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Milk fat depression in dairy ewes fed fish oil: Might differences in rumen biohydrogenation, fermentation, or bacterial community explain the individual variation?

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ABSTRACT

Dairy ewes show large individual variation in the extent of diet-induced milk fat depression (MFD) but reasons behind this variability remain uncertain. Previous results offered no convincing support for these differences being related to relevant changes in the milk fatty acid (FA) profile, including potentially antilipogenic FA, or in the transcript abundance of candidate genes involved in mammary lipogenesis. Therefore, we hypothesized that alterations in the processes of rumen biohydrogenation and fermentation, as well as in the bacterial community structure, might account for individual variation in fish oil-induced MFD severity. To test this explanation, 15 ewes received a total mixed ration without lipid supplementation (control; n = 5) or supplemented with 20 g of fish oil/kg of dry matter [10 animals divided into those showing a strong (RESPON+; −25.4%; n = 5) or a mild (RESPON−; −7.7%; n = 5) decrease in milk fat concentration] for 5 wk. Rumen fermentation parameters, biohydrogenation metabolites, and bacterial structure and diversity were analyzed in rumen samples collected before and after treatments. Although the fish oil supplementation increased the concentration of demonstrated or putative antilipogenic FA (e.g., *cis*-9 16:1, *cis*-11 18:1, or *trans*-10,*cis*-12 CLA), surprisingly, none of them differed significantly in relation to the extent of MFD (i.e., between RESPON− and RESPON+), and this was the case only for a few minor FA (e.g., *cis*-6+7 16:1 or 17:0 *anteiso*). Changes in total volatile FA, acetate, and propionate concentrations were associated with MFD severity, with higher decreases in more susceptible animals. Individual responses were not related to shifts in rumen bacterial structure but some terminal restriction fragments compatible with *Clostridiales*, *Ruminococcaceae*, *Lachnospiraceae*, and *Succiniclasticum* showed greater abundances in RESPON−, whereas some others that may correspond to *Prevotella*, *Mogibacterium*, and *Quinella*-related spp. were more abundant in RESPON+. Overall, the results suggest that individual variation in MFD severity in dairy ewes fed fish oil cannot be fully explained by differences in the processes of rumen biohydrogenation and fermentation or in the bacterial community, and further research would be necessary to elucidate the large variability in the responsiveness to MFD-inducing marine lipids.
Key words: acetate, fatty acid, marine lipid, ruminal microbiota, sheep

INTRODUCTION

Diet-induced milk fat depression (**MFD**) is commonly observed in sheep when they are fed marine lipid supplements to modulate milk fatty acid (**FA**) composition (e.g., Toral et al., 2016b; Frutos et al., 2017). However, dairy ewes, like dairy cows, show large individual variation in the extent of this condition (Reynolds et al., 2006; Weimer et al., 2010). For example, Toral et al. (2016b) observed up to 8-fold differences in milk fat decreases within a group of lactating sheep fed the same MFD-inducing diet. Yet, reasons behind this variability are uncertain.

Elucidating the cause of this different responsiveness might help to understand diet-induced MFD, which continues to be an active research area given the economic value of milk fat and associated losses (Palmquist and Jenkins, 2017). With that aim, we conducted an experiment (Frutos et al., 2017) with dairy ewes displaying either strong or just mild MFD when fed a diet containing 2% fish oil. Unexpectedly, results offered no convincing support for individual variations being linked to relevant changes in the milk FA profile, including potentially antilipogenic FA, or with the transcript abundance of candidate genes involved in mammary lipogenesis. Therefore, further research was necessary.

Bauman and Griinari (2001) postulated that MFD is related to active biohydrogenation (**BH**) intermedi-

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ates that are produced under several feeding conditions that alter rumen function, and referred to this as the BH theory of MFD. The production of these intermediates is primarily due to the rumen microbiota, especially bacteria, with no or limited contribution of other groups such as protozoa or fungi (Lourenço et al., 2010; Enjalbert et al., 2017). Nonetheless, it is still uncertain which populations are actually involved in the process (Buccioni et al., 2012; Enjalbert et al., 2017; Pitta et al., 2018). Although most studies focused on the *trans*-10,*cis*-12 18:2, whose role in marine lipid-induced MFD has been dismissed (Loor et al., 2005; Toral et al., 2012), other BH metabolites with potentially antilipogenic features have then been connected to mammary lipogenesis (Alves and Bessa, 2014; Kairenius et al., 2015; Toral et al., 2016b). For this reason, we speculated that some minor BH metabolites possibly associated with BH-induced MFD might be better detected in rumen fluid than in milk as changes occurring in the mammary gland would be excluded.

Furthermore, early theories attributed the reduction in milk fat to an acetate deficiency because this VFA is the main substrate for de novo synthesis of FA in dairy ruminants, but they were disregarded based on experiments infusing acetate to cows (see review by Bauman and Griinari, 2001). However, Urrutia and Harvatine (2017) have recently resumed research on the effect of acetate on mammary lipid synthesis and suggested that the subject would merit further investigation.

On this basis, this study was conducted to test the hypothesis that differences in the processes of rumen BH of UFA and fermentation, as well as in the bacterial community, would account for the individual variation in fish oil-induced MFD severity.

MATERIALS AND METHODS

All experimental procedures were approved and completed in accordance with European Union and Spanish 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 and Experimental Diets

Details of the experimental design and methodology were described in Frutos et al. (2017). Briefly, we used 15 lactating Assaf ewes (76.4 ± 2.66 kg of BW; 48 ± 1.4 DIM; 2.8 ± 0.15 kg of milk/d; means ± SE) that were selected from a total of 27 animals randomly allocated to 1 of 2 diets: a TMR based on alfalfa hay and a concentrate (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 to cause MFD (MFD group; n = 22). On average, experimental diets contained 138 g of starch, 180 g of CP, and 315 g of NDF/kg of DM [see Frutos et al. (2017) for further details about chemical composition, ingredients, and FA profile]. All ewes were fed the control diet for a 21-d adaptation period and then both experimental diets for 36 more days. At the end of this latter period, 10 animals out of the 22 were selected and divided in those showing a strong MFD (**RESPON+**; −25.4% decrease in milk fat concentration; n = 5) or a mild MFD (**RESPON**–; −7.7% decrease in milk fat concentration; n = 5).

Measurements and Sampling Procedures

At the end of the adaptation period and after 36 d on the experimental diets, ewes were given free access to the diets for 1 h after morning milking. Then, feeds were removed and 3 h later, samples of rumen fluid were collected from each animal (approximately 150 mL) using an oral stomach probe (Ramos-Morales et al., 2014). Immediately after collection, the fluid was strained through a nylon membrane (400 μm; Fisher Scientific S.L., Madrid, Spain); a 3-mL subsample was acidified with 3 mL of 0.2 *M* HCl for ammonia analysis, and further 0.8-mL aliquots were deproteinized with 0.5 mL of 20 g of metaphosphoric acid/L and 4 g of crotonic acid/L in 0.5 *M* HCl for VFA determinations. These samples were stored at −30°C until analysis. Further aliquots of ruminal fluid were collected (approximately 50 mL), immediately frozen at −80°C, freeze-dried, and stored again at −80°C until analyzed for FA composition and bacterial community.

Laboratory Analysis

Ruminal Fermentation Parameters. Ammonia concentration was determined by a colorimetric method (Reardon et al., 1966) and VFA by GC, using crotonic acid as an internal standard (Ottenstein and Bartley, 1971), both in centrifuged samples.

Ruminal Fatty Acid Composition. Fatty acid methyl esters of lipid in 200 mg of freeze-dried rumen digesta samples were extracted twice using 4 mL of a mixture (3:2, vol/vol) of hexane and isopropanol following the adjustment of digesta pH to 2 using 2 *M* HCl (Shingfield et al., 2003), and adding *cis*-12 13:1 (10–1301–9, Larodan Fine Chemicals AB, Solna, Sweden) as an internal standard. Organic extracts were combined and dried under nitrogen at 50°C. Lipid dissolved in 2 mL of hexane was converted to FAME using a sequential base-acid catalyzed transesterification pro-