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# Does supplemental 18:0 alleviate fish oil-induced milk fat depression in dairy ewes?

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## ABSTRACT

Supplementation of dairy ewe diet with marine lipids may be an effective strategy for modulating milk fatty acid composition but induces milk fat depression (MFD). This syndrome has been associated with a shortage of 18:0 for uptake and $\Delta^9$-desaturation that may impair the capacity of the mammary gland to achieve an adequate fluidity for milk fat secretion. On this basis, it was suggested that supplemental 18:0 may contribute to alleviate marine lipid-induced MFD in sheep. To test this hypothesis, 12 lactating ewes were allocated to 1 of 3 lots and used in a 3 × 3 Latin square design with 3 periods of 28 d each and 3 experimental treatments: a total mixed ration without lipid supplementation (control) or supplemented with 20 g/kg of DM of fish oil alone (FO) or in combination with 20 g/kg of DM of 18:0 (FOSA). Diets were offered ad libitum, and animal performance and rumen and milk fatty acid composition were studied at the end of each period. After completing the Latin square trial and following a change-over design, the in vivo digestibility of supplemental 18:0 was estimated using 6 lactating sheep. As expected, diet supplementation with fish oil increased the milk content of some potentially health-promoting fatty acids (e.g., *cis*-9,*trans*-11 18:2, *trans*-11 18:1, 20:5n-3, 22:5n-3, and 22:6n-3), but reduced milk fat concentration and yield (−20% in both FO and FOSA treatments). Thus, although reductions in milk 18:0 and *cis*-9 18:1 output caused by FO (−81 and −51%, respectively) were partially reversed with FOSA diet (−49 and −27%, respectively), the addition of 18:0 to the diet did not prove useful to alleviate MFD. This response, which could not be fully accounted for by the low digestibility coefficient of supplemental 18:0, may challenge the theory of a shortage of this fatty acid as a mechanism to explain fish oil-induced MFD in sheep. Effects of FO and FOSA on rumen and milk fatty acid composition would support that increases in the concentration of some candidate milk fat inhibitors (e.g., *cis*-9 16:1 or 10-oxo-18:0) might play a relevant role in this type of MFD.

**Key words:** mammary gland, marine lipid, rumen biohydrogenation, sheep, stearic acid

## INTRODUCTION

Nutrition is a major factor determining ruminant milk fatty acid (**FA**) composition (Lock and Bauman, 2004; Chilliard et al., 2007). Some studies in dairy ewes (Capper et al., 2007; Toral et al., 2010a; Tsiplakou and Zervas, 2013) have shown that the addition of marine lipids to the diet (≤30 g/kg of DM) may be an effective strategy for enhancing the milk content of potentially health-promoting FA, such as *cis*-9,*trans*-11 CLA, *trans*-11 18:1, and very long chain n-3 PUFA. However, the application of this feeding strategy under practical farm conditions is hindered by the appearance of milk fat depression (**MFD**; Toral et al., 2010b; Bichi et al., 2013).

The biohydrogenation (**BH**) theory represents a unifying concept to explain diet-induced MFD and attributes this syndrome to an inhibition of milk fat synthesis by specific FA produced when the ruminal environment, and consequently the BH process, are altered (Bauman and Griinari, 2001). However, reports in dairy ewes, cows, and goats provide evidence that direct inhibition by FA with confirmed or putative antilipogenic effects (e.g., *trans*-10,*cis*-12 or *trans*-9,*cis*-11 CLA) would not be a major contributor to MFD on diets containing marine lipids (Bichi et al., 2013; Kairenius et al., 2015; Toral et al., 2015); therefore, other intermediates or mechanisms should be involved. In line with this, marine lipid-induced MFD has been related to potential alterations of the milk fat fluidity (Loor et al., 2005; Shingfield and Griinari, 2007; Gama et al., 2008). Very long-chain n-3 PUFA are known to inhibit the ruminal saturation of *trans* 18:1 to 18:0 (Loor et al., 2005; Shingfield and Griinari, 2007; Toral et al., 2010c), which results in a shortage of 18:0 [melting point (**MP**) = 69.7°C)] for mammary uptake that would constrain the synthesis of *cis*-9 18:1 (MP = 16.0°C) by $\Delta^9$-desaturation. In addition, marine lipids

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increase the content of *trans* 18:1 isomers, which have higher MP (40–66°C) than their equivalent *cis* isomers. Altogether, these changes would challenge the ability of the mammary gland to maintain milk fat MP below body temperature (Timmen and Patton, 1988) and may impair the capacity to achieve an adequate fluidity for milk fat secretion, which might account for this type of MFD (Shingfield and Griinari, 2007).

On this basis, it was suggested that supplemental 18:0 could contribute to alleviate marine lipid-induced MFD in dairy sheep. To test this hypothesis, lactating ewes were fed a diet supplemented with fish oil alone or in combination with 18:0 to examine the response in terms of animal performance, and rumen and milk FA composition.

## MATERIALS AND METHODS

### Animals, Experimental Design, and Management

All procedures involving animals 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 (71.3 ± 2.68 kg of BW and 31.6 ± 1.53 DIM at the beginning of the assay) were allocated to 1 of 3 lots (n = 4), balanced according to milk production and composition, BW, DIM, and parity, and used in a 3 × 3 Latin square design to test the effects of 3 dietary treatments during 3 experimental periods of 28 d each. Diets consisted of a TMR, based on alfalfa hay and a concentrate (40:60), without lipid supplementation (control) or supplemented with 20 g of fish oil (Afampes 121 DHA; Afamsa, Mos, Spain) per kilogram of diet DM alone (**FO**) or in combination with 20 g of 18:0 (Edenor C18–98MY; Oleo Solutions, York, UK) per kilogram of diet DM (**FOSA**). 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. The experimental diets, prepared every week, were stored in the dark at room temperature (approximately 5–14°C). All ewes were fed the control diet during 3 wk of adaptation before the start of the study. The TMR was offered twice daily, at 0930 and 1830 h, to ensure ad libitum intakes (10–15% of orts). Ewes had continuous access to clean drinking water and were milked twice daily at approximately 0900 and 1800 h in a single-side milking parlor with 10 stalls.

After completing the Latin square trial, 3 ewes on FOSA and 3 on FO were housed for 7 d in individual metabolic cages to examine the in vivo digestibility of supplemental 18:0. Following a change-over design, diets received by each ewe were then switched and offered for 21 more days. The digestibility trial was repeated again on the last 7 d of this second period. Measurements and samples from sheep fed the FO treatment were used to determine the fecal concentration of 18:0 when this FA was not added to the diet. During the time the animals were kept in metabolic cages, the management was the same as described previously but portable equipment was used for milking.

### Measurements and Sampling Procedures

***Diets and Intake.*** Feed intake was recorded during the last week of each experimental period by weighing the amount of DM offered and refused by each lot over the week. Samples of the diets and orts were collected with the same frequency, stored at –30°C, and then freeze-dried before chemical analysis.

***Milk.*** Milk yield was recorded on d 25, 26, and 27 of each experimental period. With the same frequency, individual milk samples were collected and composited according to morning and evening milk yield. One aliquot was preserved with bronopol (D&F Control Systems Inc., San Ramon, CA) and stored at 4°C until analyzed for fat, CP, lactose, and TS. Milk FA composition was determined in untreated samples from each experimental lot that were composited according to individual milk yield and stored at –30°C until analysis.

***Rumen Fluid.*** On d 28 of each experimental period, ewes were milked and given free access to morning rations for 1 h. Thereafter, feeds were removed and 3 h later samples of rumen fluid were collected from each ewe using a stomach tube (Ramos-Morales et al., 2014). Immediately after collection, the fluid was strained through a nylon membrane (400 μm; Fisher Scientific S.L., Madrid, Spain) and frozen at –80°C. Samples were freeze-dried and stored at –80°C until analyzed for FA composition.

***Digestibility of Supplemental 18:0.*** After 2 d of adaptation to the metabolic cages, DMI was recorded and feces were weighed daily over 5 consecutive days. Individual samples of feeds offered and refused and of feces were collected and stored at –30°C until analyzed for DM. Lipid composition was determined in subsamples that were freeze-dried and stored at –30°C.

### Chemical Analysis

***Diets and Orts.*** Dry matter concentration in diets and orts was determined according to the ISO (1999a) standard. Diet samples were also analyzed for ash (ISO, 2002), CP (ISO, 2009), and ether extract (Ankom Technology Method 2; Ankom Technology Corp. Macedon, NY, https://www.ankom.com/). Diet NDF and ADF were determined using an Ankom