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Fish oil-induced milk fat depression and associated downregulation of mammary lipogenic genes in dairy ewes

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ABSTRACT

Several studies in dairy cows have shown a relationship between milk fat depression (MFD) and alterations caused in lipogenic gene expression by dietary nutrients. However, information on small ruminants is not only scarce but also inconsistent. Therefore, this experiment was conducted in dairy ewes to study the effect of a diet known to induce MFD on milk fatty acid (FA) composition and mRNA abundance of key candidate genes involved in mammary lipogenesis. Twelve lactating Assaf ewes (on average 63 d in milk) were randomly assigned to 2 treatments consisting of a total mixed ration based on alfalfa hay and concentrates (50:50), supplemented with 0 (control) or 17 g of fish oil/kg of diet DM (FO). Profiles of milk FA and mRNA abundance of candidate genes in biopsied mammary tissue were examined before starting the treatments and after 1 and 4.5 wk on the diets. As expected, FO induced MFD and modified milk FA composition. Compared with the control, reductions in milk fat concentration and yield were not detected on d 7, but reached up to 25 and 22%, respectively, on d 30. However, increases in confirmed or putative antilipogenic FA (*trans*-10,*cis*-12 and *trans*-9,*cis*-11 18:2, *cis*-9 16:1, *cis*-11 18:1, and oxo-FA) were already established on the early stage of the treatment and lasted until the end of the feeding period. These changes were accompanied by decreases in the mRNA abundance of genes encoding lipogenic enzymes. The coordinated nature of the downregulation, which tended to affect most studied metabolic pathways, including FA activation (*ACSS1*), de novo synthesis (*ACACA* and *FASN*), uptake and transport (*LPL* and *FABP3*), desaturation (*SCD1*), and esterification (*AGAPT6*), supports the involvement of a central regulator of milk fat synthesis. In this regard, without ruling out the potential contribution of *PPARG*, our results suggest that *SREBF1* would have a relevant role in the MFD syndrome in sheep fed FO. Among the other studied transcription factors, the tendency to a downregulation of *INSIG1* was associated with that of *SREBF1*, whereas no variation was detected for *SCAP* or *THRSP*. Fish oil had no significant effects on the transcript abundance of *CD36*, *GPAM*, *DGAT1*, *LPIN1*, and *XDH*. Overall, changes in potential antilipogenic FA and mRNA abundance of candidate lipogenic genes support a relationship between them and suggest that FO-induced MFD in dairy ewes would be mediated by transcriptional mechanisms.

Key words: fatty acid, gene expression, marine lipid, nutrigenomics, sheep

INTRODUCTION

Without ruling out the potential involvement of other causative factors, it is widely accepted that the low-milk fat syndrome is mediated by alterations of ruminal fermentation resulting in the formation of specific bioactive fatty acids (**FA**) that will exert antilipogenic effects in the mammary gland (Bauman and Griinari, 2001; Shingfield et al., 2010). However, the molecular mechanisms by which these FA are able to decrease mammary synthesis of fat remain uncertain (Bauman et al., 2011). Based on studies in dairy cows, the low-milk fat syndrome, commonly referred to as milk fat depression (**MFD**), has been hypothesized to be caused by alterations in lipogenic gene expression by dietary nutrients (Harvatine and Bauman, 2006; Angulo et al., 2012; Bionaz et al., 2015). The downregulation of the mRNA abundance of key genes involved in milk fat synthesis by *trans*-10,*cis*-12 CLA is likely the best-known example of nutrigenomics in this area (Shingfield et al., 2010; Bauman et al., 2011; Hussein et al., 2013).

Nevertheless, information about nutrigenomics and lipid metabolism in small ruminants is not only scarce but also inconsistent (Shingfield et al., 2013). For example, Hussein et al. (2013) reported that *trans*-10,*cis*-12 CLA-induced MFD in lactating ewes involves the *SREBF* family and a coordinated downregulation of genes related to mammary lipid synthesis (e.g., *ACA*-

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CA, *FASN*, *SCD1, AGPAT6*, *SREBF1*, or *INSIG1*). In contrast, several studies in dairy sheep (Dervishi et al., 2012; Bichi et al., 2013a; Castro-Carrera et al., 2015) or dairy goats (Bernard et al., 2008; Tsiplakou et al., 2009; Toral et al., 2013) observed a weak relationship between changes in milk FA production and mammary mRNA abundance of key candidate genes and transcription factors involved in lipid metabolism. Yet, Bichi et al. (2013a), who studied that relationship after 54 d on marine lipids, suggested that transcriptional regulation in dairy ewes might have been established earlier during the feeding period. This speculation was based on the findings of Invernizzi et al. (2010) in mammary tissue from dairy cows fed saturated lipids or soybean oil and fish oil, who observed that transcriptional control mechanisms altering the expression of several lipogenic genes (e.g., *PPARG*, *LPIN1*, *SREBF1*, *ACSS1*, *ACACA*, *FASN*, or *LPL*) were affected by d 7 postfeeding, but variations between diets had disappeared on d 21.

This experiment was therefore conducted in dairy ewes to study the effect of dietary supplementation with marine lipids, which is known to induce MFD in this species (Capper et al., 2007; Bichi et al., 2013b; Toral et al., 2016b), on milk fat composition and mRNA abundance of key candidate genes involved in mammary lipogenesis. Based on previous reports (Toral et al., 2010; Hussein et al., 2013), we hypothesized that changes in FA composition and gene expression would be detected early during the feeding period, whereas the milk fat decrease would only be observed later on. To test this hypothesis, transcript and FA profiles were examined before starting the assay and after 1 and 4.5 wk on a control or a fish oil supplemented diet.

MATERIALS AND METHODS

Animals, Experimental Diets, and Management

All experimental procedures were approved and completed in accordance with the Spanish Royal Decree 53/2013 for the protection of animals used for experimental purposes. Twelve lactating Assaf ewes (BW = 77.8 kg, SD = 7.81; DIM = 63, SD = 7.8; milk production = 2.5 kg/d, SD = 0.35) were housed in individual tie stalls and randomly allocated to 1 of 2 groups (n = 6). They were used to test the effects of 2 dietary treatments consisting of a TMR, based on alfalfa hay (particle size >4 cm) and concentrates (50:50), without lipid supplementation (control) or supplemented with 17 g of fish oil (Afampes 121 DHA; Afamsa, Mos, Spain)/kg of diet DM (**FO**). The ingredients and chemical composition of the experimental diets, which were prepared weekly and included molasses to reduce selection of components, are presented in Table 1. All ewes were fed the control diet during 4 wk of adaptation before the start of the study. The TMR were offered twice daily, at 0930 and 1830 h, to ensure ad libitum intakes. Ewes had continuous access to clean drinking water and were milked at approximately 0900 and 1800 h in a dedicated 1 × 10-stall milking parlor (DeLaval, Madrid, Spain).

Table 1. Formulation and chemical composition of the TMR without lipid supplementation (control) or supplemented with 17 g of fish oil/kg of DM (FO)

	TMR	
Item	Control	FO
Ingredient, g/kg of fresh matter		
Dehydrated alfalfa hay	500	493
Whole corn grain	140	138
Whole barley grain	100	99
Soybean meal solvent 440, g of CP/kg	150	148
Sugar beet pulp, pellets	50	49
Molasses, liquid	40	39
Fish oil[1]	0	15
Mineral supplement[2]	18	18
Vitamin supplement[3]	2	2
Chemical composition, g/kg of DM		
OM	904	902
CP	187	181
NDF	251	240
ADF	155	145
14:0	0.60	1.16
16:0	5.49	8.78
cis-9 16:1	0.00	0.77
18:0	1.07	2.04
cis-9 18:1	4.17	7.22
cis-11 18:1	0.31	0.89
18:2n-6	12.53	12.74
18:3n-3	3.15	3.26
20:5n-3	0.00	1.08
22:5n-3	0.00	0.27
22:6n-3	0.00	3.86
Total fatty acids	28.1	43.7

[1]Semi-refined tuna and sardine oil (Afampes 121 DHA; Afamsa, Mos, Spain); contained (g/100 g of total fatty acids): 14:0 (3.1), 16:0 (18.6), *cis*-9 16:1 (4.2), 17:0 (0.8), 18:0 (5.4), *cis*-9 18:1 (17.2), *cis*-11 18:1 (3.2), 18:2n-6 (2.3), 18:3n-3 (0.9), *cis*-11 20:1 (1.8), 20:5n-3 (5.9), 22:5n-3 (1.5), and 22:6n-3 (21.2).

[2]Declared as containing (g/kg): $CaCO_3$ (556), Ca_2HPO_4 (222), and NaCl (222).

[3]VITAFAC Ovino 0.2% AC (DSM Nutritional Products S.A., Madrid, Spain). Declared as containing: vitamin A (4,000,000 IU/kg), vitamin D_3 (1,000,000 IU/kg), vitamin E (5 g/kg), iron (17.5 g/kg), manganese (20 g/kg), cobalt (50 mg/kg), iodine (250 mg/kg), zinc (15 g/kg), selenium (100 mg/kg), sepiolite (100 g/kg), calcium (26.2 g/kg), and magnesium (6.15 g/kg).

Measurements and Sampling Procedures

Diets. Representative samples of the experimental diets, collected weekly, and the fish oil were stored at −30°C until analysis. Feed intake was measured on d 0 (pretreatment), 7, and 30 by weighing the amount of DM offered and refused by each ewe.