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Effect of fish oil and sunflower oil on rumen fermentation characteristics and fatty acid composition of digesta in ewes fed a high concentrate diet

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ABSTRACT

Studies in ruminants have shown that supplementing the diet with a mixture of fish oil (FO) and sunflower oil (SO) enhances the concentration of *cis*-9, *trans*-11 conjugated linoleic acid (CLA), 20:5 n-3, and 22:6 n-3 in milk because of alterations in ruminal biohydrogenation, but the intermediates formed under these conditions are not well characterized. Five ewes fitted with rumen cannula and fed a high concentrate diet were used to examine the effect of a mixture (30 g/kg of DM) of FO and SO (1:2, wt/wt) on temporal changes in rumen fermentation characteristics and the relative abundance of biohydrogenation intermediates in ruminal digesta collected after 0, 3, and 10 d on diet. Appearance and identification of biohydrogenation intermediates was determined based on complementary gas-liquid chromatography and Ag+-HPLC analysis of fatty acid methyl esters and gas chromatography-mass spectrometry analysis of corresponding 4,4-dimethyloxazoline derivatives. Inclusion of FO and SO in the diet had no effect on rumen pH, volatile fatty acid concentrations, or nutrient digestion, but altered the fatty acid composition of ruminal digesta, changes that were characterized by time-dependent decreases in 18:0 and 18:2 n-6 and the accumulation of *trans* 16:1, *trans* 18:1, 10-O-18:0, and *trans* 18:2. Lipid supplements enhanced the proportion of 20:5 n-3 and 22:6 n-3 in digesta and resulted in numerical increases in *cis*-9, *trans*-11 conjugated linoleic acid concentrations, but decreased the relative abundance of *trans*-10, *cis*-12 conjugated linoleic acid. Furthermore, detailed analysis revealed the appearance of several unique 20:1, 20:2, 22:1, 22:3, and 22:4 products in ruminal digesta that accumulated over time, providing the first indications of 20 and 22 carbon fatty acid intermediates formed during the biohydrogenation of long-chain unsaturated fatty acids in sheep. In conclusion, FO and SO in a high concentrate diet caused a time-dependent inhibition of the complete biohydrogenation of 16 and 18 carbon unsaturated fatty acids, and resulted in the accumulation of *trans* 16:1, *trans* 18:1, and *trans* 18:2, 20, and 22 carbon metabolites in ruminal digesta of sheep, with no evidence of a shift in ruminal biohydrogenation pathways toward *trans*-10 18:1 formation.
Key words: biohydrogenation, fish oil, sheep, *trans* fatty acid

INTRODUCTION

Studies in ruminants have shown that inclusion of fish oil (**FO**) in the diet alone or in combination with plant oils increases *cis*-9, *trans*-11 conjugated linoleic acid (**CLA**), 20:5 n-3, and 22:6 n-3 contents in milk (Shingfield et al., 2003, 2006; Toral et al., 2010a), with the level of enrichment being related to a large extent to the biohydrogenation of dietary unsaturated fatty acids (**FA**) in the rumen.

Plant oils rich in 18:2 n-6 are known to enhance the accumulation and ruminal outflow of *trans*-18:1 and *cis*-9, *trans*-11 CLA (Kucuk et al., 2004; Atkinson et al., 2006; Shingfield et al., 2008). Inclusion of FO containing 20:5 n-3 and 22:6 n-3 inhibits the complete biohydrogenation of 18 carbon unsaturated FA causing an increase in the supply of *trans*-18:1 available for incorporation in milk fat triacylglycerides and tissue lipids (Shingfield et al., 2003; Lee et al., 2008; Shingfield et al., 2010b). Even though 20:5 n-3 and 22:6 n-3 are extensively hydrogenated in the rumen, the products formed and the mechanisms involved are not known.

Measurements of temporal changes in milk FA composition have provided evidence that the inclusion of plant oils in high concentrate diets (Bauman et al., 2000; Roy et al., 2006) or FO and sunflower oil (**SO**) in TMR (Shingfield et al., 2006; Cruz-Hernandez et al., 2007) result in time-dependent changes in ruminal biohydrogenation. Recent studies in sheep have also shown that supplementing a high concentrate diet with 10 g of FO and 20 g of SO/kg of diet increases milk fat *cis*-9, *trans*-11 CLA, 20:5 n-3, and 22:6 n-3 concentrations. However, the enrichment of *cis*-9, *trans*-11 CLA in milk increased within 7 d on diet but declined thereafter

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(Toral et al., 2010a). Transient increases in milk *cis*-9, *trans*-11 CLA content were also associated with a progressive decrease in DMI and milk fat synthesis over time (Toral et al., 2010a), effects that may, at least in part, be related to the effect of lipid supplements on nutrient digestion in the rumen (Wachira et al., 2000; Fievez et al., 2003; Shingfield et al., 2003). Furthermore, the decreases in mammary lipogenesis were not explained by increases in milk fat concentrations of biohydrogenation intermediates known to inhibit milk fat synthesis in cows (Shingfield and Griinari, 2007), suggesting that other metabolites, or mechanisms, or both are involved. In the current experiment, the effect of FO and SO on temporal changes in rumen fermentation patterns and the accumulation of biohydrogenation intermediates in sheep fed high concentrate diets were examined based on the analysis of samples collected after 0, 3, and 10 d on diet. Fatty acid composition of ruminal digesta was analyzed using GLC and Ag+-HPLC analysis of FA methyl esters (**FAME**) and GC-MS analysis of corresponding 4,4-dimethyloxazoline (**DMOX**) derivatives to provide further insight into the metabolic fate of long-chain FA in the rumen.

MATERIALS AND METHODS

Animals and Diets

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. Five dry Merino ewes (approximately 3 yr old; mean BW 63.0 ± 5.99 kg) each fitted with a rumen cannula were individually housed in raised-floor pens (1.5 × 2 m). Diets comprised alfalfa hay (mean particle size >4 cm) and a concentrate supplement (35:65 forage:concentrate ratio, DM basis) containing 0 (control diet) or 30 g/kg of DM of a mixture (1:2 wt/wt) of FO and SO (**SFO**). Oils replaced other dietary ingredients on a proportionate basis. Ingredients and chemical composition of the experimental diets are shown in Table 1. Fatty acid composition of the control diet, FO, and SO is reported in Table 2. Diets were fed as a TMR to avoid selection of dietary components as 2 meals (60% at 900 h and 40% at 1800 h) at a rate of 41 g of DM/kg of $BW^{0.75}$ equivalent to proportionately 0.8 of ad libitum intake measured over a 15-d period immediately before the start of the experiment. Ration mixes were prepared every 4 d and adjusted for changes in component DM content. Semirefined tuna and sardine oil (Afampes 121 DHA, Afamsa, Vigo, Spain) and SO (Carrefour S.A., Madrid, Spain) were stored in the dark at 4°C before incorporation into daily rations. Animals had continuous access to fresh clean water.

Table 1. Ingredient and chemical composition of experimental diets[1]

Item	Control	SFO
Ingredient (g/kg of fresh matter)		
Dehydrated alfalfa hay	350	341
Whole corn grain	203	198
Soybean meal	163	158
Whole barley grain	122	119
Beet pulp	73	71
Molasses	53	51
Salt premix[2]	33	32
Minerals and vitamins[3]	4	4
Sunflower oil[4]	0	18
Fish oil[5]	0	9
Composition (g/kg of DM)		
OM	882	891
CP	172	163
NDF	269	279
ADF	156	172
Starch	274	253
Fatty acids	46	75

[1]Control = no oil supplementation; SFO = supplemented with 20 g of sunflower oil plus 10 g of fish oil/kg of DM.

[2]Contained (g/kg): 375 $NaHCO_3$; 350 $CaCO_3$; 150 Ca_2HPO_4; and 125 NaCl.

[3]INA OV1 (Evialis, Madrid, Spain). Contained: 60 g/kg of S; 30 g/kg of Mg; 12 g/kg of Fe; 10 g/kg of Zn; 10 g/kg of Mn; 6 g/kg of choline; 200 mg/kg of Co; 200 mg/kg of I; 36 mg/kg of Se; 200 mg/kg of vitamin B_1; 200 mg/kg of vitamin B_2; 1,250 IU/g of vitamin A; 250 IU/g of vitamin D_3; and 3 IU/g of DL-α-tocopheryl acetate.

[4]Carrefour S.A. (Madrid, Spain).

[5]Semirefined tuna and sardine oil (Afampes 121 DHA, Afamsa, Vigo, Spain).

Experimental Design

The experiment was conducted over 2 consecutive 11-d periods. In the first period, the control diet was fed over an 11-d adaptation period to establish a baseline and thereafter all ewes were fed the SFO diet during the second 11-d period. In vivo, in situ, and in vitro determinations were conducted before the start of oil supplementation (d 0, control) and after 3 d (**SFO_3**) and 10 d (**SFO_{10}**) on the SFO diet. Even though treatment was confounded with experimental period, this approach was used rather than a more robust switchback or change-over design to avoid possible residual carry-over effects of FO and SO on rumen fermentation and lipid metabolism (Cruz-Hernandez et al., 2006). The same experimental design has been used previously to examine the effects of FO on ruminal biohydrogenation and milk fat composition (Kitessa et al., 2001; Shingfield et al., 2003; Roy et al., 2006).

Experimental Procedures

In Vivo Studies. Dry matter intake was measured daily. Rumen fluid was collected from each sheep after 0, 3, and 10 d on the SFO diet (control, SFO_3, and SFO_{10}, respectively) at 0, 1.5, 3, 6, and 9 h after morn-