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in

Ranilla M.J. (ed.), Carro M.D. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Challenging strategies to promote the sheep and goat sector in the current global context

Zaragoza : CIHEAM / CSIC / Universidad de León / FAO
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 99

2011
pages 337-342

Milk production and fatty acid profile after three weeks of diet supplementation with sunflower oil and marine algae in dairy ewes

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Abstract. This work was conducted to investigate the effect of supplementation of diets for dairy ewe with sunflower oil (SO) and incremental levels of marine algae (MA) on animal performance and milk fatty acid (FA) profile. Fifty Assaf ewes in mid-lactation, distributed in 10 lots of 5 animals each, were allocated to 5 treatments (feeding regimes; 2 lots/treatment). All animals received a total mixed ration either without lipid supplementation (Control) or supplemented with 25 g SO/kg plus 0, 8, 16, or 24 g MA/kg (SO, $SOMA_1$, $SOMA_2$, and $SOMA_3$, respectively). Milk production and composition were recorded after 3 weeks on treatments. SO plus the highest level of algae supplement ($SOMA_3$) resulted in the highest *cis*-9 *trans*-11 CLA content of milk fat (3.89 *vs* 1.58 and 0.48 % of total FA for $SOMA_3$, SO and Control diets, respectively), while the atherogenicity index decreased in all supplemented treatments as compared with the Control (P<0.01) but with no differences between them. On the other hand, although there were no significant differences in DM intake and milk production between treatments, milk fat content decreased in all ewes fed marine algae (-21%; P<0.001). The joint action of some biohydrogenation intermediates is discussed to explain these differences.

Keywords. CLA – Milk fat depression – PUFA – Sheep.

Production laitière et profil des acides gras du lait chez des brebis trois semaines après avoir reçu des régime enrichis d'huile de tournesol et d'algues marines

Résumé. *Ce travail a été mené pour étudier l'effet d'un régime à base d'huile de tournesol (SO) et de quantités croissantes d'algues marines (MA) sur des brebis laitières ainsi que sur le profil des acides gras (FA) de leur lait. Cinquante brebis Assaf en mi-lactation, réparties en 10 groupes (n=5) ont été soumis à 5 traitements (régime: 2 groupes/traitement). Tout les animaux ont reçu une ration sans supplémentation lipidique (témoin) ou supplémentés avec 25 g SO/kg et 0, 8, 16 ou 24 g MA/kg (SO, $SOMA_1$, $SOMA_2$, et $SOMA_3$ respectivement). La production ainsi que la composition du lait ont été relevées après trois semaines de traitement. La complémentation en SO et en algues marines au niveau le plus élevé ($SOMA_3$) ont presenté la teneur la plus élevée en cis-9 trans-11 CLA dans la lait (3,89 contre 1,58 et 0,48% des FA totaux pour les régimes $SOMA_1$, SO et le contrôle, respectivement), alors que l'index d'athérogénicité a baissé avec les traitements SO, $SOMA_1$, $SOMA_2$, et $SOMA_3$, en comparaison avec le régime témoin (P<0,001). Cependant, aucune différence n'a été constatée entre les index. De l'autre côté, même s'il n'y avait pas de différence significative quant à l'ingestion de matière sèche et à la production de lait entre les traitements, le teneur en lipides du lait a chuté chez toutes les brebis recevant les algues marines (-21%; P<0,001). L'action conjointe des intermédiaires en biohydrogénation est examinée afin d'expliquer ces différences.*

Mots-clés. *CLA – Dépression du gras du lait – PUFA – Brebis.*

I – Introduction

Ovine milk production is of great importance for the economy of Mediterranean countries where, as a result of considerable improvements in dairy sheep farming, it could prove to be a suitable alternative for the dairy cattle industry. The nutritional advantages of sheep milk consumption

(Park *et al.*, 2007) may be used for its promotion, especially the fact that its content in conjugated linoleic acid (CLA), a fatty acid (FA) with potential anti-mutagenic, anti-atherosclerotic and anti-diabetic effects (Palmquist *et al.*, 2005), appears to be the highest among ruminants (>10 g/kg total FA; Park *et al.*, 2007). This is thought to be due to pasture-based systems still being common in sheep production, while milk CLA concentration is higher in grazing animals than in those fed conserved forages or cereal-based concentrates (Pulina *et al.*, 2006; Gómez-Cortés *et al.*, 2009). However, it has recently been shown that supplementation of total mixed rations with vegetable oils, such as sunflower oil (SO), can increase milk CLA content in ewes (up to 36 g/kg total FA; Hervás *et al.*, 2008) to a higher extent than grazing.

Adding marine lipids, rich in long chain n-3 polyunsaturated fatty acids (PUFA) to SO supplements, can induce further increases in milk CLA content (Palmquist and Griinari, 2006) but also milk fat depression in cows (Franklin *et al.*, 1999; Boeckaert *et al.*, 2008; Gama *et al.*, 2008). Although similar results have been observed when fish oil is included in the diet of sheep (Capper *et al.*, 2007; Toral *et al.*, 2009), the effects of marine algae (MA) are inconsistent and may depend on basal diet composition and algae dosage (Papadopoulos *et al.*, 2002; Reynolds *et al.*, 2006). In addition, the scarcity of studies on this issue makes it difficult to establish an optimal level of inclusion of MA plus a vegetable oil (e.g., SO) in the diet of ewes in order to obtain a healthier milk FA profile with no detrimental effects on animal performance.

The objective of this study was therefore to investigate the effect of the inclusion of SO plus incremental levels of MA in the diet of dairy ewes on animal performance and milk fatty acid profile.

II – Materials and methods

Fifty Assaf ewes (body weight: 84.9 ± 1.66 kg) in mid lactation (at week 14 at the beginning of the experiment) were distributed in 10 lots of 5 animals each, balanced for milk production, body weight, days postpartum and number of lactation, and allocated at random to 5 experimental feeding regimes (2 lots per diet): a total mixed ration (TMR) without lipid supplementation (Control) or supplemented with 25 g/kg of SO plus 0, 8, 16, or 24 g/kg of whole cell MA (SO, $SOMA_1$, $SOMA_2$, and $SOMA_3$ diets, respectively). The TMR was formulated (g/kg) from dehydrated alfalfa hay (485), whole maize (136) and barley (175) grains, soybean meal (97), beet pulp (49), molasses (36), and minerals and vitamins (22). The algae (DHA GOLD® Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD, USA) contained 403 g of ether extract (EE)/kg DM and the concentration of FA (g/kg total FA methyl esters) for C14:0, C16:0, C22:5 n-6, and C22:6 n-3 was 85, 232, 177, and 423, respectively. Sunflower oil FA concentration (g/kg total FA methyl esters) for C16:0, C18:0, C18:1, and C18:2 was 75, 43, 263, and 605, respectively.

The ewes were milked twice daily at 8.30 and 18.30 h in a 1 x 10 stall milking parlour (DeLaval, Madrid, Spain) and the diets, prepared each week, were offered *ad libitum* after each milking. Clean water and vitamin-mineral supplement were always available. The experiment was carried out in accordance with Spanish Royal Decree 1201/2005 for the protection of animals used for experimental purposes.

After 3 weeks on treatments, individual milk production was recorded and milk samples collected from each animal for fat, protein and total solid content analyses (ISO 9622:1999). 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 Hervás *et al.* (2008). Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of the glycerides according to ISO procedure 15884:2002. Analysis of FAME was performed on a gas chromatograph (Agilent 6890 N Network System, Palo Alto, CA, USA) onto a CP-Sil 88 fused silica capillary column (100 m x 0.25 mm, Varian, Middelburg, The Netherlands). Dry matter intake

(DMI) was recorded for each experimental lot. Samples of offered diet were analysed for DM (ISO 6496:1999), ash (ISO 5984:2002), crude protein (CP; ISO 5983-2:2005) and starch (ISO 6493:2000). Ankom Technology (Ankom Technology Corp., Macedon, NY, USA) was used to determine neutral-detergent fibre (NDF) and EE (AOCS, Official Procedure Am 5-04).

All data were analysed as a one-way analysis of variance using the GLM procedure of the SAS software package, version 9.1 (SAS Institute Inc., Cary, NC, USA). Significant differences were declared at $P<0.05$.

III – Results and discussion

The basal diet (to which the supplements were added) contained, per kg DM, 897 g organic matter, 156 g CP, 188 g starch, and 300 g NDF. EE content per kg DM was 26 g for Control, 50 g for SO, 54 g for $SOMA_1$, 57 g for $SOMA_2$, and 63 g for $SOMA_3$.

As shown in Table 1, there were no significant differences in DMI between treatments after three weeks on the diets, which agrees with previous studies in dairy sheep reporting no variations in DMI when fed a highly SO-supplemented diet (60 g/kg; Hervás *et al.*, 2008). However, inconsistent results have been observed with MA supplementation and longer trials (Papadopoulos *et al.* 2002; Reynolds *et al.*, 2006). Although in dairy cows MA supply reduces feed intake and consequently milk production (Franklin *et al.*, 1999; Offer *et al.*, 2001; Boeckaert *et al.*, 2008), no variations in the amount of milk produced were found in the present study, in accordance with the lack of differences in DMI.

Table 1. Dry matter intake (kg/d), milk production (kg/d) and composition (%) in ewes fed a total mixed ration without lipid supplementation (Control) or supplemented with 25 g/kg of sunflower oil plus 0, 8, 16, or 24 g/kg of marine algae (SO, $SOMA_1$, $SOMA_2$, and $SOMA_3$ diets, respectively), after three weeks on diets

		Diet					s.e.d.	P =
		Control	SO	$SOMA_1$	$SOMA_2$	$SOMA_3$		
Dry matter intake		3.32	3.59	3.70	3.48	3.49	0.240	0.623
Milk production		1.92	2.13	2.00	1.93	1.83	0.335	0.925
Composition	Fat	5.95[a]	6.28[a]	4.95[b]	4.89[b]	4.66[b]	0.400	<0.001
	Protein	5.17	5.19	4.94	5.03	4.92	0.200	0.551
	Total solids	16.69[a]	17.15[a]	15.51[b]	15.48[b]	15.22[b]	0.510	0.001

s.e.d. = standard error of the difference.

[a-c] Means within a row with different superscripts differ significantly ($P<0.05$).

The protein content was not significantly affected by lipid supplementation, but milk fat content was reduced in all SOMA treatments (-21% on average; $P<0.001$), even though some authors have reported that sheep seem less prone to milk fat depression (MFD) than cows when fed high-concentrate diets and free vegetal oils (Pulina *et al.*, 2006; Hervás *et al.*, 2008). This type of MFD is usually related to an increased concentration of the isomer *trans*-10 *cis*-12 CLA in dairy cow milk but there is some evidence that feeding marine lipids reduces milk fat synthesis via other mechanisms (Shingfield and Griinari, 2007). In the present study, there were no variations in the concentration of *trans*-10 *cis*-12 CLA (see Table 2), but a 6-fold increase in *trans*-9 *cis*-11 CLA was observed with MA supplementation ($P<0.001$). However, although Perfield *et al.* (2007) demonstrated a role of this isomer in MFD in cows, it explains only a 15% reduction in milk fat during diet-induced MFD, so the contribution of other biohydrogenation intermediates, in smaller

amounts, cannot be ruled out (Shingfield and Griinari, 2007). In addition, most, if not all, cases of marine lipid-induced MFD in cows are characterized by a pronounced fall in stearic (C18:0) and oleic (*cis*-9 C18:1) acid concentration in milk fat together with an increase in *trans*-C18:1 contents (Franklin *et al.*, 1999; Boeckaert *et al.*, 2008; Gama *et al.*, 2008). This is in line with the changes induced by the three SOMA diets under study and also with results observed in ewes supplemented with SO plus fish oil (Toral *et al.*, 2009). The reductions in the supply of stearic acid for endogenous oleic acid synthesis in the mammary gland, together with the increase in *trans*-C18:1 isomers, which would replace oleic acid in milk fat triacylglycerols (Shingfield and Griinari, 2007), may also be contributing to MFD. Since *trans*-isomers of FA have higher melting points than equivalent *cis*-isomers, it seems reasonable to expect that those milk fat globules with melting points higher than body temperature cannot be secreted, resulting then in MFD (Gama *et al.*, 2008). Moreover, all supplemented diets caused a significant reduction in C6:0 to C12:0 saturated FA due to the inhibitory effect of linoleic acid or its metabolites on *de novo* FA synthesis (Palmquist and Griinari, 2006; Gómez-Cortés *et al.*, 2009; Hervás *et al.*, 2008). In the specific case of the SO diet, this inhibition may have been compensated for by the increase in C18 FA yield, thus preventing a net reduction in milk fat content.

As expected, lipid supplementation induced a significant rise of the milk CLA content. Thus, addition of SO plus the highest level of algae ($SOMA_3$) resulted in the greatest rumenic acid (RA; *cis*-9 *trans*-11 CLA) content in fat (3.89 *vs* 1.58 and 0.48% of total FA for $SOMA_3$, SO and Control diets, respectively), this isomer (accounting on average for approximately 90% of total CLA) being responsible for the main health-promoting effects of CLA (Palmquist *et al.*, 2005). Nevertheless, the high levels of RA attained with SOMA diets were accompanied by a rise not only in vaccenic acid (VA; *trans*-11 C18:1; which can be converted to RA in human tissues; Palmquist *et al.*, 2005) but also in other *trans*-C18:1 isomers, such as *trans*-10. Thus, it should be pointed out that a remarkable 29-fold increase in this latter isomer was observed in ewes fed $SOMA_1$ and $SOMA_2$ diets ($P<0.001$). Although recent research suggests that *trans*-FA from ruminant-derived foods would have innocuous effects on cardiovascular disease (Lemaitre *et al.*, 2006), these findings might simply reflect the lower consumption of ruminant *trans*-FA in the human diet or isomer-specific bioactivity (Shingfield *et al.*, 2008), while the potential specific role of *trans*-10 C18:1 and other *trans*-FA is still unclear.

Notwithstanding, other changes due to lipid supplementation, such as the fall in its atherogenicity index ($P<0.01$), pointed clearly towards a healthier milk fat profile. PUFA concentrations were remarkably enhanced with increasing levels of MA, especially that of docosahexanoic acid (DHA), even though the transfer efficiency of this bioactive n-3 FA was low (on average 5%), in agreement with that observed by Offer *et al.* (2001) in cows, but lower than previously reported in dairy sheep (Papadopoulos *et al.*, 2002; Reynolds *et al.*, 2006). In any event, it is important to emphasize that a low balance of n-6/n-3 FA in foods appears to be more important than total n-3 FA in reducing the risk of many diseases (Simopoulos, 2008). Milk from ewes receiving the Control diet had a low n-6/n-3 ratio further reduced with SOMA supplementation ($P<0.01$), reaching a nadir of 1.3:1 for $SOMA_2$ and $SOMA_3$ treatments, which would potentially exert suppressive effects on the pathogenesis of highly prevalent chronic diseases (Simopoulos, 2008).

Table 2. Partial milk fatty acid (FA) profile (% of total FA) in ewes fed a total mixed ration without lipid supplementation (Control) or supplemented with 25 g/kg of sunflower oil plus 0, 8, 16, or 24 g/kg of marine algae (SO, $SOMA_1$, $SOMA_2$, and $SOMA_3$ diets, respectively), after three weeks on diets

		Diet					s.e.d.	P =
		Control	SO	$SOMA_1$	$SOMA_2$	$SOMA_3$		
C4:0		3.48^{b}	4.01^{ab}	3.94^{ab}	4.12^{ab}	4.22^{a}	0.165	0.038
C6:0		3.34^{a}	3.18^{ab}	2.71^{c}	3.03^{b}	3.11^{ab}	0.076	0.003
C8:0		3.44^{a}	2.84^{b}	2.30^{b}	2.58^{b}	2.62^{b}	0.141	0.004
C10:0		10.97^{a}	7.80^{b}	6.81^{b}	7.19^{b}	7.24^{b}	0.595	0.005
C12:0		6.13^{a}	4.20^{b}	4.26^{b}	4.02^{b}	3.93^{b}	0.356	0.007
C14:0		11.89	10.59	12.15	11.62	11.26	0.414	0.071
C16:0		25.71^{a}	21.75^{b}	24.45^{a}	24.52^{a}	26.07^{a}	0.532	0.003
C18:0		6.88^{b}	9.20^{a}	2.21^{c}	1.35^{c}	1.23^{c}	0.469	<0.001
cis-9 C18:1		13.70^{b}	17.78^{a}	9.38^{c}	8.64^{cd}	7.99^{d}	0.308	<0.001
trans-10 C18:1		0.32^{c}	0.75^{c}	9.13^{a}	9.62^{a}	5.77^{b}	0.756	<0.001
trans-11 C18:1 (VA)		0.91^{d}	3.03^{c}	6.17^{b}	6.95^{b}	8.75^{a}	0.326	<0.001
Total *trans* C18:1		1.84^{c}	5.25^{b}	16.58^{a}	17.36^{a}	15.22^{a}	0.836	<0.001
cis-9 *cis*-12 C18:2		2.29^{a}	2.21^{ab}	1.82^{bc}	1.89^{abc}	1.51^{c}	0.115	0.006
cis-9 *trans*-11 CLA (RA)		0.48^{d}	1.58^{c}	2.52^{b}	2.58^{b}	3.89^{a}	0.213	<0.001
trans-9 *cis*-11 CLA		0.02^{b}	0.04^{b}	0.17^{a}	0.19^{a}	0.16^{a}	0.014	<0.001
trans-10 *cis*-12 CLA		0.01	0.01	0.02	0.02	0.01	0.004	0.114
Total CLA		0.57^{d}	1.69^{c}	2.77^{b}	2.87^{b}	4.13^{a}	0.199	<0.001
C20:5 n-3 (EPA)		0.05^{b}	0.04^{b}	0.05^{b}	0.10^{a}	0.13^{a}	0.008	<0.001
C22:5 n-6 (DPA)		0.10^{c}	0.08^{c}	0.09^{c}	0.13^{b}	0.18^{a}	0.007	<0.001
C22:6 n-3 (DHA)		0.02^{d}	0.02^{d}	0.23^{c}	0.52^{b}	0.67^{a}	0.032	<0.001
Summary	SFA	75.37^{a}	66.35^{b}	61.58^{c}	61.07^{c}	62.38^{bc}	1.112	<0.001
	MUFA	20.08^{b}	28.03^{a}	30.92^{a}	30.50^{a}	27.80^{a}	1.099	0.001
	PUFA	4.47^{d}	5.50^{cd}	6.68^{bc}	7.44^{ab}	8.75^{a}	0.333	<0.001
n-6/n-3 ratio		3.35^{b}	4.02^{a}	2.37^{c}	1.53^{d}	1.11^{d}	0.108	<0.001
Atherogenicity index		3.25^{a}	2.04^{b}	2.06^{b}	1.98^{b}	2.06^{b}	0.152	0.002

s.e.d. = standard error of the difference; SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; Atherogenicity index = (C12:0 + 4×C14:0 + C16)/(MUFA + PUFA).
$^{a-d}$ Means within a row with different superscripts differ significantly (P<0.05).

IV – Conclusions

Diet supplementation with SO (20 g/kg) plus incremental levels of MA (8, 16 and 24 g/kg) did not affect milk production but reduced milk fat content in dairy ewes. Some changes in milk fat profile, such as a remarkable dose-dependent increase of VA, RA, and DHA contents, and reductions in the atherogenicity index and the n-6/n-3 ratio, would be in line with an improved ovine milk quality for health-conscious consumers. However, given the very high content of *trans*-10 C18:1 observed in diets $SOMA_1$ and $SOMA_2$, it would be prudent to leave these findings under question until further research confirms whether *trans*-FA from ruminant-derived foods are actually innocuous for humans.

Acknowledgements

This work was supported by the MICINN (Research Project AGL2008-04805). P.G. Toral gratefully acknowledges receipt of a pre-doctoral grant from the CSIC (I3P Programme).

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