Infusion of casein hydrolizates into the mammary gland simulates the omission of one daily milking in goats

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RIASSUNTO – Infusione degli idrolisati caseinici nella ghiandola mammaria simulano l'omissione di una mungitura giornaliera nella capra. *Quattro capre Saanen a metà lattazione sono state sottoposte per 2 giorni ad infusione in una emimammella con idrolisati caseinici (CHN) ottenuti per mezzo della plasmina (PL), mentre la controlaterale è stata infusa con un placebo (TRIS). Dopo la sospensione di 5 giorni gli stessi animali sono stati sottoposti per due giorni ad una (ODM) e due (TDM) mungiture giornaliere nelle stesse emimammelle trattate con CHN e TRIS rispettivamente. La produzione di latte dei trattamenti CHN e ODM non sono risultate diverse. CHN hanno influenzato i contenuti in proteina e CCS. CHN e ODM hanno mostrato più alti contenuti di plasmina. I risultati sembrano confermare che la riduzione della produzione nel breve periodo dovuta alla soppressione di una mungitura giornaliera sia da attribuire in parte all'azione dei CHN.*

Key words: casein hydrolizate, plasmin, milking, goat.

INTRODUCTION – Suppression of one daily milking at weekends, even though socially desirable, may reduce milk yield. These losses have been attributed to a short-term mechanism: the filling of the cistern and ductal-alveolar system with milk which contains a peptide called feedback inhibitor of lactation (FIL) (Wilde and Peaker, 1990). The FIL probably reduces the synthesis and secretion of mammary cells by blocking the potassium channel of the apical membrane (Silanikove *et al.*, 2000). Shamay *et al.* (2002) hypothesized that the FIL can be identified with the AA sequence 1-28, derived from the breakdown of β-casein by plasmin (PL). The aim of this work was to verify if the infusion of casein hydrolizates (CNH) into the mammary gland simulates the omission of one milking for two consecutive days.

MATERIAL AND METHODS – Four Saanen goats in mid lactation were used. The CNH solution was obtained as follows: commercial bovine casein (Sigma Chemical Co., St. Louis, MO) was dissolved (25 mg/ml) in 50 mM Tris-NaCl buffer (pH 8.5) and incubated at 37°C for 7 hours with 100 µl of PL (Sigma). It was then boiled for 5 min at 100°C to deactivate the PL, cooled at room temperature and acidified at pH 4.6. The surnatant containing CNH were separated by centrifuging. The pH of the CNH and control (TRIS) solutions was adjusted to pH 6.65 (physiological pH of milk). The CNH and TRIS solutions were passed through a 22 µm sterile filter to sterilize them. The trial was divided in two phases. *Phase 1*) For two days 10 ml of CNH solution was injected into the cistern of one udder half (udder T) of each goat after the morning and evening milking (i.e., four post-milking doses over 2 days); the controlateral udder (udder C) was treated with the same volume of a control solution (TRIS). Milk yields were recorded and milk samples were collected at each milking for each udder half separately on the days of treatment and for two days after treatment (recovery period). *Phase 2*) The udder T was milked once daily (ODM) and the udder C twice daily (TDM) for two days. In the next 2 days (recovery) both

udder halves received TDM treatment. Milk yields were recorded and milk samples were collected at each milking for each udder half separately. Fat and total protein contents were determined with a Milkoscan 6000. SCC was determined with a Fossomatic 360 cell counter. PL and plasminogen (PG) contents were determined following the method described by Ballou *et al.* (1995) with slight modifications.
Data were analyzed using general linear model that included the fixed effects of treatment and period and their interaction, and the random effect of the individual goat. Differences among treatment means were tested using Tukey's test. The level of significance was declared for P<0.10.

RESULTS AND CONCLUSIONS - Table 1 shows milk production traits and PL-PG activity from the udder halves of goat treated with CNH solution in phase 1 and ODM in phase 2 compared to controlateral udder halves for the treatment periods, while Table 2 shows the same data for the recovery periods. The CNH caused a reduction in milk yield (786 g/d) compared to the controlateral udder (964 g/d). This was not completely restored during recovery period (891 *vs.* 1065; P=0.09). There was a reduction of milk yield in ODM (841 g/d) when compared with TDM (1176 g/d) but this returned to normal within the recovery period (1003 *vs.* 1159; P = 0.18). The CNH effects on milk yield did not differ from the effects of ODM.

Table 1. Milk production traits and plasmin-plasminogen activity in treated udder halves during the treatment period.

	Treatment			
	CNH	ODM	TRIS	TDM
Milk, g/d	786a	841ab	964b	1176c
Fat,%	3.45	3.09	3.45	3.56
Protein,%	3.40a	3.07b	2.82c	2.98bc
SCC, Log10	4.24a	3.56b	3.30b	3.28b
Plasmin, U/ml	4.75a	5.50a	2.06b	2.56b
Plasminogen, U/ml	47.2	45.0	45.9	47.1

[a, b] P≤0.10.

Table 2. Milk production traits and plasmin-plasminogen activity in treated udder halves during the recovery period.

	Treatments			
	CNH	1X	TRIS	2X
Milk, g/d	891a	1003b	1065b	1159b
Fat,%	3.11	3.55	3.39	3.38
Protein,%	3.25a	3.16a	2.92b	3.06ab
CCS, Log10	3.64a	3.38ab	3.05b	3.23b
Plasmin, U/ml	5.47a	5.50a	2.41b	2.03b
Plasminogen, U/ml	45.80	44.10	44.28	40.60

[a, b] P≤0.10.

This suggests that the mechanism of inhibition of milk secretion is similar. During the treatment period milk yield loss with ODM (-28%) compared to TDM was higher than the milk yield loss with CNH (-18%) compared to the TRIS treated udder. This is probably because in Phase 1 the control udder was injected with TRIS solution, and this may have disturbed milk secretion. By contrast the control udder receiving a TDM in Phase 2 was not disturbed. The depressant effect of the TRIS infusion on milk production can be seen from the differences between TRIS treatment and TDM (P=0.08). Although the effect on milk yield was lower with CNH infusion than with ODM, the former seemed to cause stronger and longer lasting alterations of the secretory epithelium.

The higher content of protein in CNH treated glands may be due to CNH being injected into these udder halves (+0.2%). The higher SCC in CNH udder halves could be explained by a reaction of the mammary gland to the casein hydrolizates infused.

The PL activity was significantly higher in treated udder halves (CNH and ODM) than in the control (TRIS and TDM) udder halves during the treatment period, and these differences continued to be present during the recovery period. The PL patterns suggest that the increase of enzymes during the treatment may trigger an irreversible reaction and this may predispose the mammary gland to involution, and this mechanism should be investigated further. The results of this experiment confirm Shamay *et al.*'s (2003) hypothesis that the short-term milk losses caused by a suppression of one daily milking, could be due to the action of native CNH, which is thus a strong candidate for FIL.

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REFERENCES – **Ballou**, L.U., Pasquini, M., Bremel, R.D., Everson, T., Sommer, D., 1995. Factors affecting herd milk composition and milk plasmin at four levels of somatic cell counts. J. Dairy Sci. 78:2186-2195. **Shamay**, A, Shapiro, F, Mabjeesh, SJ, Silanikove, N., 2002. Casein-derived phosphopeptides disrupt tight junction integrity, and induced mammary involution in goats. Life Science 70:2707-2719. **Silanikove**, N, Shamay, A, Shinder, D, Moran, A., 2000. Stress down regulates milk production by a plasmin induced casein breakdown product. Life Science 67:2201-2212. **Wilde**, C.J., Peaker, M., 1990. Autocrine control in milk secretion. J. Agr. Sci. 114:235-238.