Journal of Animal and Feed Sciences, 13, Suppl. 1, 2004, 669–672

Effect of forage/concentrate ratio and oil supplementation on $C_{18:1}$ and CLA isomers in milk fat from Sarda ewes*

M. Antongiovanni[2,4], M. Mele[1], A. Buccioni[2], F. Petacchi[2], A. Serra[1], M.P. Melis[3], L. Cordeddu[3], S. Banni[3] and P. Secchiari[1]

[1]D.A.G.A.- Sezione Scienze Zootecniche, Università di Pisa, Italy
[2]Dipartimento di Scienze Zootecniche, Università di Firenze, Italy
[3]Dipartimento di Biologia Sperimentale, Università di Cagliari, Italy

ABSTRACT

Sixteen lactating Sarda ewes were fed 4 diets differing in the forage/concentrate ratio (2 diets 75/25, 2 diets 60/40, DM) and in soyabean oil supplementation (2 diets with 100 g/head/d oil, 2 diets with no oil), in a 4x4 Latin square design. The inclusion of soyabean oil in the diet resulted in a significant increase of both rumenic acid (*cis* 9 *trans,* 11 CLA) and vaccenic acid (*trans* 11 $C_{18:1}$) in milk fat, with higher increases with the 2 high forage diets. The 2 low forage diets allowed increases of *trans* 10 $C_{18:1}$ and of *trans* 10, *cis* 12 CLA, probably due to a shift of rumen biohydrogenation of linoleic acid.

KEY WORDS: sheep, milk, lipids, *trans* fatty acids, CLA

INTRODUCTION

The forage/concentrate (F/C) ratio and the quality of lipids of the diet are the major factors affecting the rate and extent of rumen biohydrogenation of unsaturated fatty acids (UFA), included those involved in the production of conjugated linoleic acid (CLA) and of vaccenic acid (*trans* 11$C_{18:1}$, VA) (Chilliard et al., 2002). Kukuk et al. (2001) demonstrated that interactions between F/C ratio and dietary UFA may occur when oils are added to the diet of lactating ewes, but no information on the milk fat composition were reported in that paper.

* Supported by Italian Ministero delle Politiche Agricole e Forestali (MIPAF) and by Agenzia Regionale per lo Sviluppo e Innovazione in Agricoltura of Tuscany (ARSIA)
[4] Corresponding author: e-mail: mauro.antongiovanni@unifi.it

Aim of the present work was to verify whether and how the supplementation with unprotected soyabean oil (SO) to diets with different F/C ratio may affect the $C_{18:1}$ and CLA isomers content in milk fat of Sarda ewes.

MATERIAL AND METHODS

The experimental design was 4×4 Latin square with 4 replicates per diet. The animals were 16 Sarda ewes in mid lactation, fed 4 different diets based on lucerne hay and a concentrate mixture of barley meal, soyabean meal, minerals and vitamins. The diets were isonitrogenous (15% CP on DM) at 2 F/C ratios (75/25 or 60/40 DM), supplemented or not with rumen unprotected SO (100 g/head/d).

Milk fat was analysed for *cis* and *trans* $C_{18:1}$ isomers by gas chromatography (Christie, 2001) and for CLA isomers by Ag^+ ion HPLC (Sehat et al., 1998).

Statistical analysis was performed using a linear model including the fixed effects of: 1. diet; 2. replicate; 3. period within replicate and 4. ewe within replicate. Contrasts for F/C ratio and SO treatment were tested for significance.

RESULTS AND DISCUSSION

All *cis* and *trans* $C_{18:1}$ isomers resulted enhanced in the milk of ewes fed the diets with SO added, but *trans* ones were increased at a higher extent (Table 1).

Table 1. Effect of F/G ratio and SO supplementation on $C_{18:1}$ isomers in milk, g/100 g total lipids

	Diets[1]				SE	Contrasts[2]	
	HF/NO	HF/O	LF/NO	LF/O		F/C	SO
trans 6, *trans* 7, *trans* 8	0.05	0.36	0.06	0.37	0.011		**
trans 9	0.10	0.44	0.12	0.36	0.015		**
trans 10	0.14	0.60	0.23	0.87	0.025	**	**
trans 11	0.73	7.75	0.77	5.95	0.192	**	**
trans 12	0.07	0.35	0.07	0.45	0.020	*	**
Total *trans* isomers	1.09	9.50	1.26	8.01	0.023	**	**
cis 7	0.15	0.33	0.13	0.38	0.016		**
cis 9	10.45	13.76	11.07	13.01	0.285		**
cis 11	0.21	0.38	0.24	0.32	0.012		**
cis 12	0.14	0.63	0.18	0.87	0.037	**	**
cis 13	0.04	0.10	0.04	0.10	0.003		**
cis 14	0.17	0.32	0.19	0.38	0.013	**	**
cis 15	0.02	0.09	0.03	0.05	0.001	**	**
Total *cis* isomers	11.18	15.60	11.89	15.12	0.003		**

[1] diets: HF/NO - high forage no oil; HF/O - high forage with oil; LF/NO - low forage no oil; LF/O - low forage with oil

[2] contrasts: F/C - forage/concentrate ratio; SO - soyabean oil

* ($P<0.05$); ** ($P<0.01$)

In particular, *trans* 11 was increased more than 10 times with diet HF/O as compared with HF/NO and almost 8 times with LF/O against LF/NO. The contrast HF vs LF was statistically significant as well. The same effect of the SO supplementation was observed for *trans* 10, but with smaller differences.

Also CLA isomers were affected by the diet composition (Table 2). *Trans* 10, *cis* 12 reached the highest level with diet LF/O, while diet HF/O accounted for the highest concentration of *cis* 9, *trans*11 (rumenic acid, RA). A similar pattern was reported by Kukuk et al. (2001) who monitored the duodenal flow of fatty acids in sheep fed SO supplemented diets with different F/C ratios; low forage diets resulted in a greater duodenal flow of UFA, including all *trans* $C_{18:1}$ isomers and *trans* 10, *cis* 12 CLA; the highest flow of RA resulted from high forage diets.

Table 2. Effect of F/C ratio and SO supplementation on CLA isomers in milk, mg/g total lipids

	Diets[1]				SE	Contrasts[2]	
	HF/NO	HF/O	LF/NO	LF/O		forage	oil
trans 11, *trans* 13	0.18	0.27	0.13	0.24	0.02		**
trans 10, *trans* 12	0.06	0.22	0.05	0.19	0.01		**
trans 9, *trans* 11	0.09	0.32	0.11	0.28	0.01		**
trans 8, *trans* 10	0.11	0.18	0.10	0.19	0.02		**
trans 7, *trans* 9	0.09	0.13	0.08	0.14	0.01		**
Other *trans*, *trans*	0.06	0.11	0.05	0.12	0.01		**
cis 13, *trans* 15	0.05	0.03	0.05	0.05	0.001		
cis 12, *trans* 14	0.01	0.02	0.01	0.02	0.001		
trans 11, *cis* 13	0.12	0.28	0.13	0.15	0.03		*
trans 10, *cis* 12	0.02	0.07	0.06	0.11	0.001	**	**
cis 9, *trans* 11	3.86	22.24	5.82	18.06	2.96		**
cis 8, *trans* 10	0.06	0.30	0.12	0.23	0.01		**
trans 7, *cis* 9	0.26	0.83	0.34	1.03	0.09		**
Total CLA isomers	4.98	25.01	7.07	20.82	3.20		**

[1] diets: HF/NO - high forage no oil; HF/O - high forage with oil; LF/NO - low forage no oil; LF/O - low forage with oil

[2] contrasts: F/C - forage/concentrate ratio; SO - soyabean oil

* ($P<0.05$); ** ($P<0.01$)

Piperova et al. (2000) reported that both RA and VA were increased only slightly in milk of dairy cows fed high concentrate diets supplemented with SO. In the present experiment, even though the F/C ratios didn't reach the extreme levels of the Piperova's experiment, a similar behaviour was observed: diet HF/O induced the highest amount of RA, probably due to the accumulation of a higher amount of VA, the mammary precursor of RA in the desaturation pathway bio-catalysed by stearoyl-CoA desaturase (Griinari et al., 2000).

The shift toward *trans* 10 isomers most probably occurred in the rumen of the ewes fed the low F/C ratio diets: actually, higher amounts of *trans* 10 $C_{18:1}$,

cis 12 $C_{18:1}$ and of *trans* 10, *cis* 12 CLA could be detected in milk fat. This is in accordance with what reported by Chilliard et al. (2002).

The levels of RA in milk from ewes fed diets with no SO supplementation resulted very low and comparable with the levels found in the milk of cows fed traditional diets in Northern Italy, based on dry forages (Secchiari et al., 2003). These data seem to indicate that when dairy ewes are fed similarly to dairy cows, the differences between the two species, as far as CLA in milk is concerned, are quite small.

CONCLUSIONS

The supplementation with SO of both HF and LF diets resulted in an important increase of RA and VA levels in milk fat, but the HF diet induced higher levels of both these acids. On the contrary, the LF diets allowed an increase of *trans* 10 $C_{18:1}$ and of *trans* 10, *cis 12* CLA, probably due to a shift of the rumen biohydrogenation pathway of linoleic acid.

In conclusion, the interaction between the F/C ratio and the lipid fraction of the diet appeared to affect the *trans* $C_{18:1}$ and CLA isomers profile of milk fat of dairy ewes, as it does in cows.

REFERENCES

Chilliard Y., Ferlay A., Loor J., Rouel J., Martin B., 2002. *Trans* and conjugated fatty acids in milk from cows and goats consuming pasture or receiving vegetable oils or seeds. Ital. J. Anim. Sci. 1, 243-254

Christie W.W., Sébédio J.L., Juáneda P., 2001. A practical guide to the analysis of conjugated linoleic acid. Inform. 12, 147-149

Griinari J.M., Corl B.A., Lacy S.H., Chouinard P.Y., Nurmela K.V.V., Bauman D.E., 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9 desaturase. J. Nutr. 130, 2285-2291

Kukuk O., Hess B.W., Ludden P.A., Rule D.C., 2001. Effect of forage/concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. J. Anim. Sci. 79, 2233-2240

Piperova L.S., Teter B.B., Bruckental I., Sampugna J., Mills S.E., Yurawecz M.P., Fritsche J., Ku K., Erdman R.A., 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130, 2658-2674

Secchiari P., Antongiovanni M., Mele M., Serra A., Buccioni A., Ferruzzi G., Paoletti F., Petacchi F., 2003. Effect of kind of dietary fat on quality of milk fat from Italian Friesian cows. Livest. Prod. Sci. 83, 43-52

Sehat N., Yurawecz M.P., Roach J.A.G., Mossoba M.M., Kramer J.K.G, Ku K., 1998. Silver ion High Performance Liquid Chromatographic separation and identification of conjugated linoleic acid isomers. Lipids 33, 217-221