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Changes of plasma insulin, urea, amino acids and rumen metabolites in somatotropin treated dairy cows

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Summary. An experiment was performed to evaluate the effects of somatotropin on plasma free amino acid, urea and insulin concentrations and rumen fermentation pattern and to assess their relationships. Four Italian Friesian dairy cows fitted with rumen cannulae were used in a switch-back design. Slow releasing recombinant bovine somatotropin (640mg/cow) was injected every 28 days for two consecutive periods. Rumen fluid and blood samples were collected before and after feeding at 0, 7 and 21 days after rbST injection. Exogenous rbST increased plasma insulin concentration and the insulin response to feeding, and decreased plasma urea and free essential and branched chain amino acid concentrations. rbST did not affect rumen fermentation pattern. No correlation was found between rumen and plasma parameters measured after feeding. Our results are consistent with the notion that the main effect of somatotropin is post-absorptive.

Keywords: Amino acids – Somatotropin – Plasma amino acids – Dairy cows

Introduction

The galactopoietic effect of both pituitary and recombinant bovine somatotropin (rbST) is well documented (McDowell et al., 1987; Peel and Bauman, 1987). rbST treatment increases milk production in lactating cows, without affecting milk composition, if energy and nitrogen balances of treated animals are positive (Chalupa and Galligan, 1989). Somatotropin increases lipolytic and gluconeogenetic processes, while lipogenesis is decreased (Knapp et al., 1992; Liesman et al., 1995). Current dogma suggest that the galactopoietic effect of rbST is partially mediated by changes in the circulating levels of hormones, such as IGF-1, promoting homeorhetic adaptations and enhancing the availability of energy yielding metabolites and the partition of nutrients to the mammary gland (Sechen et al., 1989; Vernon, 1988). Somatotropin increases the efficiency of nitrogen utilization reducing amino acid oxidation (Peel and Bauman, 1987). Decreased urinary nitrogen excretion and plasma urea levels have been observed in bST treated dairy cows (Sechen et al., 1989; Hanigan et al., 1992).

Experimental data on rbST influence on rumen fermentation pattern and nutrient digestibility seem to indicate that somatotropin has no effect at the preabsorptive level and support the concept that rbST treated cows have a digestive metabolism similar to untreated cows of similar production (Robinson et al., 1991).

The aim of this study was to evaluate the effects of rbST treatment on plasma free amino acid, urea, insulin concentrations and some ruminal metabolites and to asses their relationships.

Materials and methods

Cows: experimental design

Four lactating Italian Friesian dairy cows (191 ± 44 days of lactation, 15 kg/d of milk) fitted with rumen cannulae were used. The animals, kept in a tied-stall, were fed at 04.30 a.m. 4 kg of concentrate and 3 kg of meadow hay and at 09.00 a.m. 15 kg of maize silage (31% DM), 3 kg of meadow hay and 4 kg of concentrate given as total mixed ration (TMR). The chemical composition of the feed ingredients is reported in Table 1.

Recombinant bovine somatotropin in a slow release formulation (640 mg/cow; Somidobove Eli Lilly) was injected at the start of two 28 days consecutive periods. In the first period two animals were treated with rbST and two animals with a saline solution. In the following period the treatments were reversed.

Sample collection and analyses

Milk production was recorded weekly. Milk samples were collected weekly at consecutive morning and evening milkings and were individually analized for fat (Gerber method) and protein (Kjeldahl method) content. Feed intake was recorded weekly for three consecutive days. For each experimental period, at 0, 7 and 21 days after rbST injection, rumen fluid and blood samples from the jugular vein were collected at 09.00 a.m., before TMR administration, and at 02.00 p.m.

For volatile fatty acid (VFA) analysis, 0.8 ml of 5% (wt/vol) metaphosphoric acid was added to 4 ml of rumen fluid and stored at −20°C. VFA concentrations, using 4-methylvaleric acid as internal standard, were determined by gas chromatography with a 60/80 Carbopack S 0.3% Carbowax 20M/0.1% H_3PO_4 column.

Table 1. Chemical composition of feeds (% of dry matter)

	Maize silage	Meadow hay	Concentrate
Dry matter %	31.00	89.00	87.10
Crude protein	7.42	9.98	18.48
Crude fiber	23.54	28.57	7.46
NDF	51.20	70.12	12.80
Fat	3.20	2.90	4.47
Ash	5.52	8.04	7.46

NDF neutral detergent fiber.

Plasma insulin concentration was determined by a commercially available radioimmunoassay kit (Amersham, Italia, S.r.l.).

To measure plasma amino acid concentration (AA), samples of 200μl of plasma, with 20μl of norleucine added as an internal standard, were deproteinized with 800μl of methanol. After centrifugation, 200μl of the supernatant was collected in microfuge tubes dried under a stream of N_2-gas and resuspended in 100μl of Li citrate buffer (pH 2.4). Plasma AA concentrations were determined by HPLC with a LiCl ion exchange column. AA were post-column derivatized with o-phthaldehyde and fluorimetrically detected (Condon, 1986).

Statistical analysis

Rumen and plasma data were statistically analyzed using a least-squares ANOVA program (SAS, 1988). The statistical model included rbST treatment, day from rbST injection and sampling time as main effects. Individual AA and their combinations into total (TAA), essential (EAA), non-essential (NEAA), branched chain (BCAA), ketogenic (KETO) and glucogenic (GLUCO) amino acids were considered. Pearson's correlation analysis was carried out between rumen and plasma parameters.

Results

Overall mean daily milk yield was significantly higher in rbST-treated than in control cows (18.3 ± 1.9 *v*. 15.4 ± 1.9kg/d; $P < 0.01$). There was no effect of rbST treatment on milk protein and fat content. A peak of milk production was recorded 7 days after rbST injection (data not shown).

There was no effect of rbST on overall mean dry matter intake (17.72 ± 1.16kg/cow -control group- *v*. 17.76 ± 1.04kg/cow -rbST group-).

rbST treatment did not affect rumen VFA molar concentration and the molar proportion of acetate and propionate (Table 2). Ruminal VFA concentrations were higher, although not statistically significant, five hours after TMR administration both in control (105 ± 5 *v*. 116 ± 6mmol/l) and treated cows (117 ± 6 *v*. 124 ± 6mmol/l).

Overall mean plasma insulin concentration was significantly higher ($P < 0.01$) in rbST-treated than in control cows (Table 3). A significant post-prandial increase ($P < 0.01$) in plasma insulin concentration was found in both treated and control cows (Figure 1). At seven days after rbST injection, insulin response to feeding was amplified in treated animals (Figure 1). In rbST-treated cows, plasma insulin concentrations tended to be higher than in

Table 2. Effect of rbST on rumen parameters (lsmeans ± SEM)

Items	Control	rbST
VFA, mmol/l	110.42 ± 3.95	120.93 ± 4.09
Acetate, %	58.69 ± 1.41	58.20 ± 1.46
Propionate, %	23.99 ± 1.34	25.51 ± 1.39

VFA volatile fatty acids.

Table 3. Effect of rbST treatment on plasma parameters (lsmeans ± SEM)

	Control	rbST	P=
Insulin, mU/L	14.49 ± 4.06	31.90 ± 4.20	0.007
Urea, mmol/l	2.64 ± 0.17	1.23 ± 0.09	0.009
Amino acids, μmol/dl			
Glutamic acid	3.71 ± 0.29	3.60 ± 0.28	0.79
Serine	6.88 ± 0.44	6.64 ± 0.43	0.70
Glycine	29.13 ± 1.89	27.48 ± 2.08	0.56
Glutamine	21.58 ± 0.98	21.01 ± 1.02	0.69
Histidine	3.53 ± 0.31	2.58 ± 0.33	0.05
Threonine	6.82 ± 0.52	6.14 ± 0.53	0.37
Alanine	12.92 ± 1.22	14.82 ± 1.35	0.31
Tyrosine	4.05 ± 0.40	3.40 ± 0.42	0.28
Valine	19.67 ± 0.93	16.52 ± 0.96	0.03
Methionine	2.05 ± 0.10	2.02 ± 0.11	0.84
Isoleucine	9.50 ± 0.65	7.97 ± 0.67	0.12
Leucine	11.77 ± 1.00	9.76 ± 1.04	0.18
TAA	131.07 ± 6.00	119.01 ± 6.21	0.18
EAA	53.20 ± 3.12	44.69 ± 3.23	0.07
NEAA	77.86 ± 3.47	76.52 ± 4.04	0.80
BCAA	40.96 ± 2.49	34.27 ± 2.58	0.07
KETO	32.16 ± 2.37	27.29 ± 2.45	0.17
GLUCO	77.32 ± 3.26	72.96 ± 3.80	0.39

TAA total amino acids; *EAA* essential amino acids; *NEAA* non essential amino acids; *BCAA* branched chain amino acids; *KETO* ketogenic amino acids; *GLUCO* glucogenic amino acids.

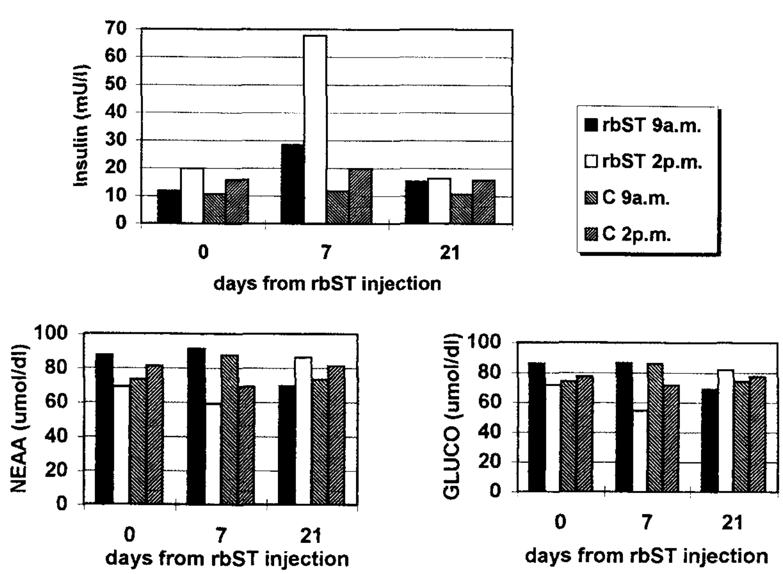

Fig. 1. Changes in plasma insulin and free amino acid concentrations in somatotropin-treated (*rbST*) and control (*C*) cows at 0, 7 and 21 days from rbST injection. 9 a.m., 2 p.m.: sampling time. *NEAA* non essential amino acids; *GLUCO* glucogenic amino acids

the control group at 09.00 a.m. (28.4 ± 8.7 *v.* 11.7 ± 6.8 mU/ml) while at 02.00 p.m., five hours after TMR, the difference was statistically significant (67.7 ± 8.4 *v.* 19.9 ± 8.7 mU/ml; $P < 0.01$). rbST treatment decreased ($P < 0.01$) plasma urea concentration (Table 3).

Overall means of plasma concentrations of some AA and their combination according to their catabolic fates are reported in Table 3. No significant effect of time of the day on plasma free AA leves was found. rbST treatment decreased plasma concentrations of His, Val ($P < 0.05$), EAA and BCAA ($P = 0.07$). This effect was statistically more significant at seven days after rbST injection, when the peak in milk production was recorded, for both EAA (54.79 ± 4.17 μmol/dl – control cows- *v.* 40.07 ± 4.50 μmol/dl -rbST-treated cows; $P < 0.05$) and BCAA (42.24 ± 3.30 μmol/dl -control cows- *v.* 31.30 ± 3.56 μmol/dl -rbST-treated cows; $P < 0.05$). In rbST-treated cows, a significant decrease in NEAA and GLUCO after TMR administration was found at seven days after rbST-injection (Figure 1). A negative correlation between insulin and NEAA was found at 02.00 p.m. ($r = -0.71$; $P < 0.06$).

Five hours after TMR administration no correlations were found between rumen and plasma parameters.

Discussion

In this study, rbST treatment increased milk production with no change in protein and fat content. Changes in milk composition of rbST-treated cows have been attributed to a status of negative energy and protein balance (Chalupa and Galligan, 1989). The weekly response in milk production showed a peak at 7 days after rbST injection in accordance with previous reports (Oldenbroek et al., 1989; Cattaneo et al., 1993).

Dry matter intake did not differ between rbST-treated and control cows. This is consistent with the results of other authors (Peel and Bauman, 1987) who reported that long-term administration of bST gradually increased feed intake, but that there appeared to be 4 to 5 week lag prior to its full expression. A substantial increase in feed consumption was found only after 12 weeks of bST supplementation (Chalupa and Galligan, 1989).

In the present study rbST treatment did not affect rumen fermentation parameters in agreement with previous reports (Dell'Orto and Savoini, 1991; Winsryg et al., 1991). A higher VFA absorption in rbST-treated cows has been described (Savoini et al., 1992). These changes may be related to the greater energy requirements associated with enhanced milk production.

Plasma insulin concentration was significantly higher in rbST-treated than in control cows in accordance with previous reports (McDowell et al., 1987; Wanderkooi et al., 1995), however no effect of somatotropin on insulin concentration has been found by other authors (Cisse et al., 1991). The increased insulin concentration in rbST-treated animals may be due to a compensatory secretion related to a reduced tissue sensitivity to insulin (Walton et al., 1986; Magri et al., 1990). This impairment of insulin action and the consequently reduced glucose uptake by peripheral tissues should facilitate the preferential utilization of glucose by the mammary gland (Ronge and Blum, 1989). The significant post-prandial increase in insulin concentrations observed in this study is in agreement with other studies and may be induced by feed intake, through the stimulation of the entero-insular axis, and VFA absorption (Morgan et al., 1988; Sutton et al., 1988). The post-prandial changes of insulin concentration were higher in treated than in control cows at seven days after rbST injection, when the peak in milk production is recorded and increased plasma somatotropin concentration is reported (Dell'Orto et al., 1993). Since feed intake and rumen fermentation pattern were not different, we might consider that the regulation of nutrient partitioning by homeorhetic and homeostatic mechanisms may be different in rbST-treated and control animals.

Plasma urea concentration was lower in treated than in control animals. The decreased urea levels in bST-treated cows may be an expression of decreased gluconeogenic use of AA and improved utilization of nitrogen (Peel and Bauman, 1987; Sechen et al., 1989; Hanigan et al., 1992). Plasma free AA concentrations were affected by bST treatment, in accordance with previous studies (Fullerton et al., 1989; Baldi et al., 1990; Hanigan et al., 1992). EAA and BCAA concentrations were lower in treated cows. Treatment-induced changes were more evident at seven days after rbST injection, when the peak in milk production was recorded. Once absorbed in the blood, AA are immediately subject to metabolism by the liver, which has a profound impact on their availabilty to the mammary gland. The liver metabolizes individual AA to varying degrees and the net liver removal of EAA and BCAA is relatively low (Reynold et al., 1994). The observed reduced plasma concentrations of EAA and BCAA in rbST-treated cows may indicate a higher uptake by the mammary gland to support higher milk protein synthesis. Synthesis and secretion of milk protein within the udder might be promoted by two processes: 1) increased quantity of AA taken up by the mammary gland and 2) improved efficiency of conversion of plasma EAA to milk protein in the secretory cells (Guinard and Rulquin, 1995). How these two factors interact is still not known. Furthermore the effect of bST in reducing plasma AA concentration may be due to an increased blood flow to the mammary gland (Davis et al., 1988). The observed significant decrease of plasma levels of NEAA and GLUCO after TMR administration in bST-treated cows may be related to the high plasma insulin concentration found.

In conclusion, bST treatment did not affect rumen metabolites, but significantly influenced plasma concentration of insulin, urea and free amino acids. Treatment-induced changes were more evident at seven days after rbST injection, when the peak in milk production was recorded. Observed changes in plasma metabolites induced by rbST appeared to be unrelated to changes in rumen metabolites. These results are consistent with the notion that the main effect of somatotropin is post-absorptive.

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