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Diet supplementation with a high dose of stearic acid to alleviate fish oil-induced milk fat depression in lactating ewes

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Abstract. Despite the benefits of using marine lipid supplements in dairy ewe diets to modulate milk fatty acid (FA) composition, this strategy causes milk fat depression (MFD), which precludes its use under farm conditions. This MFD is attributed to an impaired capacity of the mammary gland to achieve an adequate melting point for milk fat secretion. This alteration of fluidity has been linked to a shortage of available ruminal 18:0 caused by the consumption of marine lipids. However, in a previous study, we were not able to prevent the effects of fish oil supplementation through concomitant dietary addition of stearic acid (2% DM). Yet, before ruling out a mechanism based on milk fat fluidity, we decided to try with a higher dose of 18:0. Thus, this assay was conducted with 12 lactating ewes divided in 3 treatments that lasted for 4 weeks: a total mixed ration without lipid supplementation (control) or supplemented with 20 g/kg DM of fish oil alone (FO) or in combination with 40g/kg DM of 18:0 (FOSA-4). As expected, FO supplementation modified milk FA composition towards a healthier profile for consumers but, at the same time, reduced milk fat concentration. This MFD was not alleviated by the dietary addition of 4% 18:0 (FOSA-4). Depression of milk fat is discussed in relation to milk FA composition, particularly to the concentration of main metabolites responsible for changes in the melting point and fluidity of fat (i.e., 18:0 and *cis*-9 18:1).

Keywords. Fat fluidity – Fatty acid composition – Marine lipid – Ruminant nutrition – Sheep.

Supplémentation du régime avec une dose élevée d'acide stéarique pour pallier la chute du taux butyreux du lait induite par l'huile de poisson chez des brebis laitières

Résumé. *Malgré les avantages de l'introduction des huiles marines dans les régimes des brebis laitières pour moduler la composition en acides gras (AG) du lait, cette stratégie provoque une chute du taux butyreux (connu sous le nom de «* milk fat depression *» ; MFD). Ce MFD est attribué à une altération de la capacité de la glande mammaire d'atteindre un point de fusion adéquat pour sécréter des matières grasses du lait. Cette modification de la fluidité a été liée à une faible disponibilité de l'acide grasl 18:0 au niveau du rumen, causée par l'ingestion des lipides marins. Cependant, dans une étude antérieure, nous n'avons pas réussi à pallier les effets de la supplémentation du régime avec de l'huile de poisson par l'addition simultanée d'acide stéarique (2% de la matière sèche ; MS). Pourtant, avant d'exclure un mécanisme basé sur la fluidité des matières grasses du lait, nous avons décidé d'essayer avec une dose plus élevée de 18:0. Ainsi, cet essai a été effectué avec 12 brebis en lactation divisées en 3 traitements de 4 semaines de duration: une ration mixte complète sans supplémentation lipidique (témoin) ou supplémentée avec 20 g/kg MS d'huile de poisson seule (FO) ou en combinaison avec 40 g/kg MS du 18:0(FOSA-4). Comme prévu, le régime FO a modifié la composition en AG du lait vers un profil plus sain pour les consommateurs, mais, en même temps, il a induit une diminution de la teneur en matières grasses du lait. Cette MFD n'a pas été atténuée par l'addition du 4% 18:0 (FOSA-4). La chute du taux butyreux du lait est discutée par rapport à la composition en AG du lait, en particulier à la concentration des principaux métabolites responsables des modifications du point de fusion et de la fluidité du gras du lait (i.e., 18:0 et* cis*-9 18:1).*

Mots-clés. *Fluidité des matières grasses – Composition en acides gras – Nutrition des ruminants – Mouton.*

I – Introduction

Despite the benefits of including marine lipids in dairy ewe diets to modulate milk fatty acid (FA) composition towards a healthier profile for consumers, this strategy causes milk fat depression (MFD; Shingfield *et al.*, 2010; Carreño *et al.*, 2016; Toral *et al.*, 2017), which precludes its use under farm conditions.

Several theories explaining the origin of MFD have been proposed and subsequently found inadequate or incomplete (Bauman and Griinari, 2001; Shingfield and Griinari, 2007). The Biohydrogenation (BH) theory proposed by Bauman and Griinari (2001) establishes that MFD relates to an inhibition of mammary lipogenesis by specific BH intermediates that are produced under certain feeding conditions that alter rumen function. A second theory trying to explain more specifically the marine lipid-induced MFD suggests that a shortage of 18:0 for *cis*-9 18:1 synthesis in the mammary gland, would have a negative impact on the maintenance of milk fat fluidity and, consequently, on the rate of milk fat secretion, causing this syndrome (Chilliard *et al.*, 2007; Shingfield and Griinari, 2007).

However, in a previous dedicated study with lactating ewes (Toral *et al.*, 2016), we had to reject the hypothesis that the negative effects of fish oil supplementation (2% DM) would be prevented through concomitant addition of stearic acid (SA; 2% DM) to the diet. Although reductions in milk 18:0 and *cis*-9 18:1 concentration were partially reversed, supplementation with stearic acid did not prove useful to alleviate MFD. Yet, before ruling out a mechanism based on decreased ruminal production of 18:0 and subsequent alterations of milk fat fluidity, we decided to conduct this new assay with a higher dose of 18:0 (namely, 4% DM).

II – Material and methods

Twelve lactating Assaf ewes (79.0 ± 2.94 kg of body weight; 83.4 ± 2.65 days in milk at the beginning of the assay) were allocated to one of 3 groups (n = 4) balanced for milk production and composition, body weight, and days in milk. Dietary treatments consisted of a total mixed ration (TMR) containing no additional lipid (control) or 2% DM of fish oil (Afampes 121 DHA; Afamsa, Mos, Spain) alone (FO) or in combination (FOSA-4) with 4% DM of 18:0 (Edenor C18 98-100; Oleo Solutions, York, UK). The TMR was formulated (g/kg) from dehydrated alfalfa hay (400), whole maize (180) and barley (130) grains, soybean meal (150), beet pulp (70), molasses (50), and mineral and vitamin supplements (20) and was fed to the ewes for 27 days. Before that, all animals received the control TMR during 3 weeks. Diets were offered *ad libitum* twice daily, at about 9:30 and 18:30 h and clean drinking water was always available. Ewes were milked at approx. 9 and 18 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain).

On days 25, 26 and 27, milk yield was recorded and individual milk samples were collected and composited according to morning and evening milk yield. One aliquot of composite milk was preserved with bronopol and stored at 4°C until analyzed for fat concentration by infrared spectrophotometry (ISO 9622:1999). Another aliquot was untreated and stored at " 30°C until FA composition determinations.

Lipid in 1 mL of milk was extracted and converted to FA methyl esters (FAME) by base catalyzed transesterification (Shingfield *et al.*, 2003). The total FAME profile was determined using a gas chromatograph (Agilent 7890A GC System, Santa Clara, USA) equipped with a flame-ionization detector and a 100-m fused silica capillary column (CP-SIL 88, Varian Iberica, Madrid, Spain). Total FAME profile was determined using the temperature gradient program described in Shingfield *et al.* (2003). Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170°C (Shingfield *et al*., 2003). As outlined previously (e.g., Toral *et al.*, 2016), peaks were identified based on retention time comparisons with commercial standard FAME mixtures and ref-

erence samples for which the FA composition was determined based on GC analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivatives.

All data were evaluated by one-way ANOVA using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) with a model that included the fixed effect of treatment. Means were separated through the 'pdiff' option of the 'lsmeans' statement. Differences were declared significant at $P<0.05$.

III – Results and discussion

As expected, supplementation with FO modulated milk FA composition towards a profile potentially healthier for the consumer (Table 1; Chilliard *et al*., 2007). For instance, it increased the concentration of *cis*-9 *trans*-11 CLA, very long chain n-3 FA (e.g., EPA, DPA y DHA) or total polyunsaturated FA (PUFA). These changes were not only observed for FO alone, but also for FOSA-4 and agree with previous reports (Carreño *et al.*, 2016; Toral *et al.*, 2016).

At the same time, following the experiment design, diet supplementation with FO caused MFD (Carreño *et al.*, 2016; Toral *et al.*, 2017), which was characterized by a significant lower milk fat concentration in ewes on this treatment compared to those on the control. Fat production (g/day) was not significantly reduced due to the numerical (although not statistically significant) higher milk production in these animals. Very similar results were observed in ewes fed the FOSA-4 diet, showing that addition of 4% SA was not able to reverse the effect of FO. In fact, when compared to the control, decreases in milk fat concentration reached 19% in FO and 27% in FOSA-4.

TThe FO-induced MFD has been linked to a shortage of available 18:0, caused by the inhibition of the last step of ruminal BH and, consequently, a lower mammary endogenous synthesis of *cis*-9 18:1. This latter FA has a low melting point that contributes to maintain milk fat melting point below body temperature and ensure milk fat fluidity and secretion (Chilliard *et al.*, 2007; Shingfield *et al.*, 2010). In this assay, the decrease in *cis*-9 18:1 proportion due to FO was not completely reverted by FOSA-4 but its value (11.28%) was relatively close to that of the control (12.53%). However, the concentration of 18:0 fell more severely with FO (1.36%) and was still much lower in FOSA-4 (5.42%) than in control (7.18%).

The reasons underlying the lack of response to dietary 18:0 are still uncertain but may be related to the effects of a high dose of SA: a low digestibility of this FA (Boerman *et al.*, 2017) or alterations of rumen metabolism, as suggested, for example, by large increments in *trans*-10 18:1. Poor mammary uptake might also be involved (Enjalbert *et al.*, 1998). In any event, mechanisms linked to milk fat fluidity cannot be completely ruled out because this study, as well as most others, was conducted on milk fat that was successfully secreted but triacylglycerols with high melting point might accumulate in mammary epithelial cells and inhibit lipogenesis (Toral *et al.*, 2016).

As previously suspected (Toral *et al.*, 2016), results point again to the BH theory, with a significant contribution of some FA produced in the rumen to MFD. In line with this, some potentially antilipogenic FA (e.g., *trans*-10 18:1, *trans*-9 *cis*-11 CLA, and *trans*-10 *cis*-15 18:2; Shingfield and Griinari, 2007; Alves and Bessa, 2014) were remarkably increased in both FO and FOSA-4.

Table 1. Milk yield, fat concentration and yield, and composition of selected FA in milk in dairy ewes fed a total mixed ration without lipid supplementation (Control) or supplemented with 2% DM of fish oil alone (FO) or in combination with 4% of 18:0 (FOSA-4)

	Diet				
	Control	FO	FOSA-4	s.e.d.	*P*-value
Milk yield (g/d)	2133	2559	2548	378.3	0.4696
Fat (%)	5.99[a]	4.84[b]	4.35[b]	0.411	0.0089
Fat yield (g/d)	126.6	121.4	109.7	13.32	0.4624
Milk FA composition (g/100 g FA)					
18:0	7.18[a]	1.36[c]	5.42[b]	0.461	<.0001
cis-9 18:1	12.53[a]	5.91[c]	11.28[b]	0.479	<.0001
trans-10 18:1	0.36[c]	3.12[b]	5.48[a]	0.984	0.0019
trans-11 18:1	0.84[b]	3.78[a]	1.96[b]	0.663	0.0052
cis-9 *trans*-11 CLA	0.47[b]	1.88[a]	1.02[b]	0.311	0.0046
trans-9 *cis*-11 CLA	0.016[b]	0.075[a]	0.097[a]	0.0149	0.0012
trans-10 *cis*-12 CLA	0.006[b]	0.006[b]	0.010[a]	0.0013	0.0178
trans-11 *cis*-15 + *trans*-10 *cis*-15 18:2	0.04[b]	0.44[a]	0.49[a]	0.057	<.0001
20:5n-3 (EPA)	0.05[b]	0.37[a]	0.42[a]	0.048	<.0001
22:5n-3 (DPA)	0.09[b]	0.39[a]	0.47[a]	0.059	0.0003
22:6n-3 (DHA)	0.03[b]	1.10[a]	1.37[a]	0.173	<.0001
Total PUFA	5.10[b]	9.26[a]	9.03[a]	0.595	<.0001

s.e.d. = standard error of the difference.

[a-c] Different superscripts within a row indicate differences at $P<0.05$.

IV – Conclusion

Addition of stearic acid to the diet of lactating ewes (4% DM) was not able to alleviate the milk fat depression caused by the concomitant supplementation with fish oil (2% DM; strategy used to improve milk fatty acid profile). This lack of effect does not allow to accept the hypothesis suggesting that fish oil-induced MFD is mainly explained by decreased ruminal production of 18:0 and subsequent problems of milk fat fluidity.

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