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Basal levels and diurnal variations of some hormones and metabolites in blood of dairy cows treated daily with rbST in early and late lactation

Giuseppe Bertoni¹, Rosanna Lombardelli¹,
Fiorenzo Piccioli-Cappelli¹, Jurgen Blum²

¹ Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, Italy

² Institute of Animal Genetics, Nutrition and Housing. University of Berne, Switzerland

Corresponding author: Prof. Giuseppe Bertoni. Istituto di Zootecnica, Facoltà di Agraria, Università Cattolica del Sacro Cuore. Via Emilia Parmense 84, 29100 Piacenza, Italy - Tel. +39-0523-599277 - Fax: +39-0523-599276 - Email: bertoni@pc.unicatt.it

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ABSTRACT

The variations of basal value (before morning feeding at 3-4 days interval) and daily pattern (on 4th and 18th day of treatment with 8 blood collections within 24h) of hormones and metabolites were studied in four dairy cows; 2 in early and 2 in late lactation, which were alternatively injected daily for a period of 21 days with 26.3 mg rbST or saline. The rbST injection significantly increased the basal and daily levels of GH, IGF-I in both stages of lactation. Daily levels of NEFA were significantly increased by rbST in both stages of lactation and their variations, due to the effect of meals, were counteracted by the rbST; the higher level of NEFA allowed a greater sparing of glucose, the blood level of which resulted higher ($P < 0.05$) in late lactation (mainly for less lactose yield), as well as a sparing of amino acid that resulted in a lower blood urea level ($P < 0.05$, only in late lactation). Moreover, these metabolic changes in late lactating cows, and in particular the high availability of glucose, determined a significantly higher blood level of insulin and T3, and lower level of glucagon. Despite the similar GH, IGF-I and NEFA increases observed in the cows treated in early and late lactation, other metabolic and endocrine consequences resulted more evident in late lactation. Furthermore, some of these variations were affected by the forage meals. It can be concluded, therefore, that the changes occurring in the blood subsequent to a rbST treatment are not equal to those which occur at the start of lactation; moreover, they are affected by the daily feeding pattern and perhaps by the stage of lactation.

Key words: Somatotropin, Dairy cow, Metabolic hormone, Metabolite.

RIASSUNTO

VARIAZIONI DEI LIVELLI BASALI E GIORNALIERI DI ALCUNI ORMONI E METABOLITI NEL PLASMA DI BOVINE DA LATTE IN FASE INIZIALE O FINALE DELLA LATTAZIONE TRATTATE CON INIEZIONI GIORNALIERE DI rbST.

In 4 Frisone italiane, 2 in fase iniziale e 2 in fase finale della lattazione, trattate alternativamente per 21 giorni con iniezioni giornaliere di 26.3 mg di rbST o placebo, si sono studiate le variazioni basali (il mattino prima del pasto ogni 3-4 giorni) e circadiane (il 4° e 18° giorno dei trattamenti con 8 prelievi nelle 24 ore) di ormoni e metaboliti. L'iniezione di rbST ha innalzato in entrambe le fasi di lattazione i livelli di GH, sia basali (4,36 vs 5,77 µg/L, $P < 0,05$; 3,69 vs 6,34 µg/L, $P < 0,01$ rispettivamente per i gruppi di controllo e trattato, nella fase iniziale e finale della lattazione) che giornalieri (3,06 vs 22,97 µg/L, $P < 0,0001$; 2,91 vs 22,92 µg/L, $P < 0,0001$ rispettivamente per i gruppi di controllo e trattato, nella fase

iniziale e finale della lattazione), nonché i livelli delle IGF-I ed anche in questo caso sia basali (104,14 vs 140,71 $\mu\text{g/L}$, $P < 0,001$; 85,78 vs 118,06 $\mu\text{g/L}$, $P < 0,001$ rispettivamente per i gruppi di controllo e trattato, nella fase iniziale e finale della lattazione), che giornalieri (105,71 vs 155,78 $\mu\text{g/L}$, $P < 0,001$; 90,10 vs 118,95 $\mu\text{g/L}$, $P < 0,001$ rispettivamente per i gruppi di controllo e trattato, nella fase iniziale e finale della lattazione). I livelli medi giornalieri dei NEFA sono significativamente aumentati in entrambe le fasi di lattazione (0,036 vs 0,195 mmol/L , $P < 0,05$; 0,057 vs 0,251 mmol/L , $P < 0,05$ rispettivamente per i gruppi di controllo e trattato, nella fase iniziale e finale della lattazione) e le loro variazioni dovute ai pasti sono state invertite dall'iniezione di rbST; questa elevata disponibilità dei NEFA ha permesso un risparmio di glucosio, il cui livello ematico è risultato più elevato (4,16 vs 4,72 mmol/L , $P < 0,05$, rispettivamente per i gruppi di controllo e trattato) in fase avanzata della lattazione (per una minor produzione di lattosio), cosiccome un risparmio di aminoacidi, che ha determinato un minor livello di urea nel sangue, ma solo in fase avanzata di lattazione (5,94 vs 3,96 mmol/L , $P < 0,05$, rispettivamente per i gruppi di controllo e trattato). Sempre per le sole bovine in fase avanzata di lattazione, la maggiore disponibilità di glucosio dovuta al trattamento con rbST, ha determinato livelli giornalieri medi significativamente più elevati di insulina (15,91 vs 40,72, mU/L , $P < 0,01$) e T_3 (2,26 vs 2,54 nmol/L , $P < 0,01$), e minori di glucagone (77,34 vs 54,21 ng/L , $P < 0,01$). Nonostante nelle bovine trattate siano stati rilevati livelli simili di GH, IGF-I e NEFA nelle due fasi della lattazione, alcune variazioni endocrino-metaboliche sono risultate più evidenti nella fase finale della lattazione; inoltre, alcune di queste variazioni sono state modificate dalla distribuzioni dei foraggi. Si può concludere che le variazioni ematiche dovute al trattamento con rbST non sono uguali a quelle che si verificano con l'inizio della lattazione; inoltre queste variazioni ematiche sono modificate dalla sequenza dei pasti nella giornata ed anche lo stadio di lattazione pare avere un certo effetto.

Parole chiave: Somatotropina, Vacca da latte, Ormoni, Metaboliti.

Introduction

The treatment of dairy cows with recombinant bovine somatotropin (rbST) has been shown to have marked effects on milk yield (Burton *et al.*, 1994; Chilliard *et al.*, 1998; Etherton and Bauman, 1998). It is also well known that the effects of rbST are associated with, and mediated by, many physiological and metabolic changes, such as enhanced cardiac output, mammary blood flow, alveolar cell secretion activity, endocrine changes, lipolysis, glucose sparing, and gluconeogenesis (Bauman *et al.*, 1989; Burton *et al.*, 1994; Chilliard *et al.*, 1998; Etherton and Bauman, 1998). However, authors have generally concluded that only few effects on blood metabolites and hormone levels, due to the rbST treatment, can be seen (Burton *et al.*, 1994; Chilliard *et al.*, 1998). Nevertheless, looking at the experimental papers, there is evidence for marked variations in blood concentration of some metabolites and hormones, namely during the first days of rbST treatment. In addition, we have observed in dairy cows treated daily with rbST (Piccioli-Cappelli *et al.*, 1989) a significant increase in pre-feeding plasma concentrations of non-esterified fatty acids (NEFA), cholesterol and bilirubin, whereas the urea level and the activity of the lactate dehydrogenase (LDH) were significantly

reduced. Similar results have also been observed by Oldenbrock *et al.* (1989), Rosi *et al.* (1990), and consistent, but smaller variations have been shown by Sechen *et al.* (1989). Moreover, with respect to hormones, the concentrations of insulin-like growth factor (IGF-I) have been observed to increase after rbST administration (Ronge and Blum, 1989), but the rbST-IGF axis seems strictly dependent on the cow's nutritional status (Burton *et al.*, 1994; Ronge *et al.*, 1988). Insulin increments due to rbST treatment were also observed (Ronge and Blum, 1989; West *et al.*, 1991), while other authors showed variable insulin changes after rbST treatment (Davis *et al.*, 1988; de Boer and Kennelly, 1989; French *et al.*, 1990). Many other authors have reported small changes of insulin and thyroid hormone concentrations (Butterwick *et al.*, 1989; Chilliard *et al.*, 1989; Cissé *et al.*, 1991), and Johnson *et al.* (1991) observed a significant reduction of 3,5,3'- triiodothyronine (T_3) and cortisol concentrations.

Therefore, the variations of blood hormones and metabolites during rbST administration seem different for pattern and amplitude; the causes could be attributed to the very complex, and consequently easily influenced, physiological mechanisms, through which rbST acts to increase milk yield. This could be confirmed observing the dif-

ferent pattern of milk increase in dairy cows daily treated with rbST (Bertoni *et al.*, 1992) in early or late lactation (slower but higher response). In addition, the amplitude of response in milk yield to rbST treatment seems to depend on the quality of management of the herd, as reviewed by Bauman (1992), while Chilliard *et al.* (1998) indicated that best results can be obtained within 3-7 months of lactation, suggesting that pregnancy can also modify the rbST effects, as suggested by Burton *et al.* (1994) and also shown by Bertoni *et al.* (1990).

For a better understanding of these aspects, our experiment was carried out to study the effects of daily rbST treatment in early and late lactation, on blood variations of a selected number of hormones and metabolites checked on basal conditions (pre-feeding and pre-injection) and on 24-h behaviour (post-feeding and post-injection).

Material and methods

Animals, management and feeding

Four Italian Friesian cows (two pairs of twins; each pair with a cow in the 3rd and 10th month of lactation, both non-pregnant yet) were divided into two groups according to the stage of lactation, so that each group was composed of a cow in early lactation of one pair of twins and one in late lactation of the other pair.

The four cows were tied up and held at the Istituto di Zootecnica in Piacenza. They were individually fed twice daily (at 07.00 h and at 15.00 h) with 14 kg of corn-silage (as feed) and hay *ad libitum*, while the concentrate (consisting of maize, barley, wheat bran, soybean meal, beet pulps, molasses, mineral and vitamin supplements), were fed five times daily (at 07.30, 10.30, 13.30, 16.30 and 19.30) at the rate of 1 kg every 3 kg of milk.

The feed intake was recorded daily. A representative sample was taken from each feed at every batch change. All the samples, except the concentrate, were dried in a ventilated oven (65°C) for dry matter determination, ground and analysed for crude protein, crude fibre, neutral detergent fibre, ether extract, ash and starch contents (Martillotti *et al.*, 1987). Additionally, a portion of fresh silage was used to measure pH and

ammonia nitrogen. The energetic value was calculated as energy fodder unit (UFL) according to INRA (1988).

The requirements were estimated according to INRA (1988) for energy and according to NRC (1989) for crude protein.

Experimental procedure

Cows were injected daily (at 08.30 h) with 26.3 mg rbST obtained from Monsanto (St. Louis, USA) or a placebo (2.4 ml saline) in a change-over design, during 2 periods of 21 days each. Between the two periods there was a week without treatments. Blood samples for the determination of all traits, with the exception of glucagon, were taken from the jugular vein using evacuated tubes containing lithium-heparin as anti-coagulant. Blood samples were collected before the morning feeding on the day before (day 0) and on days 2, 4, 7, 11, 14, 18 and 21 after the beginning of the treatment and 4 days after the end of the treatment (day 25). Moreover, on the 4th and 18th day from the start of rbST treatment, blood samples were collected at 07.00 (before feeding), 08.00 (before injection), 09.00, 11.00, 15.00, 19.00, 23.00 h and at 03.00 and 07.00 h of the following day. Plasma was separated immediately after sampling and stored in aliquots at -20°C. For glucagon determinations, 2.5 ml of blood, withdrawn in siliconed-evacuated tubes, were added to a tube containing 2 mg of Na₂-EDTA and 1000 KIU of Trasylol (Bayer) and immediately centrifuged and the plasma stored in 2 aliquots at -20°C.

Hormone assays

Concentrations of thyroxine (T₄), triiodothyronine (T₃), cortisol, and glucagon were determined using liquid phase double antibody radioimmuno assay (RIA) kits purchased from Diagnostic Products Corp. (Los Angeles, U.S.A.). The cortisol RIA assay procedure was modified: 50 µl of plasma instead of 25 µl were used, tubes were incubated for 24 h at 4°C instead of 45 min at 37°C, and calibrators were more diluted because of the much lower normal levels in cows than in humans. Reverse T₃ (rT₃) and insulin were deter-

mined by kits purchased from Ares-Serono (Milan, Italy). The GH was determined by a solid-phase double antibody RIA. The assay utilised purified bovine GH (USDA-bGH-B-1) for standard and for iodination (Salacinski *et al.*, 1981), a rabbit anti-ovine-GH (NIDDK-anti-oGH-2), and a goat-anti-rabbit-gammaglobulin, covalently linked to sepharose gel (liso-phase columns, SCLAVO, Siena) as second antibody.

The concentration of insulin-like growth factor I (IGF-I) was determined by RIA as described by Ronge *et al.* (1988). The assay was modified by use of a monoclonal antibody and separation of bound and free fractions with an antibody against mouse γ -globulin raised in sheep.

Metabolite and enzyme assays

Blood glucose, NEFA, cholesterol, triglycerides, urea, and creatinine concentration, as well as LDH activities were processed with a clinical centrifuge-analyser (Monarch 2000, Instrumentation Laboratory, Lexington, MA, USA) using the method previously described by Bertoni *et al.* (1998).

Statistical analysis

The data were processed using the General Linear Models procedure of the S.A.S. (1989). Before processing the data for rbST effect, only the pre-feeding sampling collected during the placebo injection (control group) were processed to evaluate differences between the stage of lactation, utilising these factors: stage of lactation, cow nested inside the stage of lactation, day of bleeding. As for most of the blood parameters, significant differences were observed between the two stages of lactation, analysis to evaluate differences between placebo and rbST injection mean values was run separately for the two stages of lactation for pre-feeding and post-feeding post-rbST injection samplings using different models. With the first model the differences for the basal value (pre-feeding samplings) were evaluated utilising these factors: treatment, cow, day from treatment start and the interaction treatment*day. With the second model the difference for the daily values (post-feeding post-rbST injection samplings) has been evaluated utilising

the factors treatment, cow, hours from rbST treatment and the interaction treatment*hours. For the evaluation of differences at each sampling point (day from treatment for pre-feeding data or hour for 24-hour variation), due to the low degree of freedom available, it was not possible to use model for repeated measurements, so separate analyses were performed for single pairs of data - placebo vs rbST - within each stage of lactation (Littell *et al.*, 1998).

Results

Milk yield, dry matter intake and the energy and protein balances are shown in Table 1. Milk yield significantly ($P < 0.001$) increased after rbST treatment in both early and late lactation, but the rate of increase was different: sudden and within the first week in early lactation; prolonged to the whole treatment period in late lactation. Interestingly, the pattern of changes in early and late lactating cows was very different as already discussed in Bertoni *et al.* (1992).

Dry matter intake of both early and late lactating cows slightly increased (NS) during the treatment period, as did the milk yield, but with different patterns in the groups: quick and short increase for early lactating cows and gradual throughout the experimental period for late lactating cows. The energy and protein balances, always positive before treatment, decreased afterwards and became negative as respects for energy only in early lactating cows.

Basal blood values

Basal blood values were obtained immediately before the morning forage meal and 1 hour before the rbST injection. Pre-feeding concentrations of hormones of control groups (zero time, Tables 2 and 3) were different in early as compared to late lactation: in the former, insulin and thyroid hormones were slightly lower ($P < 0.01$ only for T_4), whereas much higher levels of glucagon ($P < 0.001$), GH (NS) and IGF-I ($P < 0.001$) were observed.

The interaction "treatment*day" has not been considered because it has never shown significant effect; in fact for most of the parameters altered by

rbST injection, changes already occurred in the first days of treatment.

During rbST treatment, plasma GH concentrations (Table 2) resulted slightly higher in the treated group in both stages of lactation than in the respective control groups, until day 11-14; nev-

ertheless, only mean values of the treatment period resulted significantly different ($P < 0.05$ in early and $P < 0.01$ in late lactation).

Plasma IGF-I concentrations (Table 2) of treated groups resulted higher than the respective control group beginning the second day from the com-

Table 1. - Weekly mean values of milk yield and main features of the diet received during the trial (body weight changes are not included in requirement computation) of 4 dairy cows (2 in early and 2 in late lactation) daily treated for 21 days, in a change-over design with 26.3 mg of rbST (rbST) of placebo (ctrl).

week from start of treatment		Pre	1	2	3	Post	overall treatment period mean	S.E. ³	
milk yield (kg/d)	early	ctrl	34.4	32.7 ***	32.1 ***	32.0 ***	32.0	32.3 ***	4.78
		rbST	33.9	36.8	37.5	38.0	32.7	37.4	
	late	ctrl	14.2	14.3 ***	14.1 ***	14.2 ***	13.9 **	14.2 ***	1.49
		rbST	13.7	16.7	20.9	22.6	17.4	20.1	
dry matter intake (kg/d)	early	ctrl	23.7	23.7	23.7	23.5	23.2	23.6	0.59
		rbST	23.6	24.2	24.6	24.5	23.3	24.4	
	late	ctrl	19.4	19.5	19.5	19.2	19.4	19.4	3.36
		rbST	18.7	18.9	19.7	20.8	19.8	19.8	
Theoretical energy balance ¹ (100=ideal)	early	ctrl	106.9	109.2	115.5	115.6	109.9	113.4 ***	32.9
		rbST	103.0	96.2	94.7	95.3	107.8	95.4	
	late	ctrl	118.5	117.8	118.6	118.5	122.3	118.3 ***	88.16
		rbST	124.0	102.0	97.9	100.9	119.7	100.3	
Theoretical total protein balance ² (100=ideal)	early	ctrl	123.7	127.3	132.0	130.3	125.5	129.9 ***	89.07
		rbST	119.9	117.8	108.7	106.4	124.7	111.0	
	late	ctrl	153.4	155.1	156.7	146.9	159.4	152.9 ***	208.98
		rbST	159.4	125.8	117.6	115.6	147.4	119.7	

Statistical differences between the treatments, marked with * if $P < 0.05$; ** if $P < 0.01$; ***if $P < 0.001$.

¹ Calculated using requirements reported in INRA (1988)

² Calculated using requirements reported in NRC (1989)

³ Pooled standard error

Table 2. Mean values of blood basal content of insulin (mU/L), GH ($\mu\text{g/L}$) and IGF-I ($\mu\text{g/L}$) in dairy cows treated daily with 26.3 mg of rbST (rbST) or placebo (ctrl) for 21 days at different days from treatment start.

Days from start of treatment	Insulin				GH				IGF-I			
	early		late		early		late		early		late	
	ctrl	rbST	ctrl	rbST	ctrl	rbST	ctrl	rbST	ctrl	rbST	ctrl	rbST
0	8.70	9.90	11.20	8.75	4.15	3.98	3.25	3.02	106.45	109.75	79.30	78.15
2	9.50	7.35	8.75 *	18.35	5.50	4.45	4.65	3.40	108.75 *	145.40	81.70 *	131.40
4	6.70	9.10	12.30 *	23.95	3.20	5.90	2.05	6.20	102.75	143.80	81.90 *	115.75
7	7.90	11.40	10.60	19.15	4.75	5.80	3.20	6.15	102.60 *	132.10	86.80	107.05
11	8.60	11.60	8.25 *	20.15	3.70	5.35	4.34	8.15	104.95 *	145.20	84.80 *	119.90
14	7.65 *	14.25	10.15 *	21.75	5.05	6.60	4.85	7.10	104.40 *	135.35	92.30	118.25
18	9.95	12.85	9.20	18.60	4.30	6.15	2.55	6.85	97.80 *	143.80	88.25 *	120.35
21	10.10	11.30	8.55	14.80	4.00	6.15	4.20	6.50	107.70 *	139.35	84.70 *	113.75
25	8.05	8.35	9.40	10.50	3.95	5.45	2.25	4.60	111.28	114.65	89.65	95.00
overall treatment period mean	8.63 *	11.12	9.69 **	19.54	4.36 *	5.77	3.69 **	6.34	104.14 ***	140.71	85.78 ***	118.06
S.E. ¹	6.76		7.48		3.92		1.51		46.71		95.69	

Statistical significant differences between control and treated group marked with: *, if $P < 0.05$, **, if $P < 0.01$, ***, if $P < 0.001$

¹ Pooled standard error

mencement of treatment, with most of the comparisons different for $P < 0.05$.

Mean values of insulin (Table 2) for the treatment period resulted higher ($P < 0.05$ and < 0.01 in early and in late lactation, respectively) in treated than in the respective control group. However, whereas in the late stage of lactation insulin levels almost doubled within 48 hours, relative to control group values ($P < 0.05$ on days 2, 4, 11 and 14), in early lactation this increase was less evident ($P < 0.05$ only on d 14).

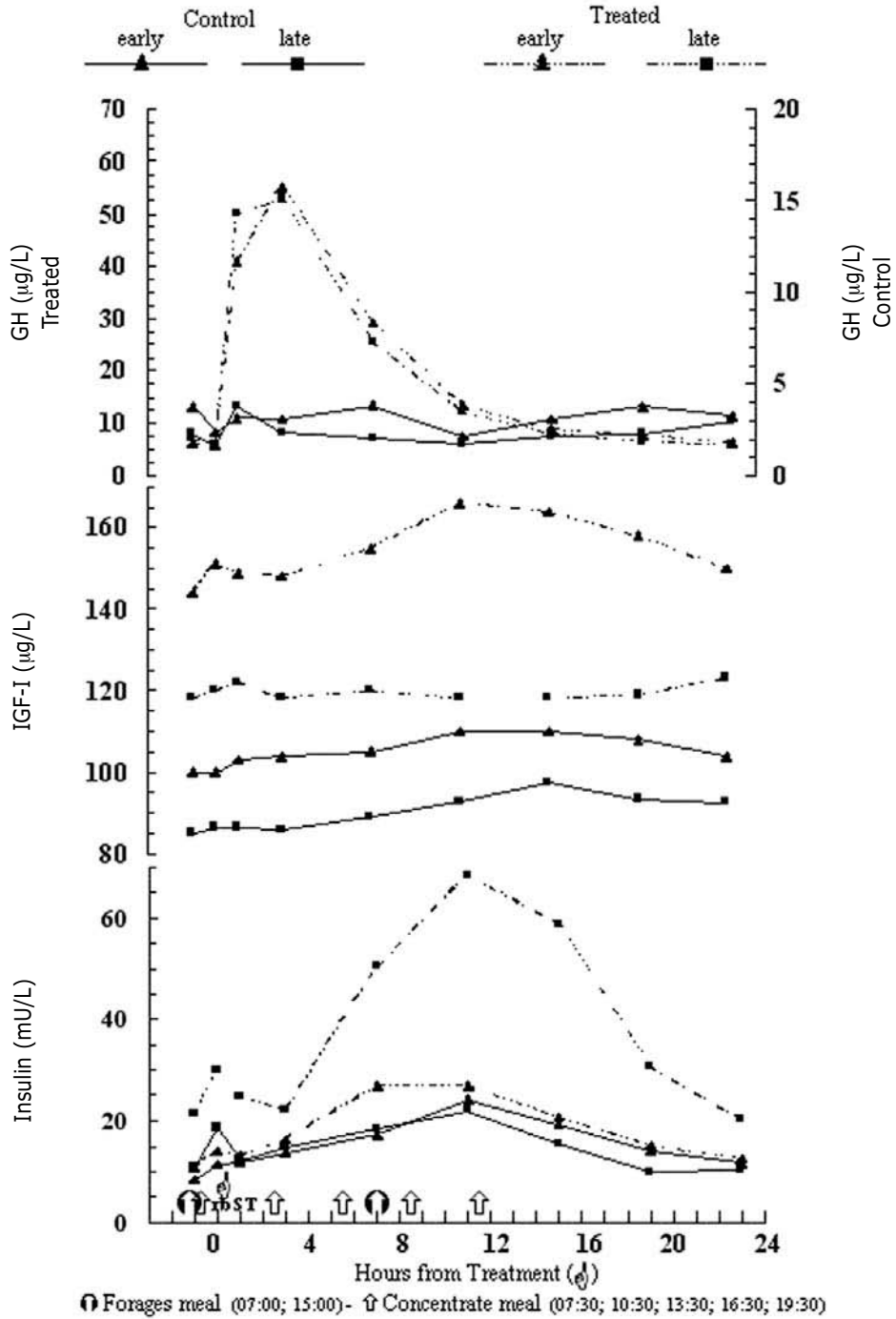
Glucagon concentrations (Table 3) were differently affected by rbST treatment, i.e., remained mostly unchanged in early lactation, but decreased slightly ($P < 0.05$) in late lactation.

Mean values of the treatment period of T_4

(Table 3) resulted slightly higher ($P < 0.05$) in the treated than in the respective control group, but only in late lactation. Changes of T_3 (Table 3) were, in general, significant at the end of the treatment period in late lactation ($P < 0.05$), but T_3 was differently affected by the treatment than T_4 . In fact, mean levels of the treatment period of T_3 resulted higher ($P < 0.001$) in late lactating and lower ($P < 0.01$) in early lactating cows (always compared to the respective control group), with an opposite trend after the end of treatment. Concentrations of rT_3 did not change (not shown).

Cortisol concentrations were always extremely variable and were not significantly affected (NS) by rbST treatment (not shown).

Figure 1. 24-h pattern of plasma GH, IGF-I and insulin concentrations of 4 dairy cows in different phases of lactation and treated daily or not with 26.3 mg of rbST in a change-over design (pooled data of collection on 4th and 18th day of treatment).



24-hour blood variations

Data obtained on days 4 and 18 of the treatment period were combined as no significant differences either for pattern of changes or level of concentrations were observed.

Concentrations of GH (Figure 1, with different axes scale for treated and control values) rapidly increased ($P < 0.001$) in both groups, reached peak concentrations 3 h after the injection and then returned towards basal values after 6-10 hours from the injection; but after 24 h values they were still markedly higher ($P < 0.05$) compared to untreated animals (6-8 vs 2-4 $\mu\text{g/L}$, respectively).

The concentrations of IGF-I (Figure 1) were markedly higher ($P < 0.001$) in treated animals than in controls, but did not follow GH variations. Only a slight increase in the middle of the day was generally observed.

Insulin concentration (Figure 1) increased continuously for 12 h after the 1st forage meal in both control and treated cows. However, while in treated cows in early lactation the rise was similar to the respective control group (only at 7 hours from the treatment the difference was significant for $P < 0.05$), in late lactation insulin rise was 3 times greater than in the respective control group ($P < 0.01$ from 7 to 19 hours after treatment start).

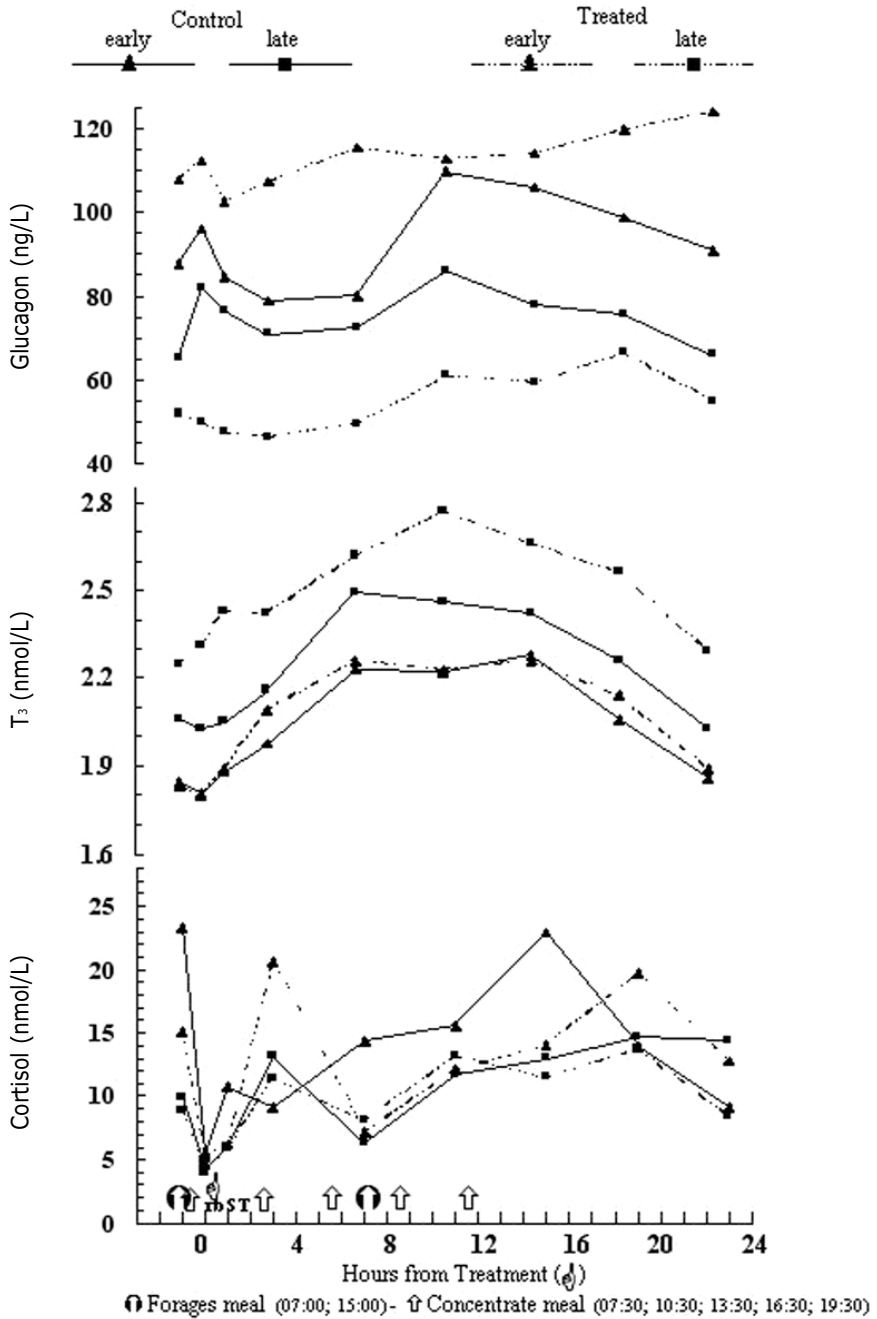
Table 3. Mean values of blood basal content of T₃ (nmol/L), T₄ (nmol/L) and glucagon (ng/L) in dairy cows treated daily with 26.3 mg of rbST (rbST) or placebo (ctrl) for 21 days at different days since the treatment start.

	T ₃				T ₄				Glucagon				
	early		late		early		late		early		late		
	ctrl	rbST	ctrl	rbST	ctrl	rbST	ctrl	rbST	ctrl	rbST	ctrl	rbST	
days from start of treatment													
0	1.93	1.83	1.97	1.97	56.82	54.18	61.92	70.56	95.50	113.00	52.50	53.50	
2	1.89	1.72	2.06	1.88	54.24	55.53	64.05	72.18	86.50	109.50	36.75	48.50	
4	1.94	1.89	2.23	2.11	58.89	58.44	67.98	81.46	90.00	111.50	51.80	46.00	
7	2.02	1.71	1.91	2.17	59.47	58.37	68.24	78.82	103.50	124.50	75.50	39.00	
11	2.11	1.83	1.76	*	2.37	61.98	56.05	64.89	75.08	94.50	119.50	53.31	37.50
14	2.08	1.85	1.97	2.28	55.34	58.05	66.69	67.60	106.50	123.50	64.50	44.00	
18	1.76	1.77	1.89	*	2.39	52.95	53.02	65.27	71.92	85.25	104.50	78.50	57.50
21	1.76	1.82	1.82	**	2.63	52.63	61.15	64.89	69.60	94.90	109.00	51.50	42.00
25	1.83	2.05	1.94	2.28	52.63	59.02	66.05	59.15	90.85	108.50	53.50	68.00	
overall treatment period mean	1.94	** 1.80	1.96	*** 2.26	57.30	58.55	65.79	* 71.89	96.42	118.68	52.76	* 41.80	
S.E. ¹	0.012		0.013		25.36		15.76		212.1		131.2		

Statistical significant differences between control and treated group marked with: *, if $P < 0.05$, **, if $P < 0.01$, ***, if $P < 0.001$

¹ Pooled standard error

Figure 2. 24-h pattern of plasma glucagon, T3 and cortisol concentrations of 4 dairy cows in different phases of lactation and treated daily or not with 26.3 mg of rbST in a change-over design (pooled data of collection on 4th and 18th day of treatment).



Treatment with rbST determined levels of glucagon (Figure 2) significantly higher in early ($P < 0.05$), and lower in late lactation ($P < 0.01$), whereas the daily pattern of changes of the hormone seemed smoothed by the treatment, as the post-feeding rise was less evident (NS) in rbST treated cows.

Of the thyroid hormones (T_3 , T_4 and rT_3), only the T_3 pattern is shown in Figure 2, because changes of T_4 and rT_3 were very similar to those of T_3 . The T_3 concentration strongly increased ($P < 0.05$ basal vs sampling from 8 to 12 hours after the morning forage meal) after forage intake in the morning, although responses were very similar in control and treated animals. However, mean levels resulted higher in rbST treated animals in late than in those in early stages of lactation (2.48 vs 2.05 nmol/L, $P < 0.001$).

Changes of cortisol (Figure 2) were similar in both groups, i.e. immediately after the morning feeding (within 1 hour) there was a sudden, transient fall ($P < 0.01$ at 1 and 2 hours after the morning meal).

Concentrations of metabolites with greatest daily changes are shown in Figure 3, while cholesterol and triglycerides, both slightly increased; other blood parameters have been already discussed in a previous paper (Piccioli-Cappelli *et al.*, 1989). In the morning, after a post-feeding decline, NEFA concentrations showed a marked increase in response to rbST ($P < 0.05$ for all the blood collection in both lactation stages), and their basal levels, in both treated groups, resulted 3-4 times higher ($P < 0.001$) than in controls. The diurnal patterns of changes of glucose and urea were unaffected by rbST treatment, nevertheless, the rise of glucose generally observed after the second meal of forages was higher after rbST treatment, and markedly so in late lactation when glucose levels resulted always higher throughout the day for $P < 0.05$. In contrast, urea concentrations were reduced in both treated groups, but with differences much greater ($P < 0.05$) in late than in early lactation (NS).

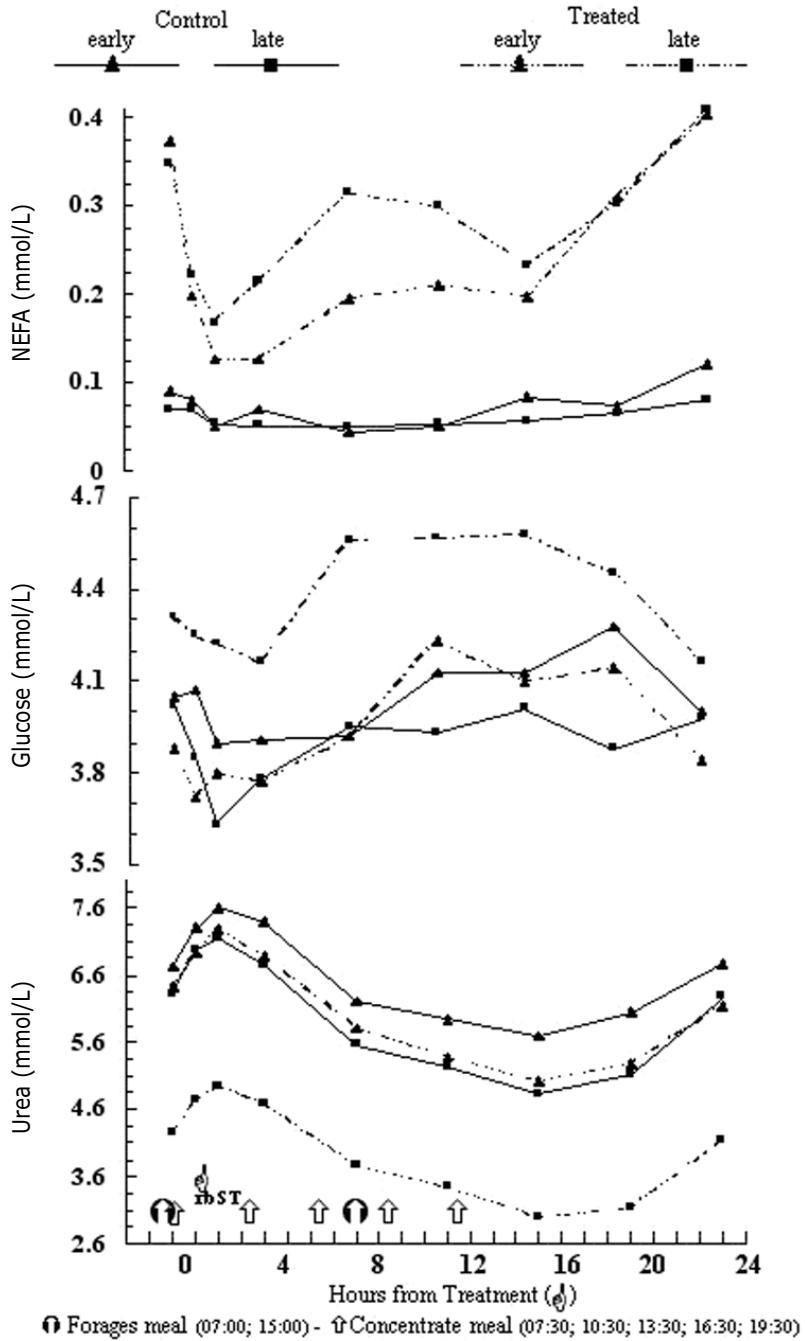
Discussion

The expected increase of GH after rbST treat-

ment, as in this study, was found by other authors (Chilliard *et al.*, 1989), but high values have been observed only within 6-10 hours after the injection. Similar blood levels have been observed between the 2nd and 10th day after a treatment with 640 mg of rbST in a sustained release formulation (Bertoni *et al.*, 1997). Such high values are presumably required for the treatment efficacy, but are greatly different from the naturally observed values in various stages of lactation (Bertoni *et al.*, 1995; Clément *et al.*, 1994; Ronge *et al.*, 1988).

However, looking at the post-treatment daily variations, the only immediate effect of rbST observed at blood level was the rise of NEFA, which counteracted the meal effect (Figure 3). The rise of NEFA was more pronounced 7-8 h after the rbST injection, but NEFA concentrations still remained elevated when GH concentrations returned to baseline values (although higher than controls: 6-8 vs 2-4 $\mu\text{g/L}$, $P < 0.05$). Nevertheless, NEFA changes still seemed related to the forage meals effect; in fact, some decline was observed after them and the highest NEFA values were observed in the treated animals 22 hours after the rbST injection - which corresponds to 16 hours from the last forage meal - though GH concentrations had already returned to baseline values many hours prior. On the contrary, insulin did not seem to modify the increased lipolysis during rbST treatment as suggested by Sechen *et al.* (1989), because very high levels of NEFA were observed while insulin was extremely high (near to 60mU/L). Moreover, although insulin levels were extremely different in early and late lactation with expected different effects on lipolysis, NEFA levels variations resulted similar. Therefore, lipolysis does not seem particularly influenced even by the energy balance, as suggested by Etherton and Bauman (1998), which in our case was nil or positive. In any case, according to Burton *et al.* (1994), Chilliard *et al.* (1998) and Etherton and Bauman (1998) lipolysis seems indirectly affected by rbST throughout an enhancement of adipocyte sensitivity to other homeostatic hormones (i.e. epinephrine). Nevertheless, if this mechanism can explain the lowering of NEFA values after the first forage meal, when epinephrine is reduced (Vernon, 1992), it cannot explain the small reduction in NEFA

Figure 3. 24-h pattern of plasma NEFA, glucose and urea concentrations of 4 dairy cows in different phases of lactation and treated daily or not with 26.3 mg of rbST in a change-over design (pooled data of collection on 4th and 18th day of treatment).



after the second forage meal, when insulin is at the highest levels and epinephrine can be assumed to be lower while GH is still slightly high. Therefore, a direct effect, at least partial, as suggested by Chilliard *et al.* (1998), of high GH values on the lipolysis seems very likely.

Many other blood changes that have been observed seem consequent to the rise in NEFA. In fact, the greater availability of NEFA may help to spare other energy-yielding substrate, i.e. glucose and amino acids (Burton *et al.*, 1994; Chilliard *et al.*, 1998; Etherton and Bauman, 1998). This was evident for blood glucose and urea concentrations; in fact, subsequent to rbST treatment, glucose was significantly increased in late lactation when less lactose has to be altogether synthesised, and this could have caused the increased insulin concentration. However, the marked rise of this latter hormone, following rbST treatment in late lactation, along with the strong rise of glucose, could be related to an increased secretion and a reduced liver clearance rate (Reynolds *et al.*, 1989), associated also with the GH induction of insulin resistance (Burton *et al.*, 1994; Chilliard *et al.*, 1998).

The concentration of IGF-I increased after a few days of rbST treatment and remained approximately 50% higher in both groups than in respective controls during the trial, showing small daily variations. The absolute values were significantly higher in the early lactating cows, but glucose levels were lower suggesting that the contribution of IGF-I to the diabetogenic effect of rbST (Burton *et al.*, 1994) is not of major importance.

However, compared to our study, the increase of IGF-I was quite delayed in experiments of Peel *et al.* (1985), and it was small in those of Elsasser *et al.* (1989) and McGuire *et al.* (1992) where animals were underfed for energy or protein (Elsasser *et al.*, 1989; McGuire *et al.*, 1992). On the contrary, in our experiment the cows were adequately fed with respect to energy and protein (Table 1) and therefore IGF-I responses to rbST were relatively marked, confirming the data reviewed in (Burton *et al.*, 1994; Chilliard *et al.*, 1998; Etherton and Bauman, 1998). Moreover, IGF-I responses to rbST were delayed and relatively small in dairy cows in early lactation, characterised by energy deficiency, compared to IGF-I responses in the same cows

during pregnancy and receiving adequate amounts of energy (Ronge and Blum, 1989).

Our study also observed a significant rise in T₃ (and partially in T₄) concentration, but only in late lactation. The fact that only T₃, and not T₄, significantly increased could be a consequence of a different liver monodeiodination activity; nevertheless, our result is the opposite of that obtained by Kahl *et al.* (1995). It can be suggested that the rise of T₃ concentration could be linked to the marked rise in glucose concentration observed in late lactation only (Bertoni *et al.*, 1983). Therefore, it is not surprising that in several studies the rbST treatment did not affect blood levels of thyroid hormones (Chilliard *et al.*, 1989; Davis *et al.*, 1988; Ronge and Blum, 1989); in fact, it may occur in some peculiar conditions only: i.e. low yielding cows in late lactation showing a strong rise of blood glucose.

In summary, some effects of rbST are confirmed to vary according to the metabolic situation of the cow, except the lipolytic activity which always resulted very high, thereby allowing the sparing of glucose. Despite this:

- in relatively early lactation glucose is immediately utilised, mainly for lactose (Chilliard *et al.*, 1998), and therefore few consequent blood changes are observed;
- in late lactation the "excess," of glucose with respect to lactose synthesis need, determines its increased level in blood with the consequent increase of insulin and T₃, while glucagon is reduced. So a high level of insulin and lower level of glucagon can justify the substantial reduction of urea (Figure 3) observed in our trial (less gluconeogenesis from amino acids). However, a higher milk protein synthesis (Bertoni *et al.*, 1992) could contribute to explain the result.

Furthermore, the constantly higher values of IGF-I in treated animals could also contribute to the second mechanism of rbST, as suggested by Etherton and Bauman (1998), the somatogenic effect at the mammary gland level (i.e. cell number, enzymes, etc.). A proliferation of cells as a consequence of IGF-I treatment has been observed by Baumruker and Stemberger (1989) and we have indirectly confirmed it showing an increase in the

DNA at mammary level after rbST treatment (Trevisi *et al.*, 1997). This obviously could be more important in late lactation when the mammary gland epithelia is less efficient, as gleaned from the different milk yield response in our trial (Table 1), slower but higher in late lactating cows.

Therefore, it is unlikely for us to accept the suggestion of Burton *et al.* (1994) that the galactopoietic effects of rbST are similar to those supporting the increase of milk yield during early lactation; in fact, there are differences and in any case it largely depends on mammary gland activity and metabolic conditions.

Finally, many hormonal and metabolite changes due to the treatment are not only affected by the physiological stage, but the distribution of daily forage meals can be of major importance (namely for NEFA, insulin, T₃ and glucose). Because the feeding and bleeding patterns are so different, the results of some authors can confound the effects of rbST and meals, and this could justify the findings in their experimental situation, i.e. the rbST treatment per se does not definitively modify the metabolic conditions of the dairy cows.

Conclusions

The cows we have utilised were not pregnant despite the fact that two of them were in very late lactation; however the energy and the protein balances were positive and only the energy one was slightly under the ideal level in the early lactating animals.

The treatment with rbST determines a somatogenic effect that seems more evident in not pregnant, low yielding, late lactating cows. In fact, the metabolic effect of treatment, with a different nutrient partitioning, can be observed much more easily. The temporary high level of GH and the constantly higher values of IGF-I are the causes of a lipolytic effect and of a sparing effect on glucose and amino acid because NEFA are highly available at muscle and adipose tissues levels. Nevertheless, the other metabolic-endocrine consequences - blood levels of glucose, urea (from amino acids catabolism), insulin, thyroid hormone - seem secondary to NEFA changes and are greatly influenced by the lactation stage (or milk yield

level) and by the daily feeding pattern.

In conclusion, it seems likely that the effects of the injected GH on milk yield could be different, at least partially, with respect to those physiologically occurring at the onset of the lactation; moreover, some of its metabolic effects can only be observed if other causes of blood changes are taken into account (stage of lactation, feeding pattern, GH level, etc).

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