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Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae

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ABSTRACT

Supplementation of ruminant diets with plant oils and marine lipids is an effective strategy for lowering saturated fatty acid (FA) content and increasing the concentration of *cis*-9,*trans*-11 conjugated linoleic acid and long-chain n-3 FA in ruminant milk. However, changes in populations of ruminal microorganisms associated with altered biohydrogenation of dietary unsaturated FA are not well characterized. Twenty-five lactating Assaf ewes were allocated at random to 1 of 5 treatments composed of dehydrated alfalfa hay and concentrates containing no additional lipid (control), or supplemented with 25 g of sunflower oil and 0 (SO), 8 ($SOMA_1$), 16 ($SOMA_2$), or 24 ($SOMA_3$) g of marine algae/kg of diet dry matter. On d 28 on diet, samples of rumen fluid were collected for lipid analysis and microbial DNA extraction. Appearance and identification of biohydrogenation intermediates was determined based on complementary gas chromatography and Ag+-HPLC analysis of FA methyl esters. Total bacteria and the *Butyrivibrio* group were studied in microbial DNA by terminal RFLP analysis, and real-time PCR was used to quantify the known *Butyrivibrio* bacteria that produce *trans*-11 18:1 or 18:0. Dietary supplements of sunflower oil alone or in combination with marine algae altered the FA profile of rumen fluid, which was associated with changes in populations of specific bacteria. Inclusion of marine algae in diets containing sunflower oil resulted in the accumulation of *trans* 18:1 and 10-O-18:0 and a marked decrease in 18:0 concentrations in rumen fluid. At the highest levels of supplementation ($SOMA_2$ and $SOMA_3$), marine algae also promoted a shift in ruminal biohydrogenation pathways toward the formation of *trans*-10 18:1 at the expense of *trans*-11 18:1. Changes in the concentration of biohydrogenation intermediates were not accompanied by significant variations in the abundance of known cultivated ruminal bacteria capable of hydrogenating unsaturated FA. However, certain bacterial groups detected by terminal RFLP (such as potentially uncultured *Lachnospiraceae* strains or *Quinella*-related bacteria) exhibited variations in their relative frequency consistent with a potential role in one or more metabolic pathways of biohydrogenation in the rumen.

Key words: lactating ewe, marine algae, ruminal bacteria, biohydrogenation intermediate

INTRODUCTION

The inclusion of plant oils rich in 18:2n-6 and fish oil or marine algae (**MA**) in the diet is an effective nutritional strategy to increase concentrations of *cis*-9,*trans*-11 conjugated linoleic acid (**CLA**) and 22:6n-3 in bovine (AbuGhazaleh et al., 2002; Shingfield et al., 2006; Invernizzi et al., 2010) and ovine milk (Reynolds et al., 2006; Toral et al., 2010a,b). However, the increases in milk fat CLA content in response to a combination of plant oils and marine lipids have often been accompanied by alterations in ruminal biohydrogenation (**BH**) pathways, leading to a shift toward the formation of *trans*-10 18:1 at the expense of *trans*-11 18:1 and a decrease in milk fat synthesis in cattle (Shingfield et al., 2006; Invernizzi et al., 2010) and sheep (Toral et al., 2010a,b). Nevertheless, relatively few studies have characterized the effect of plant oils and marine lipids in the diet on the abundance of specific FA in ruminal digesta (AbuGhazaleh et al., 2002; Toral et al., 2010c).

Traditional culture-based methods for studying the effects of diet composition on ruminal bacteria and protozoa are less sensitive and accurate compared with molecular microbial methods based on 16S/18S rRNA genes. Use of culture-independent techniques have shown that alterations in the formation of specific BH intermediates to fish oil (Kim et al., 2008; Huws et al., 2010; Liu et al., in press) or marine algae (Boeckaert et al., 2007, 2008) in the diet may involve changes in specific populations of ruminal bacteria and protozoa in cattle. However, investigations of the effect of lipid supplements on the abundance of microorganisms involved in ruminal BH in sheep are limited (Boeckaert et al., 2009; Belenguer et al., 2010).

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Previous investigations indicated that changes in the FA composition of rumen fluid in nonlactating sheep fed diets containing fish oil and sunflower oil (**SO**, *Helianthus annus*; Toral et al., 2010c) did not provide a complete explanation for the effects of these lipid supplements on milk production and milk fat composition in lactating sheep (Toral et al., 2010a). Analysis of ruminal microbial communities using terminal RFLP (**T-RFLP**) also revealed that fish oil and SO induced comparable changes in certain groups of bacteria belonging to the family *Lachnospiraceae* or to the clostridial cluster IX in lactating and nonlactating sheep, whereas the effect on other groups possibly involved in ruminal BH differed (Belenguer et al., 2010).

The objective of this study was therefore to examine and characterize the changes in the bacterial ecology and appearance of biohydrogenation intermediates in the rumen of sheep fed diets that alter milk FA composition and inhibit mammary lipogenesis.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

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. Twenty-five lactating Assaf ewes of mean parity 3.7 ± 0.10, BW 85 ± 1.7 kg, and 97 ± 1.0 DIM producing 2.27 ± 0.101 kg of milk/d were used. Ewes were allocated at random to 5 experimental treatments (5 animals/treatment): containing no additional lipid supplements (control diet) or supplemented with 25 g of SO and 0, 8, 16, or 24 g of MA/kg of diet DM (SO, **SOMA$_1$**, **SOMA$_2$**, and **SOMA$_3$** diets, respectively). Experimental diets were composed of dehydrated alfalfa hay (*Medicago sativa*) and concentrates (forage:concentrate ratio 485:515) and fed as TMR to minimizing the sorting of dietary components. Sunflower oil (Carrefour, S.A., Madrid, Spain) and MA (DHA Gold Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD) replaced other dietary ingredients on a proportional basis. Dietary ingredients and chemical composition of experimental diets are shown in Table 1.

The experiment lasted 4 wk and rations were prepared weekly and offered ad libitum twice daily at 0900 and 1900 h. Ewes were housed in tie stalls and had continuous access to fresh water. Effects of treatments on intake, milk production, and milk FA composition have been reported elsewhere (Toral et al., 2010b).

Table 1. Ingredients and chemical composition (g/kg of DM) of the experimental diets[1]

Item	Control	SO	SOMA$_1$	SOMA$_2$	SOMA$_3$
Ingredients (g/kg of fresh matter)					
Dehydrated alfalfa hay	484	474	470	466	462
Whole corn grain	136	133	131	130	129
Whole barley grain	175	170	169	168	167
Soybean meal	97	95	94	93	92
Beet pulp	49	47	47	47	46
Molasses	37	36	36	36	36
Feed supplement[2]	22	21	21	21	21
Sunflower oil[3]	0	24	24	24	24
Marine algae[4]	0	0	8	15	23
Chemical composition (g/kg of DM)					
OM	896	900	897	893	899
CP	161	159	158	159	158
NDF	308	304	296	300	293
ADF	198	195	190	191	187
Ether extract	26	50	54	57	63

[1]Refers to TMR based on dehydrated alfalfa hay and concentrates containing no additional lipid (control), or supplemented with 25 g of sunflower oil and 0 (SO), 8 (SOMA$_1$), 16 (SOMA$_2$), or 24 (SOMA$_3$) g of marine algae/kg of diet DM.

[2]Contained (g/kg) $NaHCO_3$ (333), $CaCO_3$ (311), Ca_2HPO_4 (133), mine salt (111), and mineral and vitamins (111; INA OV1, Evialis, Madrid, Spain).

[3]Contained (g/kg) 16:0 (52.7), 18:0 (42.1), *cis*-9 18:1 (347), *cis*-11 18:1 (7.7), 18:2n-6 (479), 20:0 (2.7), *cis*-11 20:1 (1.6), 22:0 (7.1), 24:0 (2.2), other (11), and total fatty acids (953).

[4]DHA Gold Animal Feed Ingredient (Martek Biosciences Corp., Columbia, MD), contained (g/kg of DM) OM (913), CP (103), and ether extract (403). Fatty acid composition (g/kg of lipid): 12:0 (2.9), 14:0 (99.3), 14:2n-3 (1.5) 15:0 (4.2), 16:0 (245), *cis*-9 16:1 (1.6), 16:2n-3 (1.2), 16:3n-3 (1.5), 18:0 (5.8), *cis*-11 18:1 (1.2), 18:3n-6 (2.1), 18:4n-3 (3.0), 20:0 (1.5), 20:3n-6 (3.7), 20:4n-3 (8.1), 20:4n-5 (1.6), 20:4n-6 (4.7), 20:4n-7 (12.5), 20:5n-3 (14.0), 22:4n-9 (2.8), 22:5n-3 (3.6), 22:5n-6 (147), 22:6n-3 (369), 26:0 (3.9), other (18.3), unidentified (5.6), and total fatty acids (955).