

SESSION

GOAT PRODUCTION

Leptin and puberty in goat

A. Vitali¹, D. Magistrelli³, J. Azevedo², U. Bernabucci¹,
B. Ronchi¹, F. Rosi³

¹ Dipartimento Produzioni Animali, Università della Tuscia, Italy

² Departamento Zootecnia, Universidade de Tràs-os-Montes e Alto Douro, Portugal

³ Istituto Zootecnia Generale, Facoltà di Agraria, Università di Milano, Italy

Corresponding Author: Andrea Vitali. Dipartimento Produzioni Animali. Via Camillo de Lellis, 01100 Viterbo, Italy – Tel: +39 0761 357440 - Fax: +39 0761 357434 – Email: vitali@unitus.it

RIASSUNTO – Leptina e pubertà nella capra. *Ventiquattro capre di razza Serrana (7,93±0,24 kg, 93±2,6 giorni di età) sono state suddivise in 3 gruppi alimentati per 11 mesi con fieno ad libitum e differenti quantità di concentrato (30, 50 e 70% della sostanza secca ingerita) fino alla pubertà. I tre gruppi hanno ingerito la stessa quantità di sostanza secca. Il gruppo alimentato con la dieta a maggior apporto di concentrato ha mostrato un anticipo di 30 giorni nella comparsa della pubertà, presentando un maggiore deposito adiposo sottocutaneo ed un maggiore peso vivo rispetto agli altri due gruppi. I livelli di leptina plasmatica sono aumentati significativamente nei 3 mesi prima della pubertà senza mostrare differenze significative tra i 3 gruppi sperimentali, sebbene siano risultati correlati (P<0,01) al peso corporeo ed allo spessore del pannicolo adiposo.*

Key words: leptin, puberty, goat.

INTRODUCTION – The onset of reproductive function involves activation of the hypothalamic-pituitary-gonadal axis and in female results in ovulation of mature oocytes (Gueorguiev *et al.*, 2001). This transition typically occurs at a genetically predetermined age. However, nongenetic variables as photoperiod, body weight (BW), and back fat depth (BFD) can modify the age at which puberty occurs (Cheung *et al.*, 2001; Garcia *et al.*, 2002). Furthermore, feed availability is an important environmental factor affecting the reproductive and somatotrophic axis. Reduced nutrition results in the suppression of gonadal activity in sheep (Nagatani *et al.*, 2000). In mammals, leptin, a satiety signal secreted from adipocyte, has been proposed as a permissive factor that links metabolic status and reproduction (Nagatani *et al.*, 2000). Indeed, functional leptin receptor and its mRNA are present in ovary, pituitary and hypothalamus of several species and fasting reduces synthesis and secretion of leptin and frequency of LH pulses (Spicer, 2001).

The aim of this study was to investigate the relationship between leptin, the onset of puberty and the quality of the diet in goat.

MATERIAL AND METHODS – Twenty four female kids, belonging to Serrana (local Portugal goat breed), were monitored from 3 months age (December) until the onset of puberty (October). At the beginning, kids were subdivided into three groups fed on hay ad libitum and different levels of concentrate: 30, 50 and 70% of dry matter intake (DMI) of concentrate for low level (LC), medium level (MC) and high level (HC), respectively. Periodically, DMI and composition were adjusted to account for growth related changes in BW. Individual DMI was registered daily, and BW was recorded weekly. Every month BFD was measured by ultrasound at the 3rd and 4th lumbar vertebra using an Aloka SSD 500V real time instrument with a 7.5 MHz linear probe, and image analysis software NIH 1.57 (National Institutes of Health). Blood samples were collected every 10 days from the jugular vein (Vacutainer system) before feeding. The samples were centrifuged and the

plasma collected and stored at -20°C. On plasma samples, leptin (multi-species leptin RIA, Linco Res. Inc., St. Charles, MO, USA) and progesterone (progesterone RIA kit, Diagno. Prod. Corp., Los Angeles, CA, USA) were detected by radioimmunoassay. Goats were considered puberal when levels of progesterone exceeded 0.5 ng/ml. Data were analyzed using the GLM procedure of SAS (SAS, 1996). Dry matter intake, live weight, backfat depth and plasma leptin concentration were evaluated utilizing a model considering the following effects: treatments (LC, NC and HC), goat within treatments, time and the error term. Significance was declared at $P < 0.05$.

RESULTS AND CONCLUSIONS – During the experimental period, the HC group grew at a higher rate, reaching a final BW higher ($P < 0.001$) than the other two groups, but the total DMI did not vary between the experimental groups (Figure 1). The BFD was always significantly higher in the HC ($P < 0.001$) compared with LC and MC groups (Figure 2). During the experimental period, mean concentrations of leptin did not differ due to diet; however, it significantly increased at puberty onset between August and October (Figure 2). Moreover, during this period plasma leptin levels were significantly correlated with both BW ($P < 0.001$) and BFD ($P < 0.01$).

Figure 1. Changes of dry matter intake (DMI: histograms) and body weight (BW: lines) in goats fed diet containing low level (LC), medium level (MC) or high level (HC) of concentrate.

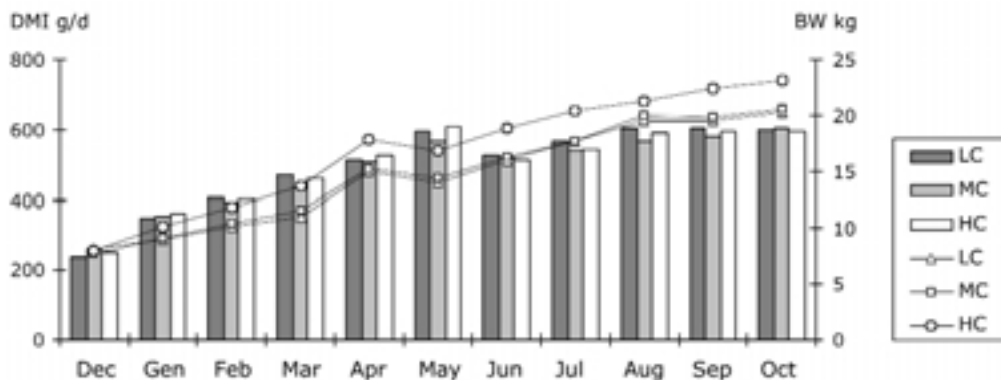
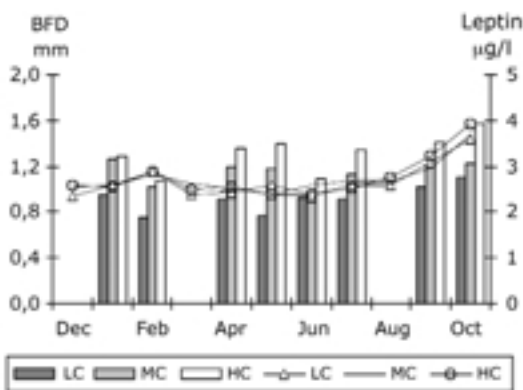


Figure 2. Changes of back fat depth BFD (histograms) and plasma leptin (lines) in goats fed diet containing low level (LC), medium level (MC) or high level (HC) of concentrate.



In ruminants, a systemic leptin level increment has been reported as puberty approaches (Spicer, 2001; Garcia *et al.*, 2003). Those authors hypothesized that this increase of leptin acts to trigger the reproductive axis at the levels of hypothalamus and pituitary. Increase in serum leptin is also reported in mature ovariectomized cows and mares during spring and summer indicating that seasonal changes in photoperiod is a factor in inducing leptin secretion (Garcia *et al.*, 2002). Those authors concluded that seasonal effects on circulating leptin could have contributed to the prepubertal rise in leptin concentration observed in developing heifers. The increase of circulating leptin observed in our study at onset of puberty might be partially due to changes in photoperiod. In the current experiment it appears that circulating leptin level was not affected by dietary treatment, although a higher not significant leptin concentration was observed in HC group in the onset of puberty. The lack of effects of the diet could depend on the absence of differences in DMI between groups, because DMI is a long-term signal that regulates plasma leptin level (Chilliard *et al.*, 1999; Marie *et al.*, 2001). But the lack of effects of the diet on plasma leptin could be due also to the effect of photoperiod that could be so strong to mask dietary effects.

Analyzing the period just before the onset of puberty, as stated by plasma progesterone levels, is noteworthy that the HC groups became sexually mature 20-27 days before, and with a BW 2 kg higher (+10%, $P < 0.001$) than the other two groups (Table 1). In contrast, leptin levels recorded during the month before the onset of the puberty were not significantly different between the three groups. The overall mean of leptin during the pubertal period was 30% higher than those previous levels (3.4 vs. 2.45 $\mu\text{g/l}$, $P < 0.001$).

Table 1. Lsmeans \pm SE of body weight (BW), age at puberty, and plasma leptin in goats fed with diet containing low level (LC), medium level (MC) or high level (HC) of concentrate at onset of puberty.

Means values	Group LC	Group MC	Group HC
BW (kg)	19.5 \pm 1.2 ^A	19.6 \pm 1.5 ^A	22.2 \pm 1.7 ^B
Age at puberty (days)	380 \pm 27 ^a	387 \pm 18 ^a	360 \pm 16 ^b
Leptin ($\mu\text{g/l}$)	3.2 \pm 0.7	3.2 \pm 0.6	3.6 \pm 0.6
BFD (mm)	1.0 \pm 0.2 ^A	1.2 \pm 0.2 ^B	1.5 \pm 0.4 ^C

a, b = $P < 0.01$; A, B, C = $P < 0.001$.

The results indicate that in the goats the body weight, body fat depot and quality of diet influence the onset of the puberty, but not plasma leptin levels. The increase of plasma leptin during the onset of the puberty probably acts as permissive signal for the development of the sexual maturity and it can be used as a diagnostic tool to predict the imminent approaching of the event.

REFERENCES – **Cheung**, C.C., Thornton, J.E., Nurani, S.D., Clifton, D.K., Steiner, R.A., 2001. A reassessment of leptin's role in triggering the onset of puberty in the rat and mouse. *Neuroendocrinology* 74(1):12-21. **Chilliard**, Y., Bocquier, F., Delavaud, C., Faulconnier, M., Bonnet, M., Guerre-Millo, M., Martin, P., Ferlay, A. 1999. La leptine chez le ruminant. Facteurs de variation physiologiques et nutritionnels. *INRA Prod. Anim.* 12:225-237. **Garcia**, M.R., Amstalden, M., Morrison, C.D., Keisler, D.H., Williams, G.L., 2003. Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age. *J. Anim. Sci.* 81(1):261-8. **Garcia**, M.R., Amstalden, M., Williams, S.W., Stanko, R.L., Morrison, C.D., Keisler, D.H., Nizielski, S.E., Williams, G.L., 2002. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. *J. Anim. Sci.* 80(8):2158-67. **Gueorguiev**, M., Goth, M.L., Korbonits, M., 2001. Leptin and puberty: a review. *Pituitary* 4(1-2):79-86. **Marie**, M., Findlay, P.A., Thomas, L., Adam, C.L., 2001. Daily patterns of plasma leptin in sheep: effects of photoperiod and food intake. *J. Endocrinol.* 170:277-286. **Nagatani**, S., Zeng, Y., Keisler, D.H., Foster, D.L., Jaffe, C.A., 2000. Leptin regulates pulsatile luteinizing hormone and growth hormone secretion in the sheep. *Endocrinology* 141(11):3965-75. **Spicer**, L.J., 2001. Leptin: a possible metabolic signal affecting reproduction. *Domest. Anim. Endocrinol.* 21(4):251-70.

Insulin and IGF-1 in goat milk: influence of the diet

D. Magistrelli, A. Valli, F. Rosi

Istituto di Zootecnia Generale, Facoltà di Agraria, Milano, Italy

Corresponding author: Fabia Rosi. Istituto di Zootecnia Generale, Facoltà di Agraria. Via Celoria 2, 20133 Milano, Italy – Tel. +39 02 50316443 – Fax: +39 02 50316434 – Email: fabia.rosi@unimi.it

RIASSUNTO – Insulina e IGF-1 nel latte di capra: influenza della dieta. *16 capre Saanen a metà lattazione sono state divise in due gruppi, alimentati con diete contenenti il 33 o il 17% di amido. La dieta ad alta concentrazione di amido ha determinato un incremento significativo dei livelli di insulina e IGF-1 nel plasma e di insulina nel latte. Sebbene la concentrazione di IGF-1 nel plasma fosse molte volte superiore a quella di insulina, la concentrazione di IGF-1 nel latte era solo il 13% di quella determinata nel plasma, mentre quella di insulina nel latte era due volte maggiore rispetto al plasma. Questi risultati indicano la possibilità di passaggi selettivi di peptidi bioattivi dal plasma al latte e la possibilità di modificare i livelli di insulina nel latte attraverso l'alimentazione materna.*

Key words: milk insulin, milk IGF-1, goat, bioactive peptides.

INTRODUCTION – Plasma IGF-1 and insulin have both growth-related and anabolic actions. The anabolic role of plasma IGF-1 consists of stimulating the uptake of amino acids and glucose by the cells, with an action similar to that of insulin. The main regulation of plasma IGF-1 and insulin is associated with food intake, especially with energy and protein intake. Moreover, insulin modulates peripheral satiety signals and directly targets the central nervous system to inhibit food intake (Sparks *et al.*, 2003; Gale *et al.*, 2004).

Also mammalian milk contains IGF-1 and insulin, as well as many other bioactive compounds, that interacting with specific receptors on the apical side of mammary epithelial cells, may regulate galactopoietics and mammary cell proliferation and differentiation (Donovan *et al.*, 1994). Additionally, receptors for various endocrine factors have been identified in the gastrointestinal mucosa of suckling animals, indicating a possible function for milk-borne growth factors in neonatal development (Blum and Baumrucker, 2002). Finally, a proportion of milk IGF-1 and insulin can be absorbed in neonatal pigs and potentially impact the endocrine asset of the suckling animal (Kinouchi *et al.*, 1998; Xu *et al.*, 2000; Sparks *et al.*, 2003).

As milk provides a basic source of nutrition for young mammals and may represent a significant source of exogenous growth-promoting factors for the neonate, it could be of interest to quantify the levels of insulin and IGF-1 in goat milk, to investigate their origin analyzing the relationships between milk and plasma levels and to evaluate if it is possible to affect the levels of milk-borne growth factors through changes in the maternal diet.

MATERIAL AND METHODS – Sixteen Saanen lactating goats were fed a diet based on mixed hay, maize, barley and soybean meal (40:60 forage to concentrate ratio) containing 17% (LS) or 33% starch (HS) at 9.00 h and 17.00 h, and milked at 8.00 h and at 18.00 h. The LS diet was formulated by replacement of two thirds of maize grain with dried beet pulp, without affecting the organic matter digestibility (Rapetti and Bava, 2004).

At 163rd day in lactation, milk production was recorded at each milking and individual samples were collected and stored at –20°C until analyzed. Milk samples, sonicated and centrifuged (2,150 xg for 30 min at 4°C), were

tested for milk insulin (Insik-5, DiaSorin S.p.A., Saluggia, Italy) and IGF-1 (IGF-1 RIA with extraction of the binding proteins, Diagnostic System Laboratories, Inc., Webster, TX, USA) content. On the same day, five ml of jugular vein blood was taken before the first feeding of the day and 4 h after the feeding. The samples were collected into EDTA tubes and centrifuged (2,150 xg for 15 min at 10°C), then plasma was stored at -20°C until analyzed for insulin and IGF-1 by the same RIA method employed for milk. These methods, using an antibody directed against human insulin and IGF-1, were validated for goat plasma and milk verifying the parallelism to the standard curve, of serial dilutions (25 to 100 µl) of plasma and milk in 100 µl buffer. Milk production, milk and blood variables were analyzed using the ANOVA 2-ways procedure of SAS (1989) and correlation computed by Pearson correlation coefficient.

RESULTS AND DISCUSSION – The dietary treatment did not affect dry matter intake and milk production (Rapetti and Bava, 2004), that was anyway 30% higher at morning milking compared to evening milking (Table 1), probably due to the longer time gap between two consecutive milkings and indicating a constant milk synthesis rate throughout the day.

High starch (HS) diet increased plasma IGF-1 as a probable consequence of a stimulatory effect on liver IGF-1mRNA (Radcliff *et al.*, 2004). As expected, high starch diet also increased plasma insulin and its post-feeding changes (Table 1). The increase in plasma insulin could depend on the higher rumen production of propionate, induced by HS diet; indeed glucogenic precursors as propionate are suggested to be important stimuli for pancreatic endocrine secretion in ruminants (Harmon, 1992).

Milk IGF-1 level was thrice higher in morning than in evening milking (Table 1). This result is surprising, and has not been reported previously. For a given peptide, numerous factors could influence milk content, including diurnal variation or time elapsed in the milk-filled mammary alveoli and ducts prior to milking. But the possibility, that these events could interfere in the levels of milk bioactive peptides, has received limited investigation, so far; however it is worthy of note that also Faulkner (1999) observed variations in goat milk IGF1 between two consecutive milkings, although lower than those here reported.

Table 1. Levels of hormones in plasma and milk at morning and evening (n=16) at 163 days in lactation.

Milk	diet			ES	Effects (P)	
		morning	evening		diet	hour
Milk g/milking		1317	1026	62	ns	<0.01
IGF-1 nM	HS	2.07	0.62	0.29	ns	<0.01
	LS	1.57	0.54			
insulin pM	HS	286	351	25.1	<0.01	0.09
	LS	212	240			
Plasma		fasting	4-h post-feeding			
IGF-1 nM	HS	9.53	11.54	1.4	<0.05	ns
	LS	6.88	8.31			
insulin pM	HS	93.5	176.5	12.2	<0.05	<0.01
	LS	75.6	139.1			

Milk insulin, but not milk IGF-1, was significantly increased by HS diet (Table 1). The levels of IGF-1 in plasma is seventy-five times higher than insulin levels, but milk IGF-1 is only 13% of blood level, whereas milk

insulin level was two/three times higher than plasma level. Similar milk to plasma differences have been reported also in other species (Donovan *et al.*, 1991; Kinouchi *et al.*, 1998; Xu *et al.*, 2000).

Insulin and IGF1 were correlated in plasma ($r=+0.51$; $P<0.01$), but not in milk; morning milk IGF-1 was correlated to morning plasma IGF-1 levels ($r=+0.88$; $P<0.01$), but not insulin ($r=+0.37$; ns).

Maternal serum is the most likely source of IGF-1 and insulin, because messenger RNA for IGF-1 was very low or undetectable in rat mammary gland isolated at mid-lactation (Donovan *et al.*, 1991) and insulin is synthesized in significant quantities only in B cells in the pancreas (Gale *et al.*, 2004). These findings and the presence of specific receptors in mammary gland suggest that IGF-1 and insulin may be sequestered from maternal serum for transfer into milk (Sparks *et al.*, 2003).

Nevertheless, the differences between IGF-1 and insulin regarding plasma and milk levels and correlations, and the different responses to plasma changes in corresponding milk levels, induce to hypothesize that the transfer processes must be different; i.e. insulin could be transferred to milk by trans- and paracellular processes through IGF-1 receptors and through its own receptors and then concentrated during secretion in milk (Britten and Kastin, 1991), whereas IGF-1 transfer is modulated by IGF-binding proteins and their related-proteins (Blum and Baumrucker, 2002).

The precise roles of various bioactive components in milk are not fully explained, but their potential biological functions do include effects of relevance for the developing neonate as well as auto/paracrine effects in the mammary gland of the mother. Bovine and caprine milk is of significant interest since it is widely consumed and used for infant formulas. The stability of growth factors in heat and acid treatment contributes to their survivability in commercial milk products and to their potential bioactivity in the gastrointestinal tract of the consumer (Campana and Baumrucker, 1995).

In the present study we conclude 1) that insulin and IGF-1 are present in goat milk and 2) that it is possible to influence the level of milk insulin through the maternal diet. Further research is needed to determine whether intraspecific or interspecific transfer can occur and whether these components from goat milk can maintain their biological activity in the gastrointestinal tract of the neonate.

REFERENCES – Blum, JW, Baumrucker CR, 2002. Colostral and milk insulin-like growth factors and related substances: mammary gland and neonatal (intestinal and systemic) targets. *Dom. Anim. Endocr.* 23, 101-10. Britton, JR, Kastin AJ, 1991. Biologically active polypeptides in milk. *Am. J. Med. Sci.* 301:124-32. Campana, WM, and Baumrucker CR, 1995. Hormones and growth factors in bovine milk. In: *Handbook of Milk Composition*. R.G. Jensen (Ed.). New York: Academic Press, pp. 476-494. Donovan, SM, Hintz RL, Rosenfeld RG, 1991. Insulin-like growth factors I and II and their binding proteins in human milk: effect of heat treatment on IGF and IGF binding protein stability. *J. Pediatr. Gastroenterol. Nutr.* 13: 242-53. Donovan, SM, Odle J, 1994. Growth factors in milk as mediators of infant development. *Ann. Rev. Nutr.* 14:147-67. Faulkner, A, 1999. Changes in plasma and milk concentrations of glucose and IGF-1 in response to exogenous growth hormone in lactating goats. *J. Dairy Res.* 66: 207-14. Gale, SM, Castracane VD, Mantzoros CS. 2004. Energy homeostasis, obesity and eating disorders: recent advances in endocrinology. *J. Nutr.* 134: 295-8. Harmon, DL, 1992. Impact of nutrition on pancreatic exocrine and endocrine secretion in ruminants: A review. *J. Anim. Sci.* 70:1290-301. Kinouchi, T, Koizumi K, Kuwata T, Yajima T, 1998. Crucial role of milk-borne insulin in the development of pancreatic amylase at the onset of weaning in rats. *Am. J. Physiol.* 275 (Reg. Int.Comp.Physiol. 44): R1958-R67. Radcliff, RP, VandeHaar MJ, Kobayashi Y, Sharma BK, Tucker HA, Lucy MC, 2004. Effect of dietary energy and somatotropin on components of the somatotrophic axis in Holstein heifers. *J. Dairy Sci.* 87: 1229-35. Rapetti, L, Bava L, 2004. Effect of grinding of maize and level of starch on digestibility and lactation performance of Saanen goats. *S. Afr. J. Anim. Sci.* 34 (Suppl. 1): 85-88. Sparks, AL, Kirkpatrick JG, Chamberlain CS, Waldner D, Spicer LJ, 2003. Insulin-like Growth Factor-I and its binding proteins in colostrum compared to measures in serum of Holstein neonates. *J. Dairy Sci.* 86: 2022-29. Xu, RJ, Wang F, Zhang SH, 2000. Postnatal adaptation of the gastrointestinal tract in neonatal pigs: a possible role of milk-borne growth factors. *Liv. Prod. Sc.* 66: 95-107.

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

G. Pulina, A. Nudda, S. Fancellu, A.M. Barbato, R. Rubattu

Dipartimento Scienze Zootecniche, Università di Sassari, Italy

Corresponding author: Giuseppe Pulina. Dipartimento Scienze Zootecniche. Via E. De Nicola 9, 07100 Sassari, Italy – Tel: +39 079 229307 – Fax: +39 079 229302 – Email: gpulina@uniss.it

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 μ l 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 μ m 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} $P \leq 0.10$.

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} $P \leq 0.10$.

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 hydrolyzates 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.

ACKNOWLEDGEMENTS - Founded by MIPAF (BENOLAT project).

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.

Effect of forage/concentrate ratio and soybean oil supplementation on milk yield and quality from dairy goats

M. Mele, A. Serra, M.R. Rafanelli, G. Conte, P. Secchiari

Dipartimento Agronomia e Gestione dell'Agroecosistema, Università di Pisa, Italy

Corresponding author: Marcello Mele. Dipartimento Agronomia e Gestione dell'Agroecosistema, Sezione Scienze Zootecniche. Via del Borghetto 80, 56124 Pisa, Italy - Tel: +39 050 599227 - Fax: +39 050 540633 - Email: mmele@agr.unipi.it

RIASSUNTO – Effetto del rapporto foraggio/concentrato e dell'integrazione con olio di soia sulla produzione e sulla qualità del latte di capra. Dodici capre di razza Saanen, in un disegno sperimentale a quadrato latino 4x4 con tre repliche, sono state alimentate con quattro diete caratterizzate da due livelli di rapporto foraggio/concentrato (alto 63/37; basso 34/66), integrate o meno con olio di soia non protetto. I risultati hanno evidenziato un significativo aumento del contenuto percentuale di grasso del latte e della secrezione giornaliera di grasso in funzione del maggior contenuto di foraggio e dell'integrazione con olio di soia. Anche la produzione giornaliera di latte è stata influenzata positivamente dall'aggiunta di olio alla dieta, mentre il tempo di coagulazione è risultato significativamente inferiore nelle diete con un maggior contenuto di foraggio.

Key words: milk, goats, quality, soybean oil.

INTRODUCTION – Dietary energy level is a limiting factor of milk production mainly in early lactation goats. Energy intake may be increased by incorporation of fat in ration. Currently, rumen protected fat is mainly adopted in goats nutrition, since the several studies have clearly confirmed that supplies of protected fat markedly improved the fat percentage of goat milk (Schmidely and Sauvant, 2001; Chilliard *et al.*, 2003). Similar results were obtained by using oilseeds (Morand-Fehr *et al.*, 2000). Moreover, the supply of protected lipids is an efficient means to reduce risks of percentage inversions between milk fat and protein in the case of diet with low fibre effectiveness or fat content (Chilliard *et al.*, 2003). Little knowledge is available about the effects of dietary supplementation with unprotected oil in goat nutrition. When dairy cows are fed low forage diets added with vegetable oils (rich in polyunsaturated fatty acids) milk fat content decrease (Bauman and Griinari, 2001), on the contrary to what observed in dairy ewes (Mele *et al.*, 2002). Aim of this paper was to determine the effects of the inclusion of unprotected soybean oil in diets at two forage/concentrate ratios on yield and composition of milk from dairy goats.

MATERIAL AND METHODS – The experimental design was 4x4 Latin square with 3 replicates per diet. The animals were 12 Saanen goats in early-mid lactation (40±3 days in milking), fed 4 different diets based on grass hay and a concentrate mixture of barley meal, soybean meal, maize meal, beet pulp, minerals and vitamins. The diets were isonitrogenous (16% CP on DM) at 2 F/C ratios (high, 63/37 or low, 34/66 DM), supplemented or not with rumen unprotected soybean oil (100 g/head/d). The composition and chemical analysis of the experimental diets are reported in table 1. Milk yield and dry matter intake (DMI) from each goat were daily recorded. At the end of each experimental period the goats were weighed and individual samples of milk and blood were collected. Milk samples were analysed for fat, protein, lactose, casein content and somatic cell count.

Linear score transformation was applied to somatic cell score data in order to normalize them. Milk reological properties were determined by Formagraph apparatus (Foss Italia, Padova). Blood samples were analysed for glucose, urea and NEFA content by spectrophotometer, using specific commercial kits. Statistical analysis was performed using a linear model including the fixed effects of: diet, replicate, period within replicate and goat within replicate. Contrasts for F/C ratio and SO treatment were tested for significance.

Table 1. Composition and chemical analysis of the experimental diets (as% DM)

	Diets			
	HF/NO	HF/O	LF/NO	LF/O
Barley	14.5	14.5	21.4	21.4
Beet pulp	7.4	7.4	21.8	21.8
Hay	63	63	34.5	34.5
Maize	7.6	3.6	11.2	7.2
Soybean meal	7.5	7.5	11.1	11.1
Soybean oil	0	4	0	4
F/C ratio	63/37	63/37	34/66	34/66
CP	16.0	15.6	16.1	15.7
CF	1.5	5.5	1.3	5.2
NDF	43.1	42.5	37.7	37.2
NSC	31.5	29.1	39.0	36.4
Ashes	8.0	7.3	5.8	5.4
Net energy (MFU/kg DM)	2.0	2.1	2.3	2.4

Diets: HF/NO = high forage no oil; HF/O = high forage with oil; LF/NO = low forage no oil; LF/O = low forage with oil; MFU: milk forage units.

RESULTS AND CONCLUSIONS – Soybean oil partially replaced maize meal in fat supplemented diets, therefore no great differences in dietary net energy concentration are detectable among diets with the same forage/concentrate ratio (table 1). All diets met the goat energy requirements; in fact the mean body weights of the experimental groups did not differ. The average daily dry matter intake (DMI) did not differ among treatments and reflected the amount of feed offered, as a consequence of the negligible amounts of feed refusal by the goats (table 2). Plasma concentration of glucose, urea and NEFA were not affected by treatments and their values are similar to those reported in literature (Brown-Crowder *et al.* 2001). The inclusion of soybean oil in the diet allowed to increase milk yield and milk fat yield and content, while milk protein and casein content and milk cheese ability were not affected by oil supplementation. These results confirm previous data about the use of supplies of protected fat or oilseeds in dairy goat nutrition as reviewed by Chilliard *et al.* (2003). Similar results in terms of milk yield and composition were observed also when Sarda dairy ewes were fed diets with two levels of forage/concentrate ratio, supplemented or not with unprotected soybean oil (Mele *et al.*, 2002). In small ruminants, therefore, the interaction between low levels of dietary fibre and fat supplies rich in polyunsaturated fatty acids (PUFA) did not induce any milk fat depression as it was very clearly observed in dairy cows (Bauman and Grinari, 2001). The reasons for these differences in dairy performance response to unprotected fat supplementation between ruminant species are not yet clear. Some differences could be related to the rate of passage of digesta that is higher in goat and sheep than in cows (Chilliard *et al.*, 2003). This could affect the behaviour of ruminal biohydrogenation processes that produce fatty acids that have an inhibitor effect on mammary lipogenesis in cows (Baumgard *et al.*, 2002). Milk fat secretion and percentage resulted enhanced also when goats were fed diets with higher levels of forage. Although milk protein and casein content did not vary among treatments, milk reological parameters resulted positively related to the higher forage/concentrate ratio, as reflected by better values of milk renneting time (table 2).

In conclusion, the inclusion of unprotected soybean oil in diet allowed to improve dairy performance of goats, regardless of the level of diet forage/concentrate, provided that the milk technological properties did not worsen. Although the experiment was carried out during early-mid lactation of goats, high levels of dietary concentrate did not allow to improve milk yield and composition. Therefore, in terms of dairy performance, the inclusion of fat (unprotected or not) in the diet of dairy goats seems to be preferable to an increase of concentrate level.

Table 2. DMI, milk yield and composition from goats fed with the experimental diets.

	Diets ¹				SE	Contrasts	
	HF/NO	HF/O	LF/NO	LF/O		Oil	Forage
DMI (kg/d)	2.43	2.41	2.43	2.42	0.02		
Body weight (kg)	64.70	63.99	63.18	63.31	0.58		
Milk yield (kg/d)	2.16	2.39	2.28	2.26	0.05	**	
Fat content (%)	3.19	3.41	3.01	3.23	0.08	**	*
Fat yield (g/d)	69.04	81.39	68.68	73.05	0.03	**	*
Protein content (%)	3.33	3.30	3.25	3.22	0.13		
Casein content (%)	2.44	2.50	2.39	2.44	0.12		
Lactose content (%)	4.40	4.45	4.38	4.48	0.03		
Linear score SCC	6.36	6.47	6.35	6.55	0.23		
r (min.:sec.)	12:56	13:16	18:09	15:06	01:21		
A30 (cm)	10.48	9.42	9.77	9.19	1.25		
Glucose (mg/dl)	57.95	58.82	57.98	53.75	2.23		
Urea (mg/dl)	46.87	44.05	40.86	44.04	4.03		
NEFA (mmol/L)	0.08	0.11	0.07	0.07	0.02		

** P≤0.01; * P≤0.05.

Diets: HF/NO = high forage no oil; HF/O = high forage with oil; LF/NO = low forage no oil; LF/O = low forage with oil; DMI: dry matter intake; NEFA: non esterified fatty acids.

ACKNOWLEDGEMENTS – Funded by the Ministry of Agricultural and Forestry Policies, Italy, D.M. 535/7303/02; 29/11/2002.

REFERENCES – **Bauman**, D.E., Griinari, J.M., 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livest. Prod. Sci.* 70: 15-30. **Baumgard**, L.H., Matitashvili, E., Corl, B.A., Dwyer, D.A., Bauman, D.E., 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. *J. Dairy Sci.* 85: 2155-2163. **Brown-Crowder**, I.E., Hart, S.P., Cameron, M., Dsahlu, T., Goetsch, A.L., 2001. Effects of dietary tallow level on performance of Alpine does in early lactation. *Small Rum. Res.* 39: 233-241. **Chilliard**, Y., Ferlay, A., Rouel, J., Lamberet, G. 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci.* 86:1751-1770. **Mele**, M., Petacchi, F., Buccioni, A., Serra, A., Ferruzzi, G., Antongiovanni, M., Secchiari, P., 2002. Qualità nel latte di pecore sarde in relazione al contenuto di grasso e di foraggio della dieta. 239. In Proc. 15th Congr. Naz. SIPAOC, Chia Laguna (CA), Italy. **Morand-Fehr**, P., Sanz Sampelayo, M.R., Fedele Sanz Sampelayo, M.R., Fedele, Y.V., Le Frileux, Y., Eknaes, M., **Schmidely**, P.H., Giger Reverdin, S., Bas, P., Rubini, R., Havrevoll, O., Sauvant, D., 2000. Effects of feeding on the quality of goat milk and cheeses. 7th Int. Conf. on Goats. Tour, France. Tome 1: 53-58. **Schmidely**, P., Sauvant, D., 2001. Taux butyreux et composition de la matière grasse du lait chez les petits ruminants: effets de l'apport de matières grasses ou d'aliment concentré. *INRA Prod. Anim.* 14: 337-354.

The transfer of conjugated linoleic acid and vaccenic acid from milk to meat in goats

A. Nudda, G. Battacone, S. Fancellu, G. Pulina

Dipartimento Scienze Zootecniche, Università di Sassari, Italy

Corresponding author: Anna Nudda. Dipartimento Scienze Zootecniche. Via Enrico De Nicola 9, 07100 Sassari, Italy – Tel: +39 079 229371 – Fax: +39 079 229302 – Email: anudda@uniss.it

RIASSUNTO – Trasferimento dell'acido linoleico coniugato (CLA) e dell'acido vaccenico (VA) dal latte alla carne nei caprini. *Quindici capretti di razza Sarda sono stati alimentati esclusivamente con latte materno e macellati a circa 50 giorni di età. Il profilo acidico del grasso del latte ha evidenziato un contenuto maggiore di acidi grassi a corta e media catena e in C18:0 e VA rispetto al Longissimus dorsi (LD). Il contenuto in CLA non è stato differente tra latte e carne. L'analisi della regressione fra latte e LD ha evidenziato relazioni positive per la maggior parte degli acidi grassi. La relazione fra VA+CLA c9,t11 nel LD e nel latte è risultata elevata ($R^2=0,80$). I risultati di questo lavoro hanno evidenziato che il profilo acidico della carne di capretto è dipendente dal profilo acidico del latte con un efficace trasferimento di VA e CLA e con un vantaggioso minore contenuto di acidi grassi a corta catena.*

Key words: kid, goat, milk, meat, fatty acid.

INTRODUCTION – Biomedical studies with animal models have demonstrated that conjugated linoleic acids (CLA) have many positive health benefits. The major sources of CLA in human diets are meat and milk products from ruminants. The content of CLA in ruminant fat depends on the rumen's production of CLA and *trans*-11 C18:1 (vaccenic acid, VA) as bio-hydrogenation intermediates and on VA by D9-desaturase. Kid's meat is a valuable and expensive product. The kids are fed exclusively with the milk of the mothers and they are slaughtered at approximately 30-45 days of age, when they reach a weight of 7-10 kg. Thus the milk component is not degraded by the rumen before it is absorbed from the intestine, and as a result the meat fatty acid (FA) content could be markedly influenced by the fatty acid composition of the milk. The aim of this work is to evaluate the transfer of fatty acids, included CLA and VA, from the milk of the goats to the meat of the kids.

MATERIALS AND METHODS – Fifteen suckling Sarda breed kids were fed exclusively on maternal milk until slaughter. The goats were fed a commercial concentrate (1.2 kg/d) supplemented with 125 g/d of oilseed and hay *ad libitum*. Four milk samples were taken from each goat during the suckling phase. Milk samples (about 100 ml) were collected in the morning before the kids started to suckle. The kids were slaughtered at approximately 50 days of age. Kids were weighted before slaughtering. The cold carcass weight (CCW) was measured after 24 hours of storage at 4°C. Twenty-four hours after slaughter the *longissimus dorsi* (LD) muscle was removed and stored at -80°C until the fatty acid could be analysed. Fat from milk was extracted using the method reported by Secchiari *et al.* (2003). Intramuscular fat from LD was extracted using the Folch method. Fatty acid methyl ester (FAME) from the triglyceride fraction was obtained using the standard FIL-IDF methylation procedure (1999). The chromatographic conditions were described by Nudda *et al.* (2005). The content of each FAME was expressed as a percentage of total FAME. Data were analyzed with one-way ANOVA to assess differences in the FAME profiles of milk and LD muscles and to detect differences in the FAME composition of LD between males and females. The relationship between the FAME content of milk and LD was estimated through regression analysis.

RESULTS AND CONCLUSION – The kids body weight (mean±SD) at slaughtering was 10.2±1.5 for males and 9.2±2.4 kg for females. The CCW was 6.6±1.0 kg for males and 5.8±1.6 for females. The FA profile of the milk and the LD are reported in Table 1. The milk FA profile, included VA and CLA, agrees with the results reported by Chilliard *et al.* (2003). In LD, the largest proportion of FA was made up of palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids, with C18:1 the most abundant. The C16:0 content in the muscle of suckling kids was lower, and C18:1 and C18:2 higher, than reported by Potchoiba *et al.* (1990). There was no consistent variation between males and females in almost all fatty acids analyzed (data not presented) with the exception of CLA content (1.05 vs. 0.63%; P<0.05). In the LD of suckling kids the content of VA was lower and CLA higher than those usually observed in the meat of various ruminant and non-ruminant species (Raes *et al.*, 2004). Todaro *et al.* (2004) analysed the CLA content of pelvic fat in suckling kids, and his results were lower than our values. This may be due either to the different tissue analyzed or to the different milk composition of the mothers. As expected, the FA profile of LD when compared to milk showed a markedly lower content of short-medium FA (C6–C14) and, consequently, an overall higher proportion of long FA, with the exception of C18:0 and VA. Goat milk fat is characterized by its high C4 – C14 FA content. This is used for energy metabolism by the kids. The lower C18:0 and VA contents in LD may be due to desaturase and isomerase activity in the muscle tissues. Regression analysis found a positive relationship between FA in milk and in meat for most of the FA. The relationship between the percentage of VA+CLAc9,t11 in LD (Y) and in milk (X) was: $Y = 0.75 + 0.31X$ ($R^2 = 0.80$). This result confirms that long chain FA' s in meat with a beneficial nutritional value, such as VA and CLA, also depend on the FA composition of the milk. In conclusion the results of this experiment found a lower content of short-medium chain FA in meat and an efficient transfer of VA and CLA from milk to meat. This enhanced the organoleptic and nutritional characteristic of kid's meat.

Table 1. Fatty acid profiles (% of total FAME) in milk of goats and in *Longissimus dorsi* (LD) of suckling kids.

	Milk	SE	LD	SE	P
C6-C14:0	24.67	0.53	4.72	0.99	**
C16:0	21.60	0.38	20.05	0.70	NS
C16:1	0.50	0.04	1.32	0.07	**
C17:0	0.59	0.02	0.59	0.03	NS
C17:1	0.20	0.02	0.38	0.03	**
C18:0	15.98	0.48	13.05	0.90	**
C18:1 t11	3.20	0.43	1.20	0.80	*
C18:1 c9	22.80	0.56	28.80	1.04	**
C18:2 c9,c12	3.71	0.21	12.14	0.39	**
C18:3	1.08	0.08	1.14	0.16	NS
CLA c9,t11	1.11	0.13	0.78	0.24	NS
CLA total	1.65	0.15	1.35	0.28	NS
C20:5 (EPA)	0.07	0.03	1.12	0.05	**
C22:6 (DHA)	0.08	0.04	1.09	0.07	**

*P<0.05; **P<0.01; NS = not significant

ACKNOWLEDGEMENTS – Work funded by MIPAF. The authors are grateful to Dr. G. Antonio Carboni of the Istituto Zooprofilattico Sperimentale della Sardegna and to Antonio Fenu and Gesumino Spanu for their invaluable assistance.

REFERENCES – **Chilliard, Y.**, Fearlay, A., Rouell, J., Lamberet, G., 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci.* 86:1751-1770. **FIL-IDF**. International Dairy Federation, 1999. Milk Fat. Preparation of fatty acid methyl esters. Standard 182:1999. IDF, Brussels, Belgium. **Nudda, A.**, McGuire, M., Battacone, G., Pulina, G., 2005. Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta. *J. Dairy Sci.* 88, 1311-1319. **Raes, K.**, Demeyer, D., De Smet, S., 2004. *Anim. Feed Sci. Techn.* 113, 199-221. **Potchoiba, M.J.**, Lu, C.D., Pinkerton, F., Sahl, T., 1990. Effects of all milk diet on weight gain, organ development, carcass characteristics and tissue composition, including fatty acids and cholesterol contents of growing male goats. *Small Rum Res.* 3:583-592. **Secchiari, P.**, Antongiovanni, M., Mele, M., Serra, A., Buccioni A., Ferruzzi, G., Paoletti, F., Petacchi, F., 2003. Effect of kind of dietary fat on the quality of milk fat from Italian Friesian cows. *Livest. Prod. Sci.* 83:43-52. **Todaro, M.**, Corrao, A., Alicata, M.L., Schinelli, R., Giaccone, P., Priolo, A., 2004. Effects of litter size and sex on meat quality traits of kid meat. *Small Rum. Res.* 54:191-196.

Effect of milk feeding system on carcass and meat quality of Frisa Valtellinese kids

E. Piasentier¹, L.A. Volpelli¹, A. Sepulcri¹, L. Maggioni², M. Corti²

¹ Dipartimento Scienze Animali, Università di Udine, Italy

² Istituto Zootecnica Generale, Facoltà di Agraria, Università di Milano, Italy

Corresponding author: Edi Piasentier. Dipartimento Scienze Animali. Via S. Mauro 2, 33010 Pagnacco, Italy – Tel: +39 0432 650110 – Fax: +39 0432 660614 – Email: edi.piasentier@uniud.it

RIASSUNTO – Effetto del sistema di allattamento sulla qualità della carcassa e della carne del capretto di razza Frisa Valtellinese - *Sedici capretti di razza Frisa Valtellinese sono stati allattati naturalmente (N) o con sostituto del latte (A) e macellati a circa 17 kg di peso. L'alimentazione artificiale ha prodotto un aumento della resa lorda di macellazione (45,6 vs. 48,6% per N e A), a causa soprattutto del minor contenuto dell'apparato digerente. Il sistema di allattamento ha avuto una scarsa influenza sulla resa in tagli, mentre la carcassa dei capretti A ha mostrato un maggior rivestimento adiposo (stato d'ingrassamento: 3,5 vs. 2,2 punti). I principali parametri di qualità della carne (pH finale, colore, perdite alla cottura, resistenza al taglio, analisi tipo) non sono stati modificati dalla dieta; tuttavia la carne dei capretti allattati naturalmente è risultata più ricca di C18:3 e C20:5, con un più favorevole rapporto n-3/n-6 (0,77 vs. 0,45).*

Key words: Frisa Valtellinese, kid, milk feeding system, carcass and meat quality.

INTRODUCTION – Frisa goat is an autochthonous breed from high Valtellina (Italian Central Alps), the territory of Sondalo and Grosio villages being regarded as its cradle. Widespread in the Province of Sondrio, it counts about 5.000 heads. Frisa Valtellinese has black coat, apart from the characteristic white stripes of the head (*frisature*) and other specific white regions, and short hair. In comparison with other similar autochthonous Alpine goat breeds it distinguishes thanks to its bigger size (80 cm at withers). The hardy constitution is coupled with some dairy traits such as deep chest, long rump and well developed udder (Corti *et al.*, 1998).

After a century long decline, the Frisa Valtellinese breeding, as well as other autochthonous Alpine goat populations, is now showing remarkable, although contradictory, recovering signals which deserve to be encouraged (Corti and Brambilla, 2003). Product characterisation and valuation is a useful way by which research may efficiently contribute. However, different farming systems have to be considered in order to fulfil this purpose. Natural suckling is the typical rearing system in extensive farming, where meat kids is usually the sole commercial output. This production system lives together with the semi-extensive one, which maintains the Alpine tradition of milking the goats, summered on mountain pastures. In the semi-extensive system, breeders often exploit intensive techniques in the permanent valley farm unit; these techniques include artificial milk feeding as well as machine milking and concentrate supplementation (Corti and Brambilla, 2003). The purpose of the present paper is to study the effects of natural *vs.* artificial milk feeding on carcass and meat quality of young Frisa Valtellinese kids.

MATERIAL AND METHODS – Sixteen Frisa Valtellinese kids, reared in a farm in Valchiavenna, were fed either on natural milk (N) suckled from their dams or on a commercial milk replacer (A), based on skimmed dry milk and milk derivatives (73%) and containing 23% powder weight of both protein and fat; the milk replacer was administered in two meals per day in a powder to water ratio variable from 15 to 18%, according to age, and in increasing quantities, up to the final 1l/head/meal. When kids averaged 17.3 (s.d. 2.65) kg live

weight, they were slaughtered according to ASPA (1991) procedures. Carcasses were halved and dissected in neck, ribs (steaks and lumbar region), flank (brisket and abdominal region), thoracic and pelvic limb. Muscle *longissimus thoracis et lumborum* (LT) was dissected from the lumbar region and analysed for Warner-Bratzler shear force (WBSF), water loss, colour, pH, proximate analysis and fatty acid composition (ASPA, 1996). Data were submitted to one-way ANOVA, comparing two milk feeding systems: natural *vs.* artificial.

RESULTS AND CONCLUSIONS – Dressing percentages (table 1) were lower than those reported by Corti and Sangiorgio (2000) for Frisa Valtellinese kids and by Manfredini *et al.* (1988) for Alpine kids with similar live weight. The administration of milk replacer allowed an increase in gross dressing percentage, as a consequence of the higher incidence of the gut content in N kids, which would consume some fibrous feed when reared under their dams. The difference in yield between rearing systems further increased considering the gross yield with head and offal, due to a higher incidence of these body parts and, especially, offal in A kids (6.44 *vs.* 8.03% for N and A, respectively): this may be due to a higher develop of liver caused by the artificial diet. The incidence of the cuts showed a significant difference only for neck, heavier in naturally fed kids. Carcass fatness, which is important for commercial evaluation (Colomer-Rocher *et al.*, 1987), was clearly higher in artificially reared kids (3.50 – good to optimum – *vs.* 2.25 – discreet to good, for A and N, respectively).

Table 1. Carcass traits.

	Milk feeding		Significance	Pooled s.d.
	Natural	Artificial		
Starved live weight (kg)	17.1	17.5	ns	2.68
Empty body weight (EBW, kg)	14.41	15.69	ns	2.499
Gut content (% EBW)	19.1	11.7	0.000	3.03
Warm carcass weight (kg)	7.81	8.50	ns	1.347
Warm carcass + head and offal (kg)	9.90	10.95	ns	1.671
Warm dressing percentage (%):				
net (on EBW)	54.3	54.2	ns	2.09
gross (on LW)	45.6	48.6	0.01	1.98
gross with head and offal	57.9	62.5	0.001	2.22
Offal (% EBW)	6.44	8.03	0.000	0.665
Empty gut (% EBW)	9.15	8.52	ns	0.984
Jointing (% carcass weight):				
neck	13.4	12.6	0.04	0.68
flank (brisket + abdominal region)	13.8	14.4	ns	0.87
thoracic limb	21.9	21.8	ns	0.73
ribs (steaks + lumbar region)	17.6	17.6	ns	0.91
pelvic limb	33.3	33.6	ns	1.02
1 st quality cuts% (1)	72.8	73.0	ns	1.08
Fatness score	2.25	3.50	0.02	0.850

(1) Sum of thoracic limb, ribs and pelvic limb.

The most important quality traits of the meat (final pH, colour, cooking loss, WBSF, chemical composition) were not affected by the two different diets (table 2). It may be noticed from WBSF values that kid meat was tender: Todaro *et al.* (2004) found higher values for meat of Nebrodi kids weighing 11 kg (6.4 kg/cm²). Some differences between the two adopted milk feeding systems were observed on meat fatty acid composition: the LT of the naturally fed kids was richer in stearic, linolenic and EPA acid, but no difference could be revealed in the incidence of saturated, mono- and poli-unsaturated fatty acids. The meat of the N kids was higher in n-3 PUFA and in n-3/n-6 ratio (0.77 *vs.* 0.45 for N and A, respectively).

In conclusion, artificial milk feeding, useful for expanding cheese production, increased the gross yield of Frisa Valtellinese kids and enabled the production of better fat covered carcasses. On the other hand, natural rearing combined its higher emotional value with some distinctive intrinsic qualities of Frisa Valtellinese kid meat, mainly linked to a higher level of n-3 PUFA, and consequently to a higher n-3/n-6 ratio, which is favourable for human health.

Table 2 Longissimus thoracis et lumborum traits.

	Milk feeding		Significance	Pooled s.d.
	Natural	Artificial		
pH	5.79	5.81	ns	0.183
Physical characteristics:				
Colour: L*	40.4	40.4	ns	2.13
a*	1.79	1.70	ns	1.112
b*	11.9	11.8	ns	0.88
Croma	12.1	11.9	ns	0.93
Hue angle	81.7	82.0	ns	5.34
Cooking loss (%)	22.8	20.4	ns	2.87
WBSF (N)	36.5	32.5	ns	6.03
Proximate analysis (%):				
Dry matter	24.6	24.8	ns	1.59
Crude fat	3.20	3.50	ns	1.180
Crude protein	19.9	19.8	ns	0.62
Ash	1.42	1.39	ns	0.210
Fatty acids (% total f.a.):				
C 18:0	12.6	10.8	0.05	1.62
C 18:3	3.0	1.6	0.03	1.06
C 20:5	2.4	1.4	0.07	1.03
saturated	38.2	36.8	ns	2.67
mono-unsaturated	35.9	39.1	ns	5.10
poli-unsaturated	25.9	24.1	ns	6.45
n-3 PUFA	10.0	6.8	0.07	3.23
n-6 PUFA	13.9	15.8	ns	4.03
n-3/n-6	0.77	0.45	0.01	0.201

ACKNOWLEDGMENTS – Regione Lombardia Funds (Valorizzazione zootecnica e ambientale delle razze caprine autoctone della Lombardia), prof. Michele Corti.

REFERENCES - ASPA, 1991. Metodologie relative alla macellazione degli animali d'interesse zootecnico e alla valutazione e dissezione della loro carcassa. ISMEA, Abete Grafica spa, Roma. ASPA, 1996. Metodiche per la determinazione delle caratteristiche qualitative della carne. Centro Stampa Univ. Studi Perugia. Colomer-Rocher, F., Morand-Feher, P., Kirton, A.H., 1987. Standard methods and procedures for goat carcass evaluation, jointing and tissue separation. Livest. Prod. Sci. 17: 149-159. Corti, M., Bruni, G., Oldrati, G., 1998. La capra in provincia di Bergamo, un allevamento che ritorna e guarda al futuro. Ferrari Edizioni. BG. Corti, M., Sangiorgio, G., 2000. Live performances and carcass characteristics of Frisa Valtellinese kids. Proc. 7th Intern. Conference on Goats, Tours, France, 669. Corti, M., Brambilla L.A., 2003. Le razze autoctone caprine dell'arco alpino e i loro sistemi di allevamento. In: L'allevamento caprino nelle Alpi: tradizioni, razze, prodotti, in sintonia con l'ambiente. Nuove Arti Grafiche, TN, 61-84. Manfredini, M., Massari, M., Cavani, C., Falaschini, A.F., 1988. Carcass characteristics of male Alpine kids slaughtered at different weights. Small Rum. Res. 1: 49-58. Todaro, M., Corrao, A., Alicata, M.L., Schinelli, R., Giaccone, P., Priolo, A., 2004. Effects of litter size and sex on meat quality traits of kid meat. Small Rum. Res. 54:191-196.

Dietary preferences and ruminal protozoal populations in roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*)

M. Trabalza Marinucci¹, A. Capecci¹, N. Riganelli²,
G. Acuti¹, C. Antonini¹, O. Olivieri¹

¹ Dipartimento Tecnologie e Biotecnologie delle Produzioni Animali, Università di Perugia, Italy

² Studio Faunistico Associato "Chiros", Macerata, Italy

Corresponding author: Massimo Trabalza Marinucci. Dipartimento Tecnologie e Biotecnologie delle Produzioni Animali. Via S. Costanzo 4, 06126 Perugia, Italy – Tel: +39 075 5857707 - Fax: +39 075 5857764 - Email: vete3@unipg.it

RIASSUNTO – Selezione alimentare e popolazione protozoaria del rumine in caprioli (*Capreolus capreolus*), daini (*Dama dama*) e mufloni (*Ovis musimon*). Sono state studiate la dieta e le caratteristiche della popolazione protozoaria del rumine su un campione di caprioli, daini e mufloni abbattuti nella provincia di Arezzo. L'analisi della dieta è stata realizzata valutando le caratteristiche istologiche dei frammenti vegetali ruminali. La concentrazione dei protozoi e la loro classificazione è stata determinata al microscopio ottico. La dieta dei caprioli è risultata composta principalmente da specie arboreo-arbustive (70%), che hanno costituito invece il 45-50% della dieta di daini e mufloni. La sovrapposizione tra diete è stata massima tra daino e muflone e minima tra muflone e capriolo. La popolazione protozoaria è risultata molto limitata nel capriolo ($2,16 \pm 0,67 \times 10^4$ /ml), più consistente nel muflone ($89,64 \pm 26,56 \times 10^4$ /ml) e nel daino ($170,72 \pm 19,95 \times 10^4$ /ml); il genere *Entodinium* è stato l'unico rinvenuto nel capriolo ed ha rappresentato la forma dominante nel daino (88%) e nel muflone (99%).

Key words: diet, rumen protozoa, wild ungulates.

INTRODUCTION – Roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*) are among the most common wild ungulates in Italy and frequently their home ranges overlap. Despite the fact that roe deer is classified as concentrate selector (Hofmann, 1989) and fallow deer and mouflons as intermediate and grass eater, respectively, the composition of the diet can be affected by other factors such as geographical area and plant communities distribution and availability. Little information is available on the feeding behaviour of cervids and mouflons in Italy (Bruno and Apollonio, 1991; Mussa *et al.*, 2003). Nevertheless, knowledge of the feeding habits is important to manage wild ungulate populations and avoid negative interactions with the domestic herbivores at pasture (Trabalza Marinucci and Polidori, 1994). In addition, diet composition can significantly affect rumen metabolism and microbial populations. Very scarce pieces of information are available on the rumen fauna of wild ungulates. Protozoal biomass interacts with the bacteria population and might play a role in the utilisation of fibrous feeds and detoxification of inhibitory substances (Dehority and Odenyo, 2003). The aim of the present study was to examine the feeding habits of roe deer, fallow deer and mouflon in central Italy, the characteristics of the rumen microbial fauna and their interrelationships with the diet.

MATERIAL AND METHODS – An evaluation of the diet and the rumen protozoal population was conducted on 36 adult animals (22 roe deer, 10 fallow deer and 4 mouflons) culled during summer in the province of Arezzo, Italy, in 2003 and 2004. This area is composed by woods (80%) dominated by *Quercus* spp., *Acer* spp. and *Arbutus unedo*, the remainder being mainly xerophyl grassland. Whole rumen contents were mixed, pH values were recorded and composite samples were collected approximately 1 h after the animals were shot. Macroscopical and microhistological analyses of plant fragments were conducted according to the procedure of Bruno and Apollonio (1991) and classified into 7 categories (table 1); within the “shrubs and trees” category, the identification of individual botanical species was carried out. The similarity index (Odum, 1983) among the three diets was calculated using data from most preferred plant species (data not shown). A sub-sample of rumen contents (50 ml) was immediately fixed with an equal volume of 50% formalin (18.5% formaldehyde) for total, subfamily, and generic counts of protozoa (Dehority, 1984). All data were subjected to analysis of variance using General Linear Model procedure of SAS (2000).

RESULTS AND CONCLUSIONS – Data showed that roe deer’s diet was mainly based on shrub and trees (70% of the total), which confirms previous investigations (Riganelli *et al.*, 2000). Both monocotyledons and dicotyledons were less than 10% of the diet. Roe deer feeds principally on the vegetative parts of herbaceous and woody plants but plant communities’ characteristics and their phenological type can influence his feeding behaviour. Mussa *et al.* (2003) found that roe deer’s diet from May to October, in the Piedmont Mountains, was largely composed by herbaceous species. Fallow deer and mouflons’ diets included a higher percentage of monocotyledons and ferns. These species are considered preferential grazers and grasses can account even for 80-90% of the annual forage intake (Poli, 1996). Ferns are unpalatable and potentially toxic in some periods of the year, but can be actively selected in some others, as shown in both red and fallow deer (Kerridge and Bullock, 1991, cited by Poli, 1996). Dietary overlap of the three ungulate species, evaluated using the similarity index, was lowest for roe deer and mouflon (0.26) (Heroldova, 1996), highest for fallow deer and mouflon (0.64) and intermediate for roe and fallow deer (0.48). A greater competition for food during the winter months can probably be observed, as all three species consume large amounts of broad-leaved sprouts.

Table 1. Diet composition (%) of roe deer, fallow deer and mouflon according to rumen plant fragments identification.

Plant categories	Roe deer		Fallow deer		Mouflon	
	Mean	SE	Mean	SE	Mean	SE
Monocotyledons	3.78 A	2.77	17.43 B	6.01	21.29 B	6.91
Dicotyledons	5.82 a	2.49	0.00 b		1.71 b	1.71
Shrubs and Trees	70.46 a	5.84	49.65 b	8.40	50.55 b	11.24
Gymnosperms	0.00		0.00		0.46	0.53
Ferns	0.42	0.39	10.50	8.51	3.24	3.48
Mushrooms	1.05	0.99	0.00		0.00	
Lichens	0.00		3.04	2.35	0.00	
Not identified	18.47	2.56	19.38	3.82	22.75	6.19

a, b: P<0.05; A, B: P<0.01.

Table 2 shows a large degree of variation among total protozoa concentrations of the three ungulates. No protozoa were detected in 10 out of the 22 roe deer’s rumen contents examined. A very low concentration was

found in the remaining samples, when compared to values obtained in mouflons and fallow deer. High-concentrate diets can decrease ruminal pH and numbers of protozoa; *Entodinium* is often the only genus detected in these cases (Dehority and Odenyo, 2003), as confirmed by the results of the present study. This can be explained also by taking into account the short rumen retention time typical of most concentrate selectors herbivores (Hofmann, 1989). The average rumen pH value recorded in roe deer was 5.7, lower than that observed in fallow deer (6.0) and mouflon (5.9). The different feeding behaviour and rumen metabolism of mouflon and fallow deer allowed a larger protozoal population and the presence of protozoa of the Diplodiniinae family and the *Epidinium* genus. Nevertheless, large variability among individuals is usually observed. Improving the knowledge of the complex interrelationships among microbial population and feeding behaviour can help researchers understand wild ungulates' rumen environment and digestive physiology.

Table 2. Total protozoal concentration and percentage generic distribution in roe deer, fallow deer and mouflon.

	Roe deer		Fallow deer		Mouflon	
	Mean	SE	Mean	SE	Mean	SE
Total protozoa (x 10 ⁴ /ml)	2.16 A	0.67	170.72 Ba	19.95	89.64 Bb	26.56
Generic distribution (%)						
<i>Entodinium</i>	100 A		87.89 B	3.29	98.98 AB	1.02
Diplodiniinae ¹	0.0 a		2.92 b	1.16	1.02 ab	1.02
<i>Epidinium</i>	0.0 A		9.19 B	2.45	0.0 B	

a, b: P<0.05; A, B: P<0.001.

¹ Subfamily. Samples were found to contain protozoa in the genera *Eudiplodinium* and *Polyplastron*.

ACKNOWLEDGMENTS – The authors wish to thank Prof. B.A. Dehority (Ohio State University, USA) for his helpful suggestions in the analysis of protozoa and Dr. L. Mattioli (Ufficio Caccia, province of Arezzo) for his cooperation and technical assistance.

REFERENCES – Bruno, E., Apollonio, M., 1991. Seasonal variations in the diet of adult male fallow deer in a submediterranean coastal area. *Rev. Ecol. (Terre Vie)* 46:349-362. Dehority, B.A., 1984. Evaluation of sub-sampling and fixation procedures used for counting rumen protozoa. *Appl. Environ. Microbiol.* 48:182-185. Dehority, B.A., Odenyo, A.A., 2003. Influence of diet on the rumen protozoal fauna of indigenous African wild ruminants. *J. Eukaryot. Microbiol.* 50(3):220-223. Heroldova, M., 1996. Dietary overlap of three ungulate species in the Palava Biosphere Reserve. *Forest Ecology and Management* 88:139-142. Hofmann, R.R., 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia* 78:443-457. Mussa, P.P., Aceto, P., Abba, C., Sterpone, L., Meineri, G., 2003. Preliminary study on the feeding habits of roe deer (*Capreolus capreolus*) in the western Alps. *J. Anim. Physiol. a. Anim Nutr.* 87:105-108. Odum, E.P., 1983. *Basic Ecology*. CBS College Publishing. SAS, 1999/2000. SAS/STAT TM. Guide for Personal Computers, Version 8.1 Edn. SAS Institute Inc., Cary, NC, USA. Poli, B.M., 1996. Feeding and nutrition in Fallow deer: a review. *Suppl. Ric. Biol. Selvaggina* 25:31-61. Riganelli, N., Trabalza Marinucci, M., Polidori, P., 2000. Selezione alimentare in cervi (*Cervus elaphus L.*) e caprioli (*Capreolus capreolus L.*) in ambiente appenninico pp. 290-297 in Proc. Congr. "Gestione degli ungulati Selvatici: Problemi e Soluzioni", Perugia, Italy. Trabalza Marinucci, M., Polidori, F., 1994. L'allevamento integrato di ruminanti selvatici e domestici a salvaguardia dell'ambiente per lo sviluppo socio-economico delle aree interne pp. 37-49 in Proc. XIII National Meeting "Allevamenti di selvaggina nella gestione del territorio", Nocera Umbra, Italy.

Blood inflammatory indices in goats around kidding

E. Trevisi¹, A. D'Angelo^{1,2}, A. Gaviraghi², L. Noé², G. Bertoni¹

¹ Istituto Zootecnica, Università Cattolica Sacro Cuore, Italy

² Istituto Sperimentale Italiano "Lazzaro Spallanzani", Lodi, Italy

Corresponding author: Erminio Trevisi. Istituto Zootecnica. Via Emilia Parmense 84, 29100 Piacenza, Italy – Tel: +39 0523 599278 – Fax: +39 0523 599276 – Email: erminio.trevisi@unicatt.it

ABSTRACT

The transition period of goats is often characterized by serious metabolic problems, mainly before parturition. These troubles are related to negative energy balance status, however all causes are not totally defined. To improve the knowledge about pathogenesis in this phase we have monitored the changes of some blood indices of lipomobilization and inflammation. Six blood samples were collected from 10 primiparous and 25 multiparous "Camosciata delle Alpi" goats after morning milking. Samples were collected around 20 and 7 days before parturition and on days 0, 3, 6 and 12 of lactation. Albumin, total protein, haptoglobin, ceruloplasmin, total cholesterol, NEFA, β -OH-butyrate (BHB), Ca, Mg and Zn were determined. Goats were grouped according to their BHB level before parturition: low (≤ 0.6 mmol/l; LOB), average (0.6-1.09; AVB) and high (≥ 1.09 mmol/l; HIB) level. Furthermore, the AVB group was divided according to plasma haptoglobin level before parturition: low (< 0.5 g/l) or high. The statistical evaluation was carried out comparing 4 groups. Nearly all the primiparous goats were allocated in LOB group (55 vs. 19% of goat of other groups), but they had the highest milk yield (n.s.). 69% of goats had typical peripartum troubles around kidding, but the prevalence was higher in HIB (100%) and AVB (65%) groups vs. LOB (44%). Goats with higher BHB before parturition had higher haptoglobin levels before as well as after parturition ($P < 0.01$). Ceruloplasmin was higher in HIB vs. LOB group, but only after parturition ($P < 0.05$). Conversely, Ca ($P < 0.05$) and Mg (only sometime significantly) were lower in HIB vs. LOB group around parturition. Total protein level was significantly higher in LOB group in comparison to HIB, from parturition to 6th day after ($P < 0.05$). AVB goats showed analogous changes of LOB or HIB, according to their levels of haptoglobin. Cholesterol and albumin showed a tendency of higher levels after kidding in groups with low haptoglobin (only sometime in significant way). Our results suggest that an increase of inflammatory indices before kidding could support the typical troubles, with higher blood ketone bodies, in goats around parturition.

Feeding behaviour of fallow deer under semi-intensive breeding conditions

E. Duranti¹, S. Diverio², A. Barone², G. Tami², C. Casoli¹

¹ Dipartimento Scienze Zootecniche, Università di Perugia, Italy

² Dipartimento Scienze Biopatologiche Veterinarie, Università di Perugia, Italy

Corresponding author: Emilia Duranti. Dipartimento Scienze Zootecniche. Via Borgo XX Giugno 74, 06121 Perugia, Italy – Tel: +39 075 5857108 – Fax: +39 075 5857122 - Email: duranti@unipg.it

ABSTRACT

Wild fallow deer present a great “trophic plasticity” and the ability to utilize different resources in relation to season, year and environment. A trial was carried out in order to assess the influence of farming conditions on fallow deer feeding behaviour. Twenty fallow deer were kept in 4 hectares natural pasture, divided into 8 paddocks for monthly rotational grazing. Food supplementation was given during winter time. Feeding behavioural data were collected by Instantaneous Sampling for a nine month period, at weekly four fixed time intervals. Ethological data were grouped in two datasets: Dataset 1 including alarm, standing, resting, moving, feeding and ruminating behaviour to assess daily and seasonal variations; Dataset 2 including feeding behaviour in relation to type of food intake (leaves, shrubs, acorns or grass). Data were statistically analysed by Principal Component Analysis (PCA) and Correspondence Analysis (CA) by SAS[®]. Main results indicated seasonal differences in feeding time budgets. Farm fallow deer spent more time feeding in winter (37% out of the total 8 hour daily observation period) than in spring (22%), but maximum feeding time was recorded in autumn (49%), when no feed supplements were given. Independently from season, feeding time variations seemed not to influence time spent moving, whereas standing (24%) and resting (20%) times increased when food integrations were given (winter period). With PCA, three factors were extracted which accounted for 80% of the common variance. Feeding behaviour belonged to the first factor, resting and standing showed high opposite loadings on the second, whereas moving was mainly represented on the third one. CA showed seasonal feed preference variations. Farm fallow deer increased grass (93%), shrubs (4%) and leaves (3%) intakes during spring time, as well as in autumn (grass 95%, shrubs 5%). Acorns and shrubs represented the first choice food during winter. Fallow deer seem to maintain their great adaptability to feed on different environmental resources also under farming conditions. However, in all seasons they showed the preference to feed on grass, followed by other food resources.

