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Effect of part-time grazing and alfalfa hay supplementation on fatty acid content of sheep's milk

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Abstract. Part-time grazing is a traditional flock management method in the Basque Country region of Northern Spain which starts in late winter or early spring according to weather conditions. Pasture feeding is supplemented indoors with forage (alfalfa and conserved forages) and concentrate to meet milk production requirements. The objective of this study was to evaluate the effect of actual grazing time on the amount of individual fatty acids in the milk obtained. The experiment was conducted during 4 weeks from late April until mid-May. Sheep were separated into 4 homogeneous groups of 12 sheep each, and randomly assigned to 3 different alfalfa hay supplements: 300 g/day (Group 1), 600 g/day (Group 2), and 900 g/day (Group 3). The control group (Group 0) received 600 g alfalfa hay and 1 kg grass hay per day and was not allowed to graze outdoors. All animals received 500 g concentrate/day at milking. Although grazing animals were given access to pasture during 4 hours a day, actual grazing time (visually monitored) was not the same: Group 3 grazed for the least amount of time ($P<0.05$); no statistically significant differences in actual grazing time were observed between Groups 1 and 2. Milk samples (evening and morning milkings combined) were taken once a week. Grazing time significantly ($P<0.05$) increased the amount of milk c9t11 CLA isomer over 3-fold (15.9 ± 3.8 mmol/g fat for Group 0, and 50.25 ± 14.49 mmol/g fat for Group 1) and that of vaccenic acid over 4.5-fold (38.2 ± 8.4 mmol/g fat for Group 0, and 176.13 ± 53.64 mmol/g fat for Group 1). Differences in total unsaturated fatty acids between Group 0 and Group 1 were also statistically significant. When the 3 different amounts of alfalfa hay were compared (Groups 1, 2 and 3), milk fat from Group 1 had the highest level of unsaturated fatty acids, c9t11 CLA isomer and vaccenic acid ($P<0.05$). Grazing increased milk production by over 30%.

Keywords. Pasture – Conjugated linoleic acid – Unsaturated fatty acids – Grazing time.

Effet du pâturage partiel supplémenté avec le foin de luzerne sur le contenu d'acides gras dans le lait de brebis

Résumé. *Le pâturage partiel est une méthode traditionnelle pour le maniement des troupeaux dans le Pays Basque au nord de l´Espagne, que selon les conditions météorologiques peut commencer à la fin de l´hiver ou au début du printemps. L'alimentation avec du pâturage est supplémenté avec du fourrage (luzerne et fourrage conservé) et du concentré quand ils sont à l´intérieur, pour améliorer la production du lait. L´objectif de cette étude a été l´évaluation de l´effet du temps du pâturage actuel sur la quantité individuelle des acides gras qui composent le lait. L´expérience a été réalisée pendant 4 semaines, de la mi-avril à la mi-mai. Les brebis ont été séparées en 4 groupes homogènes de 12 brebis chaque, avec une alimentation supplémentée avec 3 différentes quantités de foin de luzerne distribuées au hasard: 300 g/jour (Groupe 1), 600 g/jour (Groupe 2), 900 g/jour (Groupe 3). Le groupe témoin (Groupe 0) a reçu 600 g de foin de luzerne/jour et ne pouvait pas pâturer dehors. Tous les animaux ont reçu 500 g de concentré/jour pendant la traite. Bien que tous les animaux de pâturage avaient accès au pâturage pendant 4 heures par jour, le temps réel (monitoires visuellement) n'était pas le même: Le Groupe 3 le moins a pâturé ($P<0.05$); il n'y a pas eu des différences entre le Groupe1 et Groupe 2 pour le temps de pâturage. Les échantillons du lait ont été pris (traite du soir et du matin groupés) une fois par semaine. Le temps du pâturage a augmenté significativement*

(P<0.05) la quantité de l'isomère CLA c9t11, qui est multipliée par 3 (15.9 ± 3.8 mmol/g du gras pour le Groupe 0, et 50.25 ± 14.49 mmol/g du gras pour le Groupe 1) et l´acide vaccénique est multipliée par 4.5 (38.2 ± 8.4 mmol/g du gras pour le Groupe 0 et 176.13 ± 53.64 mmol/g du gras pour le Groupe 1) dans le lait. On peut observer des différences statistiquement significatives entre le Groupe 0 et le Groupe 1 pour les totaux des acides gras non-saturés. Quand les 3 différentes quantités de foin de luzerne ont été comparées (Groupes 1, 2, et 3), le gras du lait du Group 1 avait la plus grande quantité des acides gras non-saturés, l'isomère CLA c9t11 et l´acide vaccénique (P<0.05).

Mots-clés. *Pâturage – Acide linoléique conjugué – Acides gras non-saturés – Temps de pâturage.*

I – Introduction

In recent years consumers have become increasingly interested in foods that, in addition to being nutritious and good-tasting, contain compounds that provide health benefits and do not contain compounds that could have a negative health impact. The food industry, aware of these concerns, is modifying food formulations to avoid certain compounds such as *trans* fats. In these last 20 years a significant research effort has been conducted to modify the composition of milk and cheese fat in order to increase, if possible, the concentration of compounds with positive effects through the diet of the lactating animals (Jenkins and McGuire, 2006). Farmers can control this parameter which, in turn, allows them to produce milk, and cheese, with different sensory and nutritional characteristics. The majority of the published studies deal with bovine milk and/or cheeses (Chilliard and Ferlay, 2004) because of their economic importance. However, studies on ovine and caprine milk and cheeses are also gaining relevance. Diets fed to animals can be grouped in three main categories: (i) based on concentrates with supplements of vegetable oils (soya, sunflower, olive), or oil seeds (flax, sunflower), or even fish oils (Luna *et al*., 2005); (ii) based on different ratios forage: concentrate, with or without oil supplements; and (iii) pasture feeding, with or without additional concentrate and or supplements (Atti *et al*., 2006). In all these studies primary attention has been given to the amount of individual fatty acids, particularly to conjugated linoleic acid (CLA) isomers and other unsaturated fatty acids. CLA isomers are a group of 18-carbon fatty acids with two conjugated double bonds. Of the 28 possible isomers, most studies report the concentrations of the two main isomers found in milk fat, c9t11 (by far the most abundant one) and t10c12. Interest in these compounds for humans is mostly due to their positive biological activity observed in cellular and animal studies: antiatherogenicity, anticarcinogenicity, modulation of the immune response and modification of body composition (Pariza, 2004).

Part-time grazing with indoor supplementation is the traditional, most frequently used, sheep flock management system during much of the lactation period in the Basque Country region of Northern Spain, from late winter until late spring, when pastures are available (Oregui and Falagán-Prieto, 2006). Most of the milk produced during this time is used for Idiazabal cheese-making. The objective of the present study was to evaluate the effect of actual grazing time on the amount of individual fatty acids in the milk obtained.

II – Materials and methods

The experiment was conducted during 4 weeks from mid-April until mid-May with an experimental flock of sheep, with one week to allow the sheep to get accustomed to their new diets. Forty-eight multiparious *Latxa* dairy ewes between 1 and 2 months *post-partum* were separated into 4 homogeneous groups (according to live-weight, body condition score and milk production) of 12 sheep each. Initial mean values were 2nd or 3rd lactation, 40-60 day of lactation, milk yield of 1.40-1.50 L/d and 57-62 kg live weight. Groups were randomly assigned to one of the feeding regimes described in Table 1. The nutritional characteristics of the feeds are described in Table 2.

Table 1. Feeding regimes of the four groups of animals

Group	Pasture Feeding		Indoor feeding program	
	Access to pasture (h/d)	Concentrate (g/d)	Alfalfa hay (g/d)	Grass hay (g/d)
0	0	500	600	1,000
1	4	500	300	0
2	4	500	600	0
3	4	500	900	0

Table 2. Nutritional characteristics of the feeds

Feed	Crude Protein (%)	Neutral-Detergent Fibre (%)
Concentrate	22.0 ± 1.0	–
Alfalfa hay	19.6 ± 3.2	36.4 ± 6.5
Grass hay	9.3 ± 3.1	56.1 ± 5.3
Pasture†	23.4 ± 2.0	40.0 ± 3.3

† Polyphite pasture was composed mainly of *Lolium perenne, Dactylis glomerata* and *Trifolium repens*.

As described in Table 1, Group 0 was kept indoors during the entire 5 weeks with no access to pasture, whereas the other three Groups were allowed on the pasture 4 hours per day, after the morning milking. The total amount of concentrate given per day was divided into 2 equal meals and provided during the morning (250 g) and evening milking (250 g), and alfalfa hay was given after the evening milking, according to Table 1. No fresh grass was administered to Group 0. Instead, Group 0 received grass hay while Groups 1-3 were grazing. Groups 1-3 grazed on the same pasture, although in different parts of the field. Actual time spent grazing was visually monitored weekly during the 4 hours of pasture access.

During the 4 weeks milk production was daily recorded (l/d) from Monday to Thursday, and milk samples from individual sheep were taken once a week to determine the fat and protein contents (Method PE/ALVO/02, 2005). One milk sample of each Group (evening and morning milkings combined) was taken once a week for fatty acid analysis.

Whole milk was centrifuged at 2,000 *g* and 4ºC for 30 minutes in a Sorvall refrigerated high-speed centrifuge to separate the cream. Fat was extracted from the cream by the method of Folch *et al*. (1957) and fatty acids were methylated with sodium methoxide (Christie, 1982). Separation of fatty acid methyl esters was accomplished on a 100% dimethylpolysiloxane column (CP-Sil low Bleed from Agilent Technologies, 60 m x 0.25 mm x 0.25 mm film thickness) using a Hewlett Packard Gas Chromatograph (5890 Series II) equipped with a split-splitless injector, flame ionization detector and autosampler. Initial oven temperature was kept at 40ºC during 2 minutes, it was raised up to 175ºC at 10ºC/min, and kept at this temperature for 27 minutes, with a final increase up to 215ºC at 0.5ºC/min. Total analysis time to separate the isomers of interest was 122.5 minutes. Injector and detector temperatures were kept at 325ºC and 250ºC, respectively. Helium was used as carrier gas at 1 ml/min. Identification of fatty acid methyl esters was accomplished by comparing the retention times of unknown peaks with those of known standards. Quantification was done using nonanoic and heptadecanoic acids as internal standards added to the sample at the time of extraction. Chromatographic data were analysed with the HP Chemstation GC.

Statistical analysis of the data (one-way ANOVA) was done using the SPSS statistical package version 16.0. Values for the 4 weeks were averaged for each group because there were no statistically significant differences among weeks. Observed values reported are average and standard deviation.

III – Results and discussion

The amount of milk produced during the 4 weeks by grazing Groups 1-3 was fairly similar, but on average 35% greater than the amount produced by Group 0 ($P<0.05$), as seen in Table 3. However, no statistically significant differences ($P>0.05$) were observed in the milk protein and fat contents of all groups. The actual amount of time spent grazing was significantly different ($P<0.01$) and depended on the amount of alfalfa hay fed to each experimental group. These results indicate that it is possible to implement a flock management method based on part-time grazing by reducing the amount of indoors-provided supplements, increasing milk yield.

Table 3. Milk yield, gross milk composition and grazing time

	Group 0	Group 1	Group 2	Group 3
Milk yield (L/d)	1.03 ± 0.22^{a}	1.32 ± 0.32^{b}	1.36 ± 0.35^{b}	1.46 ± 0.30^{b}
Protein (%)	5.05 ± 0.34^{a}	4.88 ± 0.45^{a}	5.05 ± 0.49^{a}	4.96 ± 0.47^{a}
Fat (%)	6.98 ± 0.89^{a}	6.12 ± 1.00^{a}	6.34 ± 1.12^{a}	6.30 ± 0.95^{a}
Grazing time (min/4h)	0	228 ± 8^{a}	224 ± 6^{a}	209 ± 14^{b}

^{a,b} different superscripts in the same row indicate statistically significant differences ($p<0.05$).

The effect of grazing on the amount of the various fatty acids in the milk obtained from sheep of each experimental Group is shown in Table 4. Of the four groups, the one that received the least amount of alfalfa hay (Group 1) was clearly different from the other three. Group 1 had the highest concentrations ($P<0.05$) of total unsaturated fatty acids (and the highest percentage), as well as of the main CLA isomer (c9t11) and of vaccenic acid. The amount of c9t11 in Group 1 was over 3-fold that present in Group 0, which is a much larger proportion than that reported by other authors, both in bovine milk (Lucas *et al*., 2006) and in sheep milk (Luna *et al*., 2008).

Although the double bond in vaccenic acid is in the *trans* configuration, mammalian tissues can convert vaccenic acid to the beneficial CLA isomer c9t11 (Adlof *et al*., 2000). For this reason, some countries exclude vaccenic acid and other naturally occurring *trans* fatty acids in milk and dairy products from the total *trans* fatty acids that must be declared on food labels (Kühlsen *et al*., 2005). Although Group 1 has the highest amount of saturated fatty acids, over 42% of them are short-chain fatty acids (direct metabolic energy index) which are directly absorbed in the portal vein and metabolized in the liver to obtain metabolic energy instead of being transported by chylomicrons to the adipose tissue (German and Dillard, 2006).

The atherogenicity index of Group 1 is the lowest of all groups. This value is lower than that reported by Couvreur *et al*. (2006) for bovine milk (2.48) from animals which received 9.7 kg/d of dry matter from fresh cut grass. The atherogenicity index of milk from control cows which did not receive grass was 2.93 (Couvreur *et al*., 2006), identical to that reported in the present study.

Table 4. Milk fatty acids (mmol/g fat) and atherogenicity index for the different feeding regimes

Fatty acid(s)	Group 0	Group 1	Group 2	Group 3
Saturated	2659.69 ± 210.70^{a}	3229.66 ± 184.51^{b}	2799.30 ± 314.13^{a}	2975.64 ± 156.97^{b}
Unsaturated	849.9 ± 113.6^{a}	1186.09 ± 214.20^{b}	917.67 ± 147.13^{a}	953.69 ± 100.69^{a}
c9t11	15.88 ± 3.83^{a}	50.25 ± 14.49^{c}	34.39 ± 8.44^{b}	32.95 ± 10.32^{b}
Vaccenic	38.25 ± 8.38^{a}	176.13 ± 53.64^{c}	114.97 ± 28.67^{b}	108.25 ± 32.44^{b}
Short (C4-C10)	1020.65 ± 69.20^{a}	1368.92 ± 64.23^{c}	1171.30 ± 129.78b	1216.91 ±150.88^{b}
Medium (C12-C14)	508.74 ± 38.45^{a}	564.25 ± 12.04^{b}	522.23 ± 52.30^{a}	555.45 ± 24.81^{a,b}
Long (³ C16)	1980.53 ± 200.92^{a}	2482.58 ± 326.86^{b}	2023.43 ± 270.68^{a}	2156.98 ± 189.63^{a,b}
Total fatty acids	3509.65 ± 299.74^{a}	4415.75 ± 393.60^{b}	3716.96 ± 443.67^{a}	3929.38 ± 242.02^{a}
Direct Metabolic Energy Index†	38.41 ± 1.09^{a}	42.41 ± 0.86^{b}	41.85 ± 0.45^{b,c}	40.89 ± 0.46^{c}
Atherogenicity Index††	2.93 ± 0.32^{a}	2.25 ± 0.40^{b}	2.69 ± 0.29^{a,b}	2.72 ± 0.21^{a}

Different superscripts on the same row indicate statistically significant differences (P<0.05).

† Direct Metabolic Energy Index is calculated as follows: (short x sat^{-1}) x 100.

†† Atherogenicity Index (Ulbricht and Southgate, 1991) is calculated as follows: (C12:0 + (4 x C14:0) + C16:0) / unsaturated fatty acids.

IV – Conclusions

The results presented in this paper indicate that part-time grazing with a small amount of supplementation is an excellent alternative to indoor production. In addition to decreasing overall production costs by the use of local pasture resources, it increases milk yield over 30% without compromising its gross composition, important for milk producers (protein and fat contents). At the same time, milk produced in this manner has overall better nutritional characteristics for the consumer than milk produced in an intensive manner.

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