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G. Hervás*, P. Gómez-Cortés, M.A. de la Fuente**, A.R. Mantecón*, M. Juárez**, F.J. Giráldez* and P. Frutos***

*Estación Agrícola Experimental, CSIC, Finca Marzanas, 24346 Grulleros, León (Spain)
**Instituto del Frío, CSIC, José Antonio Novais 10, 28040 Madrid (Spain)

Abstract. This work was conducted to investigate the effect of supplementing grazing ewes on pasture with a cereal concentrate on the milk fatty acid (FA) profile. Ninety Assaf ewes in mid-lactation were distributed in 9 lots of 10 animals each and allocated to three feeding regimes: (i) P – ewes were only allowed to graze pasture (an irrigated sward of *Lolium perenne*, *Trifolium pratense* and *Dactylis glomerata*); (ii) SP – grazing ewes were supplemented with oat grain (700 g/animal per day); and (iii) TMR – ewes were fed *ad libitum* a total mixed ration (TMR; 80:20 concentrate/forage ratio). Milk yield and composition were recorded after 3 weeks. There were no significant differences in milk yield between P and SP; the highest production being observed for TMR. Milk fat from SP and TMR ewes presented similar concentrations of rumenic (*cis*-9 *trans*-11 C18:2) and vaccenic acids (*trans*-11 C18:1, the rumen-derived precursor for endogenous synthesis of CLA). Total conjugated linoleic acid (CLA) was slightly higher in SP than in TMR but far from the results observed in grazing ewes receiving no supplement (2.96, 1.71 and 1.35 for P, SP and TMR, respectively). The atherogenity index in SP milk fat was not different from that observed in milk from animals on pasture (1.45 for P, 1.61 for SP and 3.25 for TMR). It is concluded that, when pasture quality and availability are not limiting for dairy production, supplementation of grazing ewes with oat grain compromises the milk FA profile without any significant positive effect on milk production.

Keywords. Cereal supplement – CLA – Pasture – Rumenic acid – Sheep – Vaccenic acid.

Effet de la supplémentation des brebis laitières en pâturage par un concentré à base de céréales sur le profil des acides gras du lait

Résumé. *Cette expérience a été réalisée afin d'étudier l'effet de la supplémentation des brebis en pâturage avec un concentré de céréales sur le profil des acides gras du lait (FA). Quatre-vingt-dix brebis Assaf en milieu de lactation ont été réparties en 9 groupes de 10 animaux qui ont été soumis à trois régimes alimentaires : (i) P – brebis au pâturage dans une prairie irriguée de* Lolium perenne*,* Trifolium pratense *et* Dactylis glomerata*) ; (ii) SP – brebis en prairie supplémentées avec des graines d'avoine (700 g/animal et par jour) ; et (iii) TMR – brebis nourries* ad libitum *à base d'une ration mixte complète (TMR ; proportion 80:20 concentré/fourrage). La production et la composition du lait ont été mesurées après trois semaines. La supplémentation n'a pas affecté la production de lait. Cependant, le groupe TMR a produit plus de lait que les deux autres groupes (P et SP). La matière grasse du lait des brebis SP et TMR a présenté des concentrations similaires d'acides ruménique (*cis*-9* trans*-11 C18:2) et vaccénique (*trans*-11 C18:1, le précurseur dérivé du rumen pour la synthèse endogène du CLA). L'acide linoléique conjugué total (CLA) a été légèrement plus élevé dans le groupe SP que dans le groupe TMR, mais loin des résultats observés chez les brebis au pâturage non supplémentées (2,96, 1,71 et 1,35 pour P, SP et TMR, respectivement). L'indice d'athérogénicité de la matière grasse du lait SP est similaire à celui observé chez les animaux au pâturage (1,45 pour P, 1,61 pour SP et 3,25 pour TMR). En conclusion, lorsque la prairie est de bonne qualité, la supplémentation des brebis par l'avoine n'améliore pas la production de lait et a des conséquences négatives sur le profil des acides gras.*

Mots-clés. *Supplément à base de céréales – CLA – Pâturages – Acide ruménique – Brebis – Acide vaccénique.*

I – Introduction

As well known, dietary polyunsaturated fatty acids (PUFA) undergo biohydrogenation in the rumen.

Rumenic acid (*cis*-9 *trans*-11 C18:2, RA) the most important isomer of the conjugated linoleic acid (CLA) is produced as an intermediate of ruminal biohydrogenation of linoleic acid (C18:2) and mainly by endogenous synthesis from vaccenic acid (*trans*-11 C18:1; VA), via the enzyme Δ9-desaturase, in the mammary gland (Griinari *et al.*, 2000).

Pastures are rich in α-linolenic acid (C18:3 *n*-3), whose biohydrogenation in the rumen results in the production of several intermediates such as VA, *trans*-11 *cis*-13 and *trans*-11 *cis*-15 C18:2, but not in RA. A number of studies have been addressed to examine nutritional strategies to enhance the CLA content of ruminant milk. These studies have demonstrated that lactating ruminants fed conserved forages or cereal-based concentrates produce milk fat with a lower concentration of CLA and PUFA than do animals fed on rangelands and pastures (Stanton *et al.*, 2003; Pulina *et al.*, 2006). However, in many Mediterranean rangelands, grazing ewes are often supplemented with concentrates, which may change the biohydrogenation pathway of pasture fatty acids (FA) and negatively affect ewes' milk FA profile.

This work was conducted to investigate the effect of supplementing ewes on pasture with a cereal concentrate on milk production and composition, and milk FA profile. Oat grain, which is traditionally used to supplement grazing small ruminants due to its ability to maintain rumen environment more stable than other cereals (Orskov *et al.*, 1974), was chosen as the cereal concentrate. Results were also compared with ewes managed under an intensive system and fed a total mixed ration.

II – Materials and methods

1. Animals, experimental design, and milking management

Ninety Spanish Assaf ewes in mid lactation (at week 6 post-partum at the beginning of the experiment) were used in this study. Ewes were distributed in 9 lots of 10 animals, balanced for milk production, days in milk and live weight, and allocated to three feeding regimes (3 lots per treatment):

(i) P – ewes were only allowed to graze pasture (an irrigated sward of *Lolium perenne*, *Trifolium pratense* and *Dactylis glomerata*).

(ii) SP – grazing ewes were supplemented with oat grain (700 g per animal and day).

(iii) TMR – ewes were fed *ad libitum* a total mixed ration (TMR; 80:20 concentrate/forage ratio).

Clean water and vitamin-mineral blocks were always freely available for all the animals. Average sward height for the whole experimental period in the plots used by sheep on treatments P and SP was 17.5 cm. The oat supplement was offered immediately after the morning milking and ewes consumed it completely within about 15 minutes. Ewes were milked at 8.30 and 18.30 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain).

2. Measurements, sampling and chemical analysis

After 3 weeks on treatments, individual milk production was recorded and milk samples were collected from each animal for fat, protein and total solid contents analyses (AOAC, 2006) by infrared spectrophotometry, using a Milko-Scan 255 A/S N (Foss Electric, Hillerod, Denmark).

FA composition was determined in samples of milk from each experimental lot, composited according to individual milk production. Milk fat was extracted following the method described by Luna *et al.* (2005). Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) according to ISO-IDF procedure (ISO-IDF, 2002). Analysis of FAME was performed on a gas-liquid chromatograph (Agilent 6890 N Network System, Palo Alto, CA, USA) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, Varian, Middelburg, The Netherlands).

Procedures described by AOAC (2006) were used to determine dry matter, ash, Kjeldahl N and ether extract of the feeds (pasture, oat grain and TMR). Neutral-detergent fibre and acid-detergent fibre were determined using the Ankom technology (Ankom Technology, Macedon, NY, USA).

3. Statistical analyses

All data were analyzed as a one-way analysis of variance using the GLM procedure of the Statistical Analysis System program (SAS, 1999). When the effect of treatment (P *vs* SP *vs* TMR) was $P < 0.05$, means were compared using a Student-*t* test.

III – Results and discussion

Chemical composition and FA contents of feeds (pasture, oat grain and TMR) are presented in Table 1. All feeds, particularly the TMR, were good sources of linoleic acid (C18:2) and only the pasture was rich in α-linolenic acid (C18:3).

Table 1. Ingredients and chemical composition of the pasture, oat grain and total mixed ratio (TMR)

	Pasture	Oat grain	TMR
Ingredients (g/kg)			
Dehydrated alfalfa hay	–	–	200
Beet pulp	–	–	90
Whole maize grain	–	–	250
Whole barley grain	–	–	150
Soybean meal 44	–	–	200
Molasses	–	–	65
Salts	–	–	40
Minerals and vitamins	–	–	5
Chemical composition (g/kg DM)			
Organic matter	923	976	902
Crude protein	179	111	189
Neutral-detergent fibre	518	426	242
Acid-detergent fibre	259	176	127
Ether extract	27	62	21
Fatty acid composition (%)			
C16:0	18.9	22.4	17.3
C18:0	2.2	2.3	3.0
C18:1	5.2	47.2	26.2
C18:2	19.8	24.6	45.5
C18:3	31.7	1.6	3.8

Table 2 shows the milk production and composition for ewes fed pasture (P), pasture and oat grain (SP) or the total mixed ratio (TMR) after 3 weeks. There were no significant differences in milk production between P and SP treatments. The highest yield was observed for TMR but milk from animals on treatments P and SP had higher fat and total solids content. Absolute productions of milk fat, protein and total solids showed the lowest values in treatment SP, which may be related to a likely lower protein intake together with interactions among several nutritional factors (such as source of dietary neutral-detergent fibre and non-fibre carbohydrates, and energy concentration of the diets; Pulina *et al.*, 2006).

Table 2. Milk production and composition of ewes fed pasture (P), pasture + oat grain (SP) or a total mixed ratio (TMR) after 3 weeks

	Treatments			SED†	Prob.††
	P	SP	TMR		
Milk production (kg/d)	2.30[b]	1.90[b]	2.83[a]	0.202	<0.001
Composition (%)					
Fat	5.34[a]	5.56[a]	4.64[b]	0.184	<0.001
Protein	4.96	4.75	4.84	0.087	0.061
Total solids	16.28[a]	16.29[a]	15.51[b]	0.225	<0.001
Production (g/d)					
Fat	121.9	107.0	131.4	10.57	0.076
Protein	113.8[ab]	90.8[b]	136.7[a]	9.77	<0.001
Total solids	373.4[ab]	312.6[b]	438.2[a]	31.70	<0.001

†SED = standard error of the difference.
††Prob. = probability (significance level).
[a,b]In the same row, mean values with different letters differ significantly ($P < 0.05$).

Pasture intake decreased the linoleic acid (C18:2) and increased the α-linolenic acid (C18:3) content of milk fat (Table 3), in agreement with FA composition of the diets (see Table 1) and with data reported by other authors in dairy ruminants (e.g., Kelly *et al.*, 1998; Chilliard *et al.*, 2006). Increased concentrations of C18:0 and VA in the milk from ewes on pasture (P) could be explained by the supply of α-linolenic acid, which is hydrogenated into VA and thereafter C18:0 in the rumen. The same applies to the increases in oleic acid (*cis*-9 C18:1), due to its synthesis from C18:0 via mammary gland Δ9-desaturase. These differences, together with decreases in C6:0-C16:0 (because of the inhibitory effects of long-chain FA on the *de novo* lipogenesis in the mammary cells) are typically observed in milk fat from grazing cows when compared to those fed TMR diets (Chilliard *et al.*, 2001). Milk fat from ewes grazing pasture (P) had approx. 1.7 times more RA and 2 times more VA, total CLA being 2.2 fold higher than milk fat from TMR sheep. This positive effect of pasture intake on milk fat CLA content has been previously found not only in dairy cows (Kelly *et al.*, 1998) but also in dairy sheep and goats (Tsiplakou *et al.*, 2006).

Notwithstanding, when the pasture based diet was supplemented with oat grain (SP treatment), the beneficial effect of pasture intake on milk FA composition was not clear. Milk from SP ewes showed the highest concentration of C18:0 together with no increases of RA and VA compared to milk from ewes fed the TMR (and so, much lower content than animals on treatment P). Supplementation of grazing ewes with oat grain (SP *vs* P treatments) reduced the RA (–42%), VA (–49%) and total CLA (–42%) concentrations. Furthermore, it was observed a significant decrease of some ruminal biohydrogenation intermediates of α-linolenic, such as *trans*-11 *cis*-13 or *trans*-11 *cis*-15 C18:2. Total CLA was slightly higher in SP than in TMR but far from the results observed in grazing ewes receiving no supplement (P treatment).

These results suggest that the oat grain intake likely alters the major rumen biohydrogenation pathways and induces the complete hydrogenation of unsaturated FA presented in the pasture, which might be related with the effects of the cereal supplementation on ruminal pH, and subsequently with alterations in the balance of the growth and proliferation of specific ruminal bacteria involved in the biohydrogenation processes (Palmquist *et al.*, 2005). The fact that the oat grain was offered in only one meal and rapidly consumed by the ewes would account for large fluctuations in ruminal pH associated with the low meal frequency (Pitt and Pell, 1997).

Table 3. Milk FA profile (% of total fatty acids) of ewes fed pasture (P), pasture + oat grain (SP) or a total mixed ratio (TMR) after 3 weeks

	Treatments			SED†	Prob.††
	P	SP	TMR		
C4:0	4.01[b]	4.31[a]	4.00[b]	0.105	0.046
C6:0	2.58[b]	2.57[b]	3.69[a]	0.090	<0.001
C8:0	2.10[b]	1.97[b]	3.74[a]	0.144	<0.001
C10:0	5.29[b]	4.83[b]	11.76[a]	0.372	<0.001
C12:0	2.74[b]	2.67[b]	6.51[a]	0.149	<0.001
C14:0	7.81[b]	7.84[b]	11.39[a]	0.236	<0.001
C16:0	22.17[b]	23.52[b]	25.57[a]	0.553	0.002
C18:0	9.72[b]	12.09[a]	5.27[c]	0.394	<0.001
cis-9 C18:1	23.65[a]	24.55[a]	11.69[b]	0.740	<0.001
trans-10 C18:1	0.37[b]	0.46[b]	1.15[a]	0.134	0.002
trans-11 C18:1 (VA)	3.98[a]	2.02[b]	2.04[b]	0.198	<0.001
cis-9 *cis*-12 C18:2	1.90[b]	1.67[b]	2.97[a]	0.097	<0.001
cis-9 *trans*-11 C18:2 (RA)	1.37[a]	0.79[b]	0.81[b]	0.065	<0.001
trans-10 *cis*-12 C18:2	0.01	0.01	0.01	<0.001	0.702
trans-11 *cis*-13 C18:2	0.13[a]	0.05[b]	0.01[b]	0.017	0.001
trans-11 *cis*-15 C18:2	0.53[a]	0.22[b]	0.06[c]	0.032	<0.001
Total CLA	2.96[a]	1.71[b]	1.35[c]	0.103	<0.001
C18:3	1.23[a]	0.75[b]	0.40[c]	0.035	<0.001
Summary†††					
SFA	61.00[c]	64.14[b]	75.83[a]	0.775	<0.001
MUFA	32.51[a]	31.37[a]	19.14[b]	0.792	<0.001
PUFA	6.29[a]	4.31[c]	4.83[b]	0.155	<0.001
Atherogenicity index††††	1.45[b]	1.61[b]	3.25[a]	0.114	<0.001

†SED = standard error of the difference.
††Prob. = probability (significance level).
†††SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA.
††††Atherogenicity index = (C12:0 + 4×C14:0 + C16)/(MUFA + PUFA).
[a,b,c]In the same row, mean values with different letters differ significantly ($P < 0.05$).

Despite the lack of positive effects of SP treatment on milk CLA content, both pasture based diets (P and SP) significantly decreased the amount of C12:0, C14:0 and C16:0 on milk fat and increased (approx. 67%) the amount of monounsaturated FA (associated basically with the higher *cis*-9 C18:1 concentration), therefore resulting in a large decrease of the milk fat atherogenity index compared to TMR. Supplementation of grazing ewes with the cereal, however, showed the lowest value for PUFA concentration.

IV – Conclusions

Milk fat produced from ewes on pasture had a FA profile that might be deemed more favourable by consumers, compared to that from ewes under intensive systems. However, supplementation of grazing ewes with oat grain did not allow the maintenance of that desirable milk FA profile and did not increase the milk production. It is therefore suggested that, under the conditions of this study, traditional supplementation of grazing ewes with oat grain compromises the FA profile of milk fat without any significant positive effect on milk production. Notwithstanding, it is noteworthy that this experiment was conducted when pasture quality and availability were good enough to maintain animal performance (in terms of milk production and composition) and forage intake was most likely reduced as a consequence of feeding the cereal concentrate. Results would probably be different when pasture quality or availability limited dairy production and therefore further research under such conditions is recommended.

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