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Differences in nutritional quality of milk produced from ewes reared in mountain *versus* valley areas

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Abstract. In the present study, fat content and fatty acid (FA) composition of milk from ewes reared in valley and mountain pastures was investigated. Six flocks of Latxa ewes were selected (3 from valley and 3 from mountain) and milk was collected in triplicate in May and June, during the grazing period at late lactation. Mountain milk was fatter than valley milk ($p \leq 0.001$). Interaction between pasture type and sampling week was significant ($p \leq 0.05$) for saturated FA (SFA), monounsatured FA (MUFA), conjugated linolenic acid (CLA) and non-conjugated dienes, where mountain milk provided less SFA, more MUFA, and more CLA, mainly 9*cis(c)*, 11*trans(t)*-18:2. Looking closer at the nutritionally interesting FAs, in all milk samples 11*t*-18:1 was the major *trans*-18:1 isomer (average of 55.6%), while the 10*t*-/11*t*- ratio was below 1 in all milk samples. In addition, mountain milk samples provided higher values ($p \leq 0.001$) for branched-chain FA, *cis*-MUFA and polyunsaturated FAs (PUFA) content, and higher P/S ratio. In general, mountain milk provided higher contents of metabolites related to the biohydrogenation n-3 PUFA like 9*c*,11*t*,15*c*-18:3, 11*t*,15*c*-18:2 and 11*t*,13*c*-18:2 and a lower n-6/n-3 ratio. Therefore, the milk from ewes reared in the mountain grasslands had a healthier FA profile and could provide value-added cheese of commercial benefit.

Keywords. Commercial sheep flocks – Fatty acids – Grassland altitude – Grazing – Vaccenic acid.

Différences de qualité nutritionnelle pour le lait produit par des brebis élevées en zones de montagne **versus** ***zones de vallée***

Résumé. *La présente étude a examiné la teneur en matière grasse et la composition en acides gras (AG) du lait de brebis élevées sur des pâturages de vallée et de montagne. Six troupeaux de brebis de race Latxa ont été sélectionnés (3 pour la vallée et 3 pour la montagne) et le lait a été collecté en triple en mai et juin, durant la période de pâturage en fin de lactation. Le lait lié aux montagnes était plus gras que le lait des vallées ($p \leq 0{,}001$). L'interaction entre le type de pâturage et la semaine de prélèvement des échantillons était significative ($p \leq 0{,}05$) pour les AG saturés (AGS), les AG mono-insaturés (AGMI), l'acide linolénique conjugué (CLA) et les diènes non conjugués, où le lait de montagne a donné moins d'AGS, plus d'AGMI, et plus de CLA, principalement 9cis(c),11trans(t)-18:2. En regardant de plus près les AG d'intérêt nutritionnel, dans tous les échantillons de lait, le 11t-18:1 était l'isomère trans-18:1 majoritaire (en moyenne 55,6%), tandis que le ratio 10t-/11t- était inférieur à 1 dans tous les échantillons de lait. En outre, les échantillons de lait de montagne ont montré des valeurs plus élevées ($p \leq 0{,}001$) pour la teneur en AG à chaîne ramifiée, cis-AGMI et AG poly-insaturés (AGPI), et un ratio P/S plus élevé. En général, le lait de montagne apportait de plus grandes teneurs en métabolites liés aux AGPI n-3 de biohydrogénation tels que 9c,11t,15c-18:3, 11t,15c-18:2 et 11t,13c-18:2 et un ratio n-6/n-3 plus faible. Donc le lait des brebis élevées en pâturages de montagne avait un meilleur profil santé pour les AG et pourrait apporter au fromage une valeur ajoutée commercialement bénéfique.*

Mots-clés. *Troupeaux ovins commerciaux – Acides gras – Altitude des pâturages – Pâturer – acide vaccénique.*

I – Introduction

In the last years, efforts have been made by European countries to give an added value to those food products produced under specific conditions and methods, and protecting these products with quality labels like PDO (Protected Designation of Origin) and others. This is the case for Idiazabal cheese produced in the Basque Country (northern Spain) that is made exclusively from raw milk of Latxa ewes, and has a PDO label. The milking period of Latxa flocks extends from February (early lactation) to late June (late lactation), and after May flocks are managed under extensive grazing. While most flocks during lactation remain in valley grasslands close to farms, in May some flocks are taken to mountain grasslands. It has been previously reported that differences in botanical composition of grasslands located at different altitude may confer differences in milk composition when produced in these areas (Falchero *et al*., 2010). According to some studies, milk and cheese produced in mountain areas are richer in n-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acids (CLA) than those produced in lowland areas (Leiber *et al.,* 2005). In this sense, the objective of the present study was to compare the fatty acid (FA) composition of milk from Latxa ewes reared in valley and mountain grasslands.

II – Materials and methods

For the present study, six commercial flocks of 150-250 Latxa ewes each were selected, three reared in valley and three reared in mountain grasslands. Before sampling, a three week adaptation period was provided to flocks reared on mountain pastures. Tank milk was collected in triplicate and in two different weeks (May and June) during the extensive grazing period at late lactation. Total fat content of milk samples was determined by NIR (ENAC certified method No 174/LE 381; Dairy Institute of Lekunberri, Lekunberri, Spain). For the FA composition, lipids were separated by centrifugation of milk and cream as described in Luna *et al.* (2005), and FA methyl esters (FAME) were prepared using the miniaturized method described in Aldai *et al.* (2012). The FAMEs were analyzed by GC-FID combining the results obtained from a 100 m SP-2560 (175°C and 150°C programs; Kramer *et al*., 2008) and a 100 m SLB-IL111 column (Delmonte *et al*., 2011) from Supelco, Bellefonte, PA, USA. For peak identification purposes, individual FAME standards and retention times and elution orders reported in the literature were used (Alves & Bessa, 2009; Cruz-Hernandez *et al*., 2006; Delmonte *et al*., 2011; Kramer *et al*., 2008). Identifications were also confirmed using FAME fractions obtained using Ag^+-SPE (Belaunzaran *et al.,* 2014; Kramer *et al*., 2008). SPSS IBM Statistics software version 21 (New York, USA) was used for statistical analysis of variance (ANOVA) according to the following fixed effect linear model: $Y = P + S + F(P) + P*S + \varepsilon$ where P was pasture type (valley, mountain), S was sampling week (May, June), F was the flock factor, and P*S was the interaction term. Three significant figures are used to express the data.

III – Results and discussion

Milk collected from flocks reared in mountain pastures provided a higher fat content (g per 100 g of milk) in comparison to milk collected from flocks reared in valley pastures (p≤0.001; Table 1).

Regarding the FA profile of the milk, interactions and the effect of the principal factor (pasture type) affecting the composition will be discussed. The interaction was significant for the major FA groups, saturated FAs (SFAs, p≤0.01) and monounsaturated FAs (MUFA, p≤0.01), and the minor groups, CLA (p≤0.001) and non-conjugated dienes (nc-dienes, p≤0.01; Table 1). The SFA content was significantly higher in milk collected from ewes grazed on valley compared to milk from ewes grazed on mountain pastures. However, the opposite was true for the MUFA content, being higher in mountain milk. A significant interaction was evident for the *trans*-MUFA content where mountain milk provided higher levels of *trans* than valley milk. Interestingly, 11*t*- and 13*t*/14*t*- were the major *trans*-18:1 isomers

Table 1. Fat content (g/100g of milk) and fatty acid composition (g/100g fat) of milk samples from ewes reared in valley and mountain pastures and collected in May and June

	Valley		Mountain			Significance		
	May	June	May	June	SEM	P	S	P*S
Fat	7.43	7.96	8.34	8.55	0.0870	***	*	ns
SFA	62.5	58.7	56.4	55.1	0.381	***	***	**
BCFA	2.73	2.68	2.89	2.86	0.0383	***	ns	ns
MUFA	26.1	29.4	29.6	31.1	0.269	***	***	**
cis-MUFA	20.5	23.2	21.7	23.9	0.268	***	***	ns
trans-MUFA	5.58	6.18	7.95	7.21	0.104	***	ns	***
10*t*-18:1	0.389	0.412	0.404	0.380	0.00682	ns	ns	**
11*t*-18:1	2.44	2.79	4.39	3.74	0.0851	***	*	***
13*t*/14*t*-18:1	0.701	0.697	0.754	0.714	0.00890	*	ns	ns
10*t*-/11*t*-	0.171	0.152	0.0924	0.103	0.00514	***	ns	*
PUFA	3.32	3.45	4.02	4.22	0.0491	***	*	ns
n-6	2.17	2.25	2.18	2.31	0.0270	ns	*	ns
18:2n-6	1.72	1.70	1.69	1.81	0.0243	ns	ns	*
20:4n-6	0.150	0.171	0.169	0.166	0.00213	*	*	**
n-3	1.15	1.20	1.84	1.91	0.0301	***	ns	ns
18:3n-3	0.816	0.828	1.36	1.43	0.0228	***	ns	ns
22:5n-3	0.147	0.155	0.221	0.229	0.00425	***	ns	ns
n-6/n-3	1.92	1.93	1.19	1.22	0.0376	***	ns	ns
P/S	0.0539	0.0603	0.0722	0.0776	0.00110	***	***	ns
CLA	1.69	2.04	2.81	2.62	0.0561	***	ns	***
9*c*,11*t*-	1.39	1.68	2.39	2.22	0.0507	***	ns	***
7*t*,9*c*-	0.0473	0.0558	0.0555	0.0555	0.00102	*	*	*
11*t*,13*c*-	0.0679	0.0844	0.154	0.131	0.00354	***	ns	***
nc-dienes	1.31	1.45	1.77	1.71	0.0281	***	ns	**
9*c*,13*t*-18:2†	0.386	0.421	0.461	0.464	0.00677	***	*	ns
11*t*,15*c*-18:2	0.309	0.323	0.547	0.483	0.0129	***	*	***
nc-trienes	0.292	0.274	0.414	0.376	0.0105	***	*	ns
9*c*,11*t*,15*c*-18:3	0.155	0.131	0.198	0.155	0.00815	***	***	ns
9*c*,11*t*,15*t*-18:3	0.0491	0.0619	0.0830	0.0740	0.00210	***	ns	**

† Coelute with 8*t*,12*c*- and other *c*,*t*-18:2. SEM, standard error of the mean; P, pasture type (valley, mountain); S, sampling week (May, June); P*S, interaction term. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant ($p > 0.05$). SFA, saturated fatty acids; BCFA, branched-chain fatty acids; MUFA, monounsaturated fatty acids; *c, cis*; *t, trans*; PUFA, polyunsaturated fatty acids; P/S, PUFA/SFA; CLA, conjugated fatty acids; nc, non-conjugated.

(Figure 1). Expressed as relative percentages, the interaction was significant for most of the isomers except 10*t*- and 15*t*-18:1. Vaccenic acid (VA, 11*t*-18:1) was the only isomer with higher relative percentages in mountain than in valley milk. In this respect, it is well-known the relationship between pasture based diets and the higher content of VA in dairy products (Valdivielso *et al*., 2015), together with the low 10*t*-/11*t*- ratio (<1 in all cases) which is indicative of no 10*t*-shift (Bessa *et al*., 2015).

Overall, CLA content was higher in mountain than in valley milk. This is also directly associated with the VA content as rumenic acid (RA, 9*c*,11*t*-18:2), the major CLA isomer, can be synthesized by the activity of Δ9-desaturase in the mammary gland (Bauman *et al.*, 1999). Similar to the changes that occur in the *trans*-18:1 profile, 11*t*,13*c*-18:2 was the second most abundant among the CLA isomers, especially in mountain milk. In the nc-diene and triene groups, 11*t*,15*c*-18:2 and 9*c*,11*t*,15*c*-18:3 were the major metabolites, respectively, and they were significantly higher in mountain milk compared to valley milk possibly related to the metabolism of 18:3n-3 (Destaillats *et al*., 2005).

The effect of pasture type was significant ($p \leq 0.001$) for the branched-chain FA, *cis*-MUFA and PUFA content, and also associated ratios (n-6/n-3, P/S). They provided higher values in mountain compared to valley milk except the n-6/n-3 ratio. In general, the results obtained are in good agreement with the unique FA profile reported in alpine milk (Kraft *et al*., 2003) and studies on the milk and cheese composition of ruminant origin produced in mountain areas (Hauswirth *et al*., 2004; Leiber *et al*., 2005).

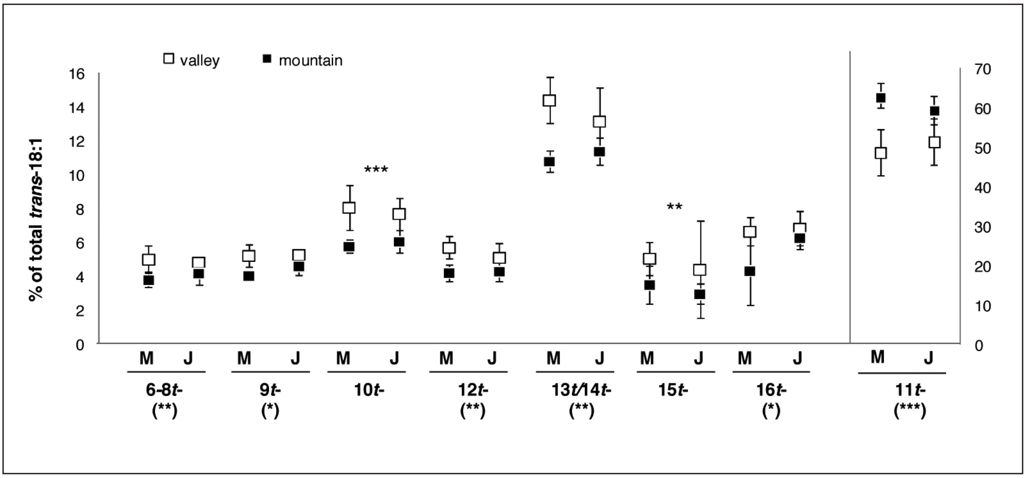

Fig. 1. Relative percentages of individual *trans*-18:1 isomers of milk samples from ewes reared in valley and mountain pastures and collected in May (M) and June (J).
In the graph, vaccenic acid (11*t*-18:1) is depicted on the right Y-axis. The effect of pasture type (valley, mountain) is indicated above the symbols while interaction effect is given in brackets along the X-axis. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

IV – Conclusions

Milk from ewes reared in mountain pastures provided a higher fat content with a healthier FA profile. Mountain milk had higher P/S but lower n-6/n-3 ratio, and higher content of VA, RA and 11*t*,13*c*-18:2 in comparison to milk produced in valley pastures. Overall, the milk FA profile was characteristic of the biohydrogenation of linolenic acid. These results could contribute to the added-value of PDO cheeses produced in mountain areas in order to reduce the progressive abandonment of shepherding in these areas.

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