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Effect of livestock stocking rate on fatty acid and tocol composition of milk from sheep managed under part-time grazing

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Abstract. Dairy sheep management system in the Basque Country (northern Spain) is based predominantly on part-time grazing from late winter to early summer. The milk produced is primarily used for cheese-making and having high nutritional quality milk at the lowest cost possible is important for cheese makers. Intensification of the system can have strong influence on farm profitability, environment, and milk and cheese quality. The aim of the present work was to investigate the effect of sheep stocking rate managed under part-time grazing on milk quality measured as fatty acid (FA) and tocol (TC) composition. Two homogeneous groups of 60 ewes each grazed on the same paddocks divided at high (H) and low (L) stocking rate, respectively. Bulk milk samples were taken once a month from mid-April to June. TC profile of milk samples was similar in both groups of animals over the sampling period although the highest contents of the major TC (α-tocopherol; mg/100 DM) were found in samples collected in June. Changes in the FA content (g FA/100 g milk fat) affected by stocking density were mainly observed in the unsaturated FA fraction. Samples from L group showed higher content than H group of α-linolenic (18:3n-3) and associated rumen biohydrogenation intermediates as rumenic (9*c*,11*t*-18:2) and vaccenic (11*t*-18:1) which are considered beneficial for human health. Changes in diet due to stocking rate seemed to be linked to differences in the milk FA composition.

Keywords. Fatty acids – Tocopherols – Sheep raw milk – Grazing management.

Effet du taux de stockage du bétail sur la composition des acides gras et des tocophérols du lait provenant de moutons gérés sous le pâturage à temps partiel

Résumé. *Le système de gestion des ovins laitiers au Pays Basque (nord de l'Espagne) repose principalement sur le pâturage à temps partiel de la fin de l'hiver jusqu'au début de l'été. Le lait produit est principalement utilisé pour la fabrication de fromage. La production du lait de bonne qualité nutritionnelle à coût aussi bas que possible est important pour les fromagers. L'intensification du système peut avoir une forte influence sur la rentabilité du troupeau, l'environnement et la qualité du lait et du fromage. L'objectif du ce travail a été d'étudier l'effet du taux de stockage des brebis géré par le pâturage à temps partiel sur la qualité du lait mesurée en tant que la composition d'acide gras (FA) et tocophérols (TC). Deux groupes homogènes de 60 brebis pâturent chacun sur les mêmes enclos divisés à un taux de stockage élevé (H) et faible (L), respectivement. Des échantillons de lait en vrac ont été prélevés une fois par mois de la mi-avril à juin. Le profil de TC des échantillons de lait était similaire dans les deux groupes d'animaux au cours de la période d'échantillonnage, bien que le plus grand contenu du TC principal (α-tocophérol ; mg/100 DM) a été trouvé dans les échantillons prélevés en juin. Les modifications de la quantité FA (g FA/100g de matière grasse du lait) affectées par la densité du stock ont été principalement observées dans la fraction non saturée de FA. Les échantillons du groupe L ont montré une quantité plus élevée que le groupe H d'acide α-linolénique (18:3n-3) et les intermédiaires de biohydrogénation du rumen associés en tant que rumenique (9c,11t-18:2) et vaccenique (11t-18:1), qui sont considérés comme bénéfiques pour la santé humaine. Les changements de régime liés au taux de stockage semblent être liés aux différences dans la composition du FA du lait.*

Mots-clés. *Acides gras – Tocophérols – Lait cru mouton – Gestion du pâturage.*

I – Introduction

Nowadays, the sustainability of sheep livestock in the Mediterranean area is seriously compromised by factors such as economic profitability, technical viability and environmental impact of the production system. In this context, innovation in management practices can be crucial to improve farm sustainability, particularly taking into account that grazing management is usual among shepherds, and that dairy sheep systems contribute to the actual landscape configuration and cultural heritage within the Mediterranean basin (Ruiz *et al.*, 2009). Livestock stocking rate is important to optimize grazing resources but little attention has been paid to grazing practices during the last decades. A non-optimal use of natural resources can sometimes generate under-grazing resulting in pasture degradation with the subsequent turning to scrub, or in other cases, overgrazing and soil erosion (Mandaluniz *et al.*, 2009; Teague *et al.*, 2011). Therefore, shepherds and farmers need to improve the utilization of grassland and forage resources, and in consequence, the sustainability of sheep production. Regarding the impact of the dairy sheep grazing regime, a previous work showed no significant differences related to stocking rate in the herbage mass or dairy production parameters. However, a higher stocking rate obtained by rotational grazing regime resulted in a higher amount of forage harvested by conservation which improves the farm forage autonomy and reduces the carbon footprint (Mandaluniz *et al.*, 2016). However, to the best of our knowledge there is very little information in the scientific literature on the composition of milk from grazing sheep managed under different livestock stocking rates. To a great extent, the traditional management of the Latxa sheep in the Basque Country (northern Spain) is based on part-time grazing from late winter to early summer. The raw milk is primarily used for cheese-making (Idiazabal – protected denomination of origin, PDO).

The aim of the present work was to investigate the effect of sheep stocking rate managed under part-time grazing on milk nutritional quality measured as fatty acid (FA) and tocol (TC) composition.

II – Material and methods

1. Experimental design

The experiment was conducted with 120 Latxa dairy sheep of the NEIKER-Tecnalia experimental flock in Vitoria-Gasteiz (Basque Country, Spain) during spring milking period following a part-time grazing. Ewes were randomly assigned into two homogeneous groups of animals (60 ewes/group) according to the month of lactation, daily milk production, live weight and body condition score. Both groups of sheep received 0.8 kg of concentrate (DM basis)/ewe/day which was offered at morning and evening milkings. After morning milking ewes grazed on pasture managed at low (L) or high (H) stocking rate, respectively. After evening milking, both groups received *ad libitum* fescue hay. The L group had 10 livestock units (LU)/ha whereas the H stocking rate was 7 times greater. The ewes of H group grazed 2-3 days/plot with 24 ± 2 days of rest periods between grazings. The ewes of L group grazed 6-10 days/plot with 15 ± 3 days of rest periods. All paddocks used by L and H sheep groups were similar in vegetation composition and production (Mandaluniz *et al.*, 2015). Replicated bulk milk samples (1.5 L) from L and H sheep groups were taken once a month from mid-April to June, and sample collection started after 2 weeks of adaptation period (early April).

2. Chemical analysis

Fat and DM content of milk samples was determined using a near infrared spectroscopy equipment. Milk FA were extracted and derivatized to FA methyl esters (FAME) following a miniaturized method previously described by Aldai *et al*. (2012). The analysis of FAME was carried out by gas-chromatography with flame ionization detector as previously described (Kramer *et al*., 2008; Delmonte *et al*., 2011). Results were expressed as g of FA/100 g milk fat. Milk TC were extracted using a one-step solid-liquid phase and analysed by high-performance liquid chromatography using fluorescence detection as previously described (Valdivielso *et al.*, 2015). Results were expressed as mg of TC/100 g of milk DM.

3. Statistical analysis

The general linear model of analysis of variance (ANOVA) was used to investigate the effect of stocking rate (low and high) and sampling period (April, May and June) on the FA and TC composition of sheep milk. Stocking rate was nested within sampling period and the following model was used:

$$Y_{ijk} = \mu + SR_i(SP_j) + SP_j + \varepsilon_{ijk}$$

where Y_{ijk} = dependent variables; μ = intercept; SR_i = stocking rate fixed effect; SP_j = sampling period fixed effect; and ε_{ijk} = random residual effects. Tukey's test was used for multiple comparisons between milk samples from different sampling months. Statistical significance was declared at $P \leq 0.05$. Three significant figures were used to express mean and standard error of the mean values.

III – Results and discussion

Table 1 shows the fat content and FA composition of milk samples from L and H stocking rates throughout the sampling period. Milk fat content did not change with stoking rate whereas a significant increase milk fat was observed from April to June. Ewes of both stocking rates were at the same lactation stage and milk nutritional composition changes over the lactation curve increasing the fat content (Leiber *et al*., 2004). Overall, the FA profile of all milk samples was the typically found in ruminants produced under grazing systems (Table 1), showing considerable content of α-linolenic acid (18:3n-3) and related rumen biohydrogenation intermediates such as vaccenic acid (11*t*-18:1), 13/14*t*-18:1, and 11*t*,15*c*-18:2. Rumenic acid (9*c*,11*t*-18:2) was the main conjugated linoleic acid (CLA) in all milk samples representing 75% of the total CLA content. The presence of n-3 polyunsaturated FA (PUFA), *iso* branched-chain FA (*iso*-BCFA), and vaccenic and rumenic acids in milk samples conferred to them potential benefits for human health (Wang *et al*., 2012; Aldai *et al*., 2013).

Milk from ewes grazed at high stocking rate (H group) showed higher content ($P \leq 0.05$) of total saturated FA (SFA) and BCFA in comparison to milk from ewes grazed at low stoking rate (L group). In fact, the content of most individual SFA (except of stearic acid, 18:0) and BCFA like *anteiso*-15:0 were higher in milk from H than L stocking rates (Table 1). Normally, short- and medium-chain SFA, including most of myristic acid (14:0) and about half of palmitic acid (16:0) are synthesized *de novo* in the mammary gland while *iso*- and *anteiso*-15:0 are mainly synthesized by rumen bacteria from leucine and isoleucine dietary amino acids (Shingfield and Griinari, 2007). Therefore, these results could be indicative that sheep from high stocking rate could have ingested a higher content of protein-rich botanical species that could favor *de novo* BCFA synthesis in the rumen.

Milk samples from ewes grazed at low stocking rate (L group) showed higher content ($P \leq 0.05$) of monounsaturated FA (MUFA), CLA, non-conjugated dienes and trienes and polyunsaturated FA (PUFA) compared to milk samples from H group (Table 1). It seemed that grazing at low stoking rate allowed sheep to specifically select botanical species that caused a higher intake of PUFA, especially α-linolenic acid, compared to sheep grazed at high stocking rate conditions. Higher dietary intake of PUFA could increase the formation of rumen biohydrogenation intermediates such as several CLA, nc-dienes and trienes and *trans*-MUFA, and could also promote the escape of PUFA (α-linolenic and linoleic (18:2n-6) acids) from rumen metabolism becoming available for the mammary gland and, simultaneously, decreasing short- and medium-chain FA *de novo* synthesis.

Table 1. Fat content (g/100 g milk) and fatty acid (FA) composition (g/100 g fat) of bulk milk samples from sheep grazed under part-time grazing at two stocking rates (low and high) and collected over three sampling peridos (April, May and June)

	Stocking rate		Sampling month			SEM	Significance
	Low	High	April	May	June		Stocking rate
Fat content	6.45	6.53	5.96^{b}	6.43^{b}	7.18^{a}	0.102	ns
16:0	19.0	19.3	19.5^{a}	19.4^{a}	18.6^{b}	0.0316	*
18:0	13.6	13.7	13.2^{c}	13.6^{b}	14.1^{a}	0.0629	ns
SFA	58.1	59.3	62.6^{a}	59.2^{b}	54.4^{c}	0.0974	*
i-16:1	0.294	0.298	0.318^{a}	0.270^{b}	0.302^{a}	0.00245	ns
i-17:0	0.337	0.337	0.315^{b}	0.304^{b}	0.391^{a}	0.00392	ns
iso-BCFA	1.14	1.14	1.09^{b}	1.03^{c}	1.29^{a}	0.00524	ns
ai-15:0	0.511	0.527	0.539^{a}	0.469^{b}	0.550^{a}	0.00237	*
ai-17:0	0.404	0.407	0.401^{b}	0.371^{c}	0.444^{a}	0.00212	ns
anteiso-BCFA	0.956	0.980	0.987^{b}	0.878^{c}	1.04^{a}	0.00405	*
BCFA	2.44	2.49	2.48^{b}	2.26^{c}	2.65^{a}	0.0124	*
9*c*-16:1	0.619	0.601	0.514^{c}	0.578^{b}	0.739^{a}	0.00376	*
9*c*-18:1	23.9	23.4	19.6^{c}	23.0^{b}	28.3^{a}	0.0697	*
cis-MUFA	26.4	25.9	22.0^{c}	25.5^{b}	30.9^{a}	0.0617	*
11*t*-18:1	1.76	1.69	1.86^{a}	1.80^{a}	1.52^{b}	0.00944	*
13/14*t*-18:1	0.806	0.755	0.882^{a}	0.834^{b}	0.626^{c}	0.00496	*
trans-MUFA	5.51	5.15	5.70^{a}	5.59^{a}	4.70^{b}	0.0429	*
MUFA	31.9	31.0	27.7^{c}	31.1^{b}	35.6^{a}	0.0698	*
7*t*,9*c*-18:2	0.0709	0.0660	0.0676	0.0686	0.0691	0.000120	ns
9*c*,11*t*-18:2	0.840	0.808	0.793^{b}	0.841^{a}	0.839^{a}	0.00591	*
CLA	1.13	1.08	1.08^{b}	1.14^{a}	1.08^{b}	0.00588	*
9*c*,13*t*-18/ 8*t*,12*c*-18:2	0.374	0.361	0.354^{b}	0.384^{a}	0.365^{ab}	0.00361	*
11*t*,15*c*-18:2	0.258	0.239	0.246^{b}	0.315^{a}	0.185^{c}	0.00399	*
nc-dienes & trienes	1.46	1.41	1.45^{ab}	1.51^{a}	1.35^{b}	0.0253	ns
18:3n-3	0.847	0.746	0.746^{c}	0.873^{a}	0.769^{b}	0.00177	*
22:5n-3	0.126	0.118	0.111^{b}	0.113^{b}	0.143^{a}	0.00264	ns
n-3	1.19	1.10	1.07^{b}	1.22^{a}	1.14^{ab}	0.0105	*
18:2n-6	1.88	1.69	1.71^{c}	1.82^{b}	1.84^{a}	0.00200	*
20:4n-6	0.155	0.142	0.146	0.140	0.160	0.00434	ns
n-6	2.28	2.13	2.15^{b}	2.20^{ab}	2.27^{a}	0.00947	*
PUFA	3.47	3.23	3.22^{b}	3.42^{a}	3.41^{a}	0.0172	*

SEM, standard error of the mean; SFA, saturated FA; BCFA, branched chain FA; MUFA, monounsaturated FA; CLA, conjugated linoleic acid; nc, non-conjugated; PUFA, polyunsaturated FA; ns, not significant ($P > 0.05$); *, $P \leq 0.05$.

^{a,b,c} Means within a row with different superscripts differ ($P \leq 0.05$) between sampling months.

As mentioned above, with few exceptions, significant changes ($P \leq 0.05$) in milk individual FA contents were observed over lactation period. Overall, the content of SFA and *trans*-MUFA decreased ($P \leq 0.05$) from April to June (late lactation), while the content of BCFA, *cis*-MUFA, n-3 and n-6 PUFA increased ($P \leq 0.05$) over the sampling period.

Tocol profile of milk samples was similar for both stocking rates (L and H) over the sampling period being α-tocopherol the main TC (around 95-99%) in all milk samples (Table 2). Very small amount of γ-tocopherol, α- and γ- tocotrienols were found in milk samples from both stocking rates. This milk TC profile was quite similar to that obtained in previous works for sheep raw milk (Valdivielso *et al.*, 2015). Not significant differences ($P > 0.05$) were found in the TC content of milk samples from H and L groups over the sampling period although the content of α-tocopherol and that of other minor TC increased from April to June (Table 2).

Table 2. Dry matter (DM) content (g /100 g milk) and tocol composition (mg/100 g of DM) of bulk milk samples from sheep grazed under part-time grazing at two stocking rates (low and high) and collected over three sampling peridos (April, May and June)

	Stocking rate		Sampling month			SEM	Significance
	Low	High	April	May	June		Stocking rate
DM content	10.5	10.4	10.3^b	10.3^b	10.5^a	0.0439	*
α-tocopherol	1.67	2.06	1.46^a	1.37^a	2.77^b	0.890	ns
α-tocotrienol	0.00757	0.00741	ND	ND	0.0224	0.0118	ns
α-tocopherol	0.0538	0.0753	0.0419	0.0474	0.104	0.0406	ns
α-tocotrienol	0.00327	0.00726	0.00537	ND	0.0104	0.00777	ns
Tocols	1.73	2.15	1.50^a	1.42^a	2.90^b	0.955	ns

SEM, standard error of the mean; ND, not detected; ns; not significant ($P > 0.05$).

[a,b,c] Means within a row with different superscripts differ ($P \leq 0.05$) between sampling months.

IV – Conclusions

Fatty acid composition of sheep milk managed under part-time grazing was slightly affected by the stocking rate conditions and the tocol content of milk samples from ewes grazed at low or high stocking rates did not change. From a nutritional milk fat quality point of view, the milk samples from ewes grazed at low stocking rate showed higher contents of fatty acids which are considered beneficial for human health. Changes in the animal selection of botanical species seemed to be associated to differences in the fatty acid composition of milk samples from ewes grazed at low or high stocking rates.

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