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Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae

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ABSTRACT

In an attempt to develop strategies for enhancing the nutritional value of sheep milk fat, dairy ewe diet was supplemented with 3 incremental levels of marine algae (MA), in combination with sunflower oil, to evaluate the effects of these marine lipids on milk fatty acid (FA) profile and animal performance. Fifty Assaf ewes in mid lactation were distributed in 10 lots of 5 animals each and allocated to 5 treatments (2 lots per treatment): no lipid supplementation (control) or supplementation with 25 g of sunflower oil/kg of DM plus 0 (SO), 8 ($SOMA_1$), 16 ($SOMA_2$), or 24 ($SOMA_3$) g of MA (56.7% ether extract)/kg of DM. Milk production and composition, including FA profile, were analyzed on d 0, 3, 7, 14, 21, and 28 of treatment. Neither intake nor milk yield were significantly affected by lipid addition, but all MA supplements decreased milk fat content from d 14 onward, reaching a 30% reduction after 28 d on $SOMA_3$. This milk fat depression might be related not only to the joint action of some putative fat synthesis inhibitors, such as *trans*-9,*cis*-11 C18:2 and probably *trans*-10 C18:1, but also to the limited ability of the mammary gland to maintain a desirable milk fat fluidity, that would have been caused by the noticeable increase in *trans*-C18:1 together with the lowered availability of stearic acid for oleic acid synthesis through Δ^9-desaturase. Furthermore, all lipid supplements, and mainly MA, reduced the secretion of de novo FA (C6:0–C14:0) without increasing the yield of preformed FA (>C16). Supplementation with sunflower oil plus MA resulted in larger increases in *cis*-9,*trans*-11 C18:2 than those observed with sunflower oil alone, achieving a mean content as high as 3.22% of total FA and representing a more than 7-fold increase compared with the control. Vaccenic acid (*trans*-11 C18:1) was also significantly enhanced (on average +794% in SOMA treatments), as was C22:6 n-3 (DHA) content, although the transfer efficiency of the latter, from the diets to the milk, was very low (5%). However, the highest levels of MA inclusion ($SOMA_2$ and $SOMA_3$) reduced the milk n-6:n-3 ratio, but MA supplements caused an important increase in *trans*-10 C18:1, which would rule out the possibility that this milk has a healthier fat profile before determining the specific role of each individual FA and ensuring that this *trans*-FA is at least innocuous in relation to cardiovascular disease risk.

Key words: conjugated linoleic acid, milk fat depression, n-3, *trans* fatty acid

INTRODUCTION

A growing epidemic of chronic disease related to dietary and lifestyle changes afflicts both developed and developing countries, with cardiovascular disease, cancer, and diabetes nowadays being among the most important causes of premature death (WHO, 2003). The discovery of potential anticarcinogenic, antiatherosclerotic, and antidiabetic effects of conjugated linoleic acid (**CLA**; Pariza et al., 2001; Shingfield et al., 2008) and the recognized role in human health of the n-3 fatty acids (**FA**; Simopoulos, 2008) have led to an increasing number of studies over the past decade seeking to enhance the content of these bioactive compounds in ruminant-derived products, mainly in cow milk. Even though consumption of ovine milk might have several nutritional advantages over bovine milk consumption, such as its higher mineral (e. g., Ca, P, and Mg), and caprylic (C8:0) and capric (C10:0) acid contents and its easier digestibility (Recio et al., 2009), research in ewes is still scarce.

In a previous study in dairy sheep, inclusion in the diet of sunflower oil (**SO**), rich in linoleic acid, induced a 4-fold increase in milk CLA content (Hervás et al., 2008), presumably through increased ruminal formation of vaccenic acid (**VA**; *trans*-11 C18:1; Palmquist et al., 2005; Chilliard et al., 2007), which serves as a substrate for endogenous synthesis of the major isomer of CLA, rumenic acid (**RA**; *cis*-9,*trans*-11 CLA) not only in the ruminant mammary gland but also in some human tissues (Palmquist et al., 2005). The use of SO

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in combination with long-chain n-3 polyunsaturated fatty acids (**PUFA**) of marine lipids, which are inhibitors of the ruminal reduction of *trans*-C18:1 to stearic acid (C18:0; Loor et al., 2005; Or-Rashid et al., 2008), would induce further increases in VA ruminal out-flow and subsequently RA mammary synthesis, as reported in cows (Shingfield et al., 2006; Cruz-Hernández et al., 2007). However, the nutritional strategy of supplying a source of linoleic acid together with marine lipids may also increase some *trans*-C18:1 in ruminant milk fat (Reynolds et al., 2006; Shingfield et al., 2006; Cruz-Hernández et al., 2007), whose potential specific role for human health is still unclear (Shingfield et al., 2008).

In addition to the effects addressed to modify milk FA profile, the inclusion of fish oil in the diet appears to affect animal performance, reducing milk fat content in both dairy ewes (Capper et al., 2007) and cows (Griinari and Bauman, 2006; Cruz-Hernández et al., 2007; Gama et al., 2008). Addition of marine algae (**MA**), on the contrary, has been reported to induce milk fat depression (**MFD**) in cows (Franklin et al., 1999; Offer et al., 2001; Boeckaert et al., 2008) but not in sheep (Papadopoulos et al., 2002; Reynolds et al., 2006) and is responsible for a greater transfer efficiency of long-chain n-3 PUFA into the milk in this species than in cattle (Papadopoulos et al., 2002; Reynolds et al., 2006; Chilliard et al., 2007). Notwithstanding, effects of MA inclusion described in the literature are quite inconsistent and might depend on several factors, such as basal diet composition and algae dosage (Reynolds et al., 2006). This fact, together with the scarcity of published studies on this issue, makes it difficult to establish an appropriate level of MA inclusion in the diet of sheep to obtain a healthier milk FA profile for human consumers, with no detrimental effects on animal performance. The objective of this study was therefore to investigate the effect of the dietary inclusion of incremental levels of MA, in combination with SO, on dairy ewes' performance and milk FA profile.

MATERIALS AND METHODS

Animals, Experimental Diets, and Management

Fifty multiparous Assaf ewes (BW = 84.9 kg; SD = 11.75) in mid lactation (at wk 14 at the beginning of the experiment; SD = 1.0) were stratified according to milk production, BW, days postpartum, and number of lactation, randomly distributed in 10 lots of 5 animals each, and allocated to 5 experimental treatments (2 lots per treatment): no lipid supplementation or supplementation with SO (Carrefour S.A., Spain), either alone or in combination with 3 increasing levels of MA (DHA Gold Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD; 567 g of ether extract/kg of DM).

The diets, prepared weekly, consisted of a TMR based on alfalfa hay (particle size >4 cm) and a concentrate (50:50) either without lipid supplementation (control diet; negative control) or supplemented with 25 g of SO/kg of DM plus 0 (SO diet; positive control), 8 (**SOMA$_1$**), 16 (**SOMA$_2$**), or 24 (**SOMA$_3$**) g of MA/kg of DM. The ingredients and chemical composition of the 5 experimental diets, which included molasses to avoid selection of dietary components, are given in Table 1. During a 3-wk adaptation period (before commencing the trial), all animals received the control diet. Clean water and a vitamin-mineral supplement were always available and fresh diets were offered daily ad libitum at 0900 and 1900 h.

The ewes were milked at approximately 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain). The experiment lasted for 4 wk and was carried out in accordance with Spanish Royal Decree 1201/2005 for the protection of animals used for experimental purposes.

Measurements, Sample Collection, and Chemical Analyses

Samples of offered and refused diets were collected once a week, stored at −30°C, and then freeze-dried. The DMI was recorded weekly for each experimental lot. Diet samples were analyzed for DM (ISO, 1999a), ash (ISO, 2002a), and CP (ISO, 2005). Neutral detergent fiber and ADF were determined as described by Ankom Technology (Ankom, 2006a,b). Neutral detergent fiber was assayed with sodium sulfite and α-amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by the Ankom Filter Bag Technology (American Oil Chemists' Society Official Procedure Am 5–04; AOCS, 2008).

Individual milk yield was recorded on d 0, 3, 7, 14, 21, and 28, both at morning and evening milkings. With the same frequency, milk samples for the analysis of fat, protein, and TS were collected from each animal, composited according to morning and evening milk yield, and treated with natamycin. The protein, fat, and TS concentrations were determined by infrared spectrophotometry (ISO, 1999b) using a MilkoScan 255 A/S N (Foss Electric, Hillerød, Denmark).

Milk FA composition was determined in untreated samples from each experimental lot and composited according to individual milk production within day. Milk fat was extracted as described by Luna et al. (2005), and FA methyl esters (**FAME**) were prepared by base-catalyzed methanolysis of the glycerides (ISO, 2002b).