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Changes in pasture and cow milk compositions during a summer transhumance in the western Italian Alps

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Summary

The changes occurring in pasture and milk compositions during summer grazing were studied following a transhumance of a dairy cattle herd in the western Italian Alps. During three consecutive grazing periods (P1, P2, and P3) the cows exploited, in sequence, mountain pastures located at 1200-1260 m a.s.l. (A1), alpine pastures at 2000-2200 m a.s.l. (A2), and then returned to A1 pastures. The botanical and nutritional compositions of pastures, as well as cow milk yield, gross composition and fatty acid (FA) profile were assessed during the transhumance.

Within the pastures, a cluster analysis allowed the recognition of five vegetation types and seven vegetation sub-types; their allocation and plant species composition differed among the exploited grazing areas. The average Pastoral Values were significantly higher in the mountain (A1P1, A1P3) than in the alpine pastures (A2P2) due to the abundance of high- and medium-quality forage species such as *Dactylis glomerata* L., *Polygonum bistorta* L., and *Festuca rubra* s.l.. Nevertheless, the nutritional quality of the herbage offered to the animals did not differ between A1P1 and A2P2, while it was significantly higher in A1P3 due to a younger vegetation phenological stage. The nutritional parameters were found to be correlated to the pasture botanical composition and phenology: organic matter digestibility and net energy for lactation were correlated negatively to the phenological stage and the Specific Contribution (SC) of *Poaceae* and positively to the SC of *Fabaceae* and *Asteraceae*.

Milk yield significantly declined while milk protein increased during the grazing season, following the advance of cows' stage of lactation. Milk fat and lactose percentages did not vary significantly among the grazing periods. The same was also observed for milk FA, with the exception of palmitic acid, whose level was lower in A2P2 if compared to the other two periods. Significant correlations were found between the percentages of some FA in milk and the SC of the main botanical families of the grazed pastures. In particular, linoleic acid was negatively correlated with the SC of *Poaceae* and positively correlated with the SC of *Fabaceae*.

Results showed that the changes in the nutritional composition of pastures depended on variations in pasture botanical composition and phenology at the time of grazing, and that such factors concurred with animal-related factors in affecting milk quality during the grazing season.

Introduction

In the Alps, semi-natural pastures are the main feed sources for ruminants during summer. They are traditionally exploited according to a vertical transhumance, following their gradual availability at growing altitudes (Dodgshon and Olsson, 2007). In recent years, semi-natural pastures have also become a key factor for the traceability and the quality of dairy products (Martin et al., 2005). This added value arises from the consideration of the multiple effects of grazing on animal performance. Milk yield and composition are affected by a combination of factors including diet (Sutton, 1989) and animal-related aspects (Coulon et al., 1991). Nevertheless, when animals graze, additional variables influence milk characteristics. Botanically diverse pastures are known to confer peculiar organoleptic and nutritional qualities to milk compared with either conserved forages or cereal-based concentrate feeds. In particular, dairy products obtained from grass-fed ruminants are very rich in beneficial fatty acids (FA), such as polyunsaturated fatty acids (PUFA) which have some interest for their positive effects on human health (Van Dorland et al., 2006). Furthermore, there is evidence that grazing in alpine areas *per se* can affect milk fat composition as well. Severe environmental conditions due to altitude, topography, and climate, along with the frequently unstable nutritional quality of herbage, lead to animals' increased energy requirements and physiological changes (i.e., increased body fat mobilisation, ruminal ecosystem alterations) which have some bearing on milk production (Zemp et al., 1989; Leiber et al., 2004).

Alpine pasture types (Jouglet et al., 1992; Cavallero et al., 2007) and the factors determining changes in their nutritional and chemical compositions (Jeangros et al., 1999; Bovolenta et al., 2008) have been described in literature. Similarly, the nutritional aspects and the chemical composition of milk produced in alpine areas have been well deepened (Collomb et al., 2001; Collomb et al., 2002a). Several studies also aimed at understanding the relationships existing between alpine pastures and milk quality during grazing (Jeangros et al., 1997; Buchin et al., 1999; Collomb et al., 2002b; De Noni and Battelli, 2008). However, to date, only few of these studies investigated the compositional changes in both pastures and milk following a dairy herd under the usual farming conditions of a transhumance and providing at the same time a full description of pastures' botanical composition (Buchin et al., 1999; De Noni and Battelli, 2008). By contrast, given that such movements of herds are widespread in all European mountain areas and considered the increasing consumers' interest in traditional dairy products, it should be important to determine which factors usually affect pasture and milk quality *in natura*. In particular, understanding the effects of changes in pasture botanical composition and timing of grazing on pasture nutritional composition could help in improving grazing management during summer and, consequently, animal performance and the quality of milk and derived products (Elgersma et al., 2006).

Therefore, the goals of this study were: i) to assess the botanical and nutritional compositions of semi-natural pastures grazed during a transhumance in the western Italian Alps, ii) to assess yield, gross composition, and FA profile of the milk produced by the herd, and iii) to determine which factors led to changes in pasture and milk compositions during the transhumance.

Materials and methods

Study sites and experimental design

This trial was carried out in the Mont Avic Natural Park (Aosta Valley, Northwest Italy) during an entire summer grazing season. A dairy farm breeding a herd of thirty-six lactating Aosta Red Pied cows was selected as representative of the traditional pasture-based farming system typical of this alpine area. All cows were multiparous, in mid lactation (176 ± 30 days in milk at the beginning of the trial), and adapted to the alpine environment. Their feed source was limited to fresh grass from pastures.

From the beginning of June to the end of July (P1), the herd exploited mountain pastures located at 1200-1260 m a.s.l. (A1). In August (P2), the cows moved to alpine pastures located at 2000-2200 m a.s.l. (A2), while in September (P3) they returned to the mountain pastures already exploited at the beginning of the trial (A1). Thus, during the grazing season three experimental periods were identified (A1P1, A2P2, and A1P3) (Tab. 1). The cows were managed according to a rotational grazing system, with paddocks grazed in sequence both in the mountain and alpine areas. The surface of paddocks and the stocking periods ranged respectively from 0.4 to 1.6 hectares and from 3 to 8 days depending on vegetation composition, herbage biomass, and site conditions (e.g., topography, stone cover).

Vegetation surveys

Pasture botanical composition was surveyed using a vertical point-quadrat method (Daget and Poissonet, 1969) along 25 m transects laid out on representative and homogeneous areas. Twenty-two surveys were performed before exploitation (A1P1 and A2P2). Five surveys were repeated in A1 during the regrowth period (P3). All plant species were identified (Pignatti, 1982) and their relative cover was computed as Specific Contribution (SC) percentage (Daget and Poissonet, 1969). Vegetation Pastoral Values (PV) were determined according to Daget and Poissonet (1972).

Vegetation sampling and analysis

Representative mixed grass samples (500 g) were collected at each grazing area to determine the herbage nutritional composition (Tab. 1). Sampling was carried out two days before exploitation following herd movements within the grazing areas. The phenological stage of the most abundant species was recorded using the Lambertin's schedule (Lambertin, 1990) and 1×1 m^2 area of each pasture was harvested to assess the herbage biomass (t DM ha^{-1}).

After collection, the samples were immediately oven-dried at 60°C for 48 hours and ground to pass a 1-mm screen using a Cyclotech mill (Tecator, Höganäs, Sweden). The samples were analyzed for dry matter (DM) (930.15; AOAC, 2000), crude protein (CP) (978.04; AOAC, 2000), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) (Van Soest et al., 1991), organic matter digestibility (OMD) (Aufrère, 1982), and ash (930.05; AOAC, 2000). The net energy for lactation (NEl) was calculated on the basis of the INRA energy system of feedstuffs evaluation (Jarrige, 1988).

Milk sampling and analysis

Milk yield recording and milk sampling started in A1P1 after a three-week period provided to allow cow rumen adaptation to summer grazing. In A2P2 and A1P3 milk yield recording and milk sampling started five days after the entrance in each grazing area to support rumen adaptation to vegetation types (Tab. 1). Milk samples to be analyzed for their gross composition were immediately transported to the laboratory in a portable refrigerator at 4°C, while the samples destined to the assessment of the FA composition were frozen until analyzed.

Milk fat, protein, and lactose percentages were assessed by infrared spectroscopy (MilkoScan 4000, Foss Electric, Hillerød, Denmark). For FA analysis, milk fat extraction was obtained by centrifugation at 8000 *g* for five minutes at -4°C. The fatty acid methyl esters (FAME) were prepared by esterification (AOAC, 2000) and determined by using a gaschromatograph (Perkin-Elmer P-E 8700, Perkin-Elmer Co., Norwalk, CT, USA) equipped with a DB-WAX (J&W Scientific Inc., Folsom, CA, USA) capillary column (60 m × 0.53 mm ID, 1.0 µm

Tab. 1: Features of the grazing season and experimental design.

Dairy herd	36 Aosta Red Pied cows		
Grazing periods	June 1st - July 27th	July 28th - August 31st	September 1st - October 6th
codes	**P1**	**P2**	**P3**
Grazing areas	Mountain pastures	Alpine pastures	Mountain pastures
codes	**A1**	**A2**	**A1**
coordinates	45°41'N, 7°36'E	45°39'N, 7°35'E	45°41'N, 7°36'E
altitude (m a.s.l.)	1200-1260	2000-2200	1200-1260
surface (ha)	9.0	9.2	6.9
notes	first grazing event	first grazing event	second grazing event
Grazing management system	Rotational grazing (paddocks)		
Fodder	Fresh grass from pasture		
Milking system	By hand (shed)		
Surveys and samplings	**A1P1**	**A2P2**	**A1P3**
Botanical surveys	on the first grass growth, before the first exploitation		on the regrowth, before the second exploitation
number	6	16	5
Vegetation sampling	2 days before grazing		
number	7	5	4
Milk sampling	3 weeks after moving into the grazing area	5 days after moving into the grazing area	
number	7	7	7

film thickness). The column temperature was held at 180°C for one minute and then raised 4°C min^{-1} to a final temperature of 225°C, where it remained for 45 minutes. The injector was maintained at 250°C and the flame ionization detector (FID) at 270°C. Peaks were identified by comparing their retention times with pure FAME standards (Restek Corporation, Bellefonte, PA, USA; Matreya Inc., Pleasant Gap, PA, USA). Milk FA composition was expressed as a percentage of each individual FA per total FA detected.

Statistical analysis

A two-level classification system, based on pasture types and sub-types, was used to describe pasture vegetation (Cavallero et al., 2007). To identify homogeneous vegetation groups – in terms of species composition – botanical data from the first grass growth (A1P1 and A2P2) were classified by cluster analysis performed using the SC (©Clustan Graphics 5.27 software; Wishart, 1987). The similarity matrix was calculated using Pearson's correlation, while Between-groups linkage was selected as agglomeration method.

One-way ANOVA was used to compare the characteristics of pastures (i.e., SC of the main botanical families, phenological stage, PV, herbage biomass), the nutritional composition of herbage, yield, gross composition, and FA profile of milk among the three experimental periods. The assumption of equal variances was assessed by Levene's homogeneity of variance test. The Bonferroni post-hoc test was performed to test the difference between each pair of means. Correlations between the herbage nutritional parameters, milk quality, and vegetation attributes (i.e., SC of the main botanical families, phenological stage) were calculated using the Pearson's correlation test. Statistical analyses were performed using ©SPSS (2007). Significance was declared at $P<0.05$.

Results

Botanical composition and attributes of pastures

A total of 145 plant species belonging to 38 botanical families were identified in the study area. Five vegetation types and seven vegetation sub-types were recognized (Fig. 1). The botanical composition of types and sub-types is shown in Tab. 2. The average herbage biomass, PV, and SC of the main botanical families in the three experimental periods are given in Tab. 3.

Poaceae, *Fabaceae*, *Asteraceae*, *Polygonaceae*, and *Cyperaceae* were the most widespread families in the pastures (78% of SC). The abundance of *Poaceae* and *Cyperaceae* did not differ significantly among the grazing areas. *Fabaceae* and *Polygonaceae* species were significantly more abundant in the mountain (A1P1 and A1P3) than in the alpine pastures (A2P2), while *Asteraceae* species were significantly more abundant in A1P3 with respect to both A1P1 and A2P2 pastures (Tab. 3). Overall, dicotyledonous species SC were significantly higher ($P<0.05$) in the mountain regrowth (60.9% in A1P3) than in the alpine pastures (37.0% in A2P2).

The mountain pastures had significantly higher PV than the alpine pastures. In fact, in the A1P1 area, vegetation sub-types with higher PV ascribable to type *Polygonum bistorta* and type *Festuca rubra* s.l. were dominant (about 77% of total A1 surface), while sub-types with lower PV ascribable to type *Bromus erectus* were confined to steep heat slopes covering few areas (about 23% of surface). By contrast, in the A2P2 pastures, sub-types with lower PV (type *Nardus stricta* and type *Carex sempervirens*) were more widespread (about 80% of total A2 surface) than those with higher PV (type *Festuca rubra* s.l.; about 20% of surface).

By comparing A1P1 and A1P3, the mountain pastures were shown not to have undergone significant changes in their PV and their botanical composition did not differ with the exception of *Asteraceae* abundance. However, the amount of herbage biomass was significantly lower at the regrowth stage than at the beginning of the season.

Nutritional values of pastures

The phenological stage and nutritional values of pastures during the three experimental periods are shown in Tab. 3.

The growth stage of pastures differed significantly among the three periods. On average, pastures were grazed by the cows at the full flowering stage in A1P1, at the end of the flowering stage in A2P2, and at the beginning of the heading stage in A1P3.

DM percentages did not differ among the experimental periods;

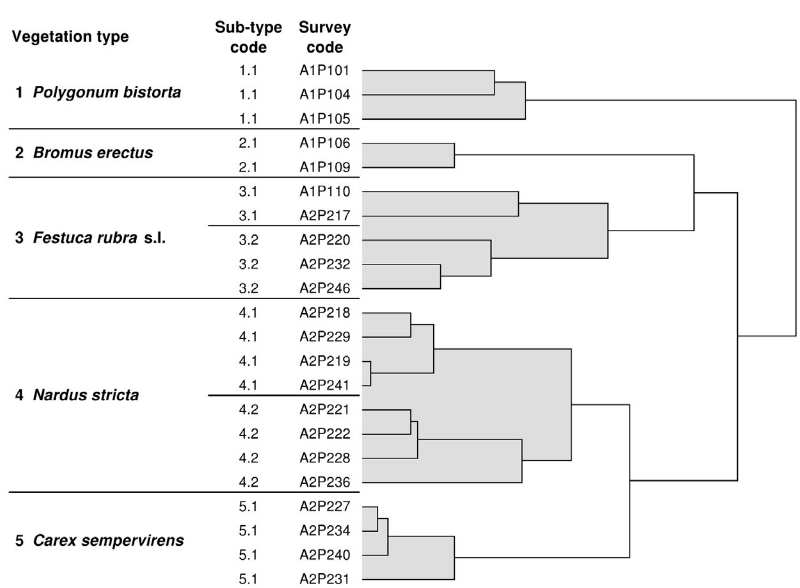

Fig. 1: Dendrogram of the vegetation data obtained through cluster analysis. For the botanical composition of vegetation types and sub-types refer to Tab. 2. The survey codes refer to areas (A1 and A2) and to periods (P1 and P2) of the study.

Tab. 2: Botanical composition of the vegetation types and sub-types in the mountain (A1P1) and alpine (A2P2) pastures, and botanical composition of A1P3 mountain pastures after the first exploitation. The mean (*x*) and Standard Deviation (SD) of Specific Contributions are given for the ten most abundant species of sub-types (the species having cumulative SC>25% are in bold font). Floristic nomenclature follows Pignatti (1982).

Area / Period of grazing	**Mountain Pastures – A1P1 (1200-1260 m a.s.l.)**									**Mountain Pastures – A1P3 (1200-1260 m a.s.l.)**					
type sub-type code	**1 - *Polygonum bistorta* 1.1**			**2 - *Bromus erectus* 2.1**			**3 - *Festuca rubra* s.l. 3.1**								
		x	**SD**		***x***	**SD**		***x***	**SD**[†]		***x***	**SD**		***x***	**SD**
	Polygonum bistorta	12.4	1.4	***Bromus erectus***	26.7	9.0	***Trisetum flavescens***	13.8	-	***Taraxacum officinale***	12.9	3.4	***Taraxacum officinale***	23.9	6.4
	Agrostis tenuis	11.3	7.3	*Festuca rubra* s.l.	9.7	3.6	***Festuca rubra* s.l.**	13.4	-	***Trisetum flavescens***	12.0	5.3	***Festuca rubra* s.l.**	9.5	7.2
	Taraxacum officinale	9.6	4.1	*Carex pallescens*	5.9	8.4	*Carex caryophyllea*	7.7	-	***Polygonum bistorta***	11.0	2.3	*Dactylis glomerata*	6.7	5.1
	Dactylis glomerata	7.2	3.3	*Lotus corniculatus*	4.5	0.8	*Bromus erectus*	6.9	-	*Trifolium repens*	8.4	1.9	*Polygonum bistorta*	6.7	7.6
	Trifolium pratense	7.0	9.7	*Trisetum flavescens*	3.8	5.4	*Luzula campestris* s.l.	6.1	-	*Dactylis glomerata*	7.9	4.6	*Holcus lanatus*	6.4	4.5
	Rumex acetosa	5.1	3.9	*Agropyron repens*	3.1	4.5	*Achillea millefolium*	5.7	-	*Agrostis tenuis*	7.0	4.9	*Achillea millefolium*	6.3	2.5
	Trisetum flavescens	5.0	2.3	*Achillea millefolium*	3.1	4.3	*Lathyrus pratensis*	3.7	-	*Ranunculus acris*	6.7	1.1	*Trifolium pratense*	6.2	6.6
	Anthoxanthum odoratum	4.9	7.8	*Ononis spinosa*	2.8	3.9	*Ranunculus bulbosus*	3.7	-	*Achillea millefolium*	5.6	3.7	*Lolium perenne*	5.2	3.8
	Achillea millefolium	4.8	3.6	*Hieracium pilosella*	2.8	3.9	*Lotus corniculatus*	3.3	-	*Galium mollugo*	4.3	3.9	*Plantago lanceolata*	3.7	1.5
	Ranunculus acris	3.7	1.3	*Brachypodium rupestre*	2.7	1.7	*Plantago lanceolata*	2.4	-	*Lathyrus pratensis*	3.9	4.5	*Brachypodium rupestre*	3.5	3.0

Area / Period of grazing	**Alpine Pastures – A2P2 (2000-2200 m a.s.l.)**														
type sub-type code	**3 - *Festuca rubra* s.l. 3.1**			**3.2**			**4 - *Nardus stricta* 4.1**			**4.2**			**5 - *Carex sempervirens* 5.1**		
		x	**SD**[†]		***x***	**SD**		***x***	**SD**		***x***	**SD**		***x***	**SD**
	***Festuca rubra* s.l.**	22.0	-	***Festuca rubra* s.l.**	18.2	4.5	***Nardus stricta***	22.4	7.8	***Anthoxanthum alpinum***	11.8	3.6	***Carex sempervirens***	16.1	6.2
	Trisetum flavescens	13.0	-	***Nardus stricta***	15.3	6.1	***Carex fusca***	22.0	3.9	***Nardus stricta***	11.6	5.9	***Plantago serpentina***	15.0	4.8
	Poa alpina	12.4	-	*Phleum alpinum*	14.3	9.8	*Anthoxanthum alpinum*	11.4	7.1	***Festuca ovina* s.l.**	10.3	2.0	*Leontodon helveticus*	14.1	3.8
	Trifolium pratense	10.7	-	*Poa alpina*	10.6	9.3	*Leontodon helveticus*	6.3	5.9	*Geum montanum*	8.1	2.2	*Nardus stricta*	6.5	5.8
	Agropyron repens	10.2	-	*Carex fusca*	3.8	6.0	*Carex stellulata*	5.8	4.7	*Carex sempervirens*	5.9	6.6	*Festuca ovina* s.l.	5.3	2.2
	Polygonum bistorta	6.8	-	*Geum montanum*	3.6	3.9	*Festuca ovina* s.l.	4.4	6.7	*Poa alpina*	5.8	2.7	*Agrostis alpina*	4.9	5.7
	Leontodon helveticus	4.0	-	*Anthoxanthum alpinum*	3.5	4.5	*Viola biflora*	3.9	2.6	*Potentilla grandiflora*	4.7	5.6	*Poa alpina*	3.2	2.5
	Anthoxanthum alpinum	3.4	-	*Trifolium pratense*	2.7	3.4	*Poa alpina*	3.1	4.0	*Plantago serpentina*	4.6	3.3	*Soldanella alpina*	3.0	3.0
	Potentilla grandiflora	3.4	-	*Soldanella alpina*	2.7	3.2	*Soldanella alpina*	2.6	3.5	*Leontodon helveticus*	4.0	4.2	*Campanula scheuchzeri*	2.7	2.4
	Phleum alpinum	2.3	-	*Potentilla grandiflora*	2.5	2.4	*Trichophorum caespitosum*	2.4	3.0	*Agrostis tenuis*	2.7	3.3	*Anthoxanthum alpinum*	2.5	1.4

[†]Sub-types including only one survey.

Tab. 3: Attributes and nutritional values of the pastures grazed by the herd during the three experimental periods.

Area / Period of grazing	A1P1	A2P2	A1P3	SEM	Inter-area / period differences†	Bonferroni post-hoc significant comparisons at $P < 0.05$‡		
Pasture attributes								
Herbage biomass (t DM ha^{-1})	3.3	3.0	1.8	0.167	F = 27.605***	A1P1 *vs* A1P3	A2P2 *vs* A1P3	
Phenological stage§	400.7	497.2	212.5	29.713	F = 42.310***	A1P1 *vs* A2P2	A1P1 *vs* A1P3	A2P2 *vs* A1P3
Pastoral Value	33.3	20.4	45.4	2.614	F = 15.708***	A1P1 *vs* A2P2	A2P2 *vs* A1P3	
Poaceae (%)	43.2	47.2	38.3	2.593	F = 0.905			
Fabaceae (%)	11.1	3.0	11.9	1.376	F = 6.892**	A1P1 *vs* A2P2	A2P2 *vs* A1P3	
Asteraceae (%)	11.3	7.8	25.6	1.862	F = 12.944***	A1P1 *vs* A1P3	A2P2 *vs* A1P3	
Polygonaceae (%)	9.7	1.7	9.2	1.254	F = 7.066**	A1P1 *vs* A2P2	A2P2 *vs* A1P3	
Cyperaceae (%)	5.4	15.6	0.0	2.106	F = 7.108			
Nutritional values								
DM (%)	25.0	29.4	21.8	1.417	F = 2.464			
CP (%DM)	10.1	10.9	13.3	0.416	F = 12.129**	A1P1 *vs* A1P3	A2P2 *vs* A1P3	
NDF (%DM)	58.1	59.4	41.6	2.115	F = 29.233***	A1P1 *vs* A1P3	A2P2 *vs* A1P3	
ADF (%DM)	37.2	33.2	29.3	1.026	F = 11.991**	A1P1 *vs* A1P3		
ADL (%DM)	6.5	4.4	6.5	0.374	F = 5.613*	A1P1 *vs* A2P2		
Ash (%DM)	5.9	4.8	9.1	0.466	F = 35.942***	A1P1 *vs* A1P3	A2P2 *vs* A1P3	
OMD (%DM)	51.1	50.5	68.0	2.184	F = 21.870***	A1P1 *vs* A1P3	A2P2 *vs* A1P3	
NEl (MJ kg DM^{-1})	3.9	3.8	5.2	0.165	F = 19.822***	A1P1 *vs* A1P3	A2P2 *vs* A1P3	

Abbreviations: DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; OMD, organic matter digestibility; NEl, net energy for lactation.
†Significance: *P<0.05; **P<0.01; ***P<0.001.
‡*vs* = *versus*.
§Lambertin phenology scale (P = *Poaceae* and *Cyperaceae*; O = Other families): 100 = P/O: vegetative stage; 200 = P: 70% of spikes in stems, O: 70% of flowering bottoms; 300 = P: 70% of spikes out of stems (spikes close to stems), O: 70% of flowering bottoms opened; 400 = P/O: full flowering stage; 500 = P: lactic corns, O: flowers withered; 600 = P: doughy corns, O: starting fructification (fruits formed); 700 = P: hard corns, O: fruits fully ripened; 800 = P/O: end of vegetation.

however, all other nutritional parameters varied significantly during the season. Most of them did not differ between A1P1 and A2P2, but the pasture quality was significantly higher in A1P3, as demonstrated by the higher CP, OMD, and NEl and the lower NDF. The phenological phase of plants was significantly correlated with all considered nutritional parameters, with the exception of the ADF and ADL percentages: it was positively correlated to DM and NDF percentages and negatively to CP, NEl, and OMD (Tab. 4). Many nutritional parameters were also correlated to the SC of *Poaceae*, *Fabaceae*, and *Asteraceae*. In particular, NEl and OMD were found to be correlated negatively to *Poaceae* SC and positively to *Asteraceae* and *Fabaceae* SC (Tab. 4).

Milk yield, gross composition and fatty acid profile

Average bulk milk yield, gross composition and FA profile in the three grazing periods are shown in Tab. 5. During the transhumance, a decrease in milk yield was observed, specifically from 380 kg $herd^{-1}$ day^{-1} at the first sampling in June to 60 kg $herd^{-1}$ day^{-1} at the last sampling in October. Average milk yield significantly differed among the three experimental periods, being about 50% lower both in A2P2 compared to A1P1 and in A1P3 compared to A2P2. The protein percentage was significantly lower in A1P1 with respect to A2P2 and A1P3. The percentage of fat showed higher values in A2P2 and A1P3 if compared to A1P1 but such variations were not significant. The lactose percentage did not vary significantly

Tab. 4: Pearson's correlation coefficients† between the nutritional values of the grazed herbage, the phenological stages, and the Specific Contributions of the main botanical families.

	DM	NDF	ADF	ADL	CP	NEl	OMD
Phenological stage	0.625**	0.784**	0.353	–0.474	–0.548*	–0.752**	–0.747**
Poaceae	0.555*	0.460	–0.139	–0.857**	–0.391	–0.504*	–0.503*
Fabaceae	–0.408	–0.531*	0.033	0.580*	0.367	0.547*	0.534*
Asteraceae	–0.540*	–0.549*	–0.111	0.699**	0.454	0.642**	0.630**
Polygonaceae	–0.356	–0.410	0.100	0.470	0.225	0.433	0.410
Cyperaceae	0.440	0.391	0.103	–0.434	–0.160	–0.448	–0.418

Abbreviations: DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; NEl, net energy for lactation; OMD, organic matter digestibility.
†Significance: *P<0.05; **P<0.01.

Tab. 5: Bulk milk yield, gross composition and fatty acid (FA) profile during the three experimental periods.

Area / Period of grazing	A1P1	A2P2	A1P3	SEM	Inter-area / period differences†	Bonferroni post-hoc significant comparisons at $P < 0.05$‡		
Milk yield (kg herd^{-1} day^{-1})	325.8	160.0	86.0	27.546	$F = 77.209$***	A1P1 *vs* A2P2	A1P1 *vs* A1P3	A2P2 *vs* A1P3
Milk gross composition (%)								
Fat	3.87	4.23	4.14	0.077	$F = 1.952$			
Protein	3.21	3.63	3.75	0.060	$F = 26.255$***	A1P1 *vs* A2P2	A1P1 *vs* A1P3	
Lactose	4.82	4.72	4.76	0.019	$F = 3.020$			
Fatty Acids (% of total FA)								
C10:0	1.97	2.38	2.26	0.110	$F = 1.164$			
C12:0	2.26	2.98	2.75	0.161	$F = 1.796$			
C14:0	9.70	8.67	9.77	0.283	$F = 1.948$			
C14:1 *c*9	1.27	1.23	1.44	0.057	$F = 1.297$			
C15 *aiso*	0.92	0.97	0.90	0.028	$F = 0.514$			
C15:0	1.62	1.74	1.97	0.065	$F = 3.088$			
C16:0	25.40	23.62	25.55	0.357	$F = 5.305$*	A1P1 *vs* A2P2	A2P2 *vs* A1P3	
C16:1 *c*9	1.35	1.47	1.37	0.105	$F = 0.121$			
C18:0	17.63	17.27	16.15	0.397	$F = 1.213$			
C18:1 *c*9	34.59	36.98	34.45	0.567	$F = 2.880$			
C18:2 *c*9*c*12	1.79	1.48	1.88	0.146	$F = 0.723$			
C18:3 *c*9*c*12*c*15	1.52	1.23	1.52	0.123	$F = 0.649$			
ΣSFA	59.49	57.61	59.35	0.630	$F = 0.981$			
ΣMUFA	37.21	39.68	37.26	0.557	$F = 2.955$			
ΣPUFA	3.30	2.71	3.40	0.263	$F = 0.716$			
ΣUFA	40.52	42.39	40.66	0.629	$F = 0.974$			

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.
†Significance: *P<0.05; ***P<0.001.
‡*vs* = *versus*.

Tab. 6: Significant Pearson's correlation coefficients between milk fatty acids and the Specific Contributions of the main botanical families in pastures.

Milk fatty acids	Botanical families (Pearson's correlation coefficient†)
C16:0	*Polygonaceae* (r = 0.614*)
C18:1 *c*9	*Polygonaceae* (r = –0.587*); *Cyperaceae* (r = 0.643*)
C18:2 *c*9*c*12	*Poaceae* (r = –0.610*); *Fabaceae* (r = 0.697*)
ΣMUFA	*Polygonaceae* (r = –0.589*); *Cyperaceae* (r = 0.603*)
ΣPUFA	*Fabaceae* (r = 0.653*)

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
†Significance: *P<0.05.

throughout the grazing season. The FA percentages of milk did not show significant differences among periods, except in the case of palmitic acid (C16:0), whose level was significantly lower in alpine (A2P2) than in mountain milk (A1P1 and A1P3).

Significant correlations were found between the percentages of some FA in milk and the SC of the main botanical families of the grazed pastures (Tab. 6). Palmitic acid was positively correlated with the SC of *Polygonaceae*. Oleic acid (C18:1 *c*9) was negatively correlated with the SC of *Polygonaceae* and positively correlated with the SC of *Cyperaceae*. Linoleic acid (C18:2 *c*9*c*12) was negatively correlated with the SC of *Poaceae* and positively correlated with the SC of *Fabaceae*. The total monounsaturated fatty acid (MUFA) percentage was negatively correlated with the SC of *Polygonaceae* and positively correlated with the SC of *Cyperaceae*. The total PUFA percentage was instead positively correlated with the SC of *Fabaceae*. No significant correlations were observed between milk FA and the SC of *Asteraceae*.

Discussion

In the alpine farming systems, dominant plant species and available herbage biomass at the time of grazing, along with the topographic features of pastures (e.g., altitude, slope), are usually the main factors taken into account to set grazing management strategies (Jouglet et al., 1992). PV, as deduced from vegetation composition, is particularly considered by pastoralists the most effective tool to assess pasture carrying capacity (Daget and Poissonet, 1972). Instead, other relevant factors such as plant phenology hardly support management decisions because of logistic constraints, with consequences on the quality of herbage actually offered to ruminants during the grazing seasons. In the study area, mountain and alpine pastures, whose vegetation types and sub-types can be considered representative of those usually grazed in the western Alps (Cavallero et al., 2007), showed significant differences in their botanical composition and PV. In particular, the average PV were higher in the mountain than in the alpine pastures due to the abundance of high- and medium-quality forage species such as *Dactylis glomerata*, *Polygonum bistorta*, and *Festuca rubra* s.l.. Nevertheless, mountain and alpine pasture first growth did not display, as expected, significant differences in herbage allowance and quality, as they were both grazed at advanced phenological stages with digestibility levels extremely low for dairy cows (Jarrige, 1988). On the contrary, the regrowth of mountain pastures in September, which did not differ from the first growth for its PV, was found to have the highest nutritional quality. During the entire grazing season plant phenology concurred strongly with plant species composition in determining such changes in pasture nutritional profiles. Plant growth normally results in increased fibre content and decreased energy values (Schubiger et al., 2001), but it is known that the extent to which nutritional values shift and their rapidity depend on the species, being generally greater in grasslands rich in *Poaceae* than in those rich in dicotyledonous (Jarrige, 1988; Farruggia et al., 2008). Moreover, regrowths are known to have a higher leaf:stem ratio and anticipated development stages, which usually determine higher digestibility values and lower productivity relative to the first growth (Schubiger et al., 2001). The differences among pastures and the correlations between nutritional values, phenological stages and the SC of *Poaceae*, *Asteraceae*, and *Fabaceae* confirmed such tendencies. Although plant senescence effects are usually well known by farmers, in alpine environments short vegetative season normally determines rapid plant development and decline in herbage quality (Van Dorland et al., 2006), while differences in site conditions and plant species composition lead to a high variability of growth rates among pastures (Farruggia et al., 2008), which altogether do not allow to easily manage the timing of grazing during the transhumances.

In the western Alps, pasture quality changes such as those observed in this study are commonly considered to affect milk yield and composition during the summer grazing season (Battaglini et al., 2003). Given that in the study area calving traditionally occurs in December (Barmaz, 1992), a drop in milk yield and some changes in milk nutritional composition were expected due to the advance in the cows' stage of lactation. However, many other factors affected milk yield and composition throughout the season, especially as a consequence of herd transfers between mountain and alpine areas. The general grazing conditions in alpine pastures (i.e., walking, altitude) coupled with low herbage quality, likely concurred in reducing milk yield by 50% in a short period as A2P2 (about one month). In fact, high altitude grazing adaptation can be generally achieved by the animals within a few weeks, but it is associated with a concomitant decline in herbage intake and milk yield due to animal stress (Van Dorland et al., 2006). Instead, further decrease in milk yield after return to A1P3 occurred mainly as cows were at the end of the lactation. Since herbage allowance was lower compared to the previous considered periods, regrowth quality probably allowed supporting lower animal requirements at the end of the grazing season (Bovolenta et al., 2008).

Concerning milk gross composition, fat was expected to increase significantly during lactation as it was observed for protein. Nevertheless, fat is known as the most sensitive parameter in milk to dietary influences (Sutton, 1989) and percentages varied notably during the entire season probably because of variations in herbage quality. In particular, although changes were not significant, milk fat values increased in A2P2 compared to A1P1 in correspondence to the drop in milk yield and the supposed increased body fat mobilisation due to energy deficits (Leiber et al., 2006). No significant variations were also observed for lactose, but such result was expected as this parameter is known to be approximately constant in milk (Sutton, 1989).

In this study, the FA levels in milk were generally consistent with those of previous reports for cows grazing natural pastures (Collomb et al., 1999, 2002a; De Noni and Battelli, 2008). However, FA percentages did not vary as expected in correspondence of variations in pasture botanical composition and quality. In fact, when cows graze grasslands rich in dicotyledonous plant species or at young growth stages, as in A1P3, higher concentrations of long-chain MUFA and PUFA (i.e. oleic, linoleic, and α-linolenic acids), along with lower concentrations of saturated fatty acids (SFA) (i.e., myristic and palmitic acids) are expected in milk fat because of the higher availability of long-chain unsaturated fatty acid (UFA) precursors in the diet (Collomb et al., 2001). Instead, only palmitic acid percentage varied significantly during the season, being lower in the alpine area in correspondence of lower abundances of dicotyledonous such as *Fabaceae* and *Polygonaceae* species. Since it is well known that feeding has a dominant role on milk FA composition if compared to animal-related factors (Palmquist et al., 1993) and that other factors (e.g., altitude, botanical diversity) can affect milk FA as well (Leiber et al., 2005), it is hypothesised that the overall conditions of the grazing areas coupled with their botanical and nutritional composition concurred to the values observed in milk FA. In particular, the synthesis of short- and medium-chain FA by the mammary gland usually declines notably when cows are in negative energy balance and when a proportionately high dietary fibre causes digestive modifications in the animals, as during alpine grazing, confirming that the A2P2 grass NEl was likely insufficient to cover animal energy needs (Palmquist et al., 1993; Khanal et al., 2008). Although animal intake was not taken into account in the present study, it is also reasonable to hypothesise that herbage intake varied during the grazing season, being lower as expected in A2P2 because of the effect of alpine grazing conditions (Leiber et al., 2006). Finally, the correlations found between milk FA composition and the SC of some botanical families support the role played by these families in the synthesis of some FA pools (Collomb et al., 2002b). In particular, *Fabaceae* and *Cyperaceae* abundances confirmed to be positively correlated, while *Poaceae* and *Polygonaceae* were negatively correlated, with some representative individual FA as well as MUFA and PUFA in milk. By contrast, no significant correlations involved other dicotyledonous families (e.g., *Asteraceae*, *Apiaceae,* and *Rosaceae*) whose presence has been reported to increase the level of PUFA in other studies (Collomb et al., 2002b; De Noni and Battelli, 2008).

In conclusion, considering pasture and milk compositions together allowed understanding which factors normally affect animal performance during traditional transhumances of dairy cow herds. In mountain and alpine areas with high-diverse pasture types, botanical and PV evaluations could be not sufficient, *per se*, to assure a constant herbage quality to dairy cows. Managing the botanical composition along with the timing of grazing can be an effective strategy for optimizing the quality of the herbage offered to ruminants throughout a grazing season, in particular for vegetation types dominated by *Poaceae* species. Early exploitation in both mountain and alpine areas would generally lead to higher herbage energy values, improving animal performances and the quality of milk and derived dairy products.

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