An approach to identify dairy cows being responsive to recombinant bovine somatotropin

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Introduction

Recombinant bovine somatotropin (rbST) is considered a very useful tool in dairy management (Molnar et al., 1990), because of its capacity of increasing milk yield (McBride et al., 1990; McGuffey et al., 1990; Nytes et al., 1990; Thomas et al., 1991; Oldenbroek et al., 1993; Dohoo et al., 2003), around 125 days in milk (DIM) (Thomas et al., 1991) or later (McBride et al., 1990). The availability of corporal reserves (Thomas et al., 1991; Oldenbroek et al., 1993) associated with a well-balanced diet (Bauman 1992; Oldenbroek et al., 1993), absence of stressful conditions or illness (Bauman 1992), as mastitis (Thomas et al., 1991), could affect the magnitude of the rbST response significantly.

Differences in responses to rbST among breeds, despite their large size difference (McBride et al.,...
1988; Oldenbroek et al., 1993), or previous level of milk production (McDaniel and Hayes, 1988; Thomas et al., 1991) have not been demonstrated. However, Leitch et al. (1990) reported that cows with low estimated production showed better response to rbST.

Despite the fact that the dairy cows treated with rbST have shown to produce antibodies against this protein, these findings had no effect on responses and also could not explain the individual variation among animals (Eppard et al., 1992). The use of rbST during four lactations has shown that treatment in previous lactations had no effect on milk yield or yield responses in subsequent lactations (Huber et al., 1997). Although rbST can be used regardless of the herd size, its cost–benefit ratio must be considered. Therefore, the early identification of responsive cows to rbST would reduce costs associated with treatment of unresponsive cows.

The objectives of the present trial were (i) to quantify how much the short period rbST response can explain the variability reached in long period rbST response and (ii) to study the effects of previous milk yield (PMY), stage of lactation and somatic cells count (SCC) before rbST treatment on the magnitude of responses of rbST-treated cows for early identification of rbST-responsive cows using a single treatment regimen.

Materials and methods

Forty-eight primiparous Holstein cows at 202 ± 59 DIM, with average daily milk of 22.0 ± 5.3 kg/cow/day at the beginning of the experiment and 28.3 ± 7.5 kg/cow/day during the peak of lactation were used in this trial. Only pregnant cows were selected for this trial, with body weight of 507.8 ± 42.3 kg. The body condition score (BCS) was evaluated at the beginning of the experiment using a 5-point scale (1 – very thin and 5 – very fat). Only cows with BCS of 3 and 4 (3.14 ± 0.26) were selected. The average SCC at the beginning of the trial was 385 000 somatic cells per millilitre of milk. This corresponds to 20.8% of cows with more than 500 000 cells/ml of milk, which is the limit proposed by the International Dairy Federation (1999) to define clinical mastitis. However, neither of the cows presented clinical sign of abnormal milk and, in addition, 385 000 cells/ml of milk observed in this trial is still below the limit required by the European Union (400 000 cells/ml of milk) and the United States of America (750 000 cells/ml of milk) for raw milk.

Animals were housed in free-stall barns in a commercial dairy herd localized in São Paulo State, Brazil. Cows were milked three times a day and fed twice daily a total mixed ration (TMR) formulated with 40% roughage (corn silage and alfalfa hay) and 60% concentrate (whole cottonseed, ground corn, soybean meal, wheat meal, hominy feed, sodium bicarbonate, NaCl and mineral premix), based on dry matter (Table 1). Diet was analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, calcium (Ca) and phosphorus (P), according to AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed according to Van Soest et al. (1991).

A composite sample of milk from each cow before treatment period was taken for SCC ($\times 10^3$ cells/ml) analysis, by flow cytometry with the equipment Somacount 500® (Bentley Instruments, Chaska, MN, USA). Cows were treated with 500 mg of rbST (Boostin-S®, LG Chemical, Daejeon, Korea), which were injected subcutaneously at a 21-day interval during a 105-day period. Individual milk yield was recorded daily in all milkings for calculating the following variables:

1. Average apparent response (AAR) for first rbST treatment was calculated by the difference between average increase in milk yield in kilogram per cow per day and the average of six daily milk yields (milk produced during 3 days before treatment and the last 3 days of 21-day period of treatment).
2. Average apparent total response (AATR) for the 105-day period was calculated as the average of AAR for the five periods of treatment.
3. Peak of apparent response (PAR) for the first treatment was calculated by the largest difference in daily milk yield found during the 21-day period and the average of three-daily milk yield before rbST treatment.

Data were analysed by linear correlation and regression analysis using PROC CORR and PROC REG

| Table 1 Composition of total mixed ration (TMR), based on DM |
|-----------------|------------------|
| Nutrient composition | Value |
| DM (g/kg) | 528.5 |
| CP (g/kg) | 190.7 |
| ADF (g/kg) | 253.2 |
| NDF (g/kg) | 425.7 |
| Ether extract (g/kg) | 36.6 |
| Ash (g/kg) | 73.4 |
| Ca (g/kg) | 7.2 |
| P (g/kg) | 5.7 |
| Metabolizable energy (MJ/kg) | 11.42 |
procedures of SAS (SAS, 1985). Data were tested for normality of distribution using the Shapiro–Wilk test, and for homogeneity of variance by chi-square test. Significance was declared at p < 0.05.

Results and discussion

Effect of first rbST response

A positive relationship between AAR during the first 21-day period after the first rbST treatment and AATR for the 105-day period was identified, as shown in Fig. 1. The coefficient of linear correlation (r) found was 0.608 (p = 0.0001) and the resulting regression equation was AATR = 1.3013 + 0.4318 AAR ($R^2 = 0.3699$; p = 0.0001). This equation suggests that for each kilogram of milk per cow per day produced by AAR supported an increase of 0.43 kg of milk per cow per day of AATR for 105-day period. However, the low coefficient of determination ($R^2 = 0.3699$) obtained for this equation indicates that other factors may influence the response to rbST during this period of lactation, which is illustrated by the large dispersion observed in this correlation (Fig. 1).

Because of the difficulties in recording individual daily milk yield in rbST-treated cows during a 21-day period for identifying which animals respond to treatment, which day of this 21-day period would be more appropriate for measuring the response to rbST administration in terms of milk yield during the first 21-day period (r = 0.8307; p = 0.0001). The linear regression equation for this correlation was AAR = 0.5383 + 0.4599 PAR ($R^2 = 0.6903$; p = 0.0001). From this relationship, it can be concluded that there is a response of 9.66 kg of milk per cow (0.4599 kg/cow/day) during the days 8, 9 and 10 compared to average milk yield during 3 days before rbST treatment. Therefore, the peak of response (average of days 8, 9 and 10) can explain 69% of the whole variation observed for AAR.

The coefficient of correlation between AAR or PAR and AATR found in the present study is higher than the coefficient of repeatability of 0.22 reported by Oldenbroek et al. (1993). These authors analysed the response to rbST treatment for six consecutive injections at a 28-day interval, reporting a repeatability of rbST response of 0.49 among lactations when data from three lactations were analysed. However, this result is lower than the repeatability of 0.75 found in that study for total milk production per cow during the three lactations. In the present study, a positive linear correlation between average milk production (r = 0.9652; p = 0.0001) or peak of

### Table 2

Coefficients of correlation (r) and probabilities (p) between average apparent total response (AATR) and the response obtained between days 5–10 PAR (peak of apparent response) after the first rbST treatment.

<table>
<thead>
<tr>
<th>Day after first rbST treatment</th>
<th>Correlation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5</td>
<td>0.1516</td>
<td>0.3036</td>
</tr>
<tr>
<td>Day 6</td>
<td>0.2914</td>
<td>0.0445</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.2993</td>
<td>0.0430</td>
</tr>
<tr>
<td>Day 8</td>
<td>0.3750</td>
<td>0.0086</td>
</tr>
<tr>
<td>Day 9</td>
<td>0.4906</td>
<td>0.0004</td>
</tr>
<tr>
<td>Day 10</td>
<td>0.4541</td>
<td>0.0012</td>
</tr>
<tr>
<td>Day 5–10*</td>
<td>0.4224</td>
<td>0.0028</td>
</tr>
<tr>
<td>Day 8–10*</td>
<td>0.5112</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*Average among those days.
milk production ($r = 0.9294; p = 0.0001$) during the 21-day period and the average milk production during the whole experimental period (105 days) was observed.

Results from the present study disagree with the report of Oldenbroek et al. (1993) in which a differentiation of good- from poor-responsive cows after a single injection of rbST was judged as being not possible. The authors reported that the response in terms of milk production depends more on the actual energy intake and body fat reserves than on the genetic potential of the cow. It must be emphasized that, in the present study, all animals were fed a well-balanced diet and presented a BCS of 3.1 at the beginning of the experiment.

**Effect of previous milk yield**

Correlation between PMY, expressed as the average of milk production during the last 3 days before rbST treatment, and AAR was studied. A positive linear correlation was identified with low coefficient of correlation ($r = 0.3814; p = 0.0075$) resulting in a regression equation of $AAR = 0.1126 + 0.0983 \cdot PMY$ ($R^2 = 0.1455; p = 0.0075$), as shown in Fig. 2. The association of PMY and AATR resulted in a quadratic effect and the regression equation calculated was $AATR = -2.4580 + 0.4146 \cdot PMY - 0.00857 \cdot PMY^2$ ($R^2 = 0.1869; p = 0.0339$). Even though the correlations between PMY and AAR or AATR were statistically significant at level of 1% and 5%, respectively, the coefficient of determination ($R^2$) of both equations was low, indicating that the apparent response to rbST is less dependent on PMY.

By deriving this quadratic equation, it was found that cows producing around 24.2 kg/day presented higher AATR in a herd with average milk yield of 22.0 ($\pm$5.3) kg/day. It is important to state that animals in this study received a diet balanced for a milk production of 25 kg/day, on average. So, it is possible to postulate that cows with lower or higher production than 24.2 kg/day produced lower response to rbST. For cows producing less than 24.2 kg/day, lower response to rbST was observed probably because of the fact that fewer numbers of epithelial cells were able to respond to IGF-I stimulus. This lack of stimulus seems to affect mainly the maintenance of epithelial cell, once Capuco et al. (2003) demonstrated that the effect of rbST on lactation persistency is because of the maintenance of mammary cell population rather than maintenance of cellular secretory rate. For cows producing more than 24.2 kg/day, the lower response to rbST treatment observed was probably owing to lower response of liver to IGF-I production (Sharma et al., 1994), observed in animals with negative energy balance. Therefore, these results seem to be in disagreement with the hypothesis that initial milk production has no effect on the response of rbST, as stated by McDaniel and Hayes (1988), Nytes et al. (1990) and Thomas et al. (1991).

**Effect of days in milk**

No association was observed between the stages of lactation of animals expressed as DIM, prior to the beginning of treatments and the apparent response to rbST. Neither AAR ($r = 0.0810; p = 0.5844$) nor AATR ($r = 0.0607; p = 0.6818$) was correlated to stage of lactation. These results, however, are different from those obtained by McBride et al. (1990) who, studying the rbST response in animals from 28 to 294 DIM (4–41 weeks), found a positive relationship between DIM and milk response to rbST treatment. Thomas et al. (1991), using dairy cows at 57–189 DIM, observed a higher response in milk yield in cows at 125 DIM. It is possible that the present study did not detect this association because there
was not enough variation of DIM among cows, once the rbST treatments started at a minimum of 123 DIM.

Effect of somatic cells count

A negative linear association \( (r = -0.2983; p = 0.0394) \) between decade logarithm of SCC \([\log_{10} \text{SCC}(\times10^3)]\) prior to the beginning of treatments and the PMY was observed. The equation obtained \( (\text{PMY} = 28.054 - 2.701 \log_{10} \text{SCC}; R^2 = 0.0890; p = 0.0394) \) indicates a decrease of 2.7 kg of milk per day per each unit of \( \log_{10} \text{SCC} \) increased in milk.

The coefficient of linear correlation between decade logarithm of SCC \([\log_{10} \text{SCC}(\times10^3)]\) prior to the beginning of treatments and AATR was \( r = -0.3489 \) \((p = 0.0162)\) and the correlation between \( \log_{10} \text{SCC} \) and AAR was \( r = -0.2429 \) \((p = 0.0999)\), as demonstrated in Fig. 3. The linear regression equation obtained for the correlation between AATR and \([\log_{10} \text{SCC}(\times10^3)]\) was: \( \text{AATR} = 3.8033 - 0.6634 [\log_{10} \text{SCC}(\times10^3)](R^2 = 0.1217; p = 0.0162). \)

In the literature there are many studies about the effects of rbST on milk SCC. According to McBride et al. (1990) and Oldenbroek et al. (1993), the use of rbST in dairy cows has a negative effect on the mammary gland health, causing an increase in SCC. According to Craven (1991) and White et al. (1994), the greater incidence of mastitis or SCC, observed for cows that received rbST, might be associated with the higher milk yield stimulated by rbST. However, Hemken et al. (1991), Nytes et al. (1990), Gibson et al. (1992), Fontes Jr. et al. (1997) and Collier et al. (2001) did not observe any effect of rbST treatment on the incidence of mastitis.

Regarding the effects of SCC on rbST response, Thomas et al. (1991) also observed a negative linear correlation between SCC and rbST response in a 12-week period study reporting a decrease of 0.515 kg of 3.5% fat corrected milk (FCM) for each \( \log_{10} \text{SCC} \) obtained prior to the beginning of treatments \( (\text{FCM} = 5.417 - 0.515 \log_{10} \text{SCC}; R^2 = 0.02, p < 0.003). \)

It is well known that growth hormone acts stimulating mammary epithelial cells proliferation (Knight et al., 1990; Forsyth 1996), through IGF-I (Dehoff et al., 1988), synthesized in the liver (Sharma et al., 1994). This adverse effect of mastitis on rbST response is in agreement with Capuco et al. (2003), who demonstrated that rbST and mastitis have antagonistic effects on mammary cell proliferation. Whereas rbST enhances persistency by increasing cell proliferation or reducing rates of cell death, instead of increasing cellular secretory rate, mastitis increases death of epithelial cells by apoptosis.

This is also in agreement with Bauman (1992), who postulated that animals under stressful conditions or presenting some illness, as mastitis, have poor responses to rbST treatment.

Conclusions

Based on the results of the present study, it can be concluded that first response to bovine somatotropin, obtained on days 8, 9 and 10 after dosing, is associated with the long-term response. However, other factors, such as milk production level and mammary gland health status prior to rbST treatment, affected this response, whereas stage of lactation after 120 DIM did not. Results from the present study demonstrated that the mammary gland health prior to the rbST treatment, as measured by SCC, had a negative impact on the milk yield response to treatment, indicating to achieve better results excluding high SCC cows from rbST treatment.

Fig. 3 (a) Relationship between decade logarithm of somatic cells count (SCC) prior to the beginning of treatments and average apparent response (AAR) for first rbST treatment (kg/day). (b) Relationship between decade logarithm of SCC prior to the beginning of treatments and average apparent total response (AATR) for the 105-day period (kg/day).
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REFERENCES


