Plasma levels of somatotropin, somatostatin and metabolites in dairy cows fed a protected fat enriched diet

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LIVELLI PLASMATICI DI SOMATOTROPINA, SOMATOSTATINA E METABOLITI IN BOVINE DA LATTE ALIMENTATE CON UNA DIETA CONTENENTE LIPIDI PROTETTI

18 bovine da latte sono state ripartite in due gruppi: gruppo C (controllo) e gruppo S (saponi di calcio). Nel concentrato somministrato al gruppo C non erano inclusi grassi esogeni mentre il concentrato distribuito al gruppo trattato (S) conteneva il 6% di sali di calcio degli acidi grassi (saponi di Ca), in sostituzione di orzo, mais e sali inorganici di calcio. La prova ha avuto una durata di 50 giorni; campioni di sangue sono stati prelevati prima della distribuzione mattutina degli alimenti, nei giorni 0, 28, 35, 42 e 49. Rispetto al gruppo C, il trattamento con saponi ha determinato un significativo ($P<0,01$) incremento dei livelli plasmatici di fosfolipidi (+31%), colesterolo totale (+29%), colesterolo libero (+37%), esteri di colesterolo (+26%), trigliceridi (+33%) e somatostatina (+23%; $P<0,05$). Il trattamento alimentare ha invece indotto una significativa diminuzione dei livelli plasmatici di urea (—14%; $P<0,05$) e del rapporto esteri di colesterolo:colesterolo totale (77,4 vs 78,8%; $P<0,01$).
I valori osservati, quando rapportati ai valori iniziali (giorno 0), hanno evidenziato un significativo incremento dei livelli di GH (+37%; $P<0,05$), NEFA (+24%; $P<0,05$) e di proteine totali (+10%; $P<0,01$) nel plasma delle bovine S. Il trattamento alimentare non ha influito sui livelli plasmatici di glucosio e fosfato nè sul rapporto GH:somatostatina.

Eighteen dairy cows were divided into two groups: group C (control) and group S (calcium soaps). Control concentrate (group C) did not contain exogenous fat, treatment concentrate (group S) contained 6% calcium soaps, replacing amounts of barley, maize and inorganic calcium salts. The trial lasted 50 days; blood samples were taken in the morning before feeding on days 0, 28, 35, 42 and 49. Group S showed significantly higher ($P<0.01$) phospholipid (+31%), total cholesterol (+29%), free cholesterol (+37%), cholesteryl ester (+26%), triglycerid (+33%) and ($P<0.05$) somatostatin (+23%) plasma levels than group C. The levels of urea (—14%; $P<0.05$) and the cholesteryl ester: total cholesterol ratio (77.4 vs 78.8%; $P<0.01$) were lowered by the treatment. Experimental data related to the baseline values (day 0) showed a significant increase in GH (+37%; $P<0.05$), NEFA (+24%; $P<0.05$) and total protein (+10%; $P<0.01$) levels in group S. The treatment did not affect plasma levels of glucose, phosphate and the GH:somatostatin ratio.

INTRODUCTION

The inclusion of protected fats in rations for dairy cows increases the dietary energy concentration and does not influence ruminal fermentations. Since a considerable amount of long chain fatty acids is transferred from the diet to the milk, protected fat enriched diets have been used both to optimize the utilization of the dietary energy and to modify the acidic composition of the milk fat (19). As GH and somatostatin play a fundamental role in the regulation of the utilization of the nutritional substances (6), the aim of this research was to study the utilization of the nutritional substances (6), the aim of this research was to study the influence of a protected fat enriched diet on certain hormones and blood parameters levels influenced by the nutrients metabolism in lactating cows.

MATERIALS AND METHODS

Eighteen Italian Friesian cows, 150 d post partum, average milk yield 24 kg/d, were divided into two groups: control [C] and calcium soaps [S]. In concentrate S a total of 6% of corn, barley and calcium salts was replaced by 6% of calcium soaps containing 33% oleic acid, 27% palmitic acid and 22% stearic acid (Tab. I). On a 12 kg milk yield basis, all the cows were daily fed fresh herbage 30 kg, meadow hay 7 kg and concentrate 4 kg; 1 kg of concentrate per 2.5 kg milk over 12 kg/d was also given.

Blood samples were collected on days 0, 28, 35, 42 and 49 in the morning prior to feeding. Vacuum test tubes containing EDTA and aprotinin to inhibit protease (Traslylol, Bayer, 50 μl/ml of blood) were used; the plasma samples were immediately separated and stored at —20°C.

Phospholipid, total cholesterol, free cholesterol, cholesteryl ester, triglycerid, NEFA, total protein, urea, phosphate and glucose levels were measured using colorimetric methods (Boehringer Mannheim, Italy). Specific radioimmunological techniques were used to measure plasma levels of GH (Innovet, Amity PG) and somatostatin (14).

Milk yield and composition were recorded on days 0, 21, 35 and 49. Fatty acids composition of both diets and milk fat have been described elsewhere (26).

The data were analyzed using analysis of variance (30) and repeatibility was calculated using the Falconer method (9).

RESULTS

Milk yield and composition data are summarized in table II.

Table III shows the mean plasma values (± residual standard error) observed in control (group C) and calcium soap (group S) groups. Cows fed calcium soaps showed significantly higher levels in the plasma of phospholipids, total cholesterol, free cholesterol, cholesteryl ester, triglycerides and somatostatin and lower levels of urea and cholesteryl ester:total cholesterol ratio than group C.

The protected fat enriched diet also increased GH (16.4%), NEFA (29%) and total proteins (4%) plasma levels. These increases were not significant, probably because the high variability between animals. The repeatibility of these former parameters was respectively: 0.73 for GH, 0,52 for NEFA and 0.64 for total proteins ($P < 10^{-6}$). When experimental data were related to the baseline values (day 0), GH as GH/GHO (+38%; $P < 0.05$), NEFA as NEFA/NEFAO (+24% $P < 0.05$) and total protein as protein/protein 0 levels (+10%; $P < 0.01$) were found significantly increased by the treatment.

TABLE I - Composition of experimental diets

a) Concentrates, %

	Group C	Group S
Corn meal	35	34
Soybean meal	20	20
Barley meal	18	14
Wheat middlings	14	14
Linseed cake	5	5
Molasses	4	4
Calcium soaps	—	6
Calcium carbonate	1,5	0,6
Dicalcium phosphate	1,0	0,9
Sodium bicarbonate	0,6	0,6
NaCl	0,4	0,4
Mineral-vitamin premix	0,5	0,5

b) Chemical composition and energy content evaluation

	Unit	Fresh herbage	Meadow hay	Concentrate group 1	Concentrate group 2
Dry matter	%	22,30	89,00	86,70	88,50
Crude protein	% d.m.	13,79	8,98	18,68	17,20
Ether extract	"	2,70	2,92	2,14	6,06
Crude fibre	"	26,18	28,57	5,41	5,16
Ash	"	8,91	8,04	6,94	6,29
N-free extract	"	48,42	51,49	66,83	65,29
Ca	"	0,58	0,37	1,09	0,94
P	"	0,39	0,30	0,65	0,63
UFL	/kg d.m.	0,80	0,67	1,08	1,14

TABLE II - Mean milk yield and composition in control (C) and calcium soaps (S) groups

	Unit	C	S	RSE	P	
Milk yield	kg/d	21,17	22,54	1,19	0,151	ns
Fat	%	3,74	3,76	0,122	0,925	ns
Protein	"	3,15	3,09	0,068	0,374	ns
Lactose	"	4,83	4,75	0,046	0,599	ns
Daily yield:						
Fat	kg/d	0,804	0,851	0,052	0,309	ns
Protein	"	0,665	0,688	0,034	0,445	ns
Lactose	"	1,07	1,00	0,053	0,353	ns

TABLE III - Mean plasma levels in control (C) and calcium soaps (S) groups

Constituent	Unit	C	S	RSE	P	
Total protein	g/l	74,7	75,7	3,14	0,96	ns
Ratio prot:prot.0		0,96	1,05	0,03	0,002	
Urea	mM	4,04	3,46	0,20	0,05	
Glucose	mM	2,30	2,33	0,10	0,98	ns
Triglyceride	mM	0,18	0,24	0,01	<0,001	
NEFA	µEq/l	95,2	123	21,0	0,077	ns
Ratio NEFA:NEFA0		0,96	1,19	0,15	0,048	
Phospholipid	mM	2,25	2,95	0,19	<0,001	
Total cholesterol	mM	4,31	5,55	0,40	<0,001	
Free cholesterol	mM	0,91	1,25	0,09	<0,001	
Cholesterol ester	mM	3,40	4,30	0,32	0,0014	
Ratio cholesterol ester:total	%	78.8	77.4	0,62	0,007	
GH	µg/l	7,62	8,87	2,11	0,75	ns
Ratio GH:GH0		0,85	1,17	0,21	0,048	
Somatostatin	pM	9,75	11,98	1,18	0,016	
Ratio GH:SS		0,82	0,78	0,18	0,99	ns
Phosphate	mM	1,68	1,77	0,12	0,31	ns

Levels of glucose, phosphate and the GH/somatostatin ratio were not, however, affected by the diet.

Daily milk yield resulted in group S 6% higher than in group C (P = 0.15). No significant differences were also found in milk fat, protein or lactose content between the two groups.

DISCUSSION AND CONCLUSION

Compared with the control diet (group C), the administration of a calcium soap enriched diet to dairy cows (group S), determined highly significant increases (Fig. 1a) in levels of total cholesterol (+29% compared with the control animals), free cholesterol (+37%), cholesteryl ester (+26%) and phospholipids (+31%) (Fig. 1b). Other authors have found up to 100% increases in circulating cholesterol due to the intake of protected fat enriched diets and no side effects (2). Savoini et al. (27) reported calcium soaps increase respectively 35% and 42% in plasma levels of total cholesterol and phospholipids in dairy cows. In lactating goats fed calcium soaps, Rosi et al. (24) also observed a highly significant increase in total cholesterol (+26%), free cholesterol (+32%), cholesteryl ester (+23%) and phospholipid (+18%) levels.

These increases could be due to an obligatory metabolic response involved in the transport of greater amounts of circulating dietary fats by lipoproteins. These carriers can be indeed considered as stable vehicles for carrying glycerides from intestine and liver to other tissues (2, 22).

The greater need for lipid-carrying molecules could also explain the rise in total protein observed in group S, compared with the baseline values (protein/protein 0) (Fig. 2).

Fat-enriched diet also increased plasma levels of triglyceride (+33%) (Fig. 3a) and NEFA level (as NEFA/NEFAO, +24%) (Fig. 3b). The increased level of plasma triglyceride might be the consequence of the higher dietary fat intake while plasma NEFA rise could be explained by a reduced utilisation as a source for milk fat synthesis. According to Palmquist and Mattos (21) under normal condition about 50% of milk fatty acids has alimentary derivation while 5-6% is endogenous but these ratios can be modified by diet. Regarding these latter

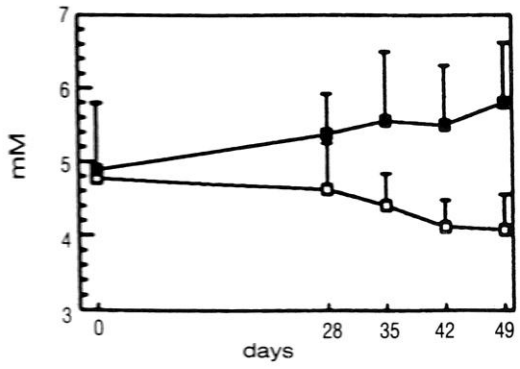

Fig. 1a - Total cholesterol. Mean values ± s.d.
□ control ■ calcium soaps

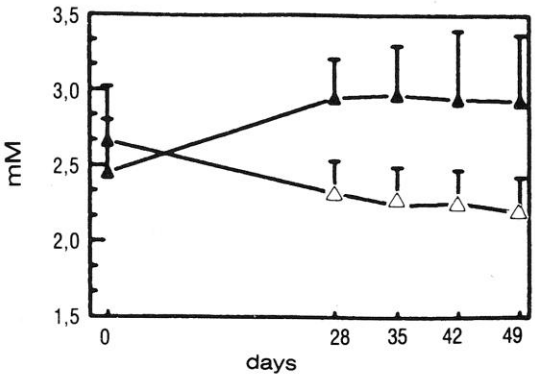

Fig. 1b - Phospholipid. Mean value ± s.d.
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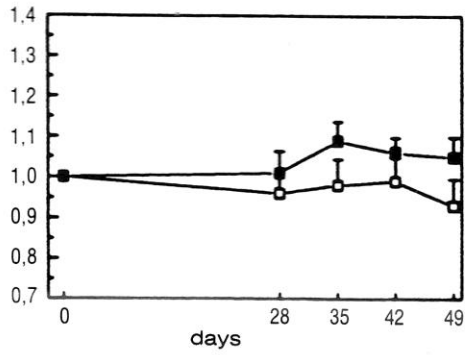


Fig. 2 - Protein/protein 0 ratio. Mean values ± s.d.
□ control ■ calcium soaps

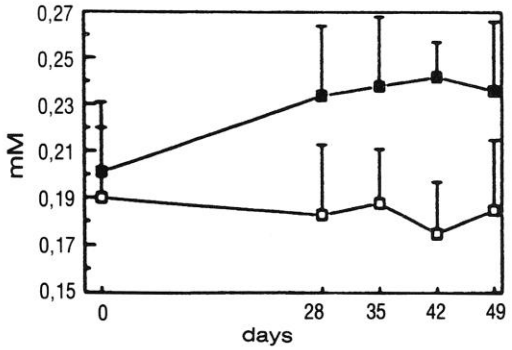


Fig. 3a - Triglyceride. Mean values ± s.d.
□ control ■ calcium soaps

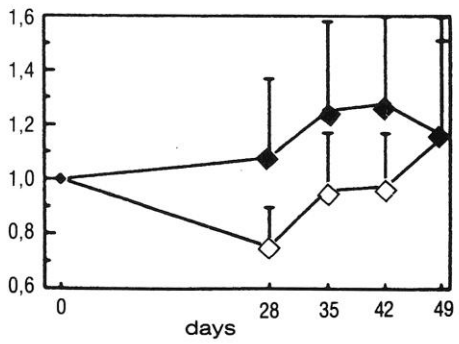


Fig. 3b - NEFA/NEFA 0 ratio. Mean values ± s.d.
◇ control ◆ calcium soaps

trends, Smith et al. (29) found similar results but other authors findings disagree (17): this can probably be ascribed either to different environmental and physiological conditions or to different experimental procedures.

The administration of a calcium soap enriched diet produced a 37% increase in the GH values compared with the baseline levels (Fig. 4). This is puzzling because an energy deficiency, like that occurring in the early lactation, was seen to determine a chronic increase in the circulating GH along with a higher mobilization of lipids from adipose tissue and consequently an increased NEFA plasma level and oxidation (4, 10, 11, 13, 25, 33). Besides that, Palmquist (18) did not observe significant variations in GH levels in cows fed fat enriched and Schneider et al. (28) reported an even negative effect of the treatment.

However, the discordance could be explained by differences in experimental conditions, including animals and procedures, since it is known that GH plasma levels are pulsatile and varing depending on the nutritional and physiological status and on the ultradian rhythm (3, 4, 11, 33).

However, the increase in GH levels in animals fed the protected fat enriched diet, could be responsible for the drop in plasma urea level (Tab. III), the increase in plasma NEFA and the milk yield. Other authors have indeed highlighted a relationship between GH, urea and NEFA or between potential milk yield and GH plasma level (10, 12, 16, 19, 25). The dietary treatment with calcium soaps affected significantly plasma level of somatostatin (+23%; $P < 0,05$) (Fig. 5); no effect on GH/somatostatin ratio (Fig. 6) was found. In previous research (27), calcium soaps showed significant increase in somatostatin level and positive correlations between milk yield, NEFA and somatostatin levels, suggesting the existence of a link between these plasma constituents.

Somatostatin, first isolated in the hypothalamus of sheep and found to inhibit GH release, was demonstrated to exert a wide range of physiological effects, mainly inhibiting both

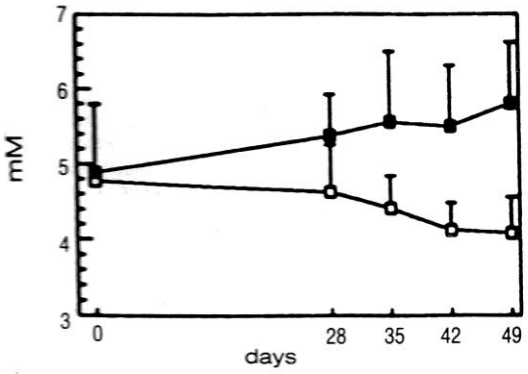


Fig. 1a - Total cholesterol. Mean values ± s.d.
□ control ■ calcium soaps

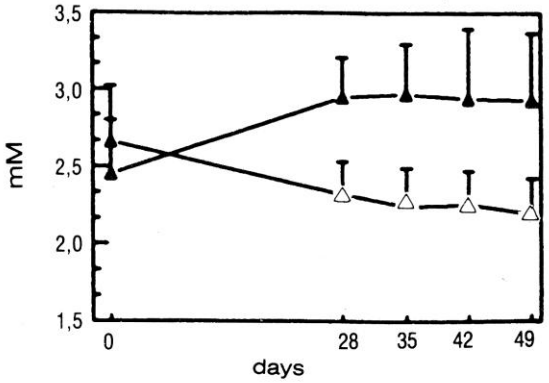
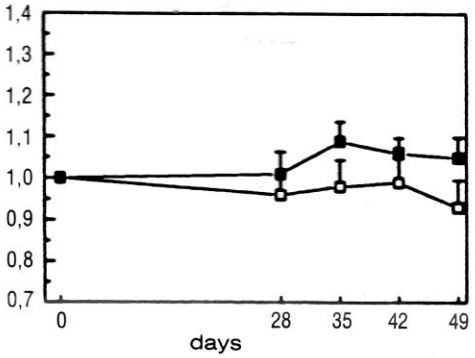


Fig. 1b - Phospholipid. Mean value ± s.d.
△ control ▲ calcium soaps



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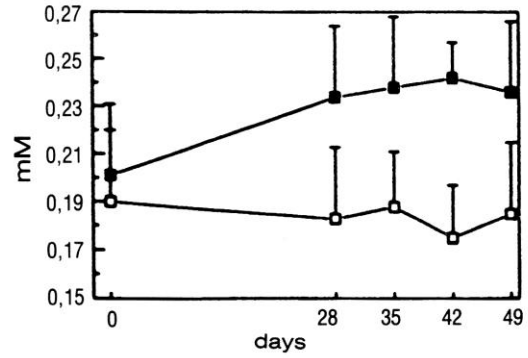

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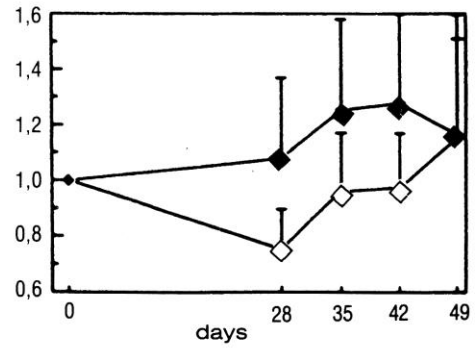

Fig. 3b - NEFA/NEFA 0 ratio. Mean values ± s.d.
◇ control ◆ calcium soaps

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