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# Effect of recombinant bovine somatotropin application intervals on Girolando cows' milk production and composition

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**The aim of this study was to evaluate the effect of recombinant bovine somatotropin (rBST) application intervals on chemical composition of milk from Girolando cows with productivity below 20 L/milk/day and animals with productivity above 20.1 liters/milk/day. The study included 30 Girolando cows with production ranging from 13 to 28 L/milk/day. Cows were submitted to two milkings: 06:00 am and 04:00 pm. Milk samples were obtained at the end of the first milking. With the aid of individual meters, 40 mL of milk were collected in bottles containing preservative bronopol. On the day of collections, the individual milk production of each animal was notes. The experiment was performed on a 3x2 factorial design. Factor A corresponded to rBST application intervals divided into three levels (intervals) of rBST application every 10, 12 and 14 days, respectively. Factor B corresponded to the production levels of animals, divided into two levels: animals with production above 20.1 L/milk/day and animals with production less than 20 L/milk/day. About 500 mg rBST were subcutaneously applied in previously disinfected ischiopubic-rectal fossa. On the first day, all animals received rBST, the aplications were respected for planned application range for each group of animals. The Application interval of 14 days was better for animals with productivity below 20 L/milk/day and for animals with higher production, the best rBST application interval was 10 and 12 days. Application interval of 10 days resulted in higher fat content in milk from animals with lower productivity, and fat and protein contents were higher for animals with lower productivity in all application intervals.**

**Key words:** Growth hormone, production increment. milk quality.

## INTRODUCTION

One of the technologies that can be used to obtain productivity gains in dairy herds is recombinant bovine somatotropin (rBST) for lactating cows (Rennó et al., 2006). Studies have shown that treatment with an

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**Abbreviations: DDE,** Defatted dry extract; **rBST,** recombinant bovine somatotropin.

extended-release formulation containing 500 mg rBST at intervals of 14 days can increase milk production from 3 to 5 kg/day, on average (Collier et al., 2001). The mechanism of action of somatotropin on the mammary gland occurs indirectly by increase factor concentration of the insulin-like growth (IGF-1), compound that plays a fundamental role in the control of metabolism and physiological processes in cattle (Gulay and Hatipoglu, 2005). rBST acts in the organism of cattle by increasing the availability of nutrients from the diet offered to the animal and directing these nutrients to the mammary gland, which can occur through a variety of rBST actions, among these, decreased lipogenesis and lipolysis stimulation, increasing the availability of lipids (Etherton and Bauman, 1998), reduced insulin activity, inhibiting gluconeogenesis in tissues (Knapp et al.,1992), providing additional glucose to the udder, increasing the mammary blood flow and improving the efficiency in the use of amino acids (Davis and Collier, 1985). The pattern of response to the use of rBST is the gradual increase in milk production a few days after application, and when rBST application ceases, milk production gradually returns to previous levels observed at the beginning of application. If treatment is continued, increased milk production is maintained (Bauman et al., 1985). Thus, rBST application in dairy cows influences milk production and the lactation curve shape (Luna-Dominguez et al., 2000). It is known that rBST application significantly increases milk production in well-nourished and healthy cows (Lucci et al., 1998). The use of rBST should begin soon after lactation peak of the animals, close to 60 days postpartum and continue until seven months of lactation. This hormone is normally administered every 14 days; however, a drastic reduction in its effect is observed in days near the subsequent application. Thus, many cattle breeders are reducing the period between applications so that this drop in productivity occurs less sharply or does not occur at all.

The aim of this study was to evaluate the rBST application intervals at 10, 12 and 14 days, and the financial viability of the application in Girolando cows with productivity below 20 L/milk/day and animals with productivity above 20.1 L/milk/day, and to evaluate the changes in chemical composition of the milk at different rBST application intervals.

## MATERIALS AND METHODS

This study was conducted on November and December 2012 in the experimental area of the Federal Institute of Goiás, campus Rio Verde, GO, Brazil, 17°48'28"S and 50°53'57"W, mean altitude 720 m. The climate is classified according to Köppen (Castro Neto, 1982) as Aw (tropical), with rain in the months of October to May and drought from June to September. The average annual temperature is 20°C at 35°C and precipitation ranges from 1500 to 1800 mm annually. The farm had handling pens, milking room with expansion tanks with individual capacity for storing 4000 L of milk and shed for storage of supplies and shelter of farming machinery.

**Table 1.** Percentage composition of diet offered to girolando cows submitted to recombinant bovine somatotropin (rBST) application.

| Total diet ingredients | % |
|---|---|
| Corn silage | 48.13 |
| Corn germ | 24.24 |
| Soybean meal 44% | 6.28 |
| Corn grits | 5.04 |
| Cotton cake | 7.14 |
| Uremax | 0.55 |
| Optigem | 1.10 |
| Cooked soybean core | 7.52 |
| Total | 100 |

The 2x6 herringbone milking room had closed circuit with high-line piping system with feeders in every containment, six teatcup sets and individual milk meters. Thirty (30) Girolando cows with production ranging from 13 to 28 L of milk/day at intermediate lactation stages participated in the study. All animals had the same feeding, water and breeding environment. Of the 30 animals selected for the experiment, 10 cows had rBST application interval of 10 days, 10 cows had rBST application interval of 12 days and 10 cows interval of 14 days. During the experiment, the animals were fed during milking (twice daily): 4 kg of feed, distributed into two milkings and 8 kg of feed and corn silage *ad libitum* in trough outside the milking parlor in the range of first for the second milking. The feed consisted of corn germ, soybean meal, corn grits, cotton cake, uremax, optigem and cooked soybean core, according to Table 1.

Cows were fed with pasture composed of Mombasa grass, with protein concentration ranging from 8 to 12%; the animals had access to pasture for a period of 10 h, that is, after the second milking the animals were allowed to graze. About 2.0 ml of a formulation containing 500 mg rBST was subcutaneously applied in the previously-sanitized ischiopubic rectal fossa. On the first day, all animals received the rBST formulation; further aplications were complied with the application interval established for each group of animals; in other words, 10 animals at interval of 10 days, 10 animals at interval of 12 days and 10 animals at interval of 14 days. Cows were milked twice daily, with the first milking at 06:00 am and the second at 04:00 pm. During milking, disposal of the first three jets in the black background mug was performed for identification of clinical mastitis. Subsequently, teats were immersed in a sodium hypochlorite solution (pre-dipping) with thorough drying using paper towel for the coupling of teatcups. After complete and uninterrupted milking, teatcups were removed and teats were immersed in 5% iodine solution (post-dipping) and release of animals for silage supply.

Milk samples were obtained at the end of the first milking. With the aid of individual meters, milk contained in the milk meter was previously agitated for five seconds; then, 40 mL of milk was collected into a flask containing preservative bronopol, previously identified with barcode corresponding to each animal. Samples were collected at intervals of one day. On the same day of collections, individual milk production of each animal was recorded and data were transferred to spreadsheets, which were subsequently used in statistical analyses. After collection, milk samples were packed in isothermal boxes containing ice and sent to the Laboratory of Animal Products of Goiás, Rio Verde Campus, to be stored at approximately 4°C. Then, flasks containing milk samples were sent to the Laboratory of Milk Quality - Food

**Table 2.** Treatments, productivity and recombinant bovine somatotropin application intervals.

| Treatment | Productivity (liters of milk/d) | Application interval |
|---|---|---|
| 1 | | 10 |
| 2 | Less than 20 L/day | 12 |
| 3 | | 14 |
| 4 | | 10 |
| 5 | More than 20.1 L/day | 12 |
| 6 | | 14 |

**Table 3.** Financial viability resulting from the recombinant bovine somatotropin application at intervals of 10, 12 and 14 days and milk productivity levels of girolando cows.

| Parameter | Milk production (Kg) | Application intervals (days) | | | CV (%) | P value |
|---|---|---|---|---|---|---|
| | | 10 | 12 | 14 | | |
| Daily average increase (liters/day) | <20 | $5.15 \pm 1.47^{Aab}$ | $3.85 \pm 1.13^{Ab}$ | $5.55 \pm 1.34^{Aa}$ | 22.64 | 0.0028 |
| | >20.1 | $3.84 \pm 1.74^{Ba}$ | $3.14 \pm 1.08^{Aa}$ | $2.63 \pm 1.33^{Ba}$ | | |
| Total increase (liters/month) | <20 | $154.52 \pm 44.02^{Aa}$ | $115.54 \pm 33.82^{Ab}$ | $166.60 \pm 40.33^{Aa}$ | 20.23 | 0.0000 |
| | >20.1 | $115.24 \pm 52.19^{Ba}$ | $94.30 \pm 32.51^{Bab}$ | $78.88 \pm 40.14^{Bb}$ | | |

Lowercase letters differ in line, capitals letters differ in column according to the Tukey test at 5% significance. For final gain calculations, the following application costs were used: Interval of 10 days = US$ 96.82; Interval of 12 days = US$ 80.68; Interval of 14 days = US$ 69.14.

Research Centre, School of Veterinary and Animal Science, Federal University of Goiás, to perform electronic analyses and issue of reports. Fat, protein, lactose, and defatted dry extract (DDE) contents were determined in MilkoScan 4000 (Foss Electric A/S. Hillerod, Denmark) equipment and results were expressed in percentage (%). The experiment was carried out in a 3x2 factorial design. Factor A corresponded to rBST application intervals divided into three levels (intervals) of rBST Application every 10, 12 and 14 days, respectively.

Factor B corresponded at production levels of animals, divided into two levels, animals that produced more than 20.1 L/milk/day and animals with production less than 20 L/milk/day, as shown in Table 2. Five animals were used per treatment, each animal was considered as repetition, totaling 30 cows. Collections were performed every other day, totaling 16 individual milk sample collections and all 16 samples were weighted to stipulate production and all animals were grouped in the same batch, so that they had common feed and environment. Productivity was assessed by measuring the amount of milk produced daily and chemical composition.

To assess the financial viability, production data of animals were grouped into each application interval and production level. Average values were used to calculate the daily production increase that was estimated in liters of milk per day, total production increase, total increase in dollar and final gain in dollar resulting from rBST application. Data were analyzed using the SISVAR statistical software (Ferreira, 2011) by performing analysis of variance and comparison by the Tukey test at 5% significance. The average increase of liters of milk per day was calculated from the difference in milk production of animals in comparison with production on day zero in all rBST application intervals (10, 12 and 14 days). The total increase in liters of milk produced within 30 days was calculated by summing up the average increases of liters of milk per day and corrected for the interval of 30 days. The total increase in dollars was calculated by estimating the amount received by the producer, which is resulting from increased milk production caused by the use of rBST, and this estimate used the value of 1.93 dollars per liter of milk, which was the price practiced in the region at the time of experiment. To calculate the final gain in dollars resulting from reducing rBST application intervals, the amount in dollars generated by the application of recombinant somatotropin minus the cost of rBST application for each interval was used, 96.81 dollars for application interval of 10 days, 80.68 dollars for application interval of 12 days and 69.14 dollars for application interval of 14 days.

## RESULTS

The total milk production during the experimental period was 18122.8 L of milk, 20.13 L/cow/day, on average. Table 3 shows the increase in daily production of each application interval and production level, total production increase in litters of milk in dollars and the final gain generated by the use of recombinant bovine somatotropin, which demonstrates the economic feasibility of reducing the rBST application interval. The daily milk production of animals with productivity below 20 L at rBST application intervals of 10 and 14 days were statistically similar, with interval of 14 days, providing increased daily production of 5.55 L/day and interval of 10 days, providing increased daily production of 5.15 L/day.

The application interval of 12 days showed significant difference only in relation to the application interval of 14

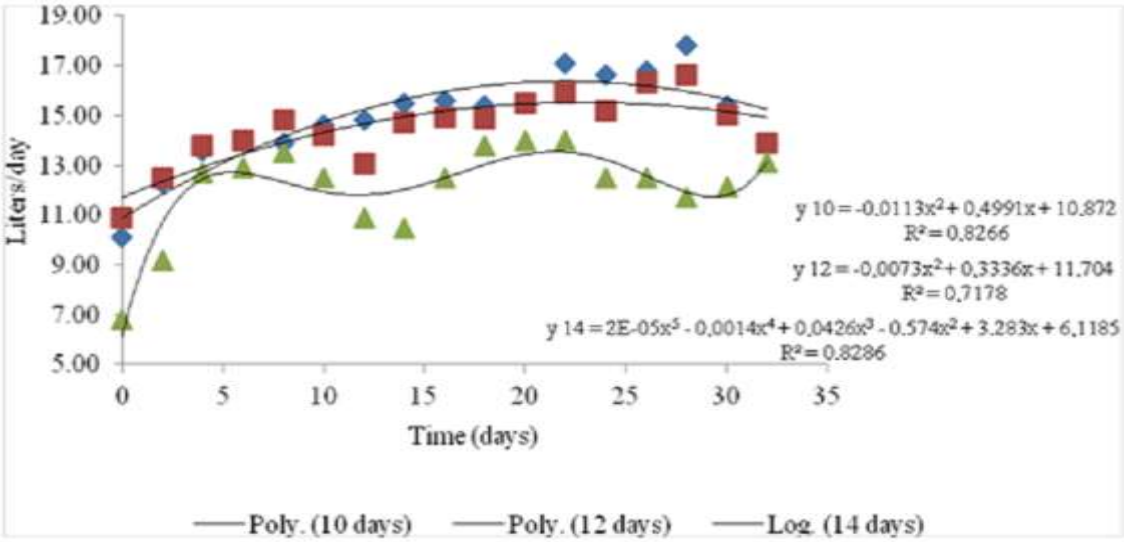


**Figure 1.** Production in litters per day at different rBST application intervals of 10, 12 and 14 days in animal with production less than 20 litres/day.

days, with no statistical difference from the application interval of 10 days, showing the lowest daily increment of 3.85 L/day/milk. For the group of animals with productivity above 20.1 L/milk/day, there was no significant difference among application intervals for the average daily increase in milk production.

In the rBST application interval of 10 days, animals produced less than 20 L responded better than the group of animals that producing above 20.1 L/milk/day, with significant difference between average daily milk productions. The average increase among animals of lower production was 5.15 L, whereas in animals of greater production, production increase was 3.84 L/day. For the application interval of 12 days, no significant difference in rBST application response to the increase in daily milk production was observed. For the application interval of 14 days, significant difference between group of lower production and group of higher production was observed, and animals of lower production showed better response, with average increase of daily milk production of 5.55 L/day, while for animals of higher production, the daily average increase in milk production was 2.63 L/milk/day.

In the total increase in dollars, animals with productivity below 20 L/milk/day showed no significant difference in relation to the reduction in the rBST application interval from 14 to 10 days, with averages of 332.98 and 308.82 dollars, respectively. The application interval of 12 days was significantly different from the other intervals, presenting the smallest increment, resulting in 230.91 dollars. In the group of animals with productivity above 20.1 L/milk/day, significant difference in the total increase in dollars response to the reduction in the rBST application interval from 14 to 10 days was observed, and the rBST application interval of 10 days showed the highest average, 230.33 dollars, while animals in the interval of 14 days showed an increase of 157.96 dollars. The application interval of 12 days was similar to application intervals of 10 and 14 days, with an increase of 188.46 dollars. Animals of lower production responded better to the effects of recombinant somatotropin in all application intervals (10, 12 and 14 days), providing greater average increase in total milk production and greater total increase in dollars, differing significantly, according to the Tukey test at 5% significance. In variable final gain in dollars, animals with lower productivity differed significantly in all rBST application intervals, and the rBST application interval of 14 days showed the highest yield, generating an additional income of 263.83 dollars per animal per month, while those in the application interval of 10 days generated additional income of 212 dollars/animal/month. The rBST application interval of 12 days showed the lowest final result, generating additional income of 150.24 dollars/animal/month. Regarding animals with productivity above 20.1 L/milk/day, no significant difference in the additional income in dollars in response to the reduction in the rBST application interval from 12 to 10 days was observed, with averages of 107.79 and 133.52 dollars/animal/month. The application interval of 14 days was significantly different in relation to the other intervals, showing the lowest additional income of 88.82 dollars/animal/month. Figure 1 shows the regression for variable production of animals with average production below 20 L per day, and it was observed that for application intervals of 10 and 12 days, there was an increase in milk production over the period of application, keeping production uniform.

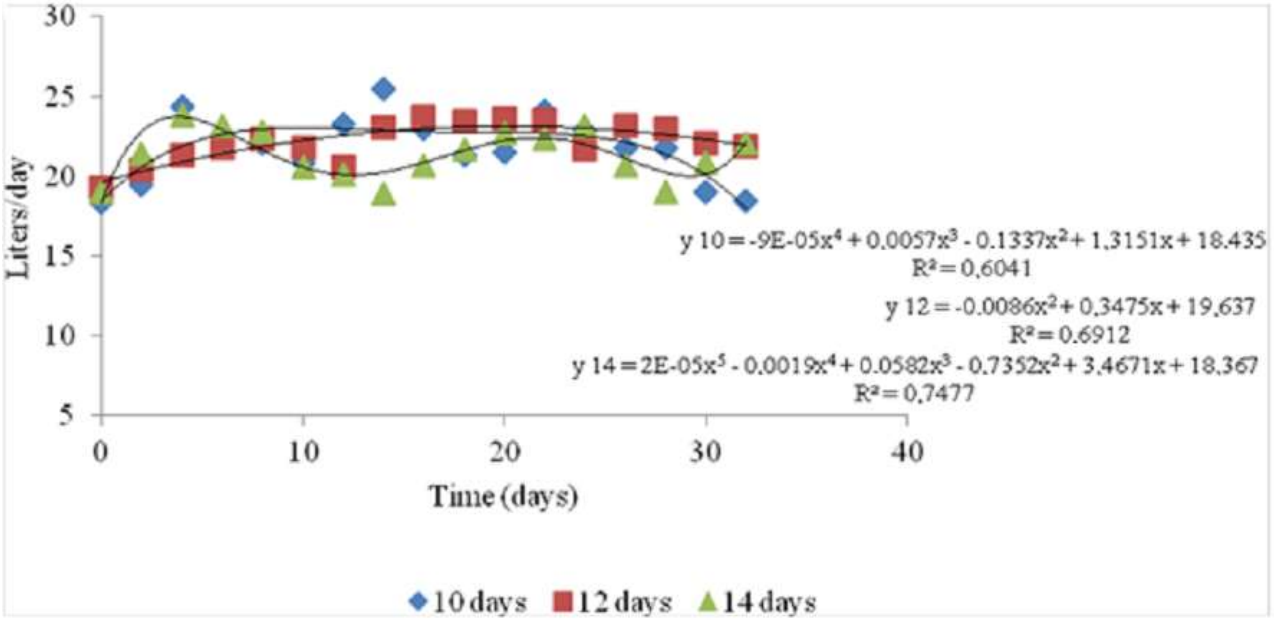


**Figure 2.** Production in litters per day at different rbst application intervals of 10, 12 and 14 days in animal with production above 20.1 L/day.

This fact can be verified by second-degree equations for application intervals of 10 and 12 days. In the rBST application interval of 14 days, milk production behaved in a less uniform manner, with reduced productivity of animals in the period next to the days of application, returning to higher production levels four days after application, so the equation that best represented such behavior was the fifth-degree equation. Figure 2 shows the regression for variable production of animals with average productivity above 20.1 L of milk per day at the different rBST application intervals. It was observed that at application interval of 10 days, there was a tendency of maintenance of animal productivity in the period corresponding to days 6 to 26, with a reduction in productivity from day 28, given that the last rBST application of this group of animals occurred on day 20. In the rBST application interval of 12 days, animal productivity resulted in a quadratic equation, with maintenance on production throughout the experimental period, which is demonstrated by the second-degree equation. For the rBST application interval of 14 days, a fluctuation in animal productivity was observed, with peak production from four to six days after recombinant bovine somatotropin application, with productivity drops always close to the days of application, thus, the equation that best represents this behavior was the fifth-degree equation. Table 4 shows the chemical composition of milk from Girolando cows with milk production below 20 L/day and above 20.1 L/day submitted to different rBST application intervals. Regarding fat content of milk, for animals with productivity below 20 L/milk/day, the rBST application interval of 10 days showed the highest average fat content of 4.92%, significantly differing from application intervals of 12 and 14 days, whose fat contents were 4.52 and 4.37%, respectively. For animal with productivity above 20.1 L/milk/day, no significant difference in fat content was observed. In all rBST application intervals, significant difference in the fat levels was observed, with higher average fat content for animals with productivity less than 20 L/milk/day, and such difference behavior can be explained by the dilution of fat in the milk of animals of higher production, thus showing lower fat percentages (Weiss et al., 2002), as shown in Table 4.

The fat levels observed in this study are high for Girolando cows, but this fact is explained by the breeding program used on the farm, where bulls are used to increase the solids content in milk, so cows produce milk with high fat and protein contents. The protein contents of milk in both groups of animals with productivity below and above 20.1 L/day showed no significant difference in none of the rBST application intervals. However, there was a significant difference in the protein percentage in all rBST application intervals in relation to animals at each application interval, with animals with productivity below 20 L/milk/day, with the highest average protein contents compared to animals of higher productivity in every application intervals of 10, 12 and 14 days, showing average protein contents of 3.59, 3.62 and 3.70%, respectively. The lactose content for animals with productivity below 20 L/milk/day showed significant difference in the application interval of 10 days, and in this interval, the average lactose content was 4.50%, while animals with application intervals of 12 and 14 days

**Table 4.** Average chemical composition values of milk from Girolando cows submitted to different recombinant bovine somatotropin application intervals.

| Composition (%) | Milk production (Kg) | Application interval (days) | | | CV (%) | P value |
|---|---|---|---|---|---|---|
| | | 10 | 12 | 14 | | |
| Fat | <20 | 4.92 ± 1.01$^{Aa}$ | 4.52 ± 0.56$^{Ab}$ | 4.38 ± 0.59$^{Ab}$ | 18.15 | 0.051 |
| | >20.1 | 3.98 ± 0.75$^{Ba}$ | 4.00 ± 0.77$^{Ba}$ | 3.98 ± 0.92$^{Ba}$ | | |
| Protein | <20 | 3.59 ± 0.42$^{Aa}$ | 3.63 ± 0.22$^{Aa}$ | 3.71 ± 0.32$^{Aa}$ | 10.58 | 0.024 |
| | >20.1 | 3.28 ± 0.48$^{Ba}$ | 3.24 ± 0.35$^{Ba}$ | 3.24 ± 0.34$^{Ba}$ | | |
| Lactose | <20 | 4.51 ± 0.39$^{Aa}$ | 4.37 ± 0.20$^{Bb}$ | 4.35 ± 0.35$^{Bb}$ | 5.87 | 0.029 |
| | >20.1 | 4.42 ± 0.28$^{Bc}$ | 4.75 ± 0.17$^{Aa}$ | 4.65 ± 0.24$^{Ab}$ | | |
| DDE | <20 | 8.10 ± 0.38$^{Aa}$ | 8.00 ± 0.30$^{Aa}$ | 8.06 ± 0.30$^{Aa}$ | 5.65 | 0.050 |
| | >20.1 | 7.70 ± 0.71$^{Bb}$ | 7.99 ± 0.40$^{Aa}$ | 7.89 ± 0.49$^{Ba}$ | | |

Lowercase letters differ in line, capitals letters differ in column according to the Tukey test at 5% significance.

showed average lactose contents of 4.37 and 4.35%, respectively. In the group of animals of higher productivity, significant difference in all application intervals was found, and the application interval of 12 days showed higher lactose percentage compared to other application intervals, with average lactose content of 4.75%, followed by application interval of 14 days, with average lactose content of 4.65%, and the lowest average lactose content was found for application interval of 10 days, with 4.42% lactose. In the application interval of 10 days, animals with productivity below 20 L/milk/day showed significant difference for the lactose content in relation to animals of higher productivity, with average lactose contents of 4.50 and 4.42% for animals with productivity below 20 L/milk/day and above 20.1 L/milk/day, respectively. For application intervals of 12 and 14 days, animals of higher productivity showed average lactose contents of 4.75 and 4.65% in both application intervals, respectively. For defatted dry solids (DDS), animal with productivity below 20 L/milk/day did not significantly differ in none of the rBST application intervals. For animals with productivity above 20.1 L/milk/day, there was no significant difference between rBST application intervals of 14 and 12 days, with average values of 7.89 and 7.99%, respectively.

## DISCUSSION

The increases in daily milk production from 3 to 5 L/day in response to rBST application were also observed in studies by several authors (Hartnell et al., 1991; Luccl et al., 1998; Collier et al., 2001; Carriquiry et al., 2008). For variable total milk increment, animals producing less than 20 L/day showed no significant difference in the milk production response in relation to decreased rBST application interval from 14 to 10 days; for rBST application interval of 12 days, significant difference compared to other intervals was observed, with production response of 115.54 L compared to 166.60 and 154.52 L for rBST application interval of 14 and 10 days, respectively. For the group of animals with production above 20.1 L/day, the response to the reduction of rBST application interval from 14 for 10 days was significant for the total increase in milk produced within 30 days, and the group of animals in the application interval of 10 days produced 115.24 more liters of milk, while animals in the application interval of 14 days produced 79.04 L of milk. Application interval of 12 days was similar to application intervals of 10 and 14 days, with an increase in production of 94.30 L of milk. The reduction in the rBST application interval from 10 and 12 days caused changes in milk production of animals of both productivity levels, and no further reduction in milk production around the time of subsequent application was observed, such as that shown in the application interval of 14 days. The fat results were lower than those found in studies by Klusmeyer et al. (2009), who evaluated the effect of rBST in the milk composition of Holstein cows. In another study with goats, no variation in fat content was found between animals that received rBST and those who did not receive the application (Barbosa et al., 2002). The higher fat content found in animals with productivity below 20 L/milk/day in rBST application interval of 10 days is due to the assumption that short rBST application intervals maintained higher rBST levels and it is known that one of the functions of somatotropin through IGF-1 is to direct more nutrients to the mammary gland, among these volatile fatty acids (Etherton and Bauman, 1998) used by the mammary gland for the formation of fat globules. Protein contents lower than those found in this experiment were reported by Gulay et al. (2004) where Holstein cows submitted to rBST application interval of 14 days showed protein percentage in milk of 2.87%. How-

ever, the protein contents were similar to those found in a study conducted by Macrina et al. (2011).

Although with no statistical difference for the lactose contents, the average values were very close for all rBST application intervals and productivity, evidenced by the low variation coefficient of 5.87%. This small variation was due to the close relationship between synthesis of lactose and amount of water drained to the milk because the lactose content of milk is the component with the lowest variation (González, 2001). Only the application interval of 10 days showed significant difference from the other intervals, with DDS content of 7.70%. This result is explained by the lower lactose content shown by this group of animals in the application interval of 10 days. In application intervals of 10 and 14 days, animals of lower productivity significantly differ for the DDS contents in relation to animals of increased productivity, with average DDS values of 8.09 and 8.06%, respectively. This variation is due to the higher protein and lactose content presented in these application intervals. In the application interval of 12 days, no significant difference in the DDS levels in different groups was observed, with average DDS value of 7.99%. The changes in the chemical composition of milk from cows to rBST application was not out of standards established by IN 62 (Brasil, 2011), which recommends minimum fat content of 3% and minimum protein content of 2.9%; however, the DDS values were lower due to the higher fat contents obtained in this experiment.

In all rBST application intervals, significant difference in the final gain in dollars was observed and animals with lower productivity provided higher additional income per month, with average of 212, 150.24 and 263.83 dollars for application intervals of 10, 12 and 14 days, respectively, while animals of higher productivity showed 133.52, 107.79 and 88.82 for intervals of 10, 12 and 14 days, respectively.

## Conclusion

In animals with productivity below 20 L/milk/day, the best rBST application intervals was 14 days, resulting in higher financial returns to milk producers. Animals with productivity over 20.1 L/milk/day, reducing the rBST application interval from 14 days to 12 and 10 days is financially feasible. The rBST application intervals of 10 days resulted in higher fat content in milk from animals of lower production. The fat and protein contents were higher for animals of lower productivity at all application intervals.

## Conflict of interests

The authors did not declare any conflict of interest.

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