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SUMMARY – The relationship between grazed plants and fatty acid content in milk and in pasture samples was studied in grazing goats under Mediterranean conditions. The experiment was carried out at Bella farm, in a native pasture of Southern Italy, at 360 m a.s.l. during the winter and summer. In each experimental period, bulk milk samples of grazing Red Syrian goats and herbage pasture samples were collected; botanical composition of pasture and herbage intake were evaluated. Fatty acid profile in milk fat and herbage samples were analysed. The percentage of Gramineae in both seasons was positively correlated with conjugated linolenic acid (CLA) and C18:1*trans* in milk fat; also, this plant family showed a negative and positive correlation with linoleic acid (LA) and linolenic acid (LNA) content in herbage pasture, respectively. Forbs exhibited a positive correlation with monounsaturated fatty acids (MUFA) and LA but a negative correlation with C18:1*trans* and LNA. *Dactylis glomerata* plant species was positively correlated with unsaturated fatty acids (UFA) during the winter and with CLA in both seasons. *Lolium perenne* showed a positive relationship with polyunsaturated fatty acids (PUFA) and LNA but a negative relationship with C16:0 and LA. In summer, a positive correlation was found between *Phleum pratense* and MUFA content in milk fat. Also, *Phleum pratense* showed a negative relationship with saturated fatty acid (SFA) and $\Sigma\omega 6$ content in milk fat. A positive correlation was observed between MUFA, $\Sigma\omega 3$ and herbage intake in both seasons. Fatty acid LA and LNA content in herbage samples exhibited a negative and positive correlation with PUFA and C18:1*trans* in milk fat, respectively.

Keywords: Botanical composition, milk fatty acid, herbage fatty acid, grazing goats.

RESUME – *"Relation entre la composition botanique du régime, le contenu en acides gras du lait et en acides gras de l'herbage pour des chèvres au pâturage". L'objectif de cette expérience était d'étudier la relation entre les plantes ingérées au pâturage par les chèvres, et la concentration des acides gras dans le lait et dans les échantillons d'herbe en milieu méditerranéen. L'expérience s'est déroulée à Bella, sur un pâturage naturel de plaine (360m d'altitude). Des échantillons de lait de masse, provenant de chèvres de race Rouge de Syrie, et en même temps des échantillons d'herbe du pâturage ont été prélevés en hiver et en été. La composition botanique du pâturage et l'ingestion ont été déterminées. La concentration des acides gras dans le lait et les échantillons d'herbe a été déterminée. Pendant les deux saisons le pourcentage de graminées a été positivement corrélé au contenu de l'acide linoléique conjugué (ALC) et de C18 :1*trans *dans la graisse du lait; cette famille de plantes a montré, respectivement, une corrélation négative et positive avec l'acide linoléique (AL) et linolénique (ALN) contenus dans l'herbe du pâturage. Les autres familles botaniques ont montré une corrélation positive avec les acides gras monoinsaturés (AGMI) et avec AL mais une corrélation négative avec C18:1*trans *et ALN. L'espèce* Dactylis glomerata *a été positivement corrélée avec les acides gras insaturés (AGI) en hiver et avec ALC pour les deux saisons. Le* Lolium perenne *a montré une relation positive avec les acides gras polyinsaturés (AGPI) et avec ALN et au contraire une corrélation négative avec C16 :0 et AL. En été, une corrélation positive a été trouvée entre* Phleum pratense *et les AGMI contenus dans la graisse du lait et une relation négative avec la même plante et les acides gras saturés (AGS) et $\Sigma\omega 6$ dans la graisse du lait. Pour les deux saisons, une relation positive entre l'ingestion d'herbe du pâturage, les AGMI et les $\Sigma\omega 3$ a été trouvée. Les acides gras AL et ALN contenus dans l'herbe du pâturage, montrent une corrélation négative et positive, respectivement, avec les AGPI et le C18:1trans dans la graisse du lait.*

Mots-clés : *Composition botanique, acides gras du lait, acides gras de l'herbage, chèvres au pâturage.*

Introduction

Forage quality and its availability have significant effects on milk quality and yield. Previous investigations in cow's milk have already shown that fodder consumed by the animals affect milk and cheese quality. In fact, forage type (Coulon *et al.,* 1996), forage conservation, botanical composition of hay, botanical composition of fresh forage and altitude influence milk quality (Bugaud *et al.,* 2001;

Collomb *et al.,* 2002a). A relationship between plant species and milk quality was found in goats and ewes grazing on pasture (Fedele *et al.,* 2000; Cabiddu *et al.,* 2003). Fatty acid composition has many effects on milk quality, including its nutritional as well as its physical properties. In addition, fatty acid (FA) profile affects the organoleptic properties of milk, due to factors such as the effect of free short-chain FA and oxidative changes in FA (Chilliard *et al.,* 2001). Factors affecting the FA profile of milk can be assigned to animal factors and feed factors (Morand-Fehr *et al*., 2000; Di Trana *et al.,* 2003). Recent studies have examined how FA composition of milk produced in lowlands, mountains and highlands is related to botanical families and plant species of pasture (Collomb *et al.,* 2002b).

The objective of the current study was to establish whether the fatty acid profile of milk fat was affected by botanical composition of natural pasture in winter and summer seasons under Mediterranean conditions. The correlation among diet botanical composition, milk fatty acids and natural pasture fatty acid content were ascertained.

Materials and methods

The experiment was carried out at Bella farm, in an herbaceous native pasture of Southern Italy, at 360 m a.s.l. (40°21' N; 15°30' E). In the pasture, grasses prevailed during winter and spring while forbs were prevalent in summer (Table 1). The study was carried out for two years during winter (February-March) and summer (June-July). In each experimental period multiparous Red Syrian goats in mid lactation (96 d and 116 d in lactation in winter and summer, respectively), homogeneous for live weight and body condition score (BCS), were used. Twenty-two bulk milk samples were collected.

Table 1. Botanical composition (%) of the pasture in the different seasons

	Winter	Summer
Botanical families		
Gramineae	92	28
Leguminosae	1	3
Forbs	7	69
Plants species		
Dactylis glomerata	25	3
Lolium perenne	60	8
Phleum pratense	5	10
Bromus ordeaceus	0	
Cyanodon dactylon		0
Festuca arundinacea		5
Other grasses	2	2
Asperula odorosa		12
Cichorium intybus		15
Convolvulus arvensis		8
Crepis sp.		9
Daucus carota		7
Plantago spp.		4
Galium verum		9
Other forbs	7	5

The contribution of the different plant species to the diet and intake was estimated in those periods. During winter and summer, on five areas of 2x2 m, randomly distributed in the pasture, the contribution of each species to the diet was estimated. This was calculated for each species from the ratio between the number of plants grazed for single species, and the number of plants, for the same species, present in the picked area before grazing. Based on this information an "artificial diet" was made. For each species, plant samples corresponding to those really browsed by goats were cut from an un-grazed area and mixed in the same estimated proportions. The so made diet was used for the chemical analyses. On five 2x2 m large sampling units, herbage intake was estimated by difference between herbage mass, measured on un-grazed area, and post-grazing herbage mass measured in experimental adjacent grazed areas.

Milk lipids were extracted with chloroform and methanol (Bligh and Dyer, 1959). Fatty acids in milk fat were converted into methyl esters and separated by gas chromatography (Varian 3800 with CP 8410 auto injector) equipped with a FID detector and a 60m x 0.25 mm (id) cyanopropyl polysiloxane (DB 23, J & W) fused silica capillary column. Operating conditions were a helium flow rate of 1.2 ml/min, a FID detector at 250°C, a split-splitless injector at 230°C with a split ratio 1:100, an injection volume of 1 µl. The temperature programme of the column was: 5 min at 60°C then increased at a rate of 14°C /min to 165°C and a subsequent increase at 2°C/min to a final temperature of 225°C and then held for 20 min. The individual fatty acid peaks were identified by comparison of retention times with those of known mixture of standard fatty acids (F.A.M.E, Sigma). The individual standard references of conjugated linolenic acid (CLA) isomers (*cis*-9*, trans*-11 97 % and *trans*-10*, cis*-12 3%) and *trans*-11 C18:1 were obtained from Larodan (Malmö, Sweden). The Gray *et al.* (1967) method was used for extraction of herbage samples lipids; methylation and gas chromatograph analysis was the same as that described for milk fatty acids. The column condition was different: 4 min at 140°C and a subsequent increase to 225°C at 4°C/min. Fatty acid identification was executed as for milk.

Statistical analysis was carried out using SYSTAT statistical package version 5.2. (Systat, 1992). Pearson's correlation coefficients were computed between groups of fatty acids or individual fatty acids of goat milk, and the botanical families or plant species of the natural pasture and some fatty acids of pasture samples.

Results and discussion

Significant Pearson's correlation coefficients concerning botanical families, individual plant species and fatty acids in milk fat and in herbage pasture samples are shown in Table 2. In pooled data of the seasons, the percentage of Gramineae was positively correlated with CLA content and C18:1*trans* in milk fat. Collomb *et al.* (2002b) stressed the relationship between plant families and groups of fatty acids. In mountain and highland pasture they found indeed positive and negative correlation between many plants families with CLA and C18:1*trans* content in cow's milk. Gramineae was positively correlated with C18:3ω3 linolenic acid (LNA) but negatively correlated with C18:2ω6 linoleic acid (LA) fatty acid content in pasture herbage. The level of LNA in herbage pasture samples increased with the percentage of the Gramineae in the diet of grazing goats. LNA is found mainly in forages and it is the most important substrate of the rumen biohydrogenation (Harfoot and Hazlewood, 1988).

No correlations were observed between Leguminosae plant family and groups of fatty acids in milk.

As concerns the forbs*,* dominant in summer pasture and characterised by numerous plant species, they were positively correlated with monounsaturated fatty acids (MUFA) but negatively related to C18:1*trans* in pooled data; furthermore, this plant group showed a positive and negative correlation with LA and LNA content in pasture samples, respectively. On the contrary, Collomb *et al.* (2002b) observed no correlation between plant families and MUFA in milk fat of cows grazing at different altitude. The relationship between forbs, LA and LNA may be attributable to a reduction of LNA and an increase of LA in plant species when stage of maturity of herbage increases (Morand-Fehr and Tran, 2001).

Concerning relationships with plant species, *Dactylis glomerata* was the only plant, which was positively correlated with CLA in pooled data. In fact, that plant contributed to the diet of grazing goats mainly in winter (Table 1). It is noteworthy that the orchardgrass showed a higher LNA content, precursor of CLA, than perennial ryegrass (Morand-Fehr and Tran, 2001). Collomb *et al.* (2002b) did not found correlation between this plant species and CLA but with other plant species; this might depend on either a less percentage of orchardgrass in the grazing sites or a limited intake of this plant species. In addition, during the winter the above-mentioned species exhibited a negative and positive correlation with saturated fatty acids (SFA) and unsaturated fatty acids (UFA), respectively. In pooled data, the proportion of *Lolium perenne* was positively correlated with polyunsaturated fatty acids (PUFA) and negatively with C16:0 in milk fat. Moreover, a relationship was detected between the aforementioned plant and fatty acids in herbage diet, in particular a positive correlation with LNA but a negative correlation with LA content was found.

Table 2. Significant Pearson's correlation coefficients (P<0.05) between botanical families, individual plant species, herbage intake and fatty acids in milk fat and natural pasture samples

	Fatty acids										
	Milk									Pasture	
	SFA	UFA	MUFA	PUFA	C16:0	∑ω3	∑ω6	CLA	C18:1*t*	LA	LNA
Botanical families											
Gramineae								0.57*	0.73	–0.64	0.61
Forbs			$\boxed{0.61^*}$						–0.70	0.69	–0.59
Plants species											
Dactylis glomerata	$\underline{-0.74}$	$\underline{0.74}$						0.76			
Lolium perenne				0.87	–0.73					–0.62	0.63
Phleum pratense	$\boxed{-0.69}$		$\boxed{0.75}$				$\boxed{-0.53}$				
Herbage intake	–0.77	0.77	0.85			0.78	$\boxed{-0.74}$				
Pasture fatty acid											
LA				–0.75	0.68				–0.81		
LNA				0.78	–0.69				0.80		

FA: Fatty acid; SFA: Saturated fatty acids; UFA: Unsaturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; CLA: Conjugated linoleic acid; LA: Linoleic acid; LNA: α-Linolenic acid.
Note: Underlined and frame coefficients are referred to winter and summer data, respectively.
*P≤ 0.08.

The relationship between perennial ryegrass and PUFA may depend on a high contribution of this plant (60% in winter) to the diet of grazing goats. A previous study proved a positive correlation of *Lolium perenne* with MUFA (Collomb *et al*., 2002b). In our study, the correlation between perennial ryegrass and LNA and LA could be attributable to the higher proportion of LNA and lower proportion of LA in this plant species than in the plant belonging to the Leguminosae (Morand-Fehr and Tran, 2001).

Throughout summer, *Phleum pratense* showed a positive relationship with MUFA but a negative correlation with SFA and ∑ω6 content in milk fat. This result may be linked to the percentage (10%) of this plant in the natural pasture in summer. It should be noticed that in lowland, mountain and highland regions, characterized by a little percentage of *Phleum pratense,* Collomb *et al.* (2002b) found a positive correlation between this species and SFA but a negative correlation between this plant and CLA and C18:1*trans*.

Herbage pasture intake showed a positive correlation with UFA, MUFA and ∑ω3 content in milk fat but a negative correlation with SFA and ∑ω6 content in milk fat. A previous study on grazing ewes showed a relationship between dry matter intake and CLA (Avondo *et al.,* 2002).

Following the analyses of the FA profile in pasture samples, in combined data of the seasons, the proportion of LNA content showed positive relationship with PUFA and C18:1 *trans* but negative with C16:0 content in milk fat. LA content in herbage pasture was negatively correlated with PUFA and C18:1 *trans* content in milk fat but positively correlated with C16:0. Conversely, at different altitudes Bugaud *et al.* (2001) observed no relationships between proportion of LNA and LA in grass samples and PUFA in milk fat. The proportion of LNA and LA is higher than other UFA in fresh herbage; they are precursors of rumenic acid and *trans* vaccenic acid, both being important for the nutritional quality of milk. The positive relation between LNA and C18:1*trans* in milk fat may be linked to its role as precursors of *trans* vaccenic acid in milk fat, Chilliard *et al.* (2003) reported this fatty acid as the main isomer of C18:1 in goat milk fat.

Conclusion

The present study was carried out under Mediterranean conditions characterised by little availability of botanical families and plant species in the pasture during winter but high variability of plant species in summer. The relationship between fatty acid profiles and botanical composition of natural pasture was found. The correlations seem to indicate that the intake of different plant families could be linked to the fatty acid profile of milk fat. The main plant species utilised by goats appear to be related to milk fatty acid profiles. The relationship between fatty acid groups and herbage intake suggests that herbage intake play the main role to improve milk quality.

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