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Browsing ratio, species intake, and milk fatty acid composition of goats foraging on alpine open grassland and grazable forestland

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Abstract

Aims of this study were to investigate diet selection of dairy goats foraging on two alpine vegetation types and to assess the related effects on milk fatty acid (FA) composition. Two enclosures laid out on an open grassland (OG) and a grazable forestland (GF) were exploited by 14 Camosciata goats. A commercial concentrate was supplemented during milking. Forty-five plots were randomly selected inside each enclosure and used to assess the species relative abundance (SRA) and phenological stage of plants, as well as goats' preferences (browsing ratio, BR) and intake (SI) for each species. Representative samples of the diet in each enclosure were built up considering the most ingested plant species and plant parts. Feed samples were analyzed for proximate, FA, and phenolic compositions. Milk samples were collected in each enclosure and analyzed for their FA profile. The enclosures showed a similar level of vegetation diversity. If compared to the GF enclosure, the OG one had higher proportion of *Poaceae* (81.2 vs 44.8% of SRA) and lower proportion of non-legume dicotyledons (14.6 vs 50.7% of SRA). The goats mostly selected eutrophic species in OG and forbs and woody species leaves in GF. The ingested vegetation was almost completely represented by grasses in OG (89.6% of SI), and by similar proportions of grasses and herbs and woody species in GF (54.9 and 45.1% of SI, respectively). The ingested forages from the OG and GF showed a comparable proximate composition; if compared to OG, the GF vegetation type was however richer in α -linolenic acid (ALA) and phenolic compounds (PC). Fatty acid analysis showed that GF milk had higher concentrations of ALA, total omega-3 FA, total *trans*-octadecadienoic acids, total conjugated linoleic acids, total *trans*-octadecenoic acids, and a reduced omega-6/omega-3 FA ratio than OG milk. Branched-chain FA were not significantly affected by treatment, suggesting that the activity of ruminal bacteria in the goats was not inhibited by the higher concentration of PC in GF plants. The main reason for the observed improvement of the FA profile in GF milk seems to be attributable to the FA profile rather than the concentration and composition of PC of the ingested plants. The abundance of herbs and woody species in the ingested vegetation was positively associated with the presence of nutritionally desirable FA in goat milk fat.

Key-words: biohydrogenation, diet selection, goat milk, grazing system, phenols.

Introduction

In north Italy, in the mountain belt, small semi-natural grasslands are interspersed within forests (Sitzia and Trentanovi, 2011; Garbarino et al., 2014). These areas had been traditionally grazed for millennia. However, during the last decades they have been broadly abandoned due to industrialization and urbanization processes, which brought to an intensification of agriculture and animal husbandry in the lowlands, determining important effects on the rural landscape (Probo et al., 2013). The resultant lack of control of shrub and tree species encroachment by animal trampling, grazing, seed transport, and nutrient redistribution has led to changes in land-cover (Celaya et al., 2010; Tocco et al., 2013; Riedel et al., 2013). Forest and pasture surface has notably increased and decreased, respectively (Falcucci et al., 2007), affecting both plant and animal diversity (Laiolo et al., 2004; Falcucci et al., 2007). At the mountain belt, where cattle rearing is not profitable anymore, small ruminant breeding plays a key role (Lombardi, 2005). In this contest, goat farms are usually very small (mainly less than 50 animals) and represent about 70% of all goat farms (ISTAT, 2010).

A lot of studies have been conducted to investigate the feeding preferences of goats in different management systems. Goat feeding preferences are very different from those of sheep and cattle, if compared under the same farming conditions. Such differences mainly regard the number of browsed species and their intake (both higher for goats than sheep and cattle) (Papachristou et al., 2005; Sanon et al., 2007; Osoro et al., 2013). Silanikove et al. (2010) reported that goats can utilize, more than other ruminants, feedstuffs rich in tannins or other plant secondary metabolites (PSM) thanks to their digestive efficiency. The majority of studies dealing with goat feeding preferences were performed indoors or compared indoor and grazing conditions (e.g., hay vs grazing, concentrate and hay vs grazing) (Morand-Fehr et al., 2007; Silanikove et al., 2010). Only in the last years some research has been conducted on goat feeding behaviour in shrublands of Mediterranean regions (Ataşoğlu et al., 2009; Delgado-Pertíñez et al., 2013; Mancilla-Leytón et al., 2013) and in rangelands of Africa (Sanon et al., 2007) or America (Foroughbakhch et al., 2013).

It is well known that fresh grass feeding positively affects the lipid fraction of goat milk by enhancing the presence of health-promoting fatty acids (FA) (e.g., rumenic and vaccenic acids, and omega-3 FA) and contemporarily lowering the omega-6/omega-3 FA ratio and the levels of specific FA considered detrimental for human health (e.g., hypercholesterolaemic medium-chain saturated FA) (Renna et al., 2012a, 2012b; Mancilla-Leytón et al., 2013). Besides that, in the last decade it has also been repeatedly demonstrated that the FA composition of milk and cheese from grazing ruminants may be significantly influenced by the botanical composition of the ingested plants (among others, Collomb et al., 2002; Di Trana et al., 2005; Renna et al., 2014a). Such influence has partly to be ascribed to the variability in unsaturated FA levels of the grazed/browsed forages (Di Trana et al., 2005; Mancilla-Leytón et al., 2013). Additionally, plant-derived bioactive molecules such as phenolic compounds (PC), other PSM (e.g., essential oils and saponins), and enzymes (e.g., polyphenol oxidase), being potentially able to significantly affect the lipolysis of dietary unsaturated fatty acids (UFA) and the pattern of their biohydrogenation (BH) occurring within the rumen, have the potential to alter the FA composition of ruminant-derived food products (Doreau et al., 2011; Buccioni et al., 2012).

In alpine regions, diet selection of goats and the effects that different vegetation composition may exert on animal performance under grazing systems are still poorly investigated. This study aims therefore to assess i) the feeding preferences of Camosciata goats grazing two alpine enclosures dominated, respectively, by an open grassland (OG) and a grazable forestland (GF), and ii) the related effects on milk composition, with particular reference to the FA profile of milk fat.

Materials and Methods

Study area and experimental design

The study was carried out during summer 2012 at Oasi Zegna, southwestern Alps, Italy (latitude 45°40' N, longitude 8°09' E). This area is characterized by an oceanic climate, with annual average air temperature of 7.2 °C and annual average precipitation of 1951 mm (Biancotti et al., 1998).

A dairy farm breeding a flock of 14 lactating Camosciata goats was selected in the area as during the summer season it managed a grazing land composed by both grasslands and forests. Two different enclosures, one dominated by an open grassland and the other by a grazable forestland, were arranged at an altitude ranging between 1250 and 1350 m a.s.l., at similar topographic conditions (mean slope: 26°; mean exposition: 315° North).

Each enclosure was exploited at the same stocking rate (on average 0.26 goat ha⁻¹ year⁻¹). A low-density grazing management was applied with the objective of maintaining high forage-to-animal ratio and encouraging selective grazing by the goats (Allen et al., 2011). The OG (0.7 ha) and GF (0.9 ha) enclosures were exploited by the goats for a grazing period of five (26 to 30 June) and six (23 to 28 July) days, respectively. The same group of goats foraged on both vegetation types to exclude confounding effects related to differences in selection behaviour among individuals (Provenza et al., 2003). To minimize the effect of the physiological stage of the animals on their intake behaviour, the time period between OG and GF exploitation was reduced at the minimum (22 days) required to adapt to such different vegetation types, especially for stabilization of FA in goat milk fat (Renna et al., 2012b). For the latter purpose, before entering the enclosures the goats grazed for three weeks two grazing lands dominated by grasses and shrubs similar to OG and GF, respectively.

The goats were manually milked indoors twice a day (at 7.00 and 18.00 h). They were allowed to graze during the milking interval whereas they were maintained indoors during the night. At each milking, the goats were supplemented with 200 g head⁻¹ of a commercial concentrate containing flaked corn, flaked barley, flaked bean, and flaked soybean. The enclosures and the stable were equipped to provide fresh water *ad libitum*.

Vegetation surveys, browsing ratio and species intake assessment

In order to assess the botanical composition of vegetation and the browsing ratio (BR) percentage of each botanical species, a dataset of 4500 points was randomly-generated by a Python plugin of Quantum Geographic Information System (QGIS, 2013) within both enclosures, and forty-five plots were randomly selected within each dataset. Their positions were corrected (± 1 meter) considering

that they had to represent a homogenous area and that the minimum distance among them allowed to avoid overlapping among neighbouring plots.

The botanical composition was surveyed using the vertical point-quadrat method along 5 m linear transects. At each transect, at every 10-cm interval, plant species touching a steel needle were identified and recorded (i.e., 50 points per transect) and species frequency of occurrence (SF=number of occurrences), which is an estimate of species canopy cover (Gallet and Roze, 2001), was recorded for each species. Species relative abundance (SRA) percentage was calculated for each species and plot in order to represent the proportion of different species, according to Daget and Poissonet (1971) and Probo et al. (2013). An average SRA was also calculated for each plant species in both enclosures.

A lot of methods have been used until now to evaluate ruminant diet selection under grazing systems [e.g., faecal analysis, esophageal fistulation (Papachristou and Nastis, 1993; Henley et al., 2006; Mellado et al., 2012), direct observation of grazing animals (Dumont and Boissy, 2000; Foroughbakhch et al., 2013; Mancilla-Leytón et al., 2013) and direct estimation of grazing damage of each species (Hejzman et al., 2008; Nagaike, 2012)]. However, in the current study the BR percentage of the whole flock on the half-long term was evaluated fitting the method proposed by Nagaike (2012) to the vertical point-quadrat method.

Ten consecutive 1m²-squares were laid out on each linear transect (five for each side) used for botanical surveys (450 squares per enclosure), and all species below 1.80 m were recorded. A value of 1 (grazed) or 0 (ungrazed) was appointed to each species present within every 1m²-square depending on its status after exploitations. The species were considered grazed when clear signs of browsing were observed on one or more plant parts (e.i., flowers, leaves, stems, buds, sprouts, or fruits). The phenological stage of all species was also recorded. Moreover, species were classified into the following functional groups: grasses (GR), legumes (LE), non-grass monocotyledons (NG), and non-legume dicotyledons (NL). The BR percentages were calculated for each species and plot as the ratio between the number of browsed quadrats (number of 1m²-squares with value 1 for the species *i*) and

the number of quadrats where the presence of the same species was recorded. An average percentage BR was also calculated for each species in both enclosures.

Considering SRA and BR percentage as the most accurate indices to represent the species phytomass and the species preference, respectively, the percentage species intake (SI) of each species in each enclosure was calculated using the following equation (1):

$$SI_i = \frac{\frac{1}{n} \sum_{j=1}^n (BR_{ij} * SRA_{ij})}{\sum_{i=1}^m \left[\frac{1}{n} \sum_{j=1}^n (BR_{ij} * SRA_{ij}) \right]} \quad (1)$$

where SI_i is the average percentage intake of the species i in the n plots ($n=45$ in each enclosure), SRA_{ij} is the species relative abundance of the species i in the plot j , and BR_{ij} is the browsing ratio of the species i in the plot j . Then the numerator represents the average intake of the species i and the denominator represents the sum of the average intake of all the species, within each enclosure.

Average SRA, average BR, and cumulated SI were calculated for each functional group within both enclosures. Cumulated SI were calculated to better highlight the differences of diet composition between the two enclosures.

At each vegetation plot, the vegetation diversity was estimated and expressed as the Shannon-Weaver diversity index (H' ; Magurran, 1988) and the evenness index (J' ; Magurran, 2004), which were calculated according to the following equations (2), (3), and (4):

$$H' = - \sum_{i=1}^m SRA_i * \log_2(SRA_i) \quad (2)$$

$$H_{MAX} = \log_2(m) \quad (3)$$

$$J' = H' / H_{MAX} \quad (4)$$

where SRA_i is the proportion of each species i of the total contacts on live plants, and m is the number of species within each vegetation plot.

Feed sampling and analysis

Two ungrazed areas, adjacent to the experimental enclosures and each constituted by the same vegetation composition of OG and GF, respectively, were selected for vegetation samples collection. Based on the observations made with the vegetation surveys, representative mixed samples for each enclosure were built up immediately after grazing considering the SI of the more ingested and widespread individual plant species. The samples (400 g) were collected using the hand-plucking method (Cook, 1964), considering the most preferred plant parts (e.i., flowers, leaves, stems, buds, sprouts, and fruits) for each species. Two homogeneous aliquots (200 g) of each sample were placed in plastic bags, immediately transported to the laboratory in a portable refrigerator at 4 °C, and then frozen at -80 °C until analysis. A sample of commercial concentrate fed to the goats during milking was also collected at the beginning of the trial.

Before chemical analysis, the first aliquot of each vegetation sample was dried at 40 °C for 24 h. Concentrate and dried mixed vegetation samples were then ground with a cutting mill to pass a 1-mm screen sieve (Pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany). AOAC (2000) procedures were used to determine dry matter (DM, method no. 930.15), ash (method no. 942.05), crude protein (CP, method no. 984.13), ether extract (EE, method no. 920.39), and acid detergent fibre (ADF, method no. 973.18) in the concentrate and mixed vegetation samples. Neutral detergent fibre (NDF) was analyzed according to Van Soest et al. (1991); α -amilase (Sigma Aldrich, Saint Louis, Missouri, USA) but no sodium sulphite was added and results were corrected for residual ash content. Starch was analyzed using a POLAX-2L polarimeter (ATAGO Co., Ltd., Japan) according to “Gazzetta Ufficiale della Repubblica Italiana” (2000). Feed chemical composition was expressed as g kg⁻¹ DM.

The second aliquot of each vegetation sample was freeze-dried (Edwards MF 1000, Milano, Italy) and ground. These aliquots and the grounded concentrate were used for the assessment of the FA composition using a combined direct *trans*-esterification and solid-phase extraction method as described by Alves et al. (2008). Separation, identification, and quantification of fatty acid methyl esters (FAME) were performed as described by Renna et al. (2014b). Feed FA results were expressed as absolute values as mg 100g⁻¹ DM.

Standard protocols were used to assess the contents of total extractable phenols (TEP) and different polyphenol fractions (non-tannin phenols, NTP; condensed tannins, CT) in the OG and GF samples, and in the commercial concentrate (Makkar, 2003). Polyphenols were extracted twice with aqueous acetone (70:30 v/v) and subjected to ultrasonic treatment for 20 min at room temperature in an ultrasonic water bath (Bransonic-21, Branson Ultrasonics, Danbury, CT, USA). Polyvinyl-pyrrolidone was used to separate NTP and total tannins (TT), according to a modified Folin-Ciocalteu method. The butanol-HCl-iron method was used to determine CT. The absorbance was recorded at 725 nm (TEP and NTP, expressed as gallic acid equivalents) and 550 nm (CT, expressed as leucocyanidin equivalents) using a UV-vis spectrophotometer (Shimadzu UVmini-1240, Shimadzu Corporation, Kyoto, Japan). Total tannins were computed as the difference between TEP and NTP. Hydrolysable tannins (HT) were estimated as the difference between TT and CT; the obtained values are of limited accuracy due to the different standards used, but such computation represents a common procedure (Kälber et al., 2011; Khiaosa-Ard et al., 2011; Willems et al., 2014).

All analyses were performed in duplicate.

Milk sampling and analysis

Within the flock grazing both OG and GF enclosures, seven multiparous lactating goats were selected considering both days in milk (DIM) and number of lactation (3.6 ± 1.3 lactations). The adopted experimental design allowed excluding a confounding significant effect of lactation stage (DIM: 126 ± 16 and 153 ± 16 at the beginning of OG and GF exploitation, respectively) on the FA profile of goat milk fat. The effect of lactation stage on the FA profile of ruminant milk fat is actually considered negligible as compared to other sources of variation. Moreover, the main changes in milk FA are known to occur in early lactation (essentially associated with modifications of the energy balance of the animals), while a relatively stable FA pattern is generally observed in mid- and late-lactation (Ataşoğlu et al., 2009; De La Fuente et al., 2009).

Individual milk yield of goats when fed in the OG and GF enclosures was recorded during two consecutive days at the end of each grazing period using graduated measuring cylinders. Individual

milk samples from the morning milking were also collected during the same two consecutive days of milk yield recording. Two aliquots of each sample were taken. The first one (50 mL) was immediately stored at 4 °C with a preservative and subsequently analyzed for fat, protein, lactose, casein, and solids non-fat (SNF) contents using an infrared analyser (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark). The second aliquot (150 mL) was frozen at -80 °C and successively analyzed for the FA composition. Lipid extraction, and FAME separation, identification and quantification were performed as detailed by Renna et al. (2012c). Briefly, milk fat extraction was obtained by centrifugation at 7300 rpm for 30 min at -4 °C. The resulting molten butter was then filtered through a hydrophobic filter (Whatman 1, Whatman International Ltd, Maidstone, England). The pure milk fat was dissolved in heptane and FAME were obtained by *trans*-esterification of glycerides using a solution of KOH in methanol. Separation, identification, and quantification of FAME were performed with a high resolution gas chromatograph (Shimadzu GC 2010 Plus; Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector, and a CP-Sil 88 capillary column (100 m × 0.25 mm ID, 0.20 µm film thickness; Varian Inc., Palo Alto, CA, USA). Nonanoic acid methyl ester was used as internal standard. The results are expressed as absolute values as g 100g⁻¹ fat. All analyses were performed in duplicate.

Statistical analysis

A non-canonical detrended correspondence analysis (DCA) (Hill and Gauch, 1980) was performed to compare species composition between OG and GF enclosures. The vegetation functional groups (GR, LE, NG, and NL) were used as passive variables to provide a better description of vegetation types.

Differences of SRA, BR, H', and J' between the two considered vegetation types were assessed using an independent-samples *t*-test. Average SRA and average BR of all the species were separately compared for each functional group, whereas the vegetation diversity indices (H' and J') were compared considering the whole list of plant species.

The effect of vegetation type on yield, gross composition, and FA profile of milk was assessed using a paired-samples *t*-test.

Prior to the analysis, data were tested for normality and homoscedasticity using the Kolmogorov-Smirnov and Levene tests, respectively, and log-transformed when necessary.

DCA analysis was performed using CANOCO 4.5 (ter Braak and Smilauer, 1998), while *t*-test analyses were performed using IBM SPSS Statistics 20.0 (IBM SPSS, 2011). Significance was declared at $P \leq 0.05$.

Results

Vegetation composition, goats feeding preferences and species intake

DCA ordination provided a clear separation between OG and GF vegetation (Fig. 1a) along the first two axes (eigenvalues (λ): axis 1=0.84; axis 2=0.16). Axis 1 explained 70.1% of total variance, helping in discriminating mainly vegetation types dominated by grasses and legumes (i.e. OG enclosure) from those dominated by non-legume dicotyledons and non-grass monocotyledons (i.e. GF enclosure) (Fig. 1b). Moreover, *t*-test analysis showed significant differences between OG and GF enclosures for all the functional groups (Table 1).

A total of eighty-four plant species were identified in the OG. This enclosure was dominated by *Poaceae* species (81.2% of SRA), followed by non-legume dicotyledons (14.6%) (Table 1). *Festuca nigrescens* (Lam. non Gaudin), *Agrostis tenuis* (Sibth.), and *Phleum alpinum* (L.) were the most abundant species in the enclosure (64.6% of SRA). Overall, *Asteraceae*, *Cyperaceae*, *Fabaceae*, *Plantaginaceae*, *Ranunculaceae*, and *Rosaceae* families accounted for 15.0% of SRA and the remaining 3.8% was constituted by other 10 families (Table 2).

A total of sixty-seven plant species were identified in the GF. This enclosure was composed by herbaceous species, shrubs, and trees. Non-legume dicotyledons were the most abundant plant functional group (50.7% of SRA) (Table 1). The most widespread families were *Poaceae*, *Ericaceae*, and *Rosaceae* (44.8%, 30.9%, and 8.6% of SRA, respectively). Overall, *Thelypteridiaceae*, *Juncaceae*, *Liliaceae*, and *Athyriaceae* families accounted for 10.7% of SRA and the remaining 5.0%

was constituted by other 13 families. *Calamagrostis arundinacea* (L. Roth.), *Rhododendron ferrugineum* (L.), and *Vaccinium myrtillus* (L.) were the most abundant species (36.4%, 17.4%, and 11.9% of SRA, respectively) (Table 3).

Neither the Shannon-Weaver diversity index (OG=2.77; GF=2.45) nor the evenness index (OG=0.59; GF=0.61) showed significant differences between the experimental enclosures.

The most selected species in the OG were *Veratrum album* (L.) (100% of BR), *Lolium perenne* (L.) (80%), *Poa annua* (L.) (69%), *Dactylis glomerata* (L.) (67%), and *F. nigrescens* (61%) (Table 2). Among species with SRA>1, *V. album* was also the most selected species in the GF enclosure (96% of BR), followed by *Senecio fuchsii* (Gmelin) (88%), *Sorbus aucuparia* (L.) (85%), *Sorbus aria* (L. Crantz) (85%), *Salix caprea* (L.) (80%), *Festuca scabriculmis* (Hackel) (75%), *Betula pendula* (Roth) (63%), *Rubus hirtus* (W. et K.) (60%), and *Luzula nivea* (L. Lam et DC.) (60%) (Table 3). However, among these species some occurred only occasionally. In OG *L. perenne*, *P. annua*, and *D. glomerata* were recorded in less than 15% of total 1m²-squares (7.1%, 11.3%, and 2.2%, respectively); similarly, in GF *S. fuchsii*, *F. scabriculmis*, and *B. pendula* were found in less than 10% of total 1m²-squares (6.4%, 2.0%, and 2.7%, respectively) (Table 2 and Table 3).

Grasses, legumes, and non-grass monocotyledons did not show significant difference in terms of BR between OG and GF enclosures, while non-legume dicotyledons showed a significantly greater BR in GF than OG enclosure (29.8% and 15.9%, respectively) (Table 1).

In the OG enclosure the three most widespread species (*F. nigrescens*, *A. tenuis*, and *Ph. alpinum*) showed high average values of BR, and consequently they were the most ingested by goats (34.0%, 29.2%, and 14.6% of SI, respectively; Table 2). In the OG enclosure diet was mainly composed by *Poaceae* (89.6% of SI), while herbs and woody species accounted only for 8.6% of SI together (Table 1). The GF diet was mainly dominated by *C. arundinacea* (50.5% of SI) because of its high SRA (36.4%) and BR (59%), but it was also characterized by a high number of shrubs and trees belonging to the *Ericaceae* and *Rosaceae* families such as *V. myrtillus* (15.2%), *R. hirtus* (6.7%), *S. aucuparia* (3.3%), *Rubus idaeus* (L.) (2.6%), and *S. aria* (1.5%) (Table 3). Overall GF diet was characterized by

grasses (54.9% of SI) and herbs and woody species (45.1%), with a broad abundance of non-legume dicotyledons (37.5%) (Table 1).

Within both enclosures, more than 65% of the species were selected by the animals one or more times during the study period (OG: 56/85 species; GF: 47/67 species). However, the bulk of diet (OG: 87.8% of SI; GF: 79.9%) was represented by only five species and 95.0% of the diet contained less than 15 species within both enclosures (OG: 9 species; GF: 13 species) (Table 2 and Table 3).

Goats showed a clear preference for flowers (38/46 species) rather than leaves (26/46) in the OG enclosure, while a more balanced preference between these plant parts (flowers: 21/26; leaves: 20/26) was detected in the GF enclosure. Goat feeding choices between flowers and leaves were assessed when both these plant parts were available, so that ferns, herbs at vegetative phenological stage and all tree species were not considered in this specific flowers/leaves preference evaluation (Table 2 and Table 3).

Proximate composition, phenolic fractions, and fatty acid profile of the ingested feeds

Both OG and GF vegetation types as well as the concentrate were of high nutritional quality (6.1, 6.7, and 9.3 MJ kg⁻¹ DM of net energy for lactation, respectively; Table 4). The vegetation types were similar in terms of protein, fat, fibre, and starch contents, while they differed for their amount and composition of PC and FA. Total extractable phenols were almost double (24.2 vs 45.5 g kg⁻¹ DM, +87.8%) in the ingested GF than OG plants. All phenolic fractions showed higher concentrations in GF than OG plants, with the greatest differences observed for TT (13.8 vs 30.2 g kg⁻¹ DM, +118.4%) and HT (8.9 vs 23.7 g kg⁻¹ DM, +166.3%). Non-tannin phenols and CT concentrations were also slightly higher in the ingested GF if compared to OG vegetation (10.4 vs 15.3 g kg⁻¹ DM, +47.1%; 5.0 vs 6.5 g kg⁻¹ DM, +30.0%, respectively). Regarding the main FA, the OG vegetation type showed approximately double concentrations of oleic (C18:1 *c*9) and linoleic (C18:2 *c*9*c*12, LA) acids, higher levels of palmitic acid (C16:0) and lower levels of α -linolenic acid (C18:3 *c*9*c*12*c*15, ALA) if compared to GF. The commercial concentrate showed low levels of PC (about 7 g kg⁻¹ DM of TEP) if compared to OG and GF vegetation types; regarding the FA profile, it was mainly rich in linoleic

(3356.5 mg 100 g⁻¹ DM) and oleic (1421.5 mg 100 g⁻¹ DM) acids (about 54% and 23% of total detected FA, respectively) (Table 4).

Milk yield, gross composition, and fatty acid profile

Milk yield, lactose and SNF contents were significantly higher when the goats fed the OG vegetation if compared to the GF one. No significant differences were instead observed between OG and GF milk if considering the fat, protein, and casein contents (Table 5).

Significant differences were observed in the FA profile of milk produced when the goats fed the OG and GF vegetation types. Regarding groups of FA (Table 6), GF milk showed significantly higher concentrations of *trans*-FA (total *trans*-octadecenoic: +27.2%; total *trans*-octadecadienoic: +33.1%; total *trans*-FA without *trans*-conjugated linoleic acids (CLA): +27.4%) and total CLA isomers (+35.1%) if compared to OG milk. The concentrations of total polyunsaturated fatty acids (PUFA) and total omega-3 FA also tended to be higher in GF if compared to OG milk (+17.1% and +40.0% for PUFA and omega-3 FA, respectively). Total saturated (SFA), monounsaturated (MUFA), branched-chain (BCFA, both *iso* and *aiso* forms), and omega-6 FA concentrations, and the atherogenicity (AI) and thrombogenicity (TI) indexes did not significantly differ between OG and GF milk. The omega-6/omega-3 FA ratio was significantly lower in GF if compared to OG milk (-29.1%).

Considering individual FA (Table 7), the majority of SFA (C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0) did not show remarkable differences between OG and GF milk. The milk obtained when the goats fed the GF vegetation type showed a significantly higher concentration of the sum of *t*6 to *t*11 octadecenoic isomers if compared to OG milk (+35.6%). The majority of detected *trans*-octadecadienoic isomers were also significantly higher (C18:2 *c*9*t*13 + *t*8*c*12: +96.5%; C18:2 *t*11*c*15: +52.5%; C18:2 *t*9*c*12: +42.3%), or tended to be higher (C18:2 *t,t*-NMID + *t*9*t*12: +19.3%; C18:2 *c,c*-MID + *t*8*c*13: +26.2%) in GF than OG milk. The sums of CLA isomers *c*9*t*11 + *t*7*c*9 + *t*8*c*10 and *t*11*c*13 + *c*9*c*11 tended to be or were significantly higher in GF if compared to OG milk samples (+36.4% and +51.4%, respectively). Other detected CLA isomers (*t*10*c*12 and *t*9*t*11) showed

comparable concentrations in OG and GF milk. Regarding individual omega-3 FA, while α -linolenic (C18:3 *c9c12c15*, ALA) and eicosapentaenoic (C20:5 *c5,c8,c11,c14,c17*, EPA) acids tended to be or were significantly more abundant in GF than OG milk (+41.3% and +33.1%, respectively), no significant differences between groups were observed for docosapentaenoic (C22:5 *c4,c7,c10,c13,c16*, DPA) and docosahexaenoic (C22:6 *c4,c7,c10, c13,c16,c19*, DHA) acids. The concentrations of linoleic acid (C18:2 *c9c12*, LA), γ -linolenic acid (C18:3 *c6c9c12*, GLA), and other detected long-chain omega-6 FA (C20:2 *c,c n6*, C20:3 *n6*, and C20:4 *n6*) did not significantly differ between OG and GF milk. Delta9-desaturase activity, estimated by the computation of a desaturase index (DI_{TOT}), was not significantly influenced by the vegetation type fed by the goats.

Discussion

Vegetation composition, goats feeding preferences, and species intake

Because of the interactions among landscape, botanical composition of pastures and animal breeds, grazing systems are more complex than indoor feeding systems and comparisons among different studies sometimes are not easy. For this reason, although during the last decades a lot of studies focused both on goat feeding preferences and quality of their products, the knowledge of these systems is just at the beginning.

The presence of two vegetation communities very different in terms of botanical composition, affected significantly goat feeding preferences. Due to the move of goats from an enclosure dominated by grasses to another one where herbs and woody species were more abundant, grass intake decreased whereas the intake of herbs and woody species increased. Goats avoided herbaceous species when herbs and woody species were accessible.

However, goats showed their ability to select specific species and plant parts rather than other within both enclosures. After exploration of each enclosure through the selection of a high number of food items one or more times, goats focused on preferred species. Indeed, the bulk of diet was represented by only few species, as found also by Papachristou *et al.* (2005) on Greek rangelands. Results about

the plant parts eaten in OG and GF enclosures highlighted the preference of goats to feed at eye-level, to reduce efforts to search other food items (Lu, 1988), and to eat parts of plants starting from the top of the sward when shrub and tree species did not occur (Del Pozo and Osoro, 1997). In the OG, where tall species (mainly grasses) were more abundant, goats usually selected more flowers than other plant parts, while rarely they reached the basal canopy of sward, where the few legumes (i.e., creeping species such as *Trifolium repens* (L.) and *Lotus alpinus* (DC. Schleicher)) were recorded. Moreover, occasionally selection of legumes (17.9% of BR and 1.8% of SI) could have been affected by the great content of saponins in *Trifolium* species (Kolodziejczyk-Czepas, 2012) and flavonoids in *Lathyrus montanus* (Bernh.) and *L. alpinus* (Ranabahu and Harborne, 1993; Wang et al., 2013). Conversely, considering all plant species and phenological stages, the most selected plant parts in GF were shrub and tree leaves, probably as a consequence of both their accessibility and the more balanced level of nutritive values of herbs and woody species throughout the year (Sanon et al., 2007; Osoro et al., 2013).

Among all the species recorded within OG and GF enclosure there were also toxin-containing feeds. The most common toxic species was *V. album*. Although toxicity of *V. album* would suggest its refusal, this species was the most selected one in both enclosures. Selection of *V. album* should not be considered fortuitous. In fact, although this species was recorded only in 1% of total 1m²-squares in OG, its great selection was confirmed in GF where it was recorded in 29.8% of total 1m²-squares (31/45 vegetation plots). Herbivores, especially goats, are able to improve their ability to detoxify compounds such as cyclopamine, jervine, cycloposine, and steroidal alkaloids, or to enhance their tolerance towards toxins consumption (Provenza et al., 2003). Indeed, in front of a high number of botanical species, goats changed their feeding selection so as to obtain a more balanced intake of nutrients such as energy, protein, and water that support ejection of toxins by urine (Provenza et al., 2003).

Milk yield, gross composition, and fatty acid profile

The stage of lactation is one of the main parameters able to influence milk production performance in dairy goats (Goetsch et al., 2011). Beyond peak of lactation, declines in milk yield and milk lactose content typically occur as the lactation period progresses (Mioč et al., 2008; Strzałkowska et al., 2009). The significant variations of milk production and composition which were observed when goats fed in the OG and GF enclosures, are at least partly to be attributed to the advance of the lactation stage. However, the experimental design was specifically set up to investigate diet selection and milk FA profile and it does not allow us to discriminate the effects of lactation stage and diet on milk yield and gross composition.

So far, available published information concerning the effects that different vegetation types fed by goats may exert on milk and meat FA profile is limited. The interpretation of the observed differences in the FA composition between OG and GF milk samples is rather difficult.

Generally, the transfer efficiency of dietary PUFA to (or their concentration in) ruminant-derived food products has been reported to be positively correlated to the vegetation diversity (Collomb et al., 2002; Lourenço et al., 2007; Willems et al., 2014), the proportion of *Fabaceae* (Cabiddu et al., 2005; Gorlier et al., 2012), and the proportion of herbs (Petersen et al., 2011; Willems et al., 2014) of the grazed pasture lands. In the current study, the experimental enclosures showed a comparable level of vegetation diversity (H' and J' indexes). In both experimental enclosures, legumes accounted for less than 3% of total cover (SRA) and less than 2% of total intake (SI), which suggests their trifling effect on goat milk FA profile. However, the concentration of total PUFA tended to be higher in milk when the goats were fed in the GF enclosure, the latter being characterized, if compared to OG, by a notably higher proportion of herbs and woody species, particularly non-legume dicotyledons, and to a lesser extent also non-grass monocotyledons.

The chemical parameters of the ingested plants which may potentially affect lipid metabolism (lipolysis and BH) in the rumen and milk FA profile are crude protein, fibre (particularly the lignified fraction), FA and PSM. Low dietary crude protein and high dietary lignin contents have been shown

to inhibit BH *in vitro* (Gerson et al., 1986; Li and Meng, 2006), thus potentially increasing the amounts of ALA, LA, and/or their BH intermediates in ruminant-derived food products. Both crude protein and lignified fibre showed however similar concentrations between the ingested vegetation types. We therefore assume that the observed differences in the FA profile between OG and GF milk should be the direct consequence of the variations in the concentrations of PSM and/or FA of the ingested feeds.

As expected, ALA was the major dietary UFA supplied by both OG and GF vegetation types. If compared to OG milk, GF milk tended to contain a higher concentration of ALA and showed higher concentrations of many of its detected recognized or alleged octadecadienoic BH intermediates [i.e., C18:2 *t11c15*, C18:2 *t11c13 + c9c11*, C18:2 *c9t13* (coeluting with C18:2 *t8c12*), C18:2 *t8c13* (coeluting with an unidentified *cis,cis*-MID), C18:2 *t9c12*, C18:2 *t9t12* (coeluting with a non identified *trans,trans*-NMID)] (Lee and Jenkins, 2011). Such result may be the consequence of a higher dietary intake of ALA, due to its higher concentration in the ingested GF plants (also considering the low level of this FA in the concentrate). Higher ALA and *trans*-octadecadienoic acids levels in milk fat were reported to occur in case of goats fed diets supplemented with ALA-rich feedstuffs, at comparable dietary ALA concentrations of the current trial (Renna et al., 2013). Several *in vivo* studies have however shown that ALA and LA contents in ruminant milk and meat may be somewhat independent of their dietary intake level (among others: Cabiddu et al., 2005; Leiber et al., 2005; Petersen et al., 2011; Willems et al., 2014). Theoretically, the fact that GF milk tended to contain a higher concentration of ALA may also suggest a higher ruminal bypass of dietary ALA in its intact form due to a reduced BH rate. In recent published trials, both ALA and LA contents in ruminant milk and meat were found to be positively correlated with type and relative abundance of dietary polyphenols (Kälber et al., 2011; Willems et al., 2014), the latter being potentially able to inhibit hydrogenating microorganisms in the rumen (Leiber et al., 2005). Tanniferous compounds were reported to possess toxic effects on several bacterial and/or protozoal species populating the rumen environment, therefore reducing their proliferation, inhibiting their activity, and consequently affecting ruminal BH (Khiaosa-Ard et al., 2009; Vasta et al., 2010). According to their molecular

structure, tannins may affect different steps of the ruminal BH pathways of dietary PUFA. In the current trial, when foraging the GF vegetation type the goats may have experienced an inhibition of the first-step hydrogenation of dietary ALA and LA due to the higher HT concentration found in the ingested GF plants. *In vitro* assays conducted by Jayanegara et al. (2011) and Buccioni et al. (2011) have in fact shown that HT may be effective in preventing the first-step of the ruminal BH of dietary ALA and/or LA. Nevertheless, at present no unequivocal conclusion concerning the effects that HT may exert on ruminal BH and the FA profile of ruminant-derived food products can be drawn, as the results of the various published studies are controversial. For example, another *in vitro* study failed at finding a positive correlation between the concentration of HT in alpine forage and ALA levels in cow rumen fluid (Khiaosa-Ard et al., 2011). In that study, however, the HT level of the forage was half than that occurred in our trial and it is well known that the dietary concentration of tanniferous compounds plays a major role for their effects on rumen fermentation. Theoretically, the hypothesis of a reduced BH rate may also explain the lack of a significant difference in LA concentration between OG and GF milk, despite the double concentration of LA found in the ingested OG plants. It seems however more plausible for such result being the consequence of the high concentration of LA found in the concentrate, which may have strongly mitigated the difference in LA concentration between the ingested experimental vegetation types.

Concerning other main ruminal BH intermediates, the sum of *trans*-6 to *trans*-11 octadecenoic acids (which are formed during the BH of dietary ALA, LA and GLA) was significantly higher in GF than OG milk. Adler et al. (2013) showed that feeding dairy cows with silages containing a high proportion of dicotyledons inhibited the terminal step of ruminal BH of dietary PUFA compared to feeding grass-based silages. Inhibitions of the penultimate and/or last step of ruminal BH (and consequently accumulations of *trans*-octadecadienoic and *trans*-octadecenoic acids in the rumen fluid) have been demonstrated to occur *in vitro* (Khiaosa-Ard et al., 2009; Vasta et al., 2009a) and *in vivo* (Vasta et al., 2009b, 2010) as a consequence of dietary supplementation of CT. In addition, Khiaosa-Ard et al. (2011) reported that *in vitro* incubations with ruminal fluid of alpine (high polyphenols) compared to lowland (low polyphenols) origin increased significantly the apparent net production and proportion

of vaccenic acid over the total FA analyzed; these authors attributed such results to the HT content of the alpine forage. We are confident enough to be able to exclude a significant effect of CT on ruminal BH and goat milk FA profile in the current trial. As previously stated for HT, the level of dietary inclusion of CT seems to be crucial for a significant impact of these PSM on the ruminal environment. It has been reported that low-level supplementation of dietary CT [e.g., 6.7 g kg⁻¹ DM of CT from quebracho (*Schinopsis lorentzii* L.) or 18 g kg⁻¹ DM of CT from French honeysuckle (*Hedysarum coronarium* L.)] did not exert significant modifications of the FA profile of cow milk fat or an accumulation of PUFA in *Longissimum dorsi* muscle of lambs, respectively (Priolo et al., 2005; Benchaar and Chouinard, 2009). More recently, another *in vivo* study showed that about 60 g kg⁻¹ DM of dietary CT were not able to determine significant increases of vaccenic and rumenic acids in milk fat of goats grazing in a Mediterranean shrubland if compared to goats fed indoors a diet containing about 10 g kg⁻¹ DM of dietary CT (Mancilla-Leytón et al., 2013). In our trial, the concentrations of CT were most probably too low and did not differ strongly between treatments (5.0 and 6.5 g kg⁻¹ DM for the ingested OG and GF plants, respectively) to affect ruminal BH and goat milk FA profile significantly. On the contrary, HT may have potentially played a role as their concentration was notably higher in the ingested GF if compared to OG vegetation types. Despite the fact that the sum of dietary ALA+LA+GLA was higher in the ingested OG than GF plants (which may suggest the occurrence of an accumulation of ruminal BH intermediates of dietary PUFA in the rumen fluid of goats when foraging the GF plants), it should be again pointed out that the concentrate may have mitigated the observed differences in the FA profile (particularly considering LA) between the ingested vegetation types.

The higher concentration of the sum of CLA isomers *c9t11* + *t7c9* + *t8c10* found in GF if compared to OG milk should be essentially attributed to the higher amount of C18:1 *t6-11* isomers escaping complete ruminal BH and being desaturated by the activity of Δ 9-desaturase within the mammary gland. The ingested vegetation types did not alter the estimated Δ 9-desaturase activity within the mammary gland. A similar result was also obtained in another trial with dairy ewes grazing on pasture lands of different botanical composition (Renna et al., 2014a). Regarding CLA *t10c12*, it was detected

only in traces and its concentration did not differ significantly between OG and GF milk. Such results are consistent to: i) the high forage and low non-fibrous carbohydrates levels of the diets, ii) the limited ruminal synthesis and mammary uptake of CLA τ 10 c 12 in goats (Bernard et al., 2009) and iii) the similar supply of LA between treatments.

The concentrations of oleic and stearic acids were numerically higher in GF than OG milk, but they did not attain statistical significance. More than 50% of oleic acid in milk fat is formed from stearic acid by Δ 9-desaturase activity within the mammary gland, while the remaining part derives from the diet. The lack of significant differences in milk oleic acid concentration between treatments can therefore be explained by: i) the concomitant lack of significant difference in the concentration of stearic acid and in the estimated Δ 9-desaturase activity and ii) the comparable oleic acid levels from OG and GF diets (considering the high concentration of oleic acid in the concentrate).

ALA is known to be the precursor of all long-chain omega-3 FA. In our trial, a higher concentration of ALA in GF milk was associated with a higher concentration of EPA. On the contrary, the concentrations of both DPA and DHA did not differ significantly between OG and GF milk samples. Comparable results have been repeatedly reported in the literature (among others, Kälber et al., 2011; Mancilla-Leytón et al., 2013) and may be ascribed to the low and step-decreasing efficiency of ALA metabolism to more unsaturated and long-chain members of the omega-3 FA family (Bernal-Santos et al., 2010). According to the lack of significant difference in LA concentration between OG and GF milk samples, long-chain omega-6 PUFA deriving from LA metabolism were also unaffected by the vegetation type fed by the goats.

In order to clarify the relative influence of dietary FA and PC upon the results obtained in this trial, milk concentration of BCFA should be taken into account. BCFA largely derive from bacteria leaving the rumen (Fievez et al., 2012). Particularly, *iso*-BCFA are synthesized by cellulolytic strains (the microorganisms mainly involved in ruminal BH; Kepler and Tove, 1967), consequently being considered valuable predictors of ruminal bacterial activity. Tanniferous compounds were reported to reduce considerably the concentration of *iso*-BCFA in the rumen (Vasta et al., 2009a, 2009b, 2010).

The lack of significant differences in the concentration of BCFA, particularly the total *iso* forms, between OG and GF milk may suggest that the ruminal microbial activity of the goats was not inhibited by the ingestion of the plants covering the GF enclosure, despite the associated higher dietary concentration of PC. Another important aspect to be considered at this regard is that the higher concentrations of ALA, *trans*-octadecadienoic and *trans*-octadecenoic acids found in GF if compared to OG milk were not achieved at cost of their corresponding products (*trans*-octadecadienoic, *trans*-octadecenoic and stearic acid, respectively) of the ruminal BH steps of dietary PUFA. Such consideration leads us hypothesize that a strong inhibition of ruminal PUFA saturation did not occur and that the higher concentration of ALA, *trans*-octadecadienoic and *trans*-octadecenoic acids in GF milk was mostly achieved through a higher intake of ALA from the diet.

The concentrations of the various detected phenolic fractions of the ingested OG and GF vegetation types (as well as the relative difference between treatments) were reported to be high enough to significantly affect rates and extents of ruminal BH and transfer of FA to milk and meat in cows and sheep (Kälber et al., 2011; Willems et al., 2014). Thanks to salivary defense mechanisms and ruminal microbial degradation of PC, goats are much less sensitive to plant antinutritional factors and are able to consume forages containing much more polyphenols than other ruminants (Silanikove, 1997). We can hypothesize that the dietary levels of PC determined in the ingested OG and GF plants in the current trial were too low to determine a significant effect on ruminal BH and milk FA composition in goats, a result which also confirms the recent findings of Mancilla-Leytón et al. (2013).

Conclusions

This study confirmed the ability of goats to change their feeding preferences in front of a different vegetation composition. Thanks to our method we assessed a specific ratio of selection for all the species recorded within both natural open grassland and grazable forestland enclosures. Therefore, for the first time it was possible to compare goat feeding selection between two different vegetation types

using the same method. This could be the first step to define a pastoral value for all the species according to goat feeding preferences.

Regarding the effects of the different vegetation types on goat milk fat quality, we may conclude that, as already reported for dairy cows and ewes, a higher ingestion of herbs (in the current study accompanied by woody species) as fresh forage positively modified the FA profile of milk fat. Such improvement was achieved through an increase in the concentration of FA, such as total omega-3, total CLA and vaccenic acid, which possess a valuable dietetic quality for human health (positive influence on brain development, reduction of blood cholesterol concentration, reduction of the incidence of cancer, diabetes and atherosclerosis) (Kliem and Givens, 2011). From a human-health perspective, another related positive result was the significant decrease of the omega-6/omega-3 FA ratio (Simopoulos, 2011), the level of which, in GF milk, fell within the optimum recommended values (2.0 - 2.5) (MacRae et al., 2005). From the results of our trial, it seems that the main reason for such improvement should be attributed to the FA profile of the ingested plants rather than the concentration and composition of their PC.

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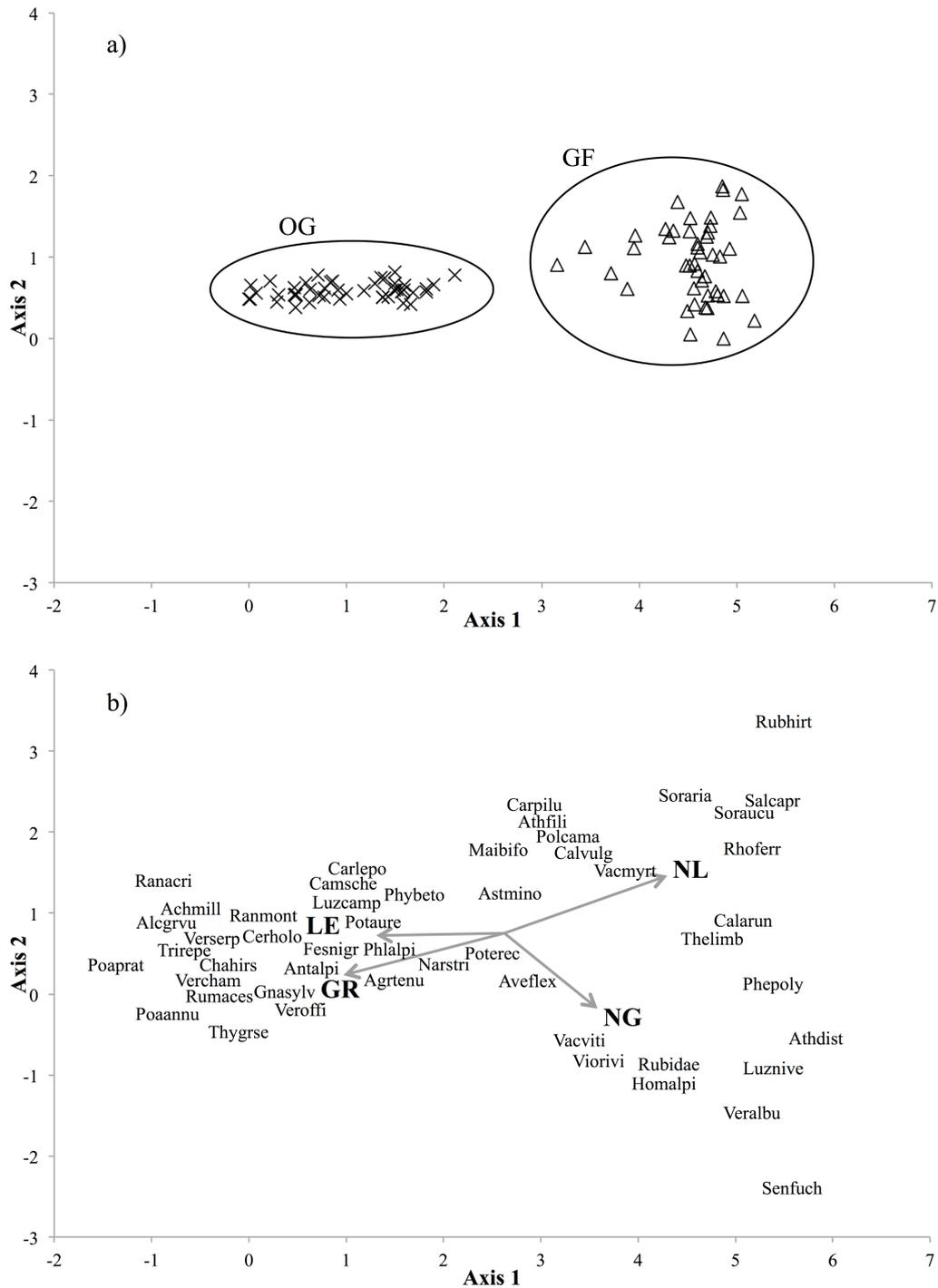


Fig. 1. DCA ordination of a) sample plots and b) species. Species labels are centred on scores, with minor adjustments to avoid text overlap.

Abbreviations:

Vegetation communities: OG, open grassland; GF, grazable forestland.

Functional groups: GR, grasses; LE, legumes; NG, non-grass monocotyledons; NL, non-legume dicotyledons.

Species codes: Achmill: *Achillea millefolium*. Agrtenu: *Agrostis tenuis*. Alegrvu: *Alchemilla vulgaris*. Antalpi: *Anthoxanthum alpinum*. Astmino: *Astrantia minor*. Athdist: *Athyrium distentifolium*. Athfili: *Athyrium filix-foemina*. Aveflex: *Avenella flexuosa*. Calarun: *Calamagrostis arundinacea*. Calvulg: *Calluna vulgaris*. Camsche: *Campanula scheuchzeri*. Carlepo: *Carex leporina*. Carpilu: *Carex pilulifera*. Cerholo: *Cerastium holosteoides*. Chahirs: *Chaerophyllum hirsutum*. Fesnigr: *Festuca nigrescens*. Gnasylv: *Gnaphalium sylvaticum*. Homalpi: *Homogyne alpina*. Luzcamp: *Luzula campestris*. Luznive: *Luzula nivea*. Maibifo: *Maianthemum bifolium*. Narstri: *Nardus stricta*. Phepoly: *Phegopteris polypodioides*. Phlalpi: *Phleum alpinum*. Phybeto: *Phyteuma betonicifolium*. Poaannu: *Poa annua*. Poaprat: *Poa pratensis*. Polcama: *Polygala chamaebuxus*. Potaure: *Potentilla aurea*. Poterec: *Potentilla erecta*. Ranacri: *Ranunculus acris*. Ranmont: *Ranunculus montanus*. Rhoferr: *Rhododendron ferrugineum*. Rubhirt: *Rubus hirtus*. Rubidae: *Rubus idaeus*. Rumaces: *Rumex acetosella*. Salcapr: *Salix caprea*. Senfuch: *Senecio fuchsii*. Soraria: *Sorbus aria*. Soraucu: *Sorbus aucuparia*. Thelimb: *Thelypteris limbosperma*. Thygrse: *Thymus gr. serpyllum*. Trirepe: *Trifolium repens*. Vacmyrt: *Vaccinium myrtillus*. Vacviti: *Vaccinium vitis-idea*. Veralbu: *Veratrum album*. Vercham: *Veronica chamaedrys*. Veroffi: *Veronica officinalis*. Verserp: *Veronica serpyllifolia*. Viorivi: *Viola riviniana*.

Table 1

Cumulated species relative abundance (SRA), average browsing ratio (BR), and cumulated species intake (SI) of grasses, legumes, non-grass monocotyledons, and non-legume dicotyledons in the open grassland (OG) and grazable forestland (GF) enclosures.

Functional plant groups	SRA (%)			BR (%)			SI (%)	
	OG	GF	P-values ^a	OG	GF	P-values ^a	OG	GF
Grasses (<i>Poaceae</i>)	81.2	44.8	***	37.7	31.8	NS	89.6	54.9
Legumes (<i>Fabaceae</i>)	2.9	0.0	***	17.1	37.5	NS	1.8	0.0
Other (herbs and woody species)								
Non-grass monocotyledons	1.3	4.5	***	22.8	29.2	NS	0.5	7.6
Non-legume dicotyledons	14.6	50.7	***	15.9	29.8	*	8.1	37.5

^a Probability: *** $P \leq 0.001$; * $P \leq 0.05$; NS, not significant ($P > 0.05$).

All the species recorded within the squares of vegetation plots but not along the vegetation linear transect were considered "rare species" and 0 value was assigned to their species relative abundance (SRA).

^a GR, grasses; LE, legumes; NG, non-grass monocotyledons; NL, non-legume dicotyledons.

^b VE, vegetative plant parts; IE, inflorescence emergence; FL, flowering; DF, development of fruit.

^c Fl, flowers; St, stems; Le, leaves; Fr, fruits; Bu, buds; Sp, sprouts.

^d Number of vegetation plots and number of 1m²-squares in which each species was surveyed on 45 vegetation plots and 450 1m²-squares, respectively.

^e Species with SRA \leq 0.1 and BR=0: *Lathyrus montanus* (Fabaceae), *Anemone nemorosa* (Ranunculaceae), *Carex pallescens* (Cyperaceae), *Leontodon helveticus* (Asteraceae), *Viola riviniana* (Violaceae), *Bromus erectus* (Poaceae), *Galeopsis tetrahit* (Lamiaceae), *Homogyne alpine* (Asteraceae), *Hieracium pilosella* (Asteraceae), *Stellaria graminea* (Caryophyllaceae), *Cruciata glabra* (Rubiaceae), *Sagina procumbens* (Caryophyllaceae).

Species with SRA=0 and BR>0: *Rumex obtusifolius* (Polygonaceae), *Cardaminopsis halleri* (Brassicaceae), *Leucanthemum vulgare* (Asteraceae), *Urtica dioica* (Urticaceae), *Juncus effusus* (Juncaceae), *Sorbus aucuparia* (Rosaceae), *Ajuga reptans* (Lamiaceae). Species with SRA=0 and BR=0: *Crocus albiflorus* (Iridaceae), *Leontodon hispidus* (Asteraceae), *Cirsium palustre* (Asteraceae), *Gentiana kochiana* (Gentianaceae), *Hieracium auricula* (Asteraceae), *Festuca varia* (Poaceae), *Hieracium sylvaticum* (Asteraceae), *Stachys pradica* (Lamiaceae), *Cerastium arvense* (Caryophyllaceae), *Galium anisophyllum* (Rubiaceae), *Oxalis acetosella* (Oxalidaceae), *Silene nutans* (Caryophyllaceae), *Arnica montana* (Asteraceae), *Danthonia decumbens* (Poaceae), *Luzula multiflora* (Juncaceae), *Vaccinium vitis-idea* (Ericaceae).

All the species recorded within the squares of vegetation plots but not along the vegetation linear transect were considered "rare species" and 0 value was assigned to their species relative abundance (SRA).

Table 3

Botanical composition, functional group (FG), phenological stage, plant parts eaten, species occurrence, average species relative abundance (SRA), species browsing ratio (BR), and species intake (SI) in the grazable forestland (GF) enclosure. Data are reported following a decreasing SI. Floristic nomenclature follows Pignatti (1982).

Species	Family	FG ^a	Phenological stage ^b	Plant parts eaten ^c	Species occurrence ^d		SRA (%)	BR (%)	SI (%)
					Plots	Squares			
<i>Calamagrostis arundinacea</i>	Poaceae	GR	FL	Fl, St, Le	45	411	36.4	59	50.5
<i>Vaccinium myrtillus</i>	Ericaceae	NL	FL	Le, Sp	44	393	11.9	48	15.2
<i>Rubus hirtus</i>	Rosaceae	NL	DF	Fl, Le, Fr	33	188	3.8	60	6.7
<i>Luzula nivea</i>	Juncaceae	NG	FL	Fl, St, Le	29	125	2.4	60	4.2
<i>Sorbus aucuparia</i>	Rosaceae	NL	DF	Le	36	169	1.5	85	3.3
<i>Veratrum album</i>	Liliaceae	NG	DF	Fl, Le, Fr	31	134	1.4	96	3.3
<i>Avenella flexuosa</i>	Poaceae	GR	FL	Fl, St, Le	38	230	6.7	10	2.9
<i>Rubus idaeus</i>	Rosaceae	NL	DF	Fl, Le, Fr	28	144	1.7	46	2.6
<i>Sorbus aria</i>	Rosaceae	NL	DF	Le	18	42	0.6	85	1.5
<i>Salix caprea</i>	Salicaceae	NL	DF	Le	21	64	0.7	80	1.5
<i>Phegopteris polypodioides</i>	Thelypteridaceae	NL	VE	Le	40	297	5.2	10	1.3
<i>Senecio fuchsii</i>	Asteraceae	NL	FL	Fl, Le	11	29	0.5	88	1.1
<i>Festuca scaberculmis</i>	Poaceae	GR	FL	Fl, Le	3	9	0.6	75	1.0
<i>Athyrium distentifolium</i>	Athyriaceae	NL	VE	Le	9	34	0.6	52	0.9
<i>Athyrium filix-foemina</i>	Athyriaceae	NL	VE	Le	23	53	0.5	37	0.6
<i>Potentilla erecta</i>	Rosaceae	NL	DF	Fl, St, Le, Fr	21	84	1.0	10	0.6
<i>Rhododendron ferrugineum</i>	Ericaceae	NL	FL	Sp	44	360	17.4	1	0.5
<i>Thelypteris limbosperma</i>	Thelypteridaceae	NL	VE	Le	13	30	0.6	37	0.5
<i>Fagus sylvatica</i>	Fagaceae	NL	DF	Bu	10	19	0.2	29	0.3
<i>Betula pendula</i>	Betulaceae	NL	DF	Le, Sp	6	12	0.2	63	0.3
<i>Danthonia decumbens</i>	Poaceae	GR	FL	Fl, St, Le	6	23	0.1	33	0.2
<i>Calluna vulgaris</i>	Ericaceae	NL	VE	Le, Sp	7	51	1.1	7	0.2
<i>Nardus stricta</i>	Poaceae	GR	FL	Fl	9	40	0.8	3	0.2
<i>Carex pilulifera</i>	Cyperaceae	NG	FL	Fl, St	12	45	0.7	7	0.1
<i>Dryopteris filix-mas</i>	Dryopteridaceae	NL	VE	Le	13	19	0.1	25	0.1
<i>Alnus viridis</i>	Betulaceae	NL	VE	Le	1	2	<0.1	100	0.1
<i>Festuca nigrescens</i>	Poaceae	GR	DF	Fl, St, Le, Fr	3	4	<0.1	33	0.1
<i>Prenanthes purpurea</i>	Asteraceae	NL	FL	Fl, St, Le	6	8	<0.1	42	0.1
<i>Astrantia minor</i>	Apiaceae	NL	DF	Fl, Fr	14	29	0.1	9	<0.1
<i>Gentiana purpurea</i>	Gentianaceae	NL	IE	Le	6	22	0.1	50	<0.1
<i>Rosa pendulina</i>	Rosaceae	NL	FL	Le, Sp	3	17	0.1	11	<0.1
<i>Agrostis tenuis</i>	Poaceae	GR	FL	Fl, St, Le	9	17	0.1	4	<0.1
<i>Homogyne alpina</i>	Asteraceae	NL	FL	Fl, St	22	89	0.4	1	<0.1
<i>Polygala chamaebuxus</i>	Polygalaceae	NL	FL	Le, Sp	12	60	0.6	1	<0.1
<i>Carex sempervirens</i>	Cyperaceae	NG	FL	Fl, St	2	5	<0.1	13	<0.1
<i>Gentiana kochiana</i>	Gentianaceae	NL	FL	Fl	6	26	<0.1	22	<0.1
<i>Euphorbia carniolica</i>	Euphorbiaceae	NL	FL	Fl, St, Le	26	62	0.1	7	<0.1
<i>Maianthemum bifolium</i>	Asparagaceae	NL	DF	Fl, Le	19	55	0.1	5	<0.1
Others ^e	-	-	-	-	-	-	-	-	0.0

^a GR, grasses; LE, legumes; NG, non-grass monocotyledons; NL, non-legume dicotyledons.

^b VE, vegetative plant parts; IE, inflorescence emergence; FL, flowering; DF, development of fruit.

^c Fl, flowers; St, stems; Le, leaves; Fr, fruits; Bu, buds; Sp, sprouts.

^d Number of vegetation plots and number of 1m²-squares in which each species was surveyed on 45 vegetation plots and 450 1m²-squares, respectively.

* Species with $SRA \leq 0.9$ and $BR=0$: *Lycopodium clavatum* (Lycopodiaceae), *Vaccinium vitis-idaea* (Ericaceae), *Viola riviniana* (Violaceae), *Festuca tenuifolia* (Poaceae), *Carex pallescens* (Cyperaceae), *Pyrola rotundifolia* (Ericaceae), *Saxifraga cuneifolia* (Saxifragaceae).

Species with $SRA=0$ and $BR>0$: *Molinia arundinacea* (Poaceae), *Blechnum spicant* (Blechnaceae), *Gentiana asclepiadea* (Gentianaceae), *Hieracium sylvaticum* (Asteraceae), *Genista germanica* (Fabaceae), *Chaerophyllum hirsutum* (Apiaceae), *Dryopteris dilatata* (Dryopteridaceae), *Daphne mezereum* (Thymelaeaceae), *Phyteuma scheuchzeri* (Campanulaceae). Species with $SRA=0$ and $BR=0$: *Oxalis acetosella* (Oxalidaceae), *Polygonatum verticillatum* (Asparagaceae), *Anthoxanthum alpinum* (Poaceae), *Anemone nemorosa* (Ranunculaceae), *Epilobium angustifolium* (Onagraceae), *Pteridium aquilinum* (Hypolepidaceae), *Scrophularia nodosa* (Scrophulariaceae), *Acer pseudoplatanus* (Aceraceae), *Arnica montana* (Asteraceae), *Aruncus dioicus* (Rosaceae), *Lathyrus montanus* (Fabaceae), *Paris quadriflora* (Liliaceae), *Veronica officinalis* (Plantaginaceae).

All the species recorded within the squares of vegetation plots but not along the vegetation linear transect were considered "rare species" and 0 value was assigned to their species relative abundance (SRA).

Table 4

Proximate composition (g kg⁻¹ DM, unless otherwise stated), phenolic fractions (g kg⁻¹ DM) and fatty acid profile (mg 100g⁻¹ DM) of the ingested vegetation of the open grassland (OG) and grazable forestland (GF) enclosures, and of the commercial concentrate.

	OG	GF	Concentrate
Proximate composition			
DM (g kg ⁻¹)	284.6	356.6	886.8
Ash	54.2	48.7	25.9
CP	114.9	119.9	140.2
EE	27.9	32.1	52.1
NDF	619.9	565.4	162.7
ADF	354.9	355.2	62.0
ADL	54.1	49.8	8.7
Starch	26.8	31.2	566.1
NSC ^a	183.1	233.9	619.1
NE _L (MJ kg ⁻¹ DM)	6.1	6.7	9.3
Phenolic fractions			
TEP ^b	24.2	45.5	7.1
NTP ^b	10.4	15.3	2.7
TT ^b	13.8	30.2	4.4
CT ^c	5.0	6.5	1.2
HT	8.9	23.7	3.2
Fatty acids			
C12:0	0.9	0.7	0.9
C14:0	5.0	7.6	0.7
C15:0	14.1	15.9	7.1
C16:0	329.0	295.3	889.1
C16:1 <i>t</i> 3	26.6	21.1	2.6
C16:1 <i>c</i> 9	2.1	1.1	6.7
C18:0	35.0	32.2	149.0
C18:1 <i>c</i> 9	96.4	51.3	1421.5
C18:1 <i>c</i> 11	10.7	5.5	74.9
C18:2 <i>c</i> 9 <i>c</i> 12 (n6) (LA)	320.6	155.2	3356.5
C20:0	22.2	21.7	18.5
C18:3 <i>c</i> 6 <i>c</i> 9 <i>c</i> 12 (n6) (GLA)	3.9	4.0	1.7
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15 (n3) (ALA)	648.3	710.8	270.6
LA + GLA + ALA	972.8	870.0	3628.8
C22:0	18.9	20.3	8.3
C20:4 <i>c</i> 5 <i>c</i> 8 <i>c</i> 11 <i>c</i> 14 (n6)	2.5	2.4	1.0
C24:0	7.8	11.6	1.9
ΣSFA	433.0	405.2	1075.4
ΣMUFA	135.9	79.1	1505.6
ΣPUFA	975.3	872.3	3629.9
Total FA	1544.2	1356.7	6211.0

^a Calculated as 1000 – (NDF + CP + EE + ash).

^b Expressed as gallic acid equivalents.

^c Expressed as leucocyanidin equivalents.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; ALA, α -linolenic acid; *c*, *cis*; CP, crude protein; CT, condensed tannins; DM, dry matter; EE, ether extract; FA, fatty acids; GLA, γ -linolenic acid; HT, hydrolysable tannins; LA, linoleic acid; MUFA, monounsaturated fatty acids; NDF, neutral detergent fibre; NE_L, net energy for lactation; NSC, nonstructural carbohydrates; NTP, non-tannin phenols; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; *t*, *trans*; TEP, total extractable phenols; TT, total tannins.

Table 5

Milk yield and gross composition of goats foraging alpine open grassland (OG) and grazable forestland (GF).

	OG	GF	SEM	P-values^a
Milk yield (kg head ⁻¹ day ⁻¹)	1.71	1.24	0.10	**
Milk composition (g kg ⁻¹)				
Fat	29.7	30.5	1.65	NS
Protein	28.7	29.2	0.62	NS
Lactose	44.2	40.5	0.72	**
Casein	21.9	22.6	0.56	NS
SNF	80.1	77.0	1.13	*

^a Probability: ** $P \leq 0.01$; * $P \leq 0.05$; NS, not significant ($P > 0.05$).

Abbreviations: SEM, standard error of the mean; SNF, solids non-fat.

Table 6

Mean contents (g 100g⁻¹ fat) of groups of fatty acids in milk fat of goats foraging alpine open grassland (OG) and grazable forestland (GF).

	OG	GF	SEM	P-values ^a
Σ short chain ^c	16.47	16.16	1.088	NS
Σ medium chain ^d	32.29	34.01	1.794	NS
Σ long chain ^e	25.16	29.53	2.719	NS
Σ saturated ^f	56.15	59.27	1.655	NS
Σ branched chain ^g	1.22	1.31	0.130	NS
Σ <i>iso</i> branched chain ^h	0.56	0.65	0.070	NS
Σ <i>aiso</i> branched chain ⁱ	0.67	0.66	0.062	NS
Σ monounsaturated ^j	15.21	17.45	1.548	NS
Σ C18:1 ^k	13.76	16.02	1.503	NS
Σ C18:1 <i>trans</i> ^l	1.57	2.00	0.166	*
Σ polyunsaturated ^m	2.61	3.05	0.193	0.060
Σ C18:2 ⁿ	1.99	2.22	0.134	NS
Σ C18:2 <i>trans</i> ^o	0.83	1.10	0.066	**
Σ <i>trans</i> without CLA ^p	4.01	5.11	0.399	*
Σ omega-3-FA ^q	0.61	0.85	0.106	0.061
Σ omega-6-FA ^r	1.89	1.92	0.153	NS
Σ omega-6/Σ omega-3	3.39	2.40	0.380	*
Σ CLA ^s	0.38	0.52	0.054	*
Σ unsaturated ^t	17.81	20.50	1.665	NS
AI ^b	2.89	2.72	0.420	NS
TI ^b	3.36	3.05	0.277	NS

^a Probability: ** $P \leq 0.01$; * $P \leq 0.05$; NS, not significant ($P > 0.05$). The P-value is shown if, thus being not significant, it shows a tendency ($0.05 < P < 0.10$).

Abbreviations: AI, atherogenicity index; CLA, conjugated linoleic acid; FA, fatty acids; TI, trombogenicity index.

^b Calculated as reported by Ulbricht and Southgate (1991).

^c C4, C5, C6, C7, C8, C10, C10:1.

^d C12, C13 *iso*, C13 *aiso*, C12:1 *c*, C13, C14 *iso*, C14, C15 *iso*, C14:1 *t*, C15 *aiso*, C14:1 *c9*, C15, C16 *iso*, C16, C17 *iso*, C16:1 *t*, C17 *aiso*, C16:1 *c9*.

^e C17, C18 *iso*, C17:1 *t*, C18 *aiso*, C18, Σ C18:1, C19, Σ C18:2, C20, C20:1 *t*, C18:3 *c6c9c12*, C20:1 *c5*, C20:1 *c9*, C20:1 *c11*, C18:3 *c9c12c15*, C20:2 *c,c* n6, C22, C20:3 n6, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA).

^f C4, C5, C6, C7, C8, C10, C12, C13, Σ branched chain, C14, C15, C16, C17, C18, C19, C20, C22.

^g C13 *iso* + *aiso*, C14 *iso*, C15 *iso* + *aiso*, C16 *iso*, C17 *iso* + *aiso*, C18 *iso* + *aiso*.

^h C13 *iso*, C14 *iso*, C15 *iso*, C16 *iso*, C17 *iso*, C18 *iso*.

ⁱ C13 *aiso*, C15 *aiso*, C17 *aiso*, C18 *aiso*.

^j C10:1, C12:1 *c*, C14:1 *ct*, C16:1 *ct*, C17:1 *t*, Σ C18:1, C20:1 *t*, C20:1 *c5*, C20:1 *c9*, C20:1 *c11*.

^k C18:1 *t5*, *t6-11*, *t12-14* + *c6-8*, *c9*, *c11*, *c12*, *c14* + *t16*.

^l C18:1 *t5*, *t6-11*, *t12-14* + *c6-8*.

^m Σ C18:2, C18:3 *c6c9c12*, C18:3 *c9c12c15*, C20:2 *c,c* n6, C20:3 n6, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA).

ⁿ C18:2 *t,t*-NMID + *t9t12*, *c9t13* + *t8c12*, *c9t12*, *c,c*-MID + *t8c13*, *t11c15*, *t9c12*, *c9c12*, *c9c15*, *c9t11* + *t7c9* + *t8c10*, *t10c12*, *t11c13* + *c9c11*, *t9t11*.

^o C18:2 *t,t*-NMID + *t9t12*, *c9t13* + *t8c12*, *c9t12*, *c,c*-MID + *t8c13*, *t11c15*, *t9c12*, *c9t11* + *t7c9* + *t8c10*, *t10c12*, *t11c13* + *c9c11*, *t9t11*.

^p C14:1 *t*, C16:1 *t*, C17:1 *t*, Σ C18:1 *t*, Σ C18:2 *t* (without CLA *trans*), C20:1 *t*.

^q C18:2 *t11c15*, C18:2 *c9c15*, C18:3 *c9c12c15*, C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA).

^r C18:1 *t12*, C18:1 *c12*, C18:2 *t,t*-NMID + *t9t12*, C18:2 *c9t12*, C18:2 *t9c12*, C18:2 *c9c12*, C18:3 *c6c9c12*, C20:2 *c,c* n6, C20:3 n6, C20:4 n6 (AA).

^s C18:2 *c9t11* + *t7c9* + *t8c10*, *t10c12*, *t11c13* + *c9c11*, *t9t11*.

^t C10:1, C12:1 *c*, C14:1 *ct*, C16:1 *ct*, C17:1 *t*, Σ C18:1, Σ C18:2, C20:1 *t*, C18:3 *c6c9c12*, C20:1 *c5*, C20:1 *c9*, C20:1 *c11*, C18:3 *c9c12c15*, C20:2 *c,c* n6, C20:3 n6, C20:4 n6 (AA), C20:5 n3 (EPA), C22:5 n3 (DPA), C22:6 n3 (DHA).

Table 7

Mean contents (g 100g⁻¹ fat) of individual fatty acids in milk fat of goats foraging alpine open grassland (OG) and grazable forestland (GF).

	OG	GF	SEM	P-values ^a
C4	2.76	3.24	0.085	***
C5	0.02	0.02	0.003	0.078
C6	2.20	2.35	0.080	NS
C7	0.04	0.02	0.003	***
C8	2.43	2.37	0.170	NS
C10	8.77	8.00	0.879	NS
C10:1 <i>c</i> 9	0.25	0.17	0.033	*
C12	3.58	2.78	0.535	NS
C13 <i>iso</i>	0.02	0.02	0.003	NS
C13 <i>aiso</i>	0.03	0.01	0.004	*
C12:1 <i>c</i> 9	0.08	0.05	0.008	**
C13	0.05	0.03	0.011	0.060
C14 <i>iso</i>	0.05	0.06	0.005	0.070
C14	7.29	7.88	0.461	NS
C15 <i>iso</i>	0.10	0.14	0.019	NS
C14:1 <i>t</i>	0.01	0.01	0.001	NS
C15 <i>aiso</i>	0.24	0.23	0.031	NS
C14:1 <i>c</i> 9	0.71	0.66	0.047	NS
C15	0.06	0.04	0.010	0.098
C16 <i>iso</i>	0.15	0.16	0.014	NS
C16	19.12	21.00	1.099	NS
C17 <i>iso</i>	0.23	0.26	0.034	NS
C16:1 <i>t</i>	0.12	0.16	0.013	*
C17 <i>aiso</i>	0.26	0.24	0.032	NS
C16:1 <i>c</i> 9	0.19	0.28	0.025	**
C17	0.42	0.48	0.036	NS
C18 <i>iso</i>	0.01	0.01	0.001	NS
C17:1 <i>t</i>	0.02	0.03	0.004	0.078
C18 <i>aiso</i>	0.14	0.18	0.013	*
C18	7.94	9.45	1.314	NS
C18:1 <i>t</i> 5	0.01	0.01	0.002	NS
C18:1 <i>t</i> 6-11	1.19	1.61	0.142	*
C18:1 <i>t</i> 12-14 + <i>c</i> 6-8	0.37	0.37	0.041	NS
C18:1 <i>c</i> 9	11.59	13.33	1.344	NS
C18:1 <i>c</i> 11	0.26	0.32	0.044	NS
C18:1 <i>c</i> 12	0.07	0.07	0.011	NS
C18:1 <i>c</i> 14 + <i>t</i> 16	0.26	0.31	0.032	NS
C19	0.02	0.02	0.002	NS
C18:2 <i>t,t</i> -NMID + <i>t</i> 9 <i>t</i> 12	0.06	0.08	0.006	0.094
C18:2 <i>c</i> 9 <i>t</i> 13 + <i>t</i> 8 <i>c</i> 12	0.02	0.03	0.005	*
C18:2 <i>c</i> 9 <i>t</i> 12	0.10	0.12	0.009	NS
C18:2 <i>c,c</i> -MID + <i>t</i> 8 <i>c</i> 13	0.14	0.18	0.018	0.086
C18:2 <i>t</i> 11 <i>c</i> 15	0.08	0.13	0.015	*
C18:2 <i>t</i> 9 <i>c</i> 12	0.04	0.06	0.005	*
C18:2 <i>c</i> 9 <i>c</i> 12 (LA)	1.15	1.11	0.087	NS
C20	0.18	0.23	0.032	NS
C20:1 <i>t</i>	0.01	0.01	0.001	0.056
C18:3 <i>c</i> 6 <i>c</i> 9 <i>c</i> 12 (GLA)	0.01	0.01	0.002	NS
C20:1 <i>c</i> 5	0.01	<0.01	0.001	0.083
C20:1 <i>c</i> 9	0.02	0.02	0.004	NS
C20:1 <i>c</i> 11	0.03	0.03	0.003	NS
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15 (ALA)	0.44	0.61	0.088	0.087
CLA <i>c</i> 9 <i>t</i> 11 + <i>t</i> 7 <i>c</i> 9 + <i>t</i> 8 <i>c</i> 10	0.35	0.48	0.054	0.055
CLA <i>t</i> 10 <i>c</i> 12	0.01	<0.01	0.002	NS

CLA <i>t11c13 + c9c11</i>	0.01	0.02	0.003	*
CLA <i>t9t11</i>	0.01	0.01	0.001	NS
C20:2 <i>c,c</i> n6	<0.01	<0.01	<0.001	0.079
C22	0.04	0.06	0.006	*
C20:3 n6	0.01	0.01	0.001	NS
C20:4 n6 (AA)	0.08	0.10	0.007	NS
C20:5 n3 (EPA)	0.02	0.03	0.002	*
C22:5 n3 (DPA)	0.04	0.05	0.006	NS
C22:6 n3 (DHA)	0.01	0.02	0.003	NS
DI ^b	0.27	0.27	0.022	NS

^a Probability: ***P≤0.001; **P≤0.01; *P≤0.05; NS, not significant (P>0.05). The P-value is shown if, thus being not significant, it shows a tendency (0.05<P<0.10).

Abbreviations: AA, arachidonic acid; ALA, α -linolenic acid; *c*, *cis*; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; DI, desaturase index; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GLA, γ -linolenic acid; LA, linoleic acid; MID, methylene interrupted diene; NMID, non methylene interrupted diene; *t*, *trans*.

^b Calculated as: (C14:1 *c9* + C16:1 *c9* + C18:1 *c9*) / (C14:0 + C14:1 *c9* + C16:0 + C16:1 *c9* + C18:0 + C18:1 *c9*).