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Effect of types of forage on terpenes content and profile in goat milk

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SUMMARY – The objective of this study was to evaluate the effect of three alimentary treatments (grazing, pasture hay and mixed hay) on Siriana goat milk terpenes content and profile. The grazing group of 15 goats grazed for 7-9 hours/day on an area of 1.2 ha. The pasture hay group (15 goats fed hay from the previous grazing area) and mixed hay group (15 goats fed hay composed of alfalfa, perennial rye grass and orchard grass) were fed indoor with hay *ad libitum*. All groups received 600 g/day of the same concentrate (150 g CP and 180 g NDF/kg dry matter). The trial lasted three weeks, after 2 weeks of adaptation to the treatment. The contribution of botanical categories to the diet was determined on hay and on grazed herbage. Once a week, for three consecutive weeks, two samples of bulk milk from each group were analysed. Volatile compounds were analysed by method of Ciccio *et al.* (2005). The highest mono and sesquiterpenes content was detected in milk from grazing goats (2,031 and 4,480 ng/l respectively) and the lowest in milk from mixed hay treatment (718 and 610 ng/l respectively). Milk from grazing and pasture hay treatment showed a profile richer in mono and sesquiterpenes compounds than milk from the mixed hay group. These differences seemed associated with a higher contribution of forbs to grazing and pasture hay diets. The natural drying process did not affect the milk terpenes profile, but only reduced the terpenes content.

Key words: Terpenes, grazing, forage diet, goat milk.

RESUME – "Effet des types de fourrages sur la teneur en terpènes et leur profil dans le lait de chèvres". L'objectif de cette étude était d'évaluer l'effet de trois traitements alimentaires (pâturage, foin de prairie et foin mixte) sur la teneur en terpènes et le profil de ces terpènes dans le lait de chèvres Siriana. Le groupe sur pâturage était constitué par 15 chèvres qui broutaient pendant 7-9 heures/jour sur une surface de 1,2 ha. Le groupe alimenté au foin de prairie (15 chèvres recevant du foin provenant du pâturage précédemment cité) et le groupe alimenté au foin mixte (15 chèvres recevant un foin composé de luzerne, ray-grass pérenne et dactyle aggloméré) recevaient l'alimentation dans l'étable avec foin *ad libitum*. Tous les groupes recevaient 600 g/jour du même concentré (150 g PB et 180 g FND/kg DM). L'essai durait trois semaines, après 2 semaines d'adaptation au traitement. La contribution des catégories botaniques à l'aliment était déterminée à partir du foin et de l'herbage pâturé. Une fois par semaine, pendant trois semaines consécutives, deux échantillons de lait en vrac provenant de chaque groupe furent analysés. Les composés volatils furent analysés par la méthode de Ciccio *et al.* (2005). La plus forte teneur en mono et sesquiterpènes avait été détectée dans le lait des chèvres au pâturage (2031 et 4480 ng/l respectivement) et la plus faible teneur dans le lait du traitement de foin mixte (718 et 610 ng/l respectivement). Le lait des traitements de pâturage et de foin de prairie a montré un profil plus riche en composés mono et sesquiterpènes que le lait du groupe de foin mixte. Ces différences semblent liées à une plus forte contribution des herbacées aux régimes de pâturage et de foin de prairie. Le processus naturel de séchage n'a pas influencé le profil des terpènes dans le lait, mais a seulement réduit la teneur en terpènes.

Mots-clés : Terpènes, pâturage, régime fourrager, lait de chèvre.

Introduction

The Italian well-informed consumers prefer cheeses from grazing systems for their pleasant sensory characteristics and, among them, those made with spring and summer milk. Cheeses made with winter milk are not so appreciated because they are not distinguishable for sensory characteristics from the commercial products. In spring and summer the animals, normally, graze more than 8 hours/day, while during winter, for the unfavourable climatic conditions, they are fed indoor with preserved forage. In winter, goat flocks are fed with hay from cultivated area (alfalfa, clover, mixes among two or plus species) or from native pasture. These preserved forages would be the cause of the sensory differences perceived by the consumers. Several study showed, in fact, that specific sensory diversity in cheese was associated with grass preservation (Urbach, 1990; Verdier-

Metz *et al.*, 2000) as hay or silage. In these differences also the botanical diversity plays an important role. Milk or cheese from animals fed grasses or dicotyledon plants showed different sensory characteristics (Bosset *et al.*, 1999; Buchin *et al.*, 1999; Bugaud *et al.*, 2002; Fedele *et al.*, 2004).

Several volatile compounds are involved in the determinism of these differences and, among them, terpenes may play a role (Fedele *et al.*, 2005) even if their implication in the determinism of cheese sensory properties is still not demonstrated. They are found in high quantity in certain botanical categories such as forbs (Mariaca *et al.*, 1997; Fedele *et al.*, 2004), and pass rapidly into the milk (Viallon *et al.*, 2000). In a previous study Fedele *et al.* (2001) observed that the panellists distinguished well goat milk and cheese from grazing systems from those of goats fed indoor with hay.

Since in Italy all goat milk is transformed in cheese and 40% or plus is produced during winter, the objective of this study was to evaluate if different forage-based diets were capable to affect milk terpenes content and profile.

Materials and methods

The trial was conducted at Bella farm of Istituto Sperimentale per la Zootecnia, located in Basilicata region (southern Italy) at 360 m.s.l. (40° 21' N; 15° 30' E).

Animal and diet

Forty-five Siriana does (mean weight 39 ± 1.92 kg) were separated in three homogeneous groups of 15 goats according to their milk production and live weight. Each group was randomly assigned to one of the three alimentary treatments: grazing, mixed hay (alfalfa, perennial rye grass and orchard grass), and pasture hay (from the previous grazing area). The grazing group grazed for 7-9 hours/day an area of 1.2 ha divided into two equal paddocks, each of them grazed by goats for a varying number of days in relationship to the herbage availability. Mixed hay and pasture hay groups were indoor fed hay *ad libitum*, simultaneously with grazing group. All groups received 600 g/day of the same concentrate (150 g of crude protein and 180 g of neutral detergent fibre/kg DM). The contribution of botanical categories to the diet was determined on hay and on grazed herbage. For the hays, the contribution was calculated in weight on DM basis as difference between botanical composition of hay offered and botanical composition of hay refused by animals. The contribution of botanical categories to the grazed herbage was estimated as ration between number of plants for each category (legumes, grasses, forbs) on five delimited areas of 2 m × 2 m, randomly located in the grazing area. Before grazing, the number of plants was evaluated for each category (legumes, grasses, forbs) present in the delimited area, while after grazing the number of plants for single category grazed by goats. For each category, the parts of plant browsed by goats were also evaluated. On the basis of these information, an artificial diet was formed. For each category, the parts of plant corresponding to those really grazed were cut, and assembled in the same proportion after weighing.

Each group received the assigned treatment for three weeks, after two weeks of adaptation to the new diet, in the period included between May and June; milk samples (3 samples in duplicate × group) were collected in the three weeks of trial at regular intervals of 7 days (1 × week). A cumulative milk sample in duplicate was collected from each group and immediately submitted to volatile compound extraction according to the method proposed by Ciccioli *et al.* (2004).

Volatile compounds analyses

Milk volatile compounds were analysed for each milk sample by a modified headspace technique described by Ciccioli *et al.* (2004). Aliquots of 100 ml were transferred in a glass container and after spiking the sample with 1 ml of methanol solution containing 100 p.p.b. by weight of perdeuterated benzene, toluene, o-xylene and 1,3,5-trimethylbenzene, the container was closed and placed in a thermostatic bath kept at 50°C. Gas extraction was performed by passing 10 l of pure helium at a rate

of 200 ml/min into the glass container. The outlet of the extraction system was connected to a sampling train comprised of two adsorption traps set in series (Tenax TA by Aldrich Chemical Co., Milwaukee, USA and Carbograph 1 by LARA S.r.l., Rome, Italy the first trap; Carbograph 1 and Carbograph 5 by LARA S.r.l., Rome, Italy the second trap). Volatile compounds retained on the adsorption traps were thermally desorbed at 250°C. The concentrated sample was then injected into the chromatographic column of Agilent 6890N gas chromatograph (Agilent Technologies, USA). Volatile compounds were identified on the basis of their mass spectra. Selected ions were used to quantify overlapping peaks on those present at trace levels. Positive identification of the various components was carried out by combining mass spectra data with Kovats retention index. The total amount of a compound dissolved in a liquid matrix is based on the assumption that the whole extraction process follows a first order kinetics.

Statistical analyses

Terpenes content was analysed by ANOVA with a model including alimentary treatment (SAS, 1987). Means were separated using least significant differences test when a significant ($P < 0.05$) effect was observed. Statistical analyses for all the terpenes identified is not reported because the differences were not significant due to the high variability.

Results

The most evident difference observed among the three diets concerned the contribution of the legumes and forbs (Fig. 1). The first category reached the highest value in the mixed hay diet (50.5%) and the lowest in grazing one (23%); on the contrary, the second category reached the maximum value in the grazing diet (37.5%) and the minimum in the mixed hay one (9%). The three botanical categories contributed at similar level to the diet of goats fed pasture hay.

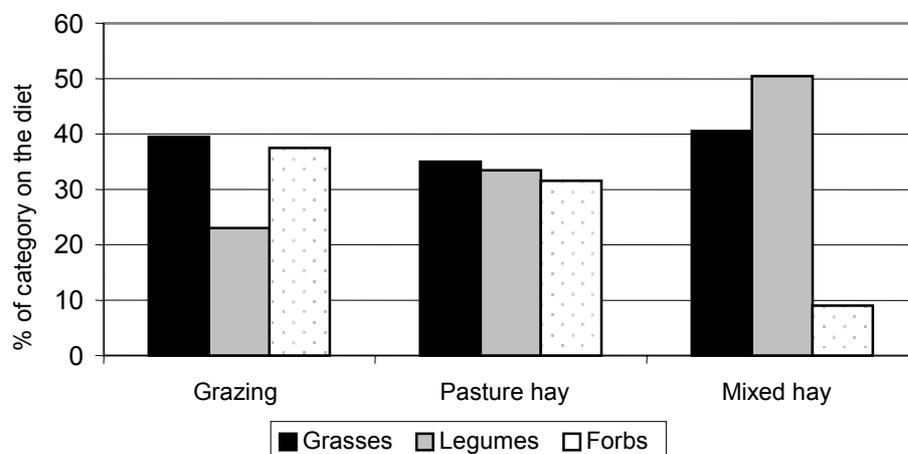


Fig.1. Contribution of botanical categories to each goat diets.

Mono and sesquiterpenes content in milk changed significantly ($P < 0.05$) according to forage-based diet (Table 1). Milk from grazing diet showed the highest mono and sesquiterpenes content (2031 and 4480 ng/l respectively), while milk from mixed hay diet the lowest one (718 and 610 ng/l respectively).

Terpenes content in milk from the two groups fed with natural resources (grazing and pasture hay) showed differences, while their monoterpene profile was almost similar (Fig. 2). On the contrary, in milk from mixed hay group, Δ -3 Carene, 4 and α + γ -Terpineol and a not identified terpenes (Ni-Terpene) were not found, while Tricyclene and α -Thujene were detected only in this group.

Table 1. Mono and sesquiterpenes content (ng/l) in milk (means \pm SD)

Feeding treatment	Grazing	Pasture hay	Mixed hay	P
Monoterpenes	2031 ^a \pm 4285	1374 ^a \pm 226	718 ^b \pm 154	<0.05
Sesquiterpenes	4480 ^a \pm 626	2334 ^b \pm 324	610 ^c \pm 152	<0.05

^{a,b,c} Letters denote the differences among means.

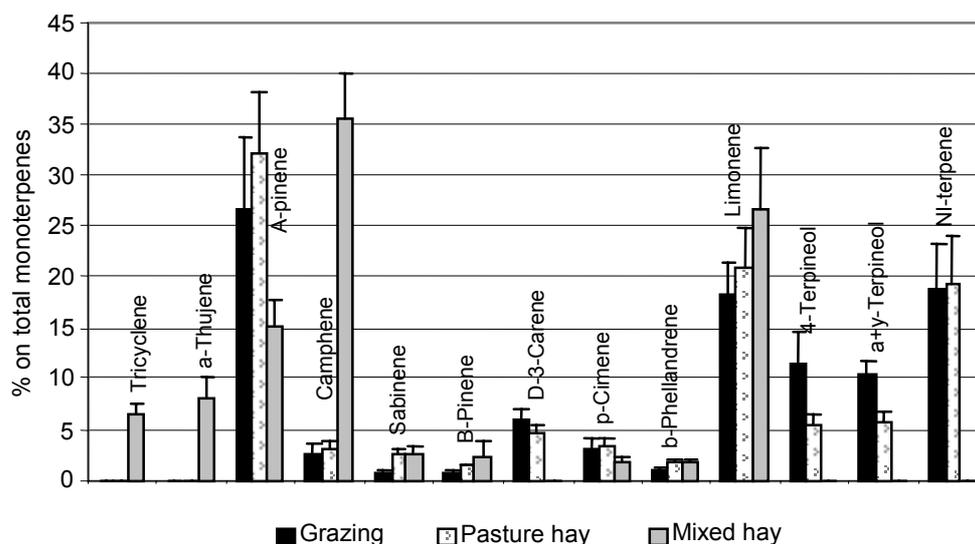


Fig. 2. Monoterpenes profile (mean values \pm SD) of goat milk from different alimentary treatments.

Differences due to forage-based diet were observed also on milk sesquiterpenes profile. Milk from goats fed mixed hay was poorer in sesquiterpenes compounds (Fig. 3). In this milk only 5 compounds were detected against 10 and 9 of the other two groups (grazing and pasture hay respectively).

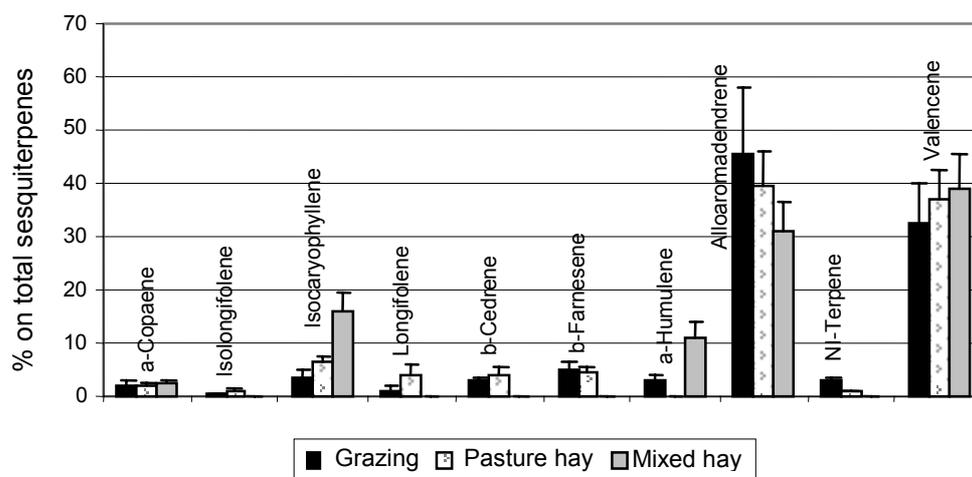


Fig. 3. Sesquiterpenes profile (mean values \pm SD) of goat milk from different alimentary treatments.

Discussions

Frequently people attribute certain effects on volatile compounds and on milk or cheeses sensory characteristics to the forage preservation methods. The thesis is based on the comparison between hays and silages, but made with different botanical species. Since every species has own specific aromatic characteristics (Mariaca *et al.*, 1997; Fedele *et al.*, 2004), it can be a forcing to separate the effects of the preservation method from those of the botanical species, especially when comparing forages made with species very different (forbs instead of grasses or legumes or mixed).

This study showed that botanical diversity of forage had a different effect from that of preservation method on the terpenes content and profile. The abundance of botanical species in the forage diet influenced especially milk terpenes profile, while grass drying-process affected milk terpenes content. Numerous studies have shown that grass species are poor in monoterpenes (Mariaca *et al.*, 1997; Bosset *et al.*, 1999; Viallon *et al.*, 2000; Fedele *et al.*, 2004), while the dicotyledons not-Leguminosae (forbs) in sesquiterpenes (Scehovich *et al.*, 1998). Mixed hay intake was composed for over 50% by legumes and 40% by grasses (orchard grass and rye grass), while the diets of the other two groups (grazing and pasture hay) by 37% of grasses and 28% of legumes in average. The forbs contributed to these diets with 35% and they were mainly represented by *Asperula odorosa*, *Galium verum*, *Cichorium intybus*, *Geranium molle*, etc., plants particularly rich in sesquiterpenes as observed by Fedele *et al.* (2004). Since the pasture hay was produced in the same area of grazing group and the milk profiles were the same, this implied that the differences observed in milk profile between goats fed natural forage (grazing and pasture hay) and mixed hay were due to this botanical category (forbs). In a comparative study between cheese made with milk from cow fed orchard grass hay (*Dactylis glomerata*) and natural pasture hay, Viallon *et al.* (1999) observed in the first cheese a profile less rich in monoterpenes compounds. With these statements, an effect of the drying-process on the profile can be excluded, it rather seemed to affect the mono and sesquiterpenes content, probably because the exposure to the sun and to the high temperature (28-32°C) for 3-5 days volatilized a quote of these compounds from the herbage.

Conclusions

On the basis of these results, it emerges that the presence in the goat diet of hay from native pasture or native herbage assures, even with the supplementation up to 600 g/head/day of concentrate, a terpenes content and profile not so different from grazing animal not supplemented.

We can hypothesise that the consumers would not perceive sensory differences due to the use of concentrate supplementation to native pasture or forage.

If this hypothesis is tested true, it means that we can improve the grazing system, through the concentrates, without impoverishing milk terpenes profile and content.

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