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Abstract

The present study investigated the influence of botanically diverse pastures grazed by dairy ewes on the fatty acid (FA) composition of Protected Designation of Origin (PDO) Murazzano cheese. Twelve multiparous Delle Langhe ewes beyond peak of lactation were blocked according to stage of lactation, parity, milk yield, gross composition, and FA profile of milk fat, and randomly assigned to two groups. The groups were allowed to graze for three weeks on two different mountain vegetation types, either dominated by *Lolium perenne* L. and *Trifolium repens* L. (LT) or by *Bromus erectus* Huds., *Festuca* gr. *rubra* L. and *Thymus serpyllum* L. (BFT); both groups were also supplemented with 0.4 kg head^{-1} day^{-1} of concentrate during milking. Each week, bulk milk obtained from the two groups was separately used on three consecutive days to produce a total of eighteen Murazzano PDO cheeses. The FA profile of cheese was significantly influenced by the pasture vegetation type. Murazzano cheese manufactured with LT milk showed higher concentrations of total polyunsaturated FA, n3 FA, n6 FA, *trans*-C18:2 and -C18:1 biohydrogenation intermediates of dietary unsaturated FA, while cheese manufactured with BFT milk was significantly richer in branched-chain FA. The botanical composition of grazed pastures, significantly influencing the FA composition and nutritional quality of cheese, appears to be a key factor in the assessment of dairy products traceability.

Keywords: Botanical composition – Grazing – Lipids – Ovine cheese – Plant secondary metabolites

Introduction

Some studies have revealed that the botanical composition of forages can significantly affect the fatty acid (FA) composition of milk and cheese (Lourenço et al., 2010). Such an effect is even more important for quality identified dairy products (e.g., Protected Designation of Origin – PDO – cheeses) because local forage-based diets strictly bind them with their geographical production site. The goal of this study was to investigate the influence of botanically diverse pastures grazed by dairy ewes on the FA profile of Murazzano PDO cheese.

Materials and methods

The experiment was carried out from May 26 (day 1) to June 23 (day 29) in a dairy sheep farm located in North-Western Italy (latitude: 44°26'46" N; longitude: 08°01'25" E). Twelve multiparous Delle Langhe ewes in mid-lactation were divided into two balanced groups according to their stage of lactation, lactation number, milk yield, milk gross composition, FA profile of milk fat, and estimated Δ9-desaturase activity. The groups were then randomly allowed to graze one of two mountain pastures, dominated by i) *Lolium perenne* L. and *Trifolium repens* L. (LT) and by ii) *Bromus erectus* Huds., *Festuca* gr. *rubra* L., and *Thymus serpyllum* L. (BFT). The ewes grazed during day and night. They were moved indoors twice daily for milking, when they were supplemented with 0.2 kg $head^{-1}$ of concentrate.

The botanical composition of LT and BFT pastures was assessed before exploitation (Daget and Poissonet, 1971). Herbage samples were collected three times during the trial (on days 14, 21, and 28) and analysed for dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), starch and FA profile as reported by Renna *et al*. (in press).

Separate bulk milk tanks (one per treatment) were used on three consecutive days for three weeks (from June 7 to June 23) to produce a total of eighteen Murazzano PDO cheeses. After a six-day ripening period, cheese samples were collected and analysed for FA profile. Cheese total lipids extraction, and fatty acid methyl esters separation, identification and quantification were performed as described by Renna *et al*. (in press).

Herbage and cheese data were subjected to analysis of variance using the GLM procedure of the SAS software package, version 9.1.3 (SAS Institute

Inc., Cary, NC, USA). Significance was declared at P≤0.05.

Results and discussion

The specific contribution (SC, percentage presence of each botanical species) in LT and BFT pastures is reported in Table 1. More than 90% of the species belonged to Poaceae (52%), Fabaceae (32%) and Geraniaceae (7%) in LT and to Poaceae (68%), Lamiaceae (17%) and Fabaceae (7%) in BFT. No significant differences were observed in DM, EE and starch contents between pastures. LT vegetation type showed higher CP (11.0 *vs* 8.6% DM; P<0.05) and lower NDF (64.4 *vs* 68.5% DM; P<0.01) content compared to BFT. The concentration of main FA also differed between pastures (C16:0, 298 *vs* 218 mg $100g^{-1}$ DM; C18:2 *c*9*c*12, 408 *vs* 299; C18:3 *c*9*c*12*c*15, 731 *vs* 564, in LT and BFT, respectively).

The FA composition of Murazzano PDO cheese was significantly affected by the pasture vegetation type (Table 2). LT cheeses were significantly richer in total polyunsaturated fatty acids (PUFA), total n3 FA, total n6 FA, total *trans*-C18:2, total *trans*-C18:1, linoleic acid (LA, C18:2 *c*9*c*12) and α-linolenic acid (ALA, C18:3 *c*9*c*12*c*15). Branched-chain fatty acids (BCFA), both *iso* and *anteiso* forms, were significantly lower in LT compared to BFT cheeses. Short- and medium-chain saturated FA (C4:0 to C16:0) and odd-chain fatty acids (OCFA, C15:0 and C17:0) showed comparable concentrations in LT and BFT cheeses.

The FA composition of milk and dairy products obtained from pasture-fed ruminants is simultaneously affected by the chemical composition of pastures and the presence and abundance of plant secondary metabolites (PSM) (e.g., polyphenol oxidase – PPO –, phenolic compounds – PC –, essential oils – EO –, etc.). Both factors are able to modify the rumen microbial ecosystem (Lourenço *et al*., 2010).

Table 1. Specific contribution (SC, %) of botanical species and plant secondary metabolites (PSM) in the main botanical species of LT and BFT vegetation types†

LT vegetation type			BFT vegetation type		
Species (Family)	**SC**	**PSM**	**Species (Family)**	**SC**	**PSM**
Lolium perenne L. (Po)	31.6	PPO	*Bromus erectus* Hudson (Po)	36.2	
Trifolium repens L. (Fa)	27.8	SA, PH	*Festuca* gr. *rubra* L. (Po)	24.1	
Bromus sterilis L. (Po)	9.0		*Thymus serpyllum* s.l. (La)	13.8	EO
Bromus hordeaceus L. (Po)	4.5		*Lotus* gr. *corniculatus* L. (Fa)	5.2	CT
Poa trivialis L. (Po)	4.5		*Bromus hordeaceus* L. (Po)	3.4	
Geranium pusillum L. (Ge)	3.8	HT	*Hypochoeris radicata* L. (As)	3.4	PH
Medicago lupulina L. (Fa)	3.8		*Poa annua* L. (Po)	3.4	
Erodium cicutarium (L.) L'Hér. (Ge)	3.0	AL, traces	*Salvia pratensis* L. (La)	3.4	EO
Plantago lanceolata L. (Pl)	3.0	PH	*Plantago lanceolata* L. (Pl)	2.6	PH
Dactylis glomerata L. (Po)	2.3	PPO	*Vicia* gr. *cracca* L. (Fa)	1.7	
Capsella bursa-pastoris (L.) Medicus (Br)	1.5				

† Only species with SC>1% are reported. Po, Poaceae; Fa, Fabaceae; Ge, Geraniaceae; Pl, Plantaginaceae; Br, Brassicaceae; La, Lamiaceae; As, Asteraceae; PPO, polyphenol oxidase; SA, saponins; PH, phenols; HT, hydrolysable tannins; CT, condensed tannins; EO, essential oils; AL, alkaloids

Table 2. Effect of vegetation type grazed by Delle Langhe ewes on selected individual FA and FA groups (g 100g^{-1} fat) detected in Murazzano PDO cheese

	Vegetation type					Vegetation type			
FA	**LT**	**BFT**	**SEM**	**P**	**FA**	**LT**	**BFT**	**SEM**	**P**
ΣSFA	55.20	56.06	1.272	ns	C18:1 *t*6-11	3.02	2.92	0.294	ns
Σ*iso*-BCFA	1.07	1.22	0.087	***	C18:1 *t*12-14+*c*6-8	1.00	0.73	0.153	***
Σ*aiso*-BCFA	1.27	1.38	0.081	***	C18:1 *t*16+*c*14	0.44	0.37	0.038	***
ΣMUFA	20.79	21.05	0.158	ns	C18:2 *t*,*t*-NMID+*t*9*t*12	0.15	0.12	0.018	***
ΣC18:1	19.65	19.88	0.806	ns	C18:2 *c*9*t*13+*t*8*c*12	0.073	0.068	0.013	*
ΣC18:1 *trans*	4.04	3.68	0.358	**	C18:2 *c*9*t*12	0.23	0.19	0.029	**
ΣPUFA	5.59	4.91	0.371	***	C18:2 *c*,*c*-MID+*t*8*c*13	0.23	0.19	0.027	**
ΣC18:2 *trans*	1.93	1.76	0.191	**	C18:2 *t*11*c*15	0.40	0.35	0.055	**
Σn3	1.61	1.36	0.180	***	C18:2 *c*9*t*11+*t*7*c*9+*t*8*c*10	0.79	0.79	0.084	ns
Σn6	4.00	3.31	0.359	***	C18:2 *t*10*c*12	<0.001	<0.001	<0.0001	ns
ΣCLA	0.84	0.84	0.093	ns	C18:2 *t*11*c*13+*c*9*c*11	0.030	0.031	0.009	ns
C4 to C16	39.50	39.58	0.965	ns	C18:2 *t*9*t*11	0.022	0.022	0.003	ns
C15:0	0.84	0.85	0.039	ns	C18:2 *c*9*c*12	2.26	1.96	0.140	***
C17:0	0.67	0.65	0.044	ns	C18:3 *c*9*c*12*c*15	0.98	0.78	0.120	***
C18:0	11.34	11.81	0.721	ns	C20:5 *c*5,*c*8,*c*11,*c*14,*c*17	0.068	0.062	0.005	***

In general, if compared to Poaceae, Fabaceae have shown a higher transfer efficiency of PUFA to milk and cheese (Cabiddu *et al*., 2010), which is consistent with the results obtained in our experiment. The lower BCFA concentration in LT cheeses suggests that the ewes' ruminal microbial activity was inhibited. This could be the consequence of the overall higher richness of biologically active PSM in the LT compared to the BFT pasture (Buccioni *et al*., 2012). The lack of significant differences in OCFA concentrations between LT and BFT cheeses may be related to specific EO (such as carvacrol, thymol and *p*-cymene) occurring in the Lamiaceae species present in the BFT pasture. The above mentioned EO, in fact, have been described to decrease rumen propionate production *in vitro* (Macheboeuf *et al*., 2008) and OCFA can be synthesised *de novo* from propionate in the mammary gland of ruminants (Vlaeminck *et al*., 2006). The significantly higher levels of total PUFA, total n6 FA, total n3 FA, total *trans*-C18:2, total *trans*-C18:1, LA and ALA in LT cheeses could be ascribed to the higher concentrations of LA and ALA in the LT pasture. In addition, *Lolium perenne* and *Dactylis glomerata* were reported to contain relatively high levels of PPO, in its active form (Lee *et al*., 2006). This enzyme, forming protein-bound phenols, can inhibit lipolysis (LP) and can therefore reduce the initial step of dietary unsaturated FA biohydrogenation (BH), leading to increased likelihood of LA and ALA passing unaltered through the rumen (Lejonklev *et al*., 2013). While non-conjugated C18:2 isomers (both methylene interrupted (MID) and non methylene interrupted (NMID) dienes) were significantly more abundant in LT than BFT cheeses, the concentration of conjugated linoleic acid (CLA) isomers was relatively low and not significantly affected by the pastures. Such result may suggest selective inhibition exerted by PSM occurring in LT botanical species on specific microorganisms involved in the ruminal formation of CLA isomers from dietary PUFA, and merits further investigation. Estimated Δ9-desaturase activity (C14:1 *c*9/C14:0 in milk samples, data not shown) was not affected by pasture type. The comparable CLA *c*9*t*11+*t*7*c*9+*t*8*c*10 concentrations in LT and BFT cheeses are consistent with the lack of differences in their precursors (C18:1 *t*7 and C18:1 *t*11) for *de novo* synthesis within the mammary gland.

Conclusions

Differences in the chemical composition and occurrence of plant secondary metabolites in the grazed forages can affect rumen lipolysis and

biohydrogenation in dairy ewes. A strong link exists between the utilization of botanically diverse and locally produced forage resources by Delle Langhe ewes and the fatty acid composition of Murazzano PDO cheese. Pasture biodiversity is a key factor for the characterization and the traceability of this cheese and for the maintenance of its tipicity in relation to the original *terroir*.

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