H, C, N and O stable isotope characteristics of alpine forage, milk and cheese

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Abstract

H, C, N, O stable isotope ratios of forage, milk and the corresponding cheese were analysed to define the isotopic characteristics of two typical mountain cheeses produced in two different terroirs with different types of vegetation. δ13C was shown to be mainly influenced by the presence of C4 plants in animal diet and, in the case of C3 plant-based forage it was below −23‰ varying according to the site and herbage type. δ15N is more related to vegetation type and confirmed to be lower than +4‰ for alpine products. The altitude of the origin area influences δ18O and δ2H. The isotopic values of milk and cheese casein were not significantly different, except δ18O, while they were both significantly different from the values of forage, except δ2H. Using the combined stable isotope ratios a good discrimination level between the products from two different types of pasture at two mountain sites was achieved.

1. Introduction

The stable isotope ratios of H, C, N, and O in animals and animal products such as milk and cheese reflect those in the diet and drinking water (Camin et al., 2008, Chesson et al., 2010, DeNiro and Epstein, 1978, DeNiro and Epstein, 1981 and Perini et al., 2009), changing according to the tissue or class of compounds considered (e.g., lipids) (Knobbe et al., 2006, McCutchan et al., 2003 and Metges et al., 1990). As the isotopic ratios of diet constituents and water are related to local environment, isotope ratio analysis has recently been used to discriminate between milk and cheese produced in different areas, with different dietary regimes and production systems (organic or conventional) (Brescia et al., 2005, Bontempo et al., 2011, Camin et al., 2004, Chesson et al., 2010, Crittenden et al., 2007, Engel et al., 2007, Kornexl et al., 1997, Manca et al., 2001, Manca et al., 2006, Molkentin, 2009, Molkentin and Giesemann, 2007, Pillonel et al., 2003 and Renou et al., 2004).

The 13C/12C isotope ratio (expressed as δ13C) of milk and cheese components is related to the quantity of maize, which is an important constituent of cows' diet in many countries, especially in intensive breeding systems, but is often also used for energy supplementation in other dairy systems. Maize is a C4 plant with higher 13C content (δ13C: −12 ÷ −14‰) than C3 plants (δ13C: −23 ÷ −30‰) (Camin et al., 2004, Camin et al., 2008, Crittenden et al., 2007, Knobbe et al., 2006, Kornexl et al., 1997, Masud et al., 1999, Metges et al., 1990, Minson et al., 1975, Molkentin, 2009, Molkentin and Giesemann, 2007, Molkentin and Giesemann, 2010 and Sacco et al., 2009). Each 10% increase in maize content in the diet corresponds to a 0.7–1.0‰ increase in the δ13C of casein and a δ13C maximum threshold value of −23.5‰ is suggested for milk casein, above which the presence of maize in the diet cannot be excluded (Camin et al., 2008).

The 15N/14N ratio (expressed as δ15N) of milk and cheese reflects agricultural conditions, e.g., different fertilisation practices, which vary between regions, closeness to the sea, drought in the area and the presence of leguminous species in the diet (Camin et al., 2004, Crittenden et al., 2007, Knobbe et al., 2006, Kornexl et al., 1997, Manca et al., 2001, Molkentin and Giesemann, 2010 and Pillonel et al., 2003). Mountain products from grass-fed cows show δ15N values lower than 4‰ (Bontempo et al., 2011, Camin et al., 2004 and Pillonel et al., 2003).

The δ18O value of milk water records the isotopic composition of drinking water and water intake from fresh forage, with minor deviations due to the contribution of food and atmosphere oxygen to body water (Chesson et al., 2010). As the 18O content of local water is related to the climatic and geographical features of an area (temperature, humidity, rainfall, distance from the sea, altitude, latitude), milk water δ18O can distinguish between milk produced in different areas (Chesson et al., 2010, Crittenden et al., 2007 and Engel et al., 2007). When cows eat fresh herbage containing water enriched in 18O due to evapo-transpiration, milk δ18O increases considerably (Kornexl et al., 1997 and Renou et al., 2004). The 2H/1H and 18O/16O ratios of dairy casein are related to the δ18O of milk water (Camin et al., 2008) and they can therefore be used to discriminate between regions (Pillonel et al., 2003), as also observed for meat proteins (Camin et al., 2007, Heaton et al., 2008 and Perini et al., 2009).
In recent years the knowledge about production chain processes has assumed a growing importance as a working basis to increase the value of local products both in terms of their qualification and traceability. Nevertheless, concerning mountain dairy products in particular, scientific research has often focused on specific segments of the production chain. This study offers an analysis of all the components involved in the whole production chain (from vegetation to milk and the relevant cheese), to investigate the relationship between the alpine environment, available vegetation types and the isotopic ratios of milk and dairy products. With this scope, two typical mountain cheeses ("Nostrale d'alpeggio" and "Asiago d'allevo") produced in two terroirs in the south-west and south-east of the Alps were investigated, considering milk and the cheese from cows grazing on different vegetation types (a terroir is a mixture of the natural environment – mainly expressed through the vegetation – farming practices and human know-how, which may lead to the production of typical cheese: Barham, 2003). This paper focuses on H, C, N, and O isotope ratios in animal forage and in milk and cheese casein and on the 18O/16O of milk water. To our knowledge, neither modification of the stable isotope ratios taking place in the process from milk to the corresponding cheese nor the impact of vegetation type in a narrow area on stable isotope ratios has been thoroughly investigated.

2. Material and methods

2.1. Sampling

The experiment was carried out at two sites: (i) Alpe Valcavera (AV, Cuneo district; latitude 44°22'54" and longitude 7°6'43", altitude: 1900–2500 m asl), located in the south-west of the Italian Alps; (ii) Malga Dosso di Sotto (MDS, Vicenza district; latitude 45°57'44" and longitude 11°24'10", altitude: 1647 m asl), located in the south-east of the Italian Alps. At each site two herds of 6–8 dairy cows (H1, H2) at late lactation adapted to alpine grazing environments grazed on two vegetation types. The breed of the cows was Italian Red Pied and Tarantaise in AV and Brown Swiss in MDS. Studies of the literature on beef, lamb and pig (as examples: Camin et al., 2007, Harrison et al., 2011, Horacek and Min, 2010, Knobbe et al., 2006, Molkentin, 2009, Molkentin and Giesemann, 2007, Molkentin and Giesemann, 2010 and Tuross et al., 2008) did not highlight a great influence of breed on the variability of stable isotope ratios of bioelements. At the AV site (details on AV vegetation are reported in Falchero et al., 2010) they fed on an oligotrophic type mainly made up of Trifolium alpinum, Nardus stricta, and Carex sempervirens (AV-T), and a mesophilous type with Festuca gr. rubra, Alchemilla xanthochlora, and Phleum alpinum (AV-F). At the MDS site (details on MDS vegetation are reported in Lombardi, Cavallero, & Bassignana, 2011) they fed on a mesophilous mesotrophic type with Agrostis tenuis, Achillea millefolium, Trifolium repens (MDS-A), and a mesophilous oligotrophic type with Festuca gr. rubra, A. tenuis, Potentilla crantzii (MDS-F). Two homogeneous areas for each vegetation type were fenced in and used by H1 and H2 following an AB/BA cross-over experimental design at each site (Kenward & Jones, 2003). The vegetation samples were collected on the first day of the adaptation (day 1 and 13) and test (day 7 and 19) periods. The herds grazed in one vegetation area (H1 in

AV-T and MDS-A; H2 in AV-F and MDS-F), milk being collected for 6 days (days 7–12) after an adaptation period of 6 days (days 1–6). Subsequently the two herds were swapped over (H1 in AV-F and MDS-F and H2 in AV-T and MDS-A) and milk was again collected (days 19–24) after 6 days of adaptation (days 13–18). The cows grazed for full days and received no supplements except mineral supplements in AV, whereas in MDS they received supplements based on maize (around 25% of dry feed matter), which were not collected and analysed in this study. Milk samples were taken daily by combining the milk from evening and morning milkings.

For each treatment, 32 forage samples, 96 milk samples and the corresponding cheese following 3-month ripening (96 samples) were collected during the summers of 2007 and 2008. In AV, the location selected for vegetation T in 2008 was at a lower altitude than in 2007, due to late vegetation growth at higher altitude. The two cheeses were cooked curd cheeses: "Nostrale d'alpeggio" from Alpe Valcavera and "Asiago d'allevo" from Malga Dosso di Sotto.

2.2. Methods

The forage was ground with 1093 Cyclotec Sample Mill (screen 1 mm, Foss, Padua, Italy). Milk and cheese were stored at −20 °C until analyses. Fat was removed from 100 mL of milk by centrifugation (ALC PK 131R, Thermo Electron Corporation, Karlsruhe, Germany; 3712 × g, 10 min) and casein was precipitated by acidification at pH 4.3 using 2 m HCl. After centrifugation, the precipitate was rinsed with water and extracted once with acetone and three times with a mixture of petrol ether:ethyl ether (2:1) using a homogeniser (Ultraturrax®, model X-620, Staufen, Germany; 14,635 × g, 3 min) for extraction and a centrifuge (3082 × g, 6 min) for separation of the solvent. The residue made of casein was lyophilised and conserved at room temperature until analysis. Four grams of cheese were extracted three times with 30 mL of the petrol ether:ethyl ether (2:1) mixture, by homogenising with the Ultraturrax device, using the centrifuge to separate the ether from the residue. The defatted cheese was washed with 20 mL of deionised water using a centrifuge (3082 × g, 3 min) to separate the water. The residue made of casein was lyophilised and conserved at room temperature until analysis.

The 13C/12C and 15N/14N ratios in forage (≈1.5 mg) and casein (≈0.5 mg) were measured simultaneously using an Isotope Ratio Mass Spectrometer (IRMS) (DELTA V, Thermo Scientific, Bremen, Germany) following total combustion in an Elemental Analyser (EA Flash 1112, Thermo Scientific), according to the procedures described in previous publications (Camin et al., 2004 and Camin et al., 2008). The 2H/1H and 18O/16O ratios in both forage (≈0.8 mg) and casein (≈0.5 mg) were measured using an IRMS (DELTA XP, Thermo Scientific) coupled with a Pyrolyser (high Temperature Conversion Elemental Analyzer, TC/EA, Thermo Scientific), following the method described elsewhere for meat protein (Perini et al., 2009). The 18O/16O in milk water was measured in 2 mL of bulk milk after equilibration with CO2 using an ISOPREP 18 (VG Isotech, Middlewich, UK) on-line preparation system that allows CO2/H2O equilibration, interfaced to an IRMS (SIRA II, VG Isogas, Middlewich, UK) according to the water equilibration method described for wine (OIV, 2009). The values were expressed in δ‰ (=[(Rsample − Rstandard)/Rstandard]*1000, where R is the ratio between the heavier isotope and the lighter one) against international standards (Vienna-Pee Dee Belemnite (V-PDB) for δ13C, Air for δ15N, Vienna-Standard Mean Ocean Water (V-SMOW) for δ2H and δ18O). For the calculation of δ‰, casein and water working in-house standards were used to calibrate against international reference materials: l-glutamic acid USGS 40 (IAEA-International Atomic Energy Agency, Vienna, Austria), fuel oil NBS-22 (IAEA) and sugar IAEA-CH-6 (IAEA) for 13C/12C; l-glutamic acid USGS 40 for 15N/14N; V-SMOW (IAEA) for 18O/16O in water and benzoic acid (IAEA-601) for casein. The 2H/1H values were corrected against the same casein standard with an assigned value of δ2H, according to the 'comparative equilibration technique' (Wassenaar & Hobson, 2003).

The uncertainty (2σ) of measurements was <0.3‰ for the δ13C and δ15N analysis, <3‰ for δ2H, <0.2‰ and 0.6‰ for δ18O in milk water and casein respectively.

2.3. Statistical analysis

The data were analysed using Statistica v 8 (StatSoft Italia srl, Padua, Italy) and SPSS for Windows, Rel. 17.0.0.2008 (SPSS Inc, Chicago, US).

Within the group, normal distribution of data was checked using the Kolmogorov–Smirnov test. Both parametric and non-parametric tests were used: General Linear Models (GLM) and Unequal N Tukey HSD (Honestly Significantly Different) to identify statistical differences between the groups, Pearson test to check the significance of correlations; Kruskall-Wallis and multiple bilateral tests, Spearman test. To limit redundancy the results of non-parametric tests are not reported as they are not different from the ones of the parametric tests.

To check if the combined isotopic parameters could be used to distinguish between milk and cheese from the four different pasture types and the two mountain areas, we carried out a multivariate canonical discriminant analysis. Discrimination robustness was tested using the following cross-validation procedure: a subset of the analysed samples marked as 'unknown' was classified using a model built on the basis of the remaining cases (Camin et al., 2010). Specifically, eight different sets of samples (three in each group) were removed from the dataset and subsequently the model was calculated each time using the remaining cases to check whether excluded samples were assigned to the correct group.

3. Results and discussion

3.1. Stable isotope composition of forage, milk and cheese

In Table 1 the mean and standard deviation of the isotopic parameters of forage, milk and cheese samples are shown, grouped by site and vegetation type.

The Kolmorgorov–Smirnov test found that the data were, in most cases, normally distributed within the groups. As parametric and non-parametric tests generally gave the same results, only the results of the parametric tests are given.

Analysis of variance performed on the dataset using the General Linear Model (GLM) identified the type of sample (forage, milk, cheese) and vegetation type (AV-T, AV-F, MDS-F, MDS-A) as significant variability factors, but not the year.

Unequal N Tukey HSD test and GLM found that the values of milk and cheese casein were not significantly ($p < 0.001$) different, except for δ18O, and were significantly correlated with each other ($r = 0.89$ for δ13C; 0.87 for δ15N; 0.84 for δ2H; 0.67 for δ18O). Moreover, they were significantly ($p < 0.001$) different from those of forage, except for δ2H. This means that in general, there is significant isotope fractionation in the animal, in comparison with the feed but there is no isotopic fractionation during cheese-making, as previously hypothesized but never verified experimentally to our knowledge. There did not seem to be a reasonable explanation for the fact that cheese and milk δ18O were shown to be statistically different (mean value: 10.5‰ vs 11.5‰). Further studies are necessary to confirm and explain these findings.

On the basis of these findings, we grouped the milk and cheese samples and the two years together for further consideration.

In Fig. 1 the Box Plot Whisker graphs and the results of the multiple comparison Unequal N Tukey HSD test for each variable are shown.

The δ13C values of milk and cheese, but not those of forage, were significantly ($p < 0.001$) influenced by vegetation type (Fig. 1a). The Tukey test showed no differences between MDS samples that were grouped together, probably due to smaller differences between MDS-A and MDS-F vegetation compared to AV-T and AV-F. The δ13C values in casein were 2.9 (±0.3)‰ and 4.2 (±0.4)‰ higher than in forage for AV and

MDS respectively. In the literature, an increase in the value of δ13C passing from the diet to the animal product in the range of 1–4‰ is reported for milk from grass-fed cows, while maize feeding results in a decrease of up to −4‰ (Camin et al., 2004, Camin et al., 2008, Knobbe et al., 2006, Masud et al., 1999, Metges et al., 1990 and Minson et al., 1975). The increase in MDS δ13C could be related both to isotope fractionation during feed protein conversion into animal and milk protein, and to maize supplementation, which is known to have a significantly higher 13C content (−12 to −14‰). Maize supplementation also justifies the higher δ13C values in MDS milk and cheese casein, as compared with AV milk and cheese casein, reversing the trend observed in the forage. In AV, where the diet did not include maize, δ13C values in casein ranged between −24.6 and −23.0‰, thus often below the δ13C maximum threshold value of −23.5‰ for casein from animals not fed with maize (Camin et al., 2008). Only in AV-T 2008 the milk and cheese samples collected at the beginning of exploitation had values above the threshold (data not shown), probably because of the influence of dietary composition during the previous period. Indeed, an adaptation period of 6 days is not sufficient for a complete turnover of C, as already observed by other authors (Camin et al., 2008 and Wilson et al., 1988).

The δ15N values of forage, milk and cheese were mainly influenced by vegetation type (Fig. 1b). The vegetation of AV-T and MDS-F contained a higher percentage of leguminous plants (mainly Trifolium species) resulting in lower δ15N values for forage, milk and cheese, as leguminous plants fix air-N, which has a δ15N equal to 0‰ (Martinelli et al., 1992 and Yoneyama et al., 1984), in agreement with values reported in the literature (Knobbe et al., 2006, Martinelli et al., 1992 and Steele et al., 1983). The δ15N values of casein were 4.6‰ ± 0.7 higher than those of forage, mainly in MDS (5.1‰, as compared with 4.1‰ in AV). The differences between δ15N values were slightly greater than reported in the literature (1–4‰: Camin et al., 2004, Camin et al., 2008, Knobbe et al., 2006, Masud et al., 1999 and Steele and Daniel, 1978). As observed for δ13C, maize in the MDS diet could result in higher δ15N values, because the content of 15N in maize (not measured here) is normally higher than in grazing vegetation (Camin et al., 2008 and Knobbe et al., 2006). Casein δ15N between 2.5‰ and 4.2‰ confirmed the maximum limit of 4‰ for mountain dairy products.

The δ18O values of milk and cheese were affected by pasture type ($p < 0.001$, Fig. 1c), although sample sharing in the groups suggests that the site was more influential than the vegetation. The same difference, i.e., lower AV values in comparison to MDS, was also observed for milk water (Fig. 1d), whose isotopic composition is related to that of the drinking water (Chesson et al., 2010), depending on latitude, altitude, distance from the evaporation source, temperature and the amount of precipitation (Clark and Fritz, 1997 and Van der Veer et al., 2009). As the two sites were at almost the same latitude, the aforementioned differences in all the δ18O values were probably due to differences in altitude at the sites, as at higher altitude there is lighter water vapour (hence with lower δ18O). Conversely vegetation did not affect the δ18O of bulk forage (Fig. 1c).

Trends similar to δ18O were observed for δ2H (Fig. 1e). In fact we found a significant relation ($p < 0.001$) between δ18O and δ2H values for forage (δ2H: 6.52 × δ18O − 251.69, R2: 0.40), milk (δ2H: 3.66 × δ18O − 152.96, R2: 0.55), and cheese (δ2H: 2.67 × δ18O − 139.77; R2: 0.32). Moreover milk casein δ2H could be predicted using the δ18O of milk water (δ2Hcas = 3.19 × δ18Omilk water − 101.16, R2 = 0.60), which also effectively predicts milk casein δ18O (δ18Ocas = 0.67 × δ18Omilk water + 13.56, R2 = 0.65), as found in previous experiments (Camin et al., 2008).

The experimental results substantiate the hypothesis that both the H and O isotope composition of casein can record geography, as observed in human hair keratin (Ehleringer et al., 2008) and in animal protein (Heaton et al., 2008 and Perini et al., 2009).

3.2. Discrimination between production area and vegetation type of alpine milk and cheese

To check if the combined isotopic parameters could be used to distinguish between milk and cheese from the four different pasture types, we carried out a multivariate canonical discriminant analysis. Milk and cheese scores for the first two canonical variables (CAN) are plotted in Fig. 2. The first two CANs account for 98% and 99% of the variability for milk (Fig. 2A) and cheese (Fig. 2B) respectively. For both, CAN1 is mainly loaded negatively by casein δ13C and δ18O and separates the two sites (AV versus MDS), whereas CAN2 is mainly determined positively by δ13C and negatively by δ15N and divides the two pasture types into each site. After cross-validation, 83% of milk samples (88% before cross-validation) and 80% of cheese samples (82% before cross-validation) were correctly classified. Considering only the site of origin, 96% of the milk and cheese samples were correctly discriminated.

4. Conclusions

The results demonstrate that the stable isotope ratios of H, C, N and O of milk and cheese are linked to the terroir, in particular to the type of vegetation and the environment. The combination of the four stable isotope ratios made it possible to separate dairy products from two alpine sites and from different pasture types within each site.

The study moreover confirms that alpine milk and cheese from grazing cows, without maize supplementation for more than two weeks before milk collection, are characterised by δ15N lower than +4‰ and δ13C lower than −23.5‰. In different mountain cheeses, δ2H and δ18O can be used to distinguish the altitude of the provenance area, δ15N to determine different grazing vegetation types and in the case of dietary supplements δ13C can determine different amounts of maize in the animal diet.

In this work, the possibility of differentiating between the two mountain sites is mainly useful in the case of milk, because the two cheeses can also be distinguished visually. On the other hand, the ability of stable isotope analysis to separate different vegetation types is to some extent advantageous for both milk and cheese.

Acknowledgements

This work was entirely funded by the Italian Ministry of Economy, University, Agriculture and Economic Development Research within the frame work of the “ProAlpe” research project.

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Table 1
Mean and standard deviation of the isotope ratios of bulk forage, milk and cheese casein and milk water.

Site/vegetation[a]	Feed/product[b]	Isotope ratios[c]				
		$\delta^{13}C$	$\delta^{15}N$	$\delta^{2}H$	$\delta^{18}O$	$\delta^{18}O_{water}$
AV-F 07	Forage	−26.9 ± 0.1	−0.2 ± 0.7	−122 ± 4	20.6 ± 0.4	
	Milk	−24.2 ± 0.1	3.4 ± 0.4	−118 ± 5	10.8 ± 1.1	−5.1 ± 0.2
	Cheese	−24.0 ± 0.2	3.2 ± 0.4	−119 ± 2	9.9 ± 0.9	
AV-T 07	Forage	−26.5 ± 0.1	−1.3 ± 0.5	−116 ± 3	21.4 ± 0.8	
	Milk	−23.9 ± 0.3	3.0 ± 0.3	−118 ± 4	11.1 ± 1.2	−3.1 ± 2.1
	Cheese	−23.9 ± 0.2	3.0 ± 0.5	−118 ± 2	10.0 ± 0.9	
MDS-F 07	Forage	−26.9 ± 0.1	−2.3 ± 0.5	−108 ± 2	21.6 ± 0.6	
	Milk	−23.1 ± 0.2	3.4 ± 0.1	−109 ± 4	12.6 ± 0.9	−2.3 ± 0.9
	Cheese	−23.0 ± 0.1	3.4 ± 0.1	−110 ± 2	11.7 ± 1.3	
MDS-A 07	Forage	−27.1 ± 0.1	−0.8 ± 0.7	−111 ± 2	21.9 ± 0.1	
	Milk	−23.3 ± 0.2	3.8 ± 0.2	−105 ± 7	13.7 ± 1.3	−0.5 ± 1.4
	Cheese	−23.2 ± 0.3	3.7 ± 0.4	−109 ± 4	12.1 ± 1.3	
AV-F 08	Forage	−26.6 ± 0.6	−1.8 ± 0.5	−109 ± 1	21.9 ± 0.7	
	Milk	−23.9 ± 0.2	3.4 ± 0.3	−116 ± 1	9.9 ± 0.6	−4.4 ± 0.3
	Cheese	−23.2 ± 0.3	3.4 ± 0.3	−115 ± 1	9.4 ± 0.4	
AV-T 08	Forage	−26.7 ± 0.4	−0.6 ± 1.3	−99 ± 5	22.4 ± 0.3	
	Milk	−23.3 ± 0.2	2.9 ± 0.2	−115 ± 2	9.8 ± 0.7	−4.8 ± 0.5
	Cheese	−23.4 ± 0.2	2.9 ± 0.1	−115 ± 3	9.3 ± 0.6	
MDS-F 08	Forage	−27.7 ± 0.4	−2.0 ± 0.7	−107 ± 4	22.1 ± 0.4	
	Milk	−23.0 ± 0.2	3.5 ± 0.3	−104 ± 2	11.9 ± 0.7	−2.2 ± 0.5
	Cheese	−23.1 ± 0.2	3.5 ± 0.3	−104 ± 2	10.6 ± 0.8	
MDS-A 08	Forage	−27.6 ± 0.2	−0.9 ± 0.5	−109 ± 5	22.0 ± 0.5	
	Milk	−23.1 ± 0.1	3.8 ± 0.2	−104 ± 2	12.4 ± 0.4	−1.3 ± 0.3
	Cheese	−23.1 ± 0.2	3.9 ± 0.2	−103 ± 2	11.5 ± 0.6	

[a] Site/vegetation key: AV, Alpe Valcavera; MDS, Malga Dosso di Sotto; F, mesophilous vegetation type; T, oligotrophic vegetation type; A, mesophilous mesotrophic vegetation type; 07, 2007 summer; 08, 2008 summer.
[b] Number of samples per item were: forage, 4; milk and cheese, 12.
[c] Values are: ‰ versus V-PDB for $\delta^{13}C$; ‰ versus AIR for $\delta^{15}N$; ‰ versus V-SMOW for $\delta^{2}H$, $\delta^{18}O$ and $\delta^{18}O_{water}$.

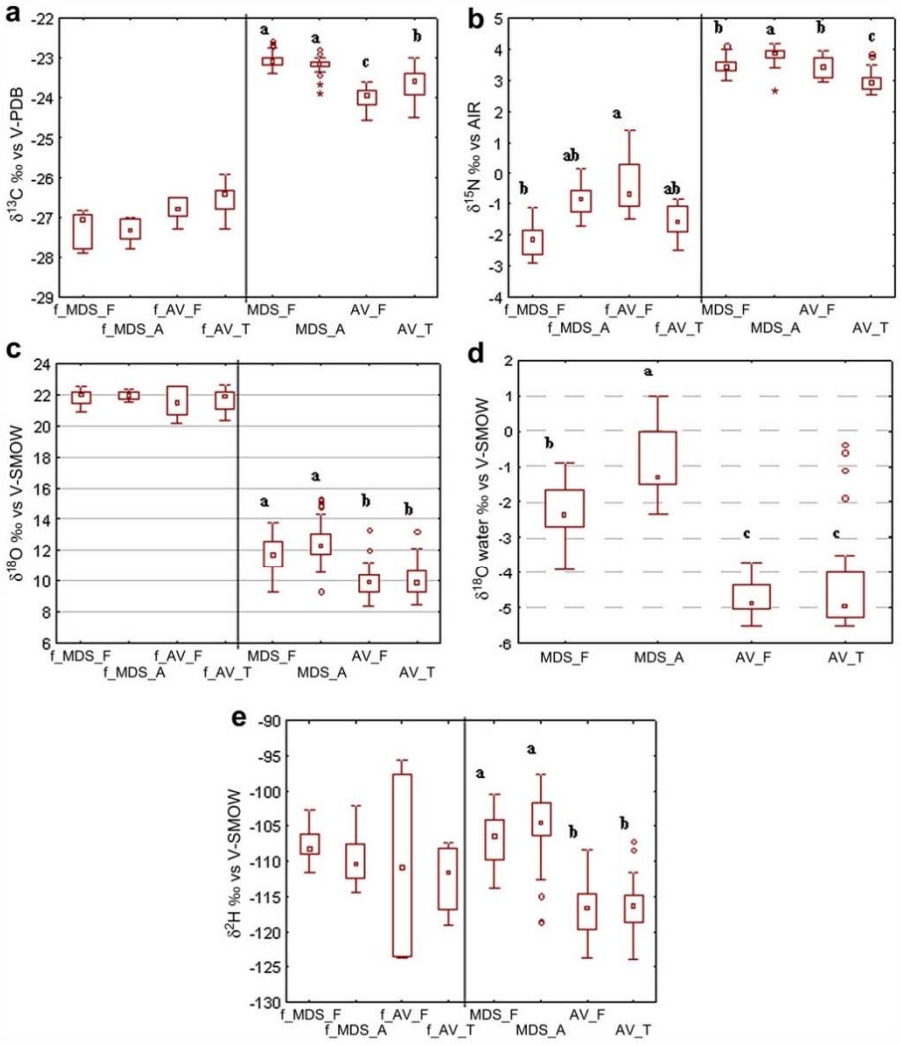

Fig. 1. Box Plot Whisker graphs and results of the Unequal *N* Tukey HSD test on the isotopic values (a, $\delta^{13}C$; b, $\delta^{15}N$; c, $\delta^{18}O_{casein}$; d, $\delta^{18}O_{water}$; e, $\delta^{2}H$) of forage (indicated by the prefix "f") and milk and cheese casein (no prefix) collected in Alpe Valcavera (AV) and Malga Dosso di Sotto (MDS) and produced on different vegetation types, mesophilous (F), oligotrophic (T), and mesophilous mesotrophic (A). Mean values with different letters are significantly different ($p < 0.001$): ▫ median; □, 25%–75%; ⊥, sp. not-outlier; ○, outlier; ✱ extreme.

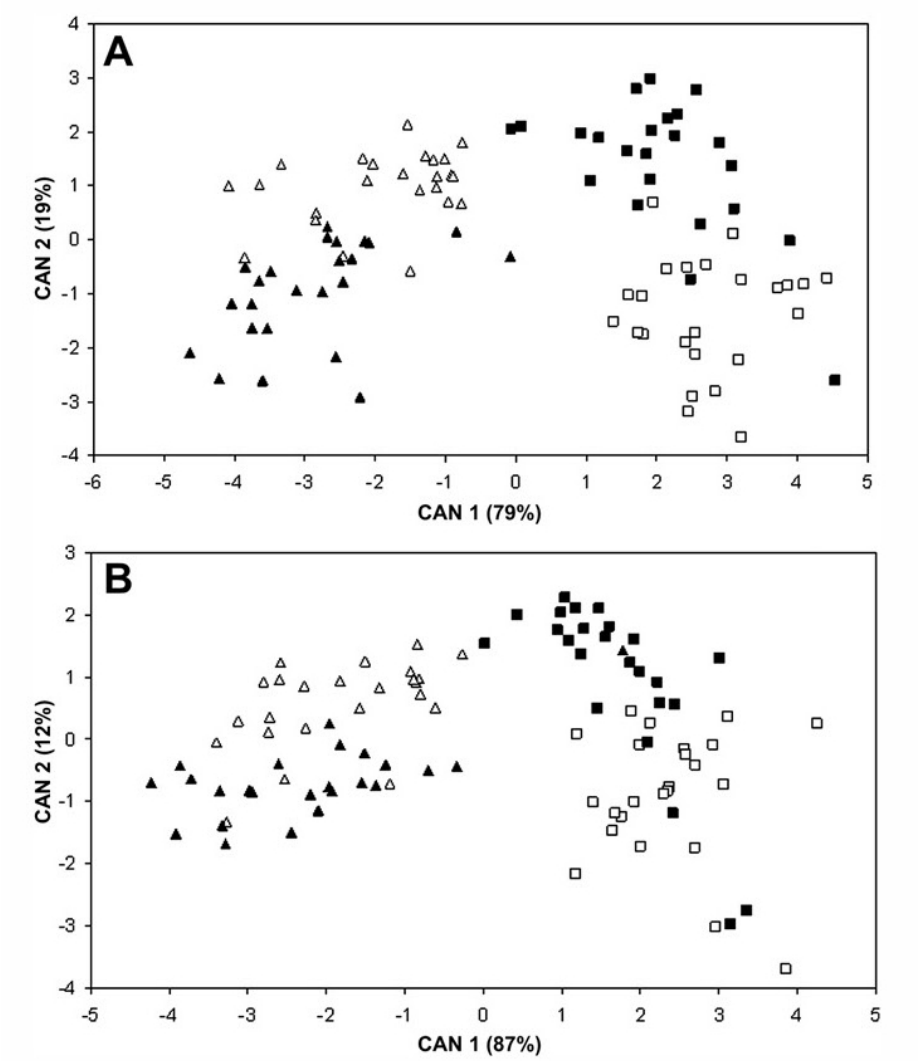

Fig. 2. Canonical Discriminant analysis of (A) milk data and (B) cheese data ($N = 96$): plot of the first two canonical variables (CAN 1 versus CAN 2) with loadings in brackets. The samples were collected in Alpe Valcavera (AV) and Malga Dosso di Sotto (MDS) and produced on different vegetation types, mesophilous (F), oligotrophic (T) and mesophilous mesotrophic (A): □, AV-F; ■, AV-T; Δ, MDS-F; ▲; MDS-A.