calcium reabsorption. Cows treated with 500 mg of rbST every 14 d, besides no effect on calcium concentration, observed an increased concentration of hydroxyproline, which is considered a bone reabsorption marker. De Morais et al. (2016) reported positive effect of rbST on milk protein synthesis. This effect seems associated with improved protein synthesis in mammary gland and higher amino acids uptake. However, in the present study, rbST showed no effects on milk protein yield (and content) and serum urea concentration.

Reinisch et al. (1996) observed that GH therapy in GH-deficient humans increased release of superoxide anion by neutrophils. In addition, it is well knowledge that insulin-like growth factor (IGF-1) modulates immune cell proliferation (Merimee et al., 1989) and inhibits lymphocytes and neutrophils apoptosis (Baserga et al., 1997).

In the present study, rbST decreased neutrophils count and no others effects were observed on cow's leukogram. However, Silva et al. (2015) supplementing dairy cows with 87.5 or 125 mg of rBST injections were administered every 7 d from −21 to 28 d relative to calving, did not observe any influence of treatments on white cell counts. In addition Silva et al. (2017) using the same dose of rBST in cows in peripartum observed decreased the incidence of uterine disorders in Holstein and Jersey dairy cows and increased yield of ECM during the first 30 DIM, despite slightly increasing the incidence of ketosis.

Besides potential positive effects on immune function, effects could be considered dose-dependent (Gohary et al., 2014). Increasing doses of rbST showed quadratic effect on cytokine production (Zarkesh-Esfahani et al., 2000), and lymphocyte proliferation (Bozzola et al., 1989). Therefore, level of the present study (250 mg) seems higher than optimal dose for optimize immune function.

Putnam et al. (1999) observed that injection of somatotropin in the pre-partum period can also lead to increased DMI in the early postpartum period. In the present study, this higher DMI is connected with increased BCS. Previous studies (Putnam et al., 1999; Gulay et al., 2003) reported that cows treated with a low dose of rbST in the peripartum has a better BCS recovery during early lactation, even though they produced more milk.

In the present study, rbST increased 2.4 kg/d (10.7%) milk yield of cows. Additionally, it was observed an 10.8% (3.7 g/kg) increase in milk fat content. Silva et al. (2017) reported greater yields of milk, fat, and energy corrected milk for cows treated with 125 mg of rbST than cows of control. Evaluating late pregnant heifer's treatment with rbST, Schneider et al. (2012) observed 2.8 kg/d increased milk yield during the first seven weeks of lactation. According to Bauman (1992), the main mechanism of rbST effect on milk yield involves increased lipolysis, nutrient prioritization to the mammary gland, and mammary gland epithelial cells proliferation. In general, treatment of pre-partum and periparturient cows with rbST has shown to increase yields of milk and energy corrected milk (Putnam et al., 1999; Gohary et al., 2014).

CONCLUSION

The supply of rbST during transition period improves dairy cows DMI and performance, with positive effects on metabolites concentration and small effects on blood cells count, therefore we recommend using rBST for cows in the transition period

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Jefferson R. Gandra - Design, administration, data analysis, writing. Tiago A. Dell Vale- data analysis, scientific writing. Caio S. Takiya- Scientific translation and writing Erika R. S. Gandra- Conception, resource management and laboratory analysis. Euclides R. Oliveira- Conception, acquisition of resources, scientific writing. Rafael H. T. B. Goes- Conception, acquisition of resources, scientific writing. Isabelle Z. Noia- Conducting an experiment on the farm, laboratory analysis, raw data processing. Cibeli A. Pedrini- Conducting an experiment on the farm, laboratory analysis, raw data processing. Giovana S. Urio- Conducting an experiment on the farm, laboratory analysis, raw data processing. Jamille D. O. Batista- Conducting an experiment on the farm, laboratory analysis, raw data processing. Giovani Antônio- Conducting an experiment on the farm, laboratory analysis, raw data processing. Anderson P. Acosta- Conducting an experiment on the farm, laboratory analysis, raw data processing.

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