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Milk fat depression induced by dietary marine algae in dairy ewes: Persistency of milk fatty acid composition and animal performance responses

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ABSTRACT

Addition of marine algae (MA) to the diet of dairy ruminants has proven to be an effective strategy to enhance the milk content of some bioactive lipids, but it has also been associated with the syndrome of milk fat depression. Little is known, however, about the persistency of the response to dietary MA in sheep. Based on previous experiments with dairy ewes fed sunflower oil plus MA, it was hypothesized that the response might be mediated by time-dependent adaptations of the rumen microbiota, which could be evaluated indirectly through milk fatty acid (FA) profiles. Animal performance and milk FA composition in response to MA in the diet were studied using 36 Assaf ewes distributed in 6 lots and allocated to 2 treatments (3 lots/treatment) consisting of a total mixed ration (40:60 forage:concentrate ratio) supplemented with 25 g of sunflower oil (SO)/kg of dry matter plus 0 (SO; control diet) or 8 g of MA/kg of dry matter (SOMA diet). Milk production and composition, including FA profile, were analyzed on d 0, 6, 12, 18, 24, 34, 44, and 54 of treatment. Diet supplementation with MA did not affect milk yield but did decrease milk fat content. Differences in the latter were detected from d 18 onward and reached −17% at the end of the experiment (i.e., on d 54). Compared with the control diet, the SOMA diet caused a reduction in milk 18:0 and its desaturation product (*cis*-9 18:1) that lasted for the whole experimental period. This decrease, together with the progressive increase in some putative fat synthesis inhibitors, especially *trans*-10 18:1, was related to the persistency of milk fat depression in lactating ewes fed MA. Additionally, inclusion of MA in the diet enhanced the milk content of *trans*-11 18:1, *cis*-9,*trans*-11 18:2, and C20–22 n-3 polyunsaturated FA, mainly 22:6 n-3. Overall, the persistency of the responses observed suggests that the ruminal microbiota did not adapt to the dietary supply of very long chain n-3 polyunsaturated fatty acids.

Key words: conjugated linoleic acid, lipid supplementation, sheep, *trans* fatty acid

INTRODUCTION

Addition of marine algae (**MA**) to the diet of dairy ruminants is an effective strategy to enhance the milk content of bioactive lipids such as n-3 PUFA or conjugated linoleic acid (**CLA**; Franklin et al., 1999; Reynolds et al., 2006). This approach in cows has also been associated with a severe decline in milk fat content (Franklin et al., 1999; Boeckaert et al., 2008a). Although the syndrome of milk fat depression (**MFD**) in ewes was not first described in response to MA (Papadopoulos et al., 2002; Reynolds et al., 2006), Toral et al. (2010b) recently observed that dietary supplementation with 2.5% sunflower oil plus incremental amounts of MA (0.8, 1.6, and 2.4%) strongly reduced milk fat content. Based on comparison with the diet supplemented with sunflower oil only, those authors attributed the MFD to the addition of MA as reflected by changes in milk FA profile. Those changes included not only an enrichment in some potentially healthy FA (e.g., *trans*-11 18:1, *cis*-9,*trans*-11 18:2, and 22:6 n-3) but also a marked increase in *trans*-10 18:1, whose effect on human health is uncertain (see review by Gebauer et al., 2011). Although some results seem contradictory (Lock et al., 2007), *trans*-10 18:1 has been proposed as a putative cause of MFD (Griinari et al., 1998; Loor et al., 2005a; Shingfield et al., 2009).

Interestingly, after 28 d on the diet supplemented with sunflower oil plus the lowest dose of MA (0.8%), Toral et al. (2010b) observed a decrease in milk *trans*-10 18:1 concentration as well as in *trans*-9,*cis*-11 18:2. Both FA arise from alternative rumen microbial biohydrogenation pathways and have been related to MFD in dairy cows (Perfield et al., 2007; Shingfield et al., 2009). This occurred together with a slight but significant increase

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in milk fat content. Analysis of the FA composition of the ruminal digesta (Toral et al., 2012) confirmed a lower content of *trans*-10 18:1 and suggested an adaptation of the rumen bacterial community to the consumption of MA and a potential reestablishment of the main pathway of biohydrogenation. If this is true and the response to marine lipid supplementation varies over time, a longer-term study would be necessary to evaluate time-dependent variations. Therefore, the main objective of this study was to evaluate animal performance and milk FA composition in dairy ewes over a 54-d period, with the aim of studying the persistency of the response to the addition of MA to a diet rich in linoleic acid.

MATERIALS AND METHODS

Animals, Experimental Diets, and Management

All experimental procedures were performed in accordance with the Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes. Thirty-six Assaf ewes (84.5 ± 1.68 kg of BW) in mid lactation (82 ± 0.9 DIM at the beginning of the experiment) were stratified according to milk yield, BW, days postpartum, lactation number, and milk fat content, and randomly distributed in 6 lots (6 ewes/lot), which in turn were assigned to 1 of 2 dietary treatments (3 lots/treatment). Diets consisted of a TMR based on alfalfa hay and a concentrate (40:60) supplemented with 25 g of sunflower oil (Carrefour S.A., Madrid, Spain)/kg of DM plus 0 (**SO**; control diet) or 8 g of marine algae (DHA Gold Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD)/kg of DM (**SOMA** diet). The ingredients and chemical composition of the experimental diets, which were prepared weekly and included molasses to reduce selection of dietary components, are presented in Table 1. Ewes were fed the control diet during 20 d of adaptation before the start of the study. Fresh diets were offered daily ad libitum at 0900 and 1900 h, and clean water was always available. Ewes were milked twice daily at 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain) throughout the 54 d experiment.

Measurements and Sampling

Intake of DM was recorded every 4 d for each experimental lot by weighing the amount of DM offered and refused by each lot. Samples of the diets and refusals were collected with the same frequency, stored at −30°C, and then freeze-dried.

Individual daily milk yields were recorded on d 0, 6, 12, 18, 24, 34, 44, and 54. Milk samples were collected with the same frequency from each ewe and composited according to morning and evening milk yield. One aliquot was stored at 4°C with natamycin (D&F Control Systems Inc., Dublin, CA) until analyzed for fat, protein, lactose, and TS. Milk FA composition was determined in untreated samples from each experimental lot, which were composited according to individual milk yield and stored at −30°C until analysis.

Chemical Analysis

Samples of TMR were analyzed for DM (ISO 6496; ISO, 1999a), ash (ISO 5984; ISO, 2002), and CP (ISO 5983-2; ISO, 2009). Neutral and acid detergent fiber were determined as described by Mertens (2002) and AOAC International (2006; method 973.18), respectively, using an Ankom^{2000} fiber analyzer (Ankom Technology Corp., Macedon, NY). 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 (AOCS, 2008; Procedure Am 5-04). Milk CP, lactose, fat, and TS contents were determined by infrared spectrophotometry (ISO 9622; ISO, 1999b), using a MilkoScan 255 A/S N (Foss Electric, Hillerød, Denmark).

Fatty acid methyl esters (**FAME**) in freeze-dried samples of TMR were prepared in a one-step extraction-transesterification procedure using chloroform and 2% (vol/vol) sulfuric acid in methanol (Shingfield et al., 2003), with tridecanoic acid (Sigma-Aldrich, Madrid, Spain) as an internal standard. For milk FA composition analysis, lipids in 1 mL of milk were extracted using diethyl ether:hexane (5:4, vol/vol) and transesterified to FAME using freshly prepared methanolic sodium methoxide, as outlined by Shingfield et al. (2003), with tridecanoic acid as an internal standard. Methyl esters were separated and quantified using a gas chromatograph (7890A GC System, Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and a 100-m fused-silica capillary column (0.25 mm i.d., 0.2 μm film thickness; CP-SIL 88, Chrompack 7489, Varian Ibérica S.A., Madrid, Spain) and He as the carrier gas. Total FAME profile in a 2-μL sample volume at a split ratio of 1:50 was determined using a temperature gradient program (Shingfield et al., 2003). Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield et al., 2003). Peaks were identified based on retention time comparisons with authentic standards (from Nu-Chek Prep., Elysian, MN; Sigma-Aldrich; and Larodan Fine Chemicals AB, Malmö, Sweden). Identification of FA was verified based on FAME standard mixtures when available, chromatograms reported in the litera-

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