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Individual variation of the extent of milk fat depression in dairy ewes fed fish oil: Milk fatty acid profile and mRNA abundance of candidate genes involved in mammary lipogenesis

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ABSTRACT

Dairy ewes are less prone than cows to milk fat depression (MFD) but suffer from this syndrome when marine lipids are added to their diet to modulate milk fatty acid (FA) profile. However, there are large individual differences in MFD extent, and the reasons behind this variability are uncertain. On this basis, a study was conducted in lactating sheep to test the hypotheses that individual susceptibility to the low-fat milk condition may be explained by differences in (1) the milk concentration of some FA, particularly antilipogenic FA, or (2) the transcriptional regulation of mammary lipogenesis. For 5 wk, 15 ewes received a total mixed ration supplemented with 0 (control; n = 5) or 20 g of fish oil/kg of dry matter [10 animals selected out of 22 and divided into those showing marked (RESPON+; n = 5) or mild (RESPON−; n = 5) MFD]. Milk production and composition, including a comprehensive FA profile, were examined on 3 consecutive days before and after treatments. Candidate gene expression was also analyzed before the start of the trial and at its end using RNA isolated from milk somatic cells. According to the experimental design, the fish-oil-induced decrease in milk fat concentration was much stronger in RESPON+ (–25.4%) than in RESPON− (–7.7%). Milk from all ewes fed the supplemented diet showed rather uniform changes in the proportion of potentially healthy FA (such as *cis*-9,*trans*-11 18:2, *trans*-11 18:1, or 20:5n-3) and of those with confirmed or putative antilipogenic effects (e.g., *cis*-9 16:1, *trans*-10 and *cis*-11 18:1, *trans*-9,*cis*-11 18:2, and 10-oxo-18:0), without significant variation between RESPON+ and RESPON−. It was not possible to relate the very few exceptions to this general trend (e.g., in *cis*-7 16:1 and 22:6n-3) to responsiveness. Major mechanisms involved in mammary lipogenesis, specifically the uptake and de novo synthesis of FA, appeared to be unequally inhibited in ewes displaying different degrees of MFD, with molar yields of >16C FA being unaffected in RESPON−. However, this was not reflected in candidate gene expression. Supplementation with fish oil showed a tendency to lower the mRNA abundance of lipogenic genes such as *ACSS2*, *FASN*, *LPIN1*, *FADS2*, and *INSIG1*, but only *SCD* and *GPAT4* tended to differ between RESPON− and RESPON+. Overall, these results offer no convincing support for the initial hypotheses, so further research must be pursued to explain the individual variation in MFD severity.

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

INTRODUCTION

Bauman et al. (2011) stated that the basis for diet-induced milk fat depression (**MFD**) had perplexed scientists for over a century. This perplexity continues today and extends not only to cows but also to small ruminants (Carreño et al., 2016; Toral et al., 2017). For several years, dairy sheep were considered to be less prone to diet-induced MFD (Shingfield et al., 2010), but their susceptibility to dietary addition of marine lipids or *trans*-10,*cis*-12 CLA is nowadays demonstrated (Capper et al., 2007; Hussein et al., 2013; Toral et al., 2015).

We have conducted several studies in ewes fed marine lipids rich in n-3 PUFA (e.g., Bichi et al., 2013; Carreño et al., 2016; Toral et al., 2015, 2016a, 2017) with the primary goal of modulating milk composition toward a healthier fatty acid (**FA**) profile. To some extent, we were also interested in the mechanism underlying the low-fat milk syndrome induced by marine lipids. Because most ovine milk is destined for cheese manufacturing (Haenlein, 2007), feeding these supplements may be challenging under practical conditions due to economic losses associated with MFD. Remarkably, we always observed individual differences in the responsiveness to marine lipids. For example, decreases in milk fat concentration ranged from 17 to 28% in ewes fed 0.8% marine algae (Bichi et al., 2013), from 14 to

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32% in animals that consumed 1.7% fish oil (Carreño et al., 2016), or from 4 to 33% with diets supplemented with 2% fish oil (Toral et al., 2016a).

Individual variability might also explain some inconsistencies found in the literature about consumption of marine lipids and MFD in sheep. For instance, Capper et al. (2007) reported a clear fish-oil-induced MFD, whereas others show no effects or only a mild MFD. The latter include several trials conducted by Reynolds et al. (2006) in dairy ewes receiving a supplement consisting of a mixture of soybean and marine algae oils (4%; 2:1 wt/wt, respectively). Their findings related to milk fat percentage contain increases, no significant differences, or a tendency toward mild MFD (−13%) with corn silages as the basal forage. Papadopoulos et al. (2002) found no significant variations in milk fat when n-3 PUFA-rich marine algae were added to the diet at concentrations of 2.4 or 4.7%. Mozzon et al. (2002) and Tsiplakou and Zervas (2013) also reported no effect or slight but not significant decreases in milk fat concentration with fish oil supplementation. Cows have also been shown to display very different degrees of diet-induced MFD (Weimer et al., 2010).

On this basis, we conducted an experiment with dairy ewes fed fish oil to try to elucidate the reasons behind the individual variation in MFD extent, and we posed 2 hypotheses for testing. The first was that differences in the milk concentration of some FA, particularly potentially antilipogenic FA, may account for that individual variability. It is widely accepted that MFD is related to an inhibition of mammary lipogenesis by active biohydrogenation (**BH**) intermediates that are produced under certain feeding conditions that alter rumen function (Bauman and Griinari, 2001). Although only *trans*-10,*cis*-12 CLA has been unequivocally demonstrated to exert antilipogenic effects, some other BH metabolites have more recently been suggested to be able to impair milk fat synthesis (Shingfield et al., 2010; Bauman et al., 2011).

The second hypothesis was that differences in the transcriptional regulation of mammary lipogenesis would be responsible for individual variations in the susceptibility to MFD. Both hypotheses may be linked because it has been proposed that alterations, particularly downregulation, in the expression of genes involved in milk fat synthesis may be mediated by dietary nutrients, including some FA with antilipogenic characteristics (Harvatine and Bauman, 2006; Bionaz et al., 2015; Toral et al., 2017).

MATERIALS AND METHODS

All experimental procedures were approved and completed in accordance with Spanish and European Union regulations [R.D. 53/2013 (BOE, 2013), and Council Directive 2010/63/EU (EU, 2010)] for the protection of animals used for experimental purposes.

Animals, Experimental Diets, and Management

Fifteen lactating Assaf ewes (BW = 76.4 ± 2.66 kg; DIM = 48 ± 1.4; parity = 2.4 ± 0.42; milk yield = 2.8 ± 0.15 kg/d; means ± SE) were used in this study. They were selected from a total of 27 animals housed in individual tie stalls and randomly allocated to 1 of 2 diets: a TMR based on alfalfa hay (particle size >4 cm) and concentrates (50:50) without lipid supplementation (control group; n = 5) or supplemented with 20 g of fish oil (Afampes 121 DHA, Afamsa, Mos, Spain)/kg of diet DM (MFD group; n = 22). The ingredients of the experimental diets, which were prepared weekly and included molasses to reduce selection of components, are shown in Table 1. All ewes were fed the control diet for a 3-wk adaptation period, and then both experimental diets for 5 more weeks, which allowed achievement of a stable MFD. At the end of this latter period, 10 ewes out of the 22 in the MFD group were selected to represent those showing a marked response in terms of MFD (**RESPON+**; n = 5) or just a mild response (**RESPON−**; n = 5) to the dietary addition of fish oil. The selection was carried out on the basis of decreases in milk fat concentration and differences between the 3 groups are shown in Table 2.

Ewes were milked twice daily at approximately 0830 and 1830 h in a single-side milking parlor with 10 stalls (DeLaval, Madrid, Spain). The diets were offered daily ad libitum after the morning milking, and clean water was always available.

Measurements and Sampling Procedures

Diets. Representative samples of the experimental diets were collected weekly and stored at –30°C until analysis. Feed intake was individually measured 4 times a week by weighing the amounts of DM offered and refused by each animal.

Milk. At the end of the adaptation period (d –5, –4, and –3) and after 31, 32, and 33 d on treatments, milk yield was recorded and individual milk samples were collected and composited according to morning and evening milk yields. One aliquot of composite milk was preserved with bronopol (D&F Control Systems Inc., San Ramon, CA) and stored at 4°C until analysis for fat, CP, and TS concentrations. Milk FA composition was determined in untreated samples stored at –30°C until analysis.

Milk Somatic Cells. Total RNA was isolated from milk somatic cells, which has been shown to re-