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Research article

Productive, biochemical, behavioral, and feeding parameters of Holstein cows treated with two formulations of recombinant bovine somatotropin

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ABSTRACT: This work aimed to compare the efficiency of two commercial recombinant bovine somatotropin (rbST) formulations on productive, metabolic, behavioral, and feeding parameters of mid-lactation Holstein cows. Eighteen secondiparous cows were randomly assigned to two groups to be treated with injectable rbST (rbST-Fast, Boostin®, MSD Saúde Animal; and rbST-Slow, Lactotropin®, Agener União Saúde Animal). Cows were rbST-treated during five cycles of 14 days each, totaling 70 days. Blood samples were collected thrice in each cycle to assess metabolic markers. Daily, automatic feeders and individual monitoring collars measured behavior and feed intake. Milk samples were collected weekly to evaluate milk composition and somatic cell count. The rbST-Fast group had higher milk production (p = 0.03) and tended to present greater feed intake (p = 0.07). In addition, animals treated with rbST-Fast had higher (p < 0.01) concentrations of non-esterified fatty acids (NEFA) and tended (p = 0.09) to have lower serum glucose values. As for the variation in body weight, cows treated with rbST-Fast lost approximately three times more weight (p < 0.01) than rbST-Slow cows. Regarding milk components, cows from the rbST-Fast group produced milk with a higher lactose content (p = 0.05). In conclusion, rbST-Fast treated cows produced more milk, had higher feed intake, and showed a higher degree of lipid mobilization, demonstrated through the higher body weight loss and higher NEFA concentrations.

Keywords: feed intake, growth hormone, milk production, non-esterified fatty acids

Introduction

Recombinant bovine somatotropin (rbST) is used mainly to increase the productivity of dairy cattle (van Amburgh et al., 1997; St-Pierre et al., 2014). The mechanisms by which this hormone induces galactopoiesis are complex, and involve multiple factors. For instance, rbST produces a homeorhetic effect involving metabolic shifts mainly in the adipose, hepatic, and mammary gland tissues.

Treatment with rbST induces hepatic gluconeogenesis and causes a shift in glucose usage from peripheral tissues to lactose synthesis in the mammary gland (Baumgard et al., 2017). Conversely, changes in the adipose tissue depend on the energy balance of the animal at the beginning of the rbST treatment. Lipolysis induction or lipogenesis reduction can occur; however, both effects are usually observed (Lanna et al., 1995). Moreover, rbST affects proliferation and apoptosis in the mammary gland, leading to a more pronounced persistency and extended lactation after its peak. Accordingly, rbST applications are most frequently initiated after peak of lactation (Bauman, 1992; Valente et al., 2011).

The dry matter intake (DMI) increase in rbST supplemented animals has been demonstrated (Dohoo et al., 2003; Paula and Silva, 2011). Metabolic adaptations are, therefore, possible in this context. Since commercial rbST products have distinct delivery mechanisms, the peak of milk yield and DMI within the rbST cycle also varies. Therefore, it is essential to know the action mechanism of each product and the moments when metabolic and behavioral repercussions occur (Gulay et al., 2004).

Two rbST formulations commercially available in Brazil differ mainly in the vehicle. Recombinant bovine somatotropin rbST-Fast (Boostin®, MSD Saúde Animal) contains lecithin and vitamin E, making it an aqueous solution with a faster release and prompter action than rbST-Slow (Lactotropin®, Agener União Saúde Animal), which contains sesame oil and zinc, which promotes a slower release and a later action due to its lipid nature (Gómez et al., 2022).

The present study aimed to compare two commercial formulations of rbST on milk yield, milk composition, feed intake, feeding behavior, and serum biochemical parameters of mid-lactation Holstein cows. Our hypothesis is that cows treated with rbst-Fast have greater milk production, which is partially explained by the higher DMI as well as by a higher degree of adipose tissue mobilization.

Materials and Methods

Animal housing

All animal procedures were approved by the Animal Ethics and Experimentation Committee of the Universidade Federal de Pelotas under code 14131.

The experiment was conducted on a dairy farm with approximately 400 lactating cows in the municipality of Rio Grande, located in the southernmost part of Rio Grande do Sul state (Brazil) (coordinates 32°16′ S, 52°32′ W, 7 m altitude). The herd is milked twice a day in a her-ringbone milking parlor. The animals are kept in a compost barn system, receiving a total mixed ration (TMR) twice a day. The experimental diet and its composition are described in Table 1.

Experimental animals

For this trial, the experimental animals initially had to meet some selection criteria: Holsteins, second lactation, and between 90 and 210 days in milk (DIM). Experimental cows were blocked by average milk production in the previous two weeks and by DIM. Body condition score (BCS), body weight (BW), and reproductive status (pregnant/not pregnant) were very similar between the experimental groups. The rbST-Fast group had 36.1 kg d^{-1}, 147 DIM, 655 kg BW, 3.22 BCS, and two pregnant and seven inseminated animals at the beginning of the trial, whereas the rbST-Slow group had 36.1 kg d^{-1}, 152 DIM, 676 kg BW, 3.56 BCS, and two pregnant and seven inseminated animals.

Based on these criteria, we selected 18 secondiparous cows between 90 and 210 DIM, with an average milk production of 36.1 kg d^{-1}, two weeks before the beginning of the experiment. Experimental animals were distributed randomly into two treatment groups differing only in the rbST brand that was administered (rbST-Fast [n = 9] or rbST-Slow [n = 9]). Both rbST-Fast and rbST-Slow groups received a dose of 500 mg rbST every 14 days (35.7 mg d^{-1}), totaling five applications and 70 days of trial. The injections were applied subcutaneously in the ischiorectal fossa.

Clinical and productive evaluations

Clinical exams were carried out once every 14 days on all animals, which included BW and BCS determinations, heart and respiratory rates, rumen motility, rectal temperature, visual evaluations of mucosal coloration, and time to capillary filling.

The BCS was obtained from two trained independent evaluators using the 1 to 5 scale (1 = thin and 5 = obese) (Wildman et al., 1982). The BW was estimated using a cattle weight band positioned behind the scapulohumeral joint to determine the thoracic perimeter. The first rbST cycle was used as a covariable for BW and BCS variables.

Daily milk production was measured electronically for each animal using the DelPro™ (DeLaval®) software throughout the 70 days of this trial. Composite milk samples were obtained on days 4 and 11 after each rbST injection and stored at 4 °C in flasks with milk preservative bronopol. The samples were sent to the Laboratório de Análise de Leite (PARLEITE) of the Associação Paranaense de Criadores de Bovinos da Raça Holandesa (APCBRH). The milk components fat, total protein, lactose, total solids, and casein contents, milk urea nitrogen (MUN), and somatic cell count (SCC) were analyzed in a NexGen® (Bentley®) automated equipment.

Table 1 – Ingredients and nutrient composition of experimental diet.

Item	Experimental diet
Ingredient, % of DM	
Corn silage	49.33
Ryegrass haylage	7.18
Soybean meal	10.61
Corn grain ground	9.52
Soy hulls	8.79
Wheat bran	3.49
Expeller soybean meal[1]	2.83
Defatted rice bran	2.49
Sorghum grain, high-moisture	2.00
Rumen-inert fat[2]	0.97
Limestone	1.20
Sodium bicarbonate	0.55
Urea	0.39
Salt	0.30
Mineral-vitamin premix[3]	0.14
Magnesium oxide	0.078
Dicalcium phosphate	0.046
Mycotoxins' adsorbent[4]	0.046
Live yeast[5]	0.046
Ionophore[6]	0.007
Nutrients, % of DM	
DM	52.55
NDF	36.97
peNDF, > 1.18 mm	21.29
ADF	20.14
CP	15.94
RDP	10.09
RUP	5.85
MP, g d^{-1}	2,921
NFC	38.38
Starch	25.83
Fat	3.47

[1]SoyPass (Cargill®). [2]Nutri Gordura Lac (Nutricorp®). [3]Contained a minimum of 210 g kg^{-1} Ca, 210 mg kg^{-1} Co, 10,000 mg kg^{-1} Cu, 500 mg kg^{-1} I, 25,000 mg kg^{-1} Mn, 250 mg kg^{-1} Se, 42,000 mg kg^{-1} Zn, 2,500 UI kg^{-1} of vitamin A, 638 kUI kg^{-1} of vitamin D_3, 17,041 UI kg^{-1} of vitamin E, and 867 mg kg^{-1} of biotin. [4]Mycosorb (Alltech®). [5]Milk-Sacc Plus (Alltech®). [6]Rumensin 20 (Elanco Brasil®). DM = dry matter; peNDF = physically effective neutral detergent fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein; RDP = rumen degradable protein; RUP = rumen undegradable protein; MP = microbial protein; NFC = non-fibrous carbohydrate.

Biochemical analyses

Blood samples were obtained on days 1, 4, and 7 post rbST injections after cows were milked in the morning. Blood samples on days 4 and 7 were chosen because a previous reference (Almeida and Viechnieski, 2011)

showed that the peak milk yield within the rbST cycle occurs on the fourth day for rbST-Fast cows and on the seventh day for rbST-Slow cows. Samples were collected from the coccygeal vein in two tubes containing a clot activator for the analyses of non-esterified fatty acid (NEFA), albumin, and beta-hydroxybutyrate (BHB). Samples were harvested in sodium fluoride tubes for blood glucose measurements. All blood samples were centrifuged at 58.33 Hz for 10 min immediately after collection to separate serum and plasma. Each sample fraction was stored in 1.5 mL Eppendorf tubes at –20 °C.

The colorimetric analyses of albumin were carried out using the commercial kit Labtest Diagnóstica S.A. NEFA, and BHB were measured according to the method described by Ballou et al. (2009) using the kits Wako NEFA-HR (Wako Chemicals) and Ranbut (Randox® Laboratories Ltd), respectively. The glucose analyses were carried out using the colorimetric method with enzymatic kits (Labtest Diagnóstica S.A.) according to the methodology specified by the manufacturer. These metabolites were determined using the automatic biochemical analyzer (LabmaxPlenno®).

Intake and eating behavior data

The amount of feed consumed by each animal was measured daily and individually using smart feeding devices (Intergado®). Animal behavior was evaluated with monitoring collars (ChipInside®) that indicate activity, rumination, and resting time (min d^{-1}) of each animal.

Feed sampling and bromatological analyses

To estimate the dry matter (DM) content, 100 g of corn silage and haylage were collected daily, whereas TMR samples were collected twice daily (morning and afternoon meals). The DM content was analyzed using the Koster Moisture Tester (Koster Moisture Tester Inc).

During the experimental period, silage and haylage samples (± 300 g) were obtained twice weekly by sampling five different points for bromatological analysis. A small portion of TMR was sampled twice daily to compose the weekly pool for the bromatological analyses.

Feed samples were sent to the Laboratório de Nutrição of the Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC-UFPel). Samples were pre-dried in a forced air incubator at 55 °C for 72 h and then ground in a Wiley Miller stationary mill 1-mm sieve. To analyze DM content, a fraction of the ground sample was dried at 105 °C for 8 h in kiln (Easley et al., 1965). For the organic and mineral matter analysis, another fraction of the ground sample was weighed and placed in a muffle furnace for 2 h at 600 °C. After reaching room temperature, the sample was weighed again (AOAC, 1995).

Total protein content was determined using a modified Kjeldhal method (AOAC, 1995) to measure the nitrogen amount. The modifications comprised the use of a 4 % (p v^{-1}) boric acid solution as the free ammonium receptor during distillation, a 0.2 % (p v^{-1}) bromocresol green solution with 0.1 % methyl red as the indicator, and a standard sulfuric acid solution for titration as described by Kozloski et al. (2003). The method Van Soest and Robertson (1985) described was used to analyze neutral detergent fiber and acid detergent fiber corrected for ash and acid detergent lignin. Neutral detergent insoluble nitrogen and acid detergent insoluble nitrogen were determined according to Licitra et al. (1996).

Core body temperature and temperature-humidity index

The internal temperature was measured using a portable thermometer (iButton DS1922L, Embedded Data Systems) attached to an intravaginal device that registered temperature in a 30 min interval. In the first cycle, intravaginal thermometers were introduced to 18 animals. However, due to farm management requirement, at each end of the cycle, the thermometers were removed, cleaned and reintroduced to five cows that did not have vaginitis after clinical examination, throughout the five cycles.

An automated weather station was installed near the animal housing to monitor environmental conditions. The registrations of ambient temperature (AT) and relative humidity (RH) were used to calculate the Temperature-humidity index (THI), provided 24 h d^{-1} by the software Cowmed® (C-tech/Chip Inside Engineering and Technology).

Statistical analysis

Data were analyzed by the MIXED procedure for repeated measures in the SAS software (SAS v.9.4 Institute Inc). We included treatment, time (days, weeks, or cycles), and their interactions as fixed effects. Blocks and cows within treatment were included as random effects. Four structures of the error covariance matrices (auto-regressive of first-order [AR(1)], compound symmetry [CS], unstructured [UN], and Toeplitz [TOEP]) were tested for each dependent variable and the best fit was chosen based on the lowest Bayesian information criteria. The Univariate procedure was used to assess data normality. Variables with non-normal distributions were converted to a log scale. Data collected before the experiment began was used as covariable. Effects were considered when $p \leq 0.05$ and tendency when $0.05 < p \leq 0.10$.

Results

The timeline of all experimental measurements was demonstrated over an application cycle (Figure 1). Animals in the rbST-Fast group produced more milk (*p*

= 0.03), averaging 40.75 ± 0.47 kg of milk d^{-1} compared to the rbST-Slow group, which averaged 39.08 ± 0.45 kg d^{-1} (Figure 2). There was a trend (p = 0.07) in DMI with the rbST-Fast group showing a tendency for higher DMI (25.98 ± 0.33 kg d^{-1}) than the rbST-Slow group (25.02 ± 0.33 kg d^{-1}) (Figure 3).

The rbST-Fast treated animals showed higher (p < 0.01) plasma NEFA concentrations in comparison to rbST-Slow, 0.61 vs 0.43 ± 0.03 mmol L^{-1} (Table 2 and Figure 4). Conversely, glucose concentrations tended toward lower values (p = 0.09) in the rbST-Fast group than rbST-Slow, 59.99 vs 62.34 ± 0.86 mg dL^{-1} (Table 2 and Figure 5). Albumin and BHB concentrations evaluated were not different (p > 0.10) between groups. However, albumin and BHB concentrations showed a tendency and effect between groups over time, (Table 2).

Animals treated with either rbST-Fast or rbST-Slow presented similar (p > 0.10) behavioral patterns (Table 3). However, rest and rumination times showed an effect between groups over time.

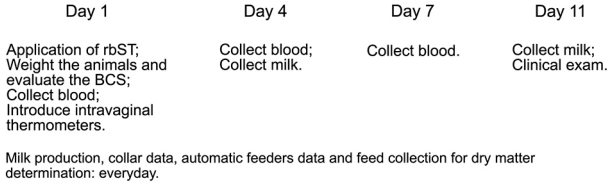

Figure 1 – Timeline of all experimental measurements over an application cycle. rbST = recombinant bovine somatotropin; BCS = body condition score.

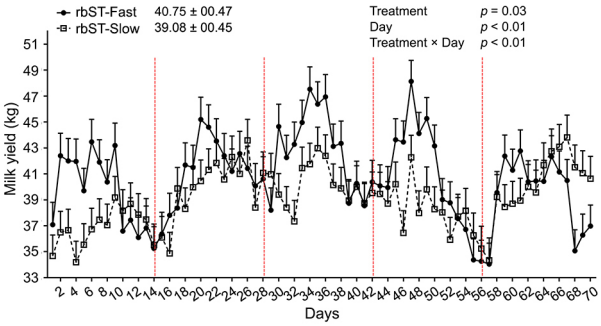

Figure 2 – Means ± standard errors for milk production in animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

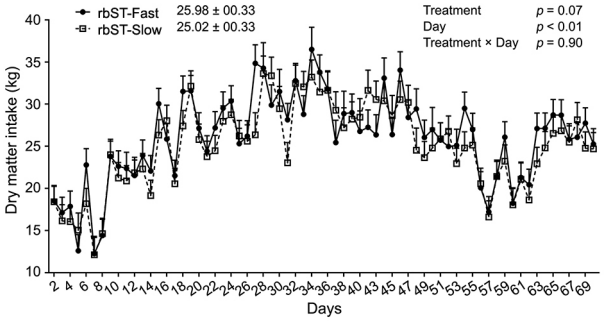

Figure 3 – Means ± standard errors for dry matter intake in animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

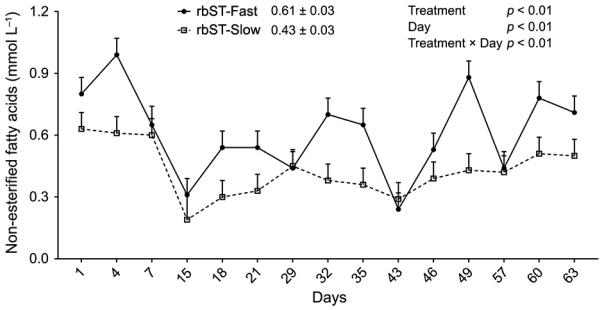

Figure 4 – Means ± standard errors for plasma non-esterified fatty acids concentrations of animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

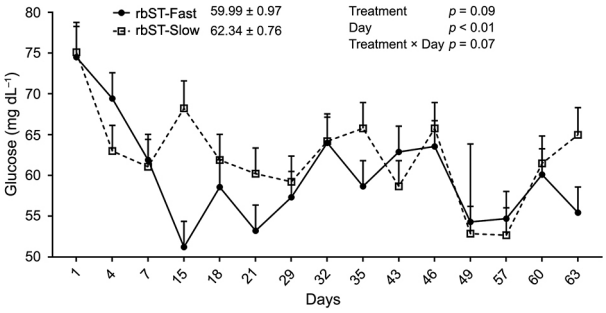

Figure 5 – Means ± standard errors for plasma glucose concentrations of animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

Table 2 – Means ± standard errors of biochemical parameters assessed in animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

	Treatment		SEM	*p*-value		
	rbST-Fast	rbST-Slow		Treatment	Day	Treatment × Day
NEFA	0.61	0.43	0.03	< 0.01	< 0.01	< 0.01
BHB	0.47	0.46	0.03	0.69	< 0.01	< 0.01
Glucose	59.99	62.34	0.86	0.09	< 0.01	0.07
Albumin	3.37	3.22	0.09	0.29	< 0.01	< 0.01

SEM = standard error of mean; NEFA = non-esterified fatty acids = mmol L^{-1}; BHB = β-hydroxybutirate = mmol L^{-1}; Glucose = mg dL^{-1}; Albumin = g dL^{-1}.

Table 3 – Means ± standard errors for activity, rumination, and resting times of animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

	Treatment		SEM	*p*-value		
	rbST-Fast	rbST-Slow		Treatment	Day	Treatment × Day
Activity	209.3	172.7	18.0	0.17	< 0.01	0.77
Rest	527.6	576.3	22.4	0.14	< 0.01	< 0.01
Rumination	667.4	689.2	20.2	0.46	< 0.01	< 0.01

SEM = standard error of mean; Activity, rest, and rumination = min d^{-1}.

Table 4 – Means ± standard errors of BW and BCS of animals treated with 500 mg of two commercial recombinant bovine somatotropin (rbST) formulations every 14 days for 70 days.

	Treatment		SEM	*p*-values		
	rbST-Fast	rbST-Slow		Treatment	Cycle	Treatment × Cycle
BW, kg	687.10	704.75	5.14	0.05	< 0.01	0.63
BCS, unit	3.01	3.11	0.10	0.56	0.19	0.77
Change in BW	–20.67	–5.44	3.02	< 0.01	-	-
Change in BCS	–0.28	–0.22	0.04	0.30	-	-

SEM = standard error of mean; BW = body weight = kg; BCS = body condition score = 1 to 5 scale, with 0.25 intervals; Change in BW = body weight variation; Change in BCS = BCS variation.

Table 5 – Means ± standard errors of milk components, MUN and SCS linear score for rbST-treated animals.

	Treatment		SEM	*p*-values		
	rbST-Fast	rbST-Slow		Treatment	Week	Treatment × Week
Fat	4.44	4.06	0.19	0.20	0.32	0.72
Protein	3.17	3.24	0.05	0.37	< 0.01	0.08
Lactose	4.50	4.37	0.04	0.05	< 0.01	0.16
Total Solids	13.06	12.58	0.23	0.18	0.07	0.69
Casein	2.51	2.58	0.04	0.22	< 0.01	0.44
MUN	12.39	12.38	0.45	0.99	< 0.01	0.99
SCS	3.45	4.37	0.63	0.33	0.95	0.39

rbST = recombinant bovine somatotropin; SEM = standard error of mean; Fat, protein, lactose, total solids and casein = g 100 g^{-1}; MUN = milk urea nitrogen = mg dL^{-1}; SCS = somatic cell score = 0 to 9 scale.

Cows from the rbST-Slow group were heavier (p = 0.05) than rbST-Fast animals; 704.8 vs 687.1 ± 5.1 kg. The rbST-Fast treated animals lost more weight (p < 0.01) than rbST-Slow treated animals; –20.7 vs –5.4 ± 3.0 kg. Nonetheless, BCS varied similarly (p = 0.30) between both groups (Table 4).

Milk from rbST-Fast treated cows had higher (p = 0.05) lactose concentrations than rbST-Slow treated animals; 4.50 vs 4.37 ± 0.04 g 100 g^{-1}. The remaining milk components were similar (p > 0.10) between groups (Table 5).

Mean internal temperatures were similar (p = 0.29) between groups, 38.83 ± 0.05 °C for rbST-Fast and 38.75 ± 0.05 °C for rbST-Slow cows.

Discussion

In this experiment, milk production was 1.67 kg d^{-1} higher in the rbST-Fast group than in the rbST-Slow group. This data is consistent with Almeida and Viechnieski (2011) results. Conversely, milk yield increased in rbST-Slow treated animals compared to rbST-Fast and non-rbST supplemented cows (De Morais et al., 2017).

The standard response to rbST is an immediate increase in milk production a few days after injection, possibly due to the homeorhetic effects of this hormone, which culminate in the redistribution of nutrients to target tissues (Bauman et al., 1987). Typically, especially in rbST-Fast treated cows, a maximum response is observed within the first week after injection. Milk production returns to its original levels 14 days after the hormone delivery (Rennó et al., 2006).

The higher DMI by rbST-Fast treated animals can be partially explained by the fact that these animals had a higher milk production than rbST-Slow treated cows; 25.98 vs 25.02 kg DM d^{-1}, respectively. This result was expected since an increase in productivity causes an increase in energy demand, leading to higher feed intake (Soliman and El-Barody, 2014).

To our knowledge, this is the first trial that compared DMI from cows treated with the two commercial rbST formulations. According to the meta-analysis, which evaluated 53 studies with rbST treatment, feed consumption in treated cows increased by approximately 1.5 kg d^{-1} in relation to untreated cows (Dohoo et al. (2003). Similarly, milk production was higher in treated animals, accompanied by higher feed intake (Paula and Silva, 2011). In the experiment, individual feed intake was not measured (De Morais et al., 2017); however, there was no change in feed

consumption after hormonal treatment with 56 to 700 mg for 14 days (Downer et al., 1993) or 29 mg d^{-1} (Binelli et al., 1995).

Other studies reported increased feed intake using rbST a few days (Almeida and Viechnieski, 2011) or weeks (Chalupa et al., 1996) post-injection. The increase milk production depends on the diet density. The results of this trial are consistent with the meta-analysis, which states that cows treated with rbST increase voluntary feed intake to sustain the increase in milk production (St-Pierre et al., 2014). Unlike other studies that estimate feed intake, this experiment provides actual and daily intake values. This was achieved using intelligent feeding devices that allow individual, uninterrupted, and precise feed intake measurements during the entire experiment.

Consistent with the higher milk yield, animals in the rbST-Fast group also showed higher plasma NEFA concentrations, particularly in the first week after rbST injection. This data indicates a higher lipid mobilization due to the nutritional demand on the mammary gland, particularly in rbST-Fast supplemented cows (Bauman, 1992).

Any increase in milk productivity raises energy demand, which in turn impacts the animal's energy balance. Thus, lipid mobilization becomes a viable alternative when the animal is in a negative energy balance (Bauman, 1992).

Lipid mobilization is considered high when blood concentrations of NEFA are above 0.7 mmol L^{-1} for cows immediately after calving (Ospina et al., 2010; Chapinal et al., 2012). However, this mobilization of adipose tissue is physiological when milk production increases faster than feed intake at the beginning of lactation (Reist et al., 2003; Walsh et al., 2007; Wathes et al., 2007). The high NEFA concentrations in the experimental cows, notably on rbST-Fast treated animals (mean 0.61 mmol L^{-1}), are particularly interesting since they suggest an unusual, although modest, lipid mobilization for mid-lactation cows.

A similar effect of rbST was observed since the hormonal treatment culminated in decreased body fat and increased NEFA concentrations in the bloodstream, as found by Binelli et al. (1995). rbST modifies the nutrient distribution among tissues, leading to increased productivity in dairy cows (Bauman et al., 1985). The lipolysis rate is directly affected since rbST reduces glucose usage by peripheral tissues, prioritizing its use by the mammary gland for lactose production (Peel and Bauman, 1987). Decreasing glucose availability increases fatty acid mobilization from the adipose tissue; therefore, it can be used by other tissues (Gluckman et al., 1990). Somatotropin stimulates lipolysis and gluconeogenesis, providing higher glucose availability in the mammary gland (Capuco and Akers, 2011). This process is highlighted in our results.

The current experiment showed no increase in BHB without differences between treatments, indicating that the liver could oxidize the circulating NEFA completely or export VLDL satisfactorily, implying moderate lipid mobilization in treated animals. Furthermore, rbST treatment promoted a slightly negative energy balance in the treated animals but without causing accumulation of ketone bodies, which could, in turn, lead to a subclinical ketosis state. The low concentrations of BHB (< 0.5 mmol L^{-1}) observed for both treatments are consistent with this statement. Therefore, using rbST seems to gradually shift lipid metabolism directing the additional energy provided by increased feed intake toward prioritized tissues.

An increase in milk production raises energy demand, which induces the animal to use its reserves to maintain production (NRC, 2001). Reduced glucose concentrations in supplemented animals are expected since it is essential for milk production once the mammary gland uses 60 to 80 % of the glucose derived from gluconeogenesis (Bauman, 1992). Thus, we consider the lower glucose concentrations observed in rbST-Fast treated animals a consequence of increased milk production in this group. This data is consistent with a previous experiment with a similar result (Abdelrahman et al., 2010).

The nutritional state is the main factor interfering with the effectiveness of bST (Bauman et al., 1992). We observed a higher weight loss in rbST-Fast than in rbST-Slow treated animals (20.7 vs 5.4 kg, respectively), although without an equivalent change in the BCS. This result is partially in agreement with the meta-analysis, which states that the BW of rbST-treated animals is lower than the BW of control animals (Dohoo et al., 2003). Conversely, other studies observed no effect of rbST treatment on BW (Huber et al., 1997; Tarazon-Herrera et al., 2000; De Morais et al., 2017). Furthermore, these authors report the challenges of identifying BW variations in experiments with rbST, since positive energy balance is one of the prerequisites to the success of rbST supplementation.

We expected BCS variations to match the higher BW losses observed in rbST-Fast treated animals. Therefore, the BCS may be skewed in our experiment, due to the subjectivity of the method used to evaluate this parameter. Both BW loss and BCS reduction may occur due to increased nutritional demand imposed by the surge in milk production (NRC, 2001). An increase in milk production leads to a negative energy balance in dairy cattle, followed by lipid mobilization (Xu et al., 2020).

The milk composition also varied between the two groups in this experiment. Higher lactose concentrations were observed in the rbST-Fast treated animals compared to rbST-Slow treated animals (4.50 vs 4.37 g 100 g^{-1}, respectively). Although both values are lower than we expected, they may be associated with the intermediate SCC values from both groups. These results diverge from previous studies in which milk components were not affected by rbST treatment (Binelli et al., 1995; Chalupa et al., 1996).

Nevertheless, given the small number of experimental animals and weekly milk sampling, this experiment was not designed to detect differences in milk composition between groups. Future studies should address these questions more thoroughly. Mainly, subsequent experiments could elucidate whether the non-difference (approximately 0.38 %) in milk fat content we observed between groups could be reproducible. Moreover, investigations should be carefully designed to detect increases in the long-chain fatty acid content of the milk, mainly C18:1 cis-9, which is usually associated to increases in lipid mobilization (Hanuš et al., 2018).

The behavioral patterns (activity, rest, and rumination time) of animals were not affected by either form of commercial rbST. Dado and Allen (1994) suggest that rumination time is the main factor positively associated to milk yield; however, Stone et al. (2017) observed a weak correlation between these two variables.

Internal temperatures remained within physiologically acceptable values (38.83 °C in the rbST-Fast group vs 38.75 °C in the rbST-Slow group). There is a strong correlation between milk production and increased internal temperatures (Kadzere et al., 2002). Consequently, the risk for heat stress is elevated in highly productive animals in relation to those with lower yields (Collier et al., 1982). Therefore, rbST ultimately leads to elevated body temperatures since it induces feed intake and metabolic activity, increasing milk production (Fike et al., 2002). Nevertheless, rbST-Fast animals in the current experiment did not experience elevated body temperatures, even though they ingested more feed and produced more milk. This is possibly due to the suitable conditions for thermal comfort in the animal housing facilities, even considering that this trial was conducted during summer.

In conclusion, rbST-Fast treated cows produced more milk, had higher feed intakes, and showed elevated levels of lipid mobilization, which was demonstrated through the higher weight loss and higher NEFA concentrations.

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Authors' Contributions

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