

Variation in terpene content and profile in milk in relation to the dominant plants in the diet of grazing goats

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Abstract

The objective of this study was to evaluate the effect of the diet of grazing goats on the concentration and the profile of terpenes in their milk. This was tested during three seasons of the year using the two most preferred plant species in each season. Fifteen non-supplemented lactating goats grazed the pastures during the different seasons for 8 hours/day to measure their preference of specific plant species. The terpene concentration of the milk of six grazing lactating goats fitted with rumen cannulae were determined. For six consecutive days, 500 g/day of fresh herbage were introduced through the cannulae into their rumens, *Lolium perenne* and *Dactylis glomerata* in winter, *Geranium molle* and *Asperula odorosa* in spring and *Cichorium intybus* and *Galium verum* in summer. The results showed that each plant species modified the profile and the concentration of mono and sesquiterpenes of milk. In every season sesquiterpenes appeared the most abundant compounds. In summer the sesquiterpenes reached the highest levels, enriching goat milk most pronouncedly.

Keywords: Terpenes, goat, milk, plant

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Introduction

Terpene content and profile in milk and dairy products are influenced by feed and especially by grazed herbage. This relationship could be used to distinguish milk or cheese originating from grazing or no grazing systems (Fedele *et al.*, 2000; Rubino *et al.*, 2002), or to trace their geographical origin, or production site (Viallon *et al.*, 2000).

The diet of the more selective species such as goats is very diverse from one season to another. Goats graze grasses more willingly during winter, while the relative intake of legumes and forbs increases from spring to summer (Fedele *et al.*, 1993). During summer forbs contribute to over 50% of the diet dry matter. Generally, the dicotyledons enrich dairy products more than the monocotyledons (Mariaca *et al.*, 1997). While knowledge of these general aspects increases continuously, there is little information on the effect exerted by the single plant on milk composition. The knowledge of this relationship could allow using these plants as markers of cheese origin, of cheese sensory characteristics, of grazing season and finally to know which are the plants that best characterize the sensory properties of dairy products in order to increase their proportion into a pasture. The objective of this study was to evaluate, during three different seasons of the year, the effect of the two most ingested plants by goats on the concentration and profile of terpenes in their milk.

Materials and Methods

A native herbaceous pasture in a Basilicata valley (Southern Italy) at 360 m.s.l. was used for this study. From March to July 15 non-supplemented lactating goats (G) grazed for 8 hours/day an area of 1.2 ha. During March, May and July the contribution of each plant species to the diet was estimated on five areas of 2x2 m, randomly distributed throughout the pasture. The ratio was calculated between the number of plants grazed per single species and the number of plants from the same species present in a delimited area before grazing. In order to evaluate the effect of the two preferred plant species on milk terpenes, six grazing lactating goats fitted with rumen cannulae were used. For six consecutive days, 500 g/day of fresh herbage of the following species were infused through the cannulae: *Lolium perenne* (Lp) and *Dactylis glomerata* (Dg) in winter; *Geranium molle* (Gm) and *Asperula odorosa* (Ao) in spring and *Cichorium intybus* (Ci) and *Galium verum* (Gv) in summer. The two herbage species within a season were introduced into the rumen of three goats per herbage (250 g/day in the morning and 250 g/day in the afternoon). Terpene analyses were performed on three cumulative milk samples for each season, using a modified headspace technique (Fedele *et al.*, 2000). VOC were analysed by HRGC-MS and identified on the basis of their mass spectra.

Results and Discussion

During winter the goats preferred *Lolium* (44.5%) and *Dactylis* (14%). Milk from these two treatments showed lower terpene concentrations (100 ng/L and 132 ng/L, respectively) than milk from the grazing goats (11889 ng/L). Differences were observed also in the terpene profile (Figure 1). *Dactylis* milk was the poorest in monoterpenes (6 molecules), while milk from Lp and G goats was the richest (9 and 12 molecules, respectively). In the two treated groups the dominant terpenes were the Limonene (56.8% Lp and 45.8% Dg), and in the G group α -Pinene (28.6%). The general profile from G milk was almost similar to that from Lp. The high contribution of this species (44.5%) probably exerted an influence on the G milk profile. In the last milk samples, two molecules, Δ -3- Carene and an unidentified terpene (mono NI-a), absent in the other milk, were found. The low capacity of Orchard grass to enrich the dairy products in monoterpenes was also observed by Coulon *et al.* (2000). They observed that cheese from Dg hay was lower in monoterpenes than cheese from pasture.

At the approach of spring legumes and forbs in the diet increased, but monoterpenes in G milk decreased (343 ng/L). During this season Ao (8.0%) and Gm (6.0%) were the two most grazed species. These two species, in comparison to winter species, increased milk monoterpenes concentration (284 ng/L and 227 ng/L, respectively) and also modified its profile. The α -Pinene (76.7%) was the dominant terpenes in Gm milk, an unidentified terpene (mono NI-b) was dominant (47.2%) in Ao and Limonene (55.2%) in G milk. In this season the profile of G milk was almost similar to that of the dominant species in the diet (Ao). The fact that the mono NI-a terpenes were found only in G winter, G spring and Ao milk, suggests that Ao in the diet was the source.

Figure 1 Monoterpene profile in milk from Lp, Dg and winter G diet

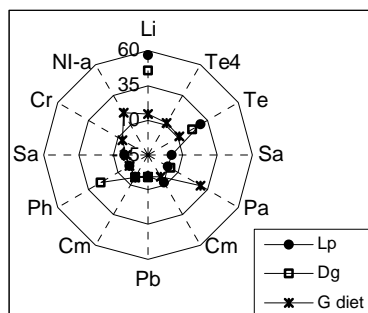


Figure 2 Monoterpene profile in milk from Gm, Ao and spring G diet

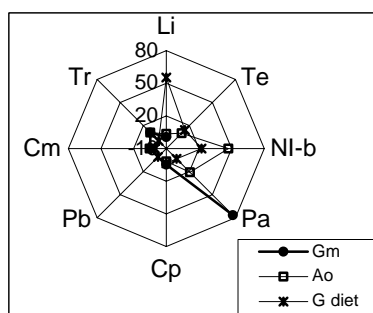
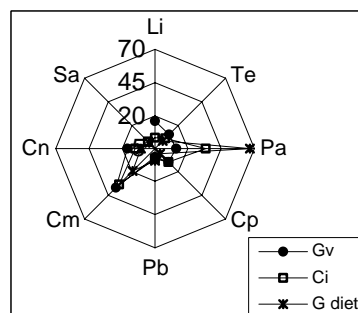


Figure 3 Monoterpene profile in milk from Ci, Gv and summer G diet



Cr= Δ -3-Carene; Cp=Camphene; Li=Limonene; NI-a, NI-b=Not Identified; P α = α -Pinene; P β = β -Pinene; a=Sabinene; Te= α + γ Terpineol; Te4=4-Terpineol; Sa=Sabinene; Cm=p-Cymene; Ph= β -Phellandrene; Tr=Tricyclene

During summer goats selected especially Ci (14.5%) and Gv (9%). The feeding of these two species decreased the terpene concentration (respectively 76 ng/L and 116 ng/L) in the milk to the same level as in winter, while the concentration in milk of the non-treated group (478 ng/L) increased slightly. The terpene profile of G milk was also similar to the species most grazed (Figure 3). The p-Cymene was the most representative terpenes in milk from Ci and Gv treatment (33.2% and 36.8%, respectively), while α -Pinene was the most representative in G milk. In all seasons the enrichment of diets by plant infusion into the rumen induced a decrease of monoterpenes in milk in comparison to non-infused goats (G). At the moment there is no explanation for this phenomenon. It is probable that the excess of herbage in the rumen altered the digestive and metabolic process, limiting the absorption of terpenes by the rumen wall, or that a portion of the more volatile terpenes escaped through the rumen cannulae, impoverishing the rumen environment. Sesquiterpene concentration in all milk samples was higher than the monoterpene concentration. The plant infusion into the rumen caused a decrease only in winter. Milk from non-treated goats (G) showed the lowest value in spring (1183 ng/L) and the highest concentrations in summer (16484 ng/L).

In winter (Figure 4) α -Copaene was the dominant terpene in both treated groups, while Alloaromadendrene was dominant in G. Similar sesquiterpene profiles were observed for Lp and G milk, as observed for the monoterpenes, but in the G milk Valencene and β -Farnesene concentrations were found

which were not recovered in the other milk samples. In spring the sesquiterpenes concentration and the number of molecules decreased (4 in Dg milk and 5 in both Lp and 1 in G). In all milk samples (Figure 5) β -Caryophyllene, α -Copaene and Sequi NI-d were the dominant terpenes. The Sequi NI-e terpenes were recovered only in the Ao treatment, probably because only this species contained them. During summer the sesquiterpenes reached the maximum concentration and their profile was more diversified. The concentration ranged from 16.484 ng/L of G milk to 41.484 ng/L of Gv milk.

Figure 4 Sesquiterpene profile in milk from Lp, Dg and winter G diet

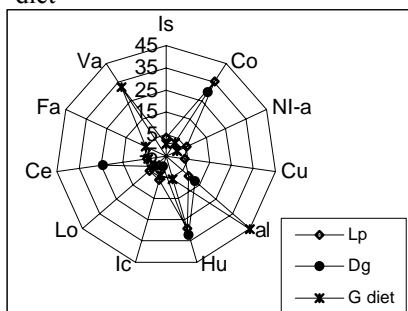


Figure 5 Sesquiterpene profile in milk from Gm, Ao and spring G diet

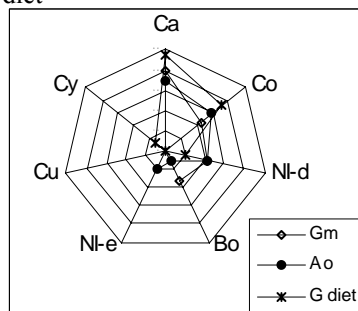
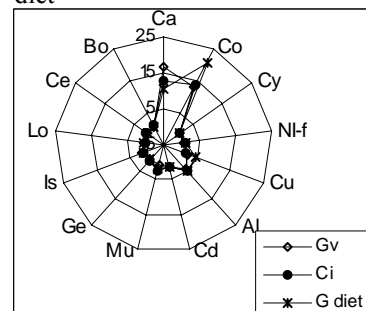


Figure 6 Sesquiterpene profile in milk from Gv, Ci and summer G diet



Al=alloaromadendrene; Bo=Boubonene; Ca= β -Caryophyllene; Cd=Cadidene; Ce= β -Cedrene; Co= α -Copaene; Cu=Cubebene; Cy=cycloisantivene; Fa= β -Farnesene; Ge=Germanene; Hu= α -Humulene; Ic=isocaryophyllene; Is=Isolongifilene; Lo=Longifilene; Mu=Muurulene; NI-c, NI-d, NI-e= Not identified; Va=Valencene

No substantial differences in the sesquiterpene profile were observed and in all milk samples β -Caryophyllene and α -Copaene were the dominant terpenes. Since Ci and Gv were more than 25% in the G diet it is probable that this level is sufficient to characterize the sesquiterpene profile, or possibly that the other grazed plants had the same effect as that of the tested one.

Conclusions

By changing the contribution of herbage species in the goat diet, the terpene concentration and profile in the milk can be changed. At the quantitative level these results showed that the sesquiterpenes in every season and for every tested species assumed a greater importance than the monoterpenes. This last category seemed to depend especially on grasses. Some emerged phenomena are not simple to explain in the light of the general knowledge. The impoverishment in spring of terpenes in all milk samples, in spite of the diets being rich in terpene species, was similar to the effect observed when the plants were infused into the rumen. The higher herbage intake in spring (30-40% more than in the other seasons) probably modified the digestive and metabolic processes. Consequently, the terpenes were less available for absorption. In summer when intake generally decreased and the herbage was less fermentable, sesquiterpenes reached the highest concentrations and the profile was very enriched.

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