Instituto de Ganadería de Montaña (IGM)

SÍNTESIS ENDÓGENA DE ÁCIDOS GRASOS EN LA GLÁNDULA MAMARIA Y SÍNDROME DE BAJA GRASA EN LA LECHE EN OVEJAS

Elena Bichi Ruspoli Forteguerri

León, Noviembre 2015

SÍNTESIS ENDÓGENA DE ÁCIDOS GRASOS EN LA GLÁNDULA MAMARIA Y SÍNDROME DE BAJA GRASA EN LA LECHE EN OVEJAS

ENDOGENOUS SYNTHESIS OF FATTY ACIDS IN THE MAMMARY GLAND AND MILK FAT DEPRESSION IN DAIRY EWES

SINTESI ENDOGENA DI ACIDI GRASSI NELLA GHIANDOLA MAMMARIA E SINDROME DI SCARSO GRASSO NEL LATTE DI PECORA

Memoria de Tesis Doctoral presentada por
Elena Bichi Ruspoli Forteguerri

dirigida por
Dra. Pilar de Frutos Fernández
y Dr. Gonzalo Hervás Angulo

para acceder al grado de Doctor, dentro del programa de Doctorado "Alimentación de rumiantes" de la Universidad de León.

León, Noviembre de 2015

La autora de esta memoria ha disfrutado de una ayuda del Programa JAE Predoc para la formación de personal investigador del Consejo Superior de Investigaciones Científicas (CSIC) cofinanciada por el Fondo Social Europeo.

Los trabajos que componen esta memoria han sido financiados por los Ministerios de Ciencia e Innovación (MICINN; AGL2008-04805) y de Economía y Competitividad (MINECO; AGL2011-23700).

Deseo expresar mi agradecimiento a todas las personas e instituciones que han puesto a nuestra disposición sus conocimientos y los medios necesarios para la realización de este trabajo.

A *Los Tres Mosqueteros*, sin duda

e a Marta Monari, in ricordo.

CONTENIDOS

ÍNDICE

Página

INDEX

Page

ABREVIATURAS

ADN ..... ácido desoxirribonucleico
AG ..... ácido graso
ARN ..... ácido ribonucleico
ARNm ..... ácido ribonucleico mensajero
BH ..... biohidrogenación
CLA ..... ácido linoleico conjugado
DHA ..... ácido docosahexaenoico
EPA ..... ácido eicosapentaenoico
MA ..... microalgas marinas
MFD ..... síndrome de baja grasa en la leche
MFGM ..... membrana del glóbulo graso de la leche
PCR ..... reacción en cadena de la polimerasa
RA ..... ácido ruménico
SCD ..... estearoil-CoA desaturasa o Δ^9-desaturasa
TG ..... triglicéridos
VA ..... ácido vaccénico

ABBREVIATIONS

ADF ..... acid detergent fiber
ANOVA ..... analysis of variance
BHBA ..... ß-hydroxybutyrate
BW ..... body weight
CLA ..... conjugated linoleic acid
CP ..... crude protein
d ..... day
D ..... diet
DIM ..... days in milk
DM ..... dry matter
DMI ..... dry matter intake
DNA ..... deoxyribonucleic acid
FA ..... fatty acid
FAME ..... fatty acid methyl esters
FO ..... fish oil
GC ..... gas chromatography
GC-MS ..... gas chromatography-mass spectrometry
GH ..... growth hormone
IGF ..... insulin-like growth factor
LCFA ..... long-chain fatty acids
MA ..... marine algae
MFD ..... milk fat depression
MUFA ..... monounsaturated fatty acids

NEFA ..... non esterified fatty acids
NDF ..... neutral detergent fiber
ns ..... non significant
OM ..... organic matter
P ..... probability
PCR ..... polymerase chain reaction
PUFA ..... polyunsaturated fatty acids
RNA ..... ribonucleic acid
mRNA ..... messenger ribonucleic acid
qPCR ..... quantitative polymerase chain reaction
SCD ..... stearoyl-CoA desaturase or Δ^9-desaturase
SED ..... standard error of the difference
SEM ..... standard error of the mean
SFA ..... saturated fatty acids
SO ..... sunflower oil
SOMA ..... sunflower oil plus marine algae
T ..... time
TAG ..... triacylglycerol
TMR ..... total mixed ration
TS ..... total solids
wk ..... week

RESUMEN

La grasa es el componente más variable de la leche de los rumiantes, dependiendo su contenido y composición básicamente de la dieta consumida. Así, el perfil de ácidos grasos (AG) es el resultado de una compleja interacción entre los nutrientes y el metabolismo ruminal y mamario. Entre los AG de la grasa láctea de oveja cabe destacar el ácido linoléico conjugado (CLA) por sus efectos potencialmente beneficiosos en la salud humana. El *cis*-9, *trans*-11 CLA que aparece en la leche tiene un doble origen: por una parte ruminal, mediante la acción de la microbiota local, y por otra endógena en la glándula mamaria, por medio de la enzima Δ^9-desaturasa (o SCD). En la oveja, al igual que en otros rumiantes lecheros, la Δ^9-desaturasa sería la principal responsable de la síntesis de los AG de cadena larga con doble enlace en posición *cis*-9 que se encuentran en la leche (p. ej., el *cis*-9 18:1 a partir de 18:0 y el *cis*-9, *trans*-11 18:2 a partir de *trans*-11 18:1). Sin embargo, la información al respecto es todavía escasa.

Con el objetivo de intentar cuantificar la síntesis endógena de estos AG en la glándula mamaria del ovino, se llevó a cabo una prueba en la que se administró ácido estercúlico, un potente y específico inhibidor de la SCD, a un grupo de 6 ovejas en lactación. Los animales se controlaron durante un periodo experimental de 15 días, dividido en tres de 5 días cada uno: pretratamiento, tratamiento y postratamiento. Durante el periodo de tratamiento, las ovejas recibieron una dosis diaria de 0,5 g de ácido estercúlico, que se administró por vía endovenosa cada 6 horas (i. e., 4 veces al día). A lo largo de todo el ensayo experimental, los animales se alimentaron con pasto fresco para proporcionar una dieta rica en ácido linolénico y minimizar el aporte ruminal de *cis*-9, *trans*-11 18:2. Los resultados mostraron una reducción en el contenido de aquellos AG que se originan por acción de la Δ^9-desaturasa (p. ej., *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 y *cis*-9, *trans*-11 18:2), junto con un aumento de aquellos que constituyen su sustrato (p. ej., 16:0, 18:0 y *trans*-11 18:1). La concentración de otros AG, que hasta el momento no se conocían como sustratos de la SCD (p. ej., el *cis*-15 18:1 y el *trans*-11, *trans*-15 18:2), también aumentó tras la administración de ácido estercúlico, mientras que disminuyó la de sus productos (el *cis*-9, *cis*-15 18:2 y el *cis*-9, *trans*-11, *trans*-15 18:3). La inhibición de la Δ^9-desaturasa alcanzó un 70% y permitió estimar que la síntesis endógena del *cis*-9 18:1 representaba el 63% de lo que aparecía en la leche y la del *cis*-9, *trans*-11 18:2 el 74%.

Otro aspecto relacionado con el *cis*-9 18:1 es el papel que este AG juega en el

mantenimiento de la fluidez de la grasa de la leche. De hecho, se considera que la escasez de 18:0 para la síntesis endógena de *cis*-9 18:1 podría explicar la depresión de la grasa láctea (MFD) que se observa en animales que consumen lípidos de origen marino, ya que la caída de este isómero 18:1 aumentaría el punto de fusión, dificultando así la secreción de la grasa en la leche. Los AG n-3 de cadena muy larga, abundantes en los lípidos marinos, son conocidos inhibidores del último paso de la biohidrogenación ruminal, lo cual provoca una disminución de la cantidad de 18:0 que abandona el rumen y que representa el sustrato necesario para la formación de *cis*-9 18:1 en la glándula mamaria.

Por otro lado, basándose en resultados previos obtenidos en ovejas en lactación cuya dieta se suplementaba con microalgas marinas (MA), se planteó la hipótesis de una adaptación de la microbiota ruminal al aporte de lípidos marinos que permitiera una recuperación gradual del porcentaje de grasa de la leche.

A partir de estos puntos, se llevó a cabo una segunda prueba experimental para estudiar la persistencia de la respuesta de 36 ovejas en lactación a la suplementación de su dieta con MA. Dicha respuesta se midió en términos de rendimiento productivo y composición de la leche, con especial interés en el perfil lipídico en general y en los AG relacionados con la MFD en particular. Los animales fueron divididos en 6 lotes de 6 ovejas cada uno y asignados a 2 tratamientos experimentales: 3 lotes recibieron la dieta control, que consistió en una ración completa mezclada con una relación forraje:concentrado 40:60 y que contenía 25 g de aceite de girasol/kg de materia seca, y los otros 3 recibieron la misma dieta control pero suplementada con 8 g MA/kg de materia seca. La producción, composición y perfil lipídico de la leche se analizaron antes (día 0) y tras 6, 12, 18, 24, 34, 44 y 54 días de tratamiento.

La suplementación de la dieta con microalgas no afectó a la producción de leche, pero redujo su contenido de grasa, lo cual resultó evidente a partir del día 18 y alcanzó un -17% al final del experimento (día 54). Comparado con el grupo control, el consumo de MA causó una reducción en la concentración de 18:0 y del producto de su desaturación, el *cis*-9 18:1, que persistió durante todo el ensayo. Además, en la leche procedente de las ovejas alimentadas con MA se observó un aumento de AG de conformación *trans*, especialmente del *trans*-10 18:1 (lo cual se relacionó con la persistencia de la MFD), así como del *trans*-11 18:1, el *cis*-9, *trans*-11 18:2, el *trans*-10, *cis*-12 18:2 y el *trans*-9, *cis*-11 18:2, junto con el aumento de AG n-3 de 20-22C, principalmente el 22:6 n-3. En conjunto, la persistencia de la respuesta no permitió

aceptar la hipótesis de una adaptación de la microbiota ruminal al aporte dietario de AG n-3 de cadena muy larga.

Por otra parte, aunque la nutrición representa el principal factor regulador de la producción de grasa láctea, es aún muy escasa la información disponible sobre la posible relación entre la dieta y la regulación de los genes implicados en el metabolismo lipídico en ovejas lecheras. Esta relación se ha estudiado en el vacuno durante la MFD, pero es todavía muy desconocida en el ovino, especialmente cuando el síndrome ha sido inducido por el consumo de lípidos marinos.

Por lo tanto, a partir de los animales de la segunda prueba, se planteó un estudio para analizar los cambios en la abundancia de ARNm de los principales genes candidatos involucrados en el metabolismo lipídico en el tejido mamario, la grasa subcutánea y el hígado, en respuesta a la MFD causada en ovejas lecheras por la ingestión de MA durante 8 semanas. Para ello, se utilizaron 11 animales, 5 del tratamiento control y 6 del MA, que fueron sacrificados al final del ensayo (día 54). Se tomaron muestras de glándula mamaria, tejido adiposo e hígado y los análisis se llevaron a cabo mediante PCR cuantitativa con transcriptasa inversa.

No se observó ninguna diferencia entre los dos tratamientos en la abundancia de ARNm de los genes que codifican las principales proteínas de la glándula mamaria y del tejido adiposo necesarias para la captación y activación de los AG (*ACSS2*, *LPL*), transporte intracelular (*FABP3*, *FABP4*), síntesis *de novo* (*ACACA*, *FASN*), esterificación (*DGAT1*, *DGAT2*, *LPIN1*), desaturación (*SCD*), elongación (*ELOVL6*), formación de la gota lipídica (*ADFP*, *BTN1A1*, *XDH*) y regulación transcripcional (*INSIG1*, *MED1*, *PPARG*, *RXRA*, *SCAP*, *SREBF1*, *THRSP*). En el tejido hepático, el consumo de MA no afectó a la expresión de los genes responsables de la ß-oxidación y de la síntesis de lipoproteínas (*ACOX1*, *APOB*, *CPT1A*, *PPARA*, *RXRA*), si bien indujo un aumento en la expresión de *HMGCS2*, un gen relacionado con la cetogénesis. Por su parte, tampoco las concentraciones plasmáticas de ß-hidroxibutirato, AG libres, glucosa, triglicéridos, hormona del crecimiento, factor de crecimiento insulínico tipo I, insulina y leptina se vieron afectadas de forma significativa por la ingestión de microalgas durante 54 días. En todo caso, no se puede descartar que los cambios en el transcriptoma se produjeran relativamente pronto tras el consumo de MA, al igual que ocurrió con el perfil lipídico de la leche (segunda prueba) y fuera más complicado detectarlos a largo plazo.

En conjunto, los resultados muestran la complejidad de los factores determinantes del desarrollo del síndrome de baja grasa en la leche y subrayan la importancia de continuar la investigación en el ovino lechero, ya que las incertidumbres al respecto son aún numerosas.

SUMMARY

Fat is the most variable component of milk in dairy ruminants, its amount and composition being affected basically by the consumed diet. Thus, its fatty acid (FA) profile is the result of a complex interaction between nutrients and ruminal and mammary metabolism. Among FA present in ewe milk fat, it is worth mentioning the conjugated linoleic acid (CLA) because of its potential beneficial effects on human health. The *cis*-9, *trans*-11 CLA present in milk has a double origin: on the one side, ruminal through the action of local microbiota, and on the other side, endogenous in the mammary gland, through the Δ^9-desaturase enzyme (SCD). In the ewe, as well as in other dairy ruminants, the Δ^9-desaturase would be the main responsible for the synthesis of milk long-chain FA with a double bound in the *cis*-9 position (e.g., *cis*-9 18:1 from 18:0, and *cis*-9, *trans*-11 18:2 from *trans*-11 18:1). However, information on this topic is still scant.

With the aim of trying to determine the endogenous synthesis of those FA in the ovine mammary gland, it was carried out a trial in which sterculic acid, a powerful and specific Δ^9-desaturase inhibitor, was administered to a group of 6 lactating ewes. Animals were monitored for a 15-day experimental period, which included a 5-day pretreatment period, a 5-day treatment period, and a 5-day posttreatment period. During the treatment period, ewes received 0.5 g/day of sterculic acid, delivered intravenously in 4 equal doses at 6-hour intervals. Throughout the whole experiment, animals were fed pasture to supply mainly α-linolenic acid and minimize the amount of milk *cis*-9, *trans*-11 18:2 of ruminal origin. Results showed a decrease in the milk content of those FA arising from SCD action (e.g., *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1, and *cis*-9, *trans*-11 18:2) together with an increase in the enzyme substrates (e.g., 14:0, 18:0, and *trans*-11 18:1). Concentration of some other milk FA, further to previously reported substrates of SCD (e.g., *cis*-15 18:1, and *trans*-11, *trans*-15 18:2) were also increased by sterculic acid administration, whereas concentration of its products were decreased (e.g., *cis*-9, *cis*-15 18:2, and *cis*-9, *trans*-11, *trans*-15 18:3). The inhibition of the Δ^9-desaturase reached 70% and allowed to estimate that 63% of *cis*-9 18:1 and 74% of *cis*-9, *trans*-11 18:2 present in milk fat arise from endogenous synthesis.

Another interesting aspect related to the *cis*-9 18:1 is the role that this FA plays in the maintenance of milk fat fluidity. In fact, the lack of 18:0 for the endogenous synthesis of *cis*-9 18:1 has been indicated to be able to explain the milk fat depression (MFD) observed in animals fed marine lipids, because the decrease of this 18:1

isomer would increase the melting point, making milk fat secretion more complicated. Very long-chain n-3 FA, abundant in marine lipids, are well known inhibitors of the last step of ruminal biohydrogenation, which causes a decrease in the amount of 18:0 leaving the rumen that is the substrate for *cis*-9 18:1 synthesis in the mammary gland.

Furthermore, based on previous results in lactating ewes receiving a diet that was supplemented with marine microalgae (MA), it was hypothesized the possibility of an adaptation of the rumen microbiota to the consumption of marine lipids that allowed a gradual recovery of milk fat percentage.

Based on these points, a second experimental trial was conducted to study the persistency of the response of 36 lactating ewes to the supplementation of their diet with MA. This response was measured in terms of animal performance and milk composition, with especial focus on milk FA profile in general and on those FA related to MFD in particular. Animals were distributed in 6 lots of 6 ewes/lot and allocated to 2 treatments: 3 lots were fed the control diet, consisting of a total mixed ration (40:60 forage:concentrate ratio) supplemented with 25 g of sunflower oil/kg of dry matter, and the remaining 3 lots were fed the same diet but supplemented with 8 g of MA/kg of dry matter. Milk production and composition, including FA profile, were analyzed before (day 0) and after 6, 12, 18, 24, 34, 44, and 54 days of treatment. Diet supplementation with MA did not affect milk yield, but decreased milk fat content. Differences in the latter were detected from day 18 onward and reached -17% at the end of the experiment (i.e., on day 54). Compared with the control diet, MA consumption caused a reduction in milk 18:0 and its desaturation product, *cis*-9 18:1, that lasted for the whole experimental period. Additionally, inclusion of MA in the diet enhanced the milk content of *trans* FA, particularly *trans*-10 18:1 (which was related to the persistency of MFD), and *trans*-11 18:1, *cis*-9, *trans*-11 18:2, *trans*-10, *cis*-12 18:2, *trans*-9, *cis*-11 18:2, and C20-22 n-3 polyunsaturated FA, mainly 22:6 n-3. Overall, the persistency of the responses did not allow to accept the original hypothesis of the ruminal microbiota adaptation to the dietary supply of very long-chain n-3 polyunsaturated FA.

Even though nutrition represents the main factor affecting milk fat yield, there is still very scant available information on the potential relationship between diet and regulation of genes involved in lipid metabolism in dairy ewes. This relationship has been studied in the bovine during MFD, but is still unknown in the ovine, especially when the MFD has been induced by marine lipid consumption.

Therefore, using the animals involved in the second trial, a study was conducted to investigate changes in the mRNA abundance of candidate genes involved in lipid metabolism in the mammary gland, the subcutaneous adipose tissue and the liver in response to long-term (8 weeks) MFD induced by MA. To this end, 11 animals, 5 from the control and 6 from the MA treatments, were euthanized at the end of the study (day 54), and samples of mammary gland, adipose tissue, and liver were harvested and analyzed by quantitative reverse transcription-PCR.

There was no effect of MA on mammary and adipose tissue expression of genes encoding proteins required for FA uptake and activation (*ACSS2*, *LPL*), intracellular FA transport (*FABP3*, *FABP4*), de novo FA synthesis (*ACACA*, *FASN*), esterification (*DGAT1*, *DGAT2*, *LPIN1*), desaturation (*SCD*), elongation (*ELOVL6*), lipid droplet formation (*ADFP*, *BTN1A1*, *XDH*), and transcriptional regulation (*INSIG1*, *MED1*, *PPARG*, *RXRA*, *SCAP*, *SREBF1*, *THRSP*). In the hepatic tissue, addition of MA did not affect the expression of ß-oxidation- and lipoprotein-related genes (*ACOX1*, *APOB*, *CPT1A*, *PPARA*, *RXRA*), but it up-regulated hepatic *HMGCS2*, which controls ketogenesis. The concentration of plasma ß-hydroxybutyrate, NEFA, glucose, triacylglycerol, growth hormone, insulin-like growth factor 1, insulin, and leptin was not different between groups at d 54. However, it cannot be discarded that transcriptional changes were established during earlier stages of the feeding treatment, similarly to what occurred in the milk FA profile (Trial II), and it was more complicated to detect them in the long-term.

Overall, the results show the complexity of the key factors involved in the development of MFD and highlight the need for further research in dairy ewes, because the topic is still full of uncertainties.

RIASSUNTO

Il grasso è la componente più variabile del latte dei ruminanti, e il suo contenuto e la sua composizione dipendono fondamentalmente dalla dieta assunta. Inoltre, il profilo degli acidi grassi (AG) è il risultato di una complessa interazione fra i nutrienti e il metabolismo ruminale e mammario. Fra gli AG del grasso del latte di pecora vale la pena menzionare l'acido linoleico coniugato (CLA) per i suoi effetti potenzialmente benefici sulla salute umana. Il *cis*-9, *trans*-11 CLA presente nel latte ha una doppia origine: da una parte ruminale, attraverso l'azione delle micropopolazioni locali, e dall'altra parte endogena, per mezzo dell'enzima Δ^9-desaturasi (o SCD). Nella pecora, come anche negli altri ruminanti da latte, la Δ^9-desaturasi sarebbe la principale responsabile della sintesi di AG a lunga catena con un doppio legame in posizione *cis*-9 presenti nel latte (per esempio, *cis*-9 18:1 dal 18:0 e *cis*-9, *trans*-11 18:2 dal *trans*-11 18:1). Tuttavia, le informazioni al riguardo sono ancora piuttosto scarse.

Con l'obiettivo di cercare di quantificare la sintesi endogena di questi AG nella ghiandola mammaria dell'ovino, si è condotta una prova (Prova I) nella quale si è somministrato acido sterculico, un potente e specifico inibitore dell'SCD, a un gruppo di 6 pecore in lattazione. Gli animali furono controllati durante tutto il periodo sperimentale di 15 giorni, diviso in tre periodi di 5 giorni ciascuno: pre-trattamento, trattamento, e post-trattamento. Durante il periodo di trattamento, le pecore ricevettero una dose quotidiana di 0.5 g di acido sterculico, che fu somministrata per via endovenosa ogni 6 ore (4 volte al giorno). Lungo tutto il periodo sperimentale, gli animali furono alimentati con foraggio fresco per fornire una dieta ricca di acido linolenico e minimizzare l'apporto ruminale di *cis*-9, *trans*-11 18:2. I risultati mostrarono una riduzione del contenuto di quegli AG che originano dall'azione della Δ^9-desaturasi (per esempio, *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 e *cis*-9, *trans*-11 18:2), oltre all'aumento di quegli AG che ne costituiscono il substrato (per esempio, 14:0, 18:0 e *trans*-11 18:1). Anche la concentrazione di altri AG, che fino al momento non erano conosciuti come substrato dell'SCD (per esempio, *cis*-15 18:1 e *trans*-11, *trans*-15 18:2) aumentò a seguito della somministrazione di acido sterculico, mentre diminuì quella dei suoi prodotti (per esempio, *cis*-9, *cis*-15 18:2 e *cis*-9, *trans*-11, *trans*-15 18:3). L'inibizione della Δ^9-desaturasi raggiunse il 70% e ha permesso di calcolare che la sintesi endogena del *cis*-9 18:1 rappresentava il 63% del totale presente nel latte e quella del *cis*-9, *trans*-11 18:2 il 74%.

Un altro aspetto relativo al *cis*-9 18:1 riguarda il ruolo che questo AG ricopre nel mantenimento della fluidità del grasso del latte. Di fatto, si considera la scarsa disponibilità di 18:0 per la sintesi endogena di *cis*-9 18:1 come possibile meccanismo per spiegare la sindrome di scarso grasso nel latte (MFD) che si osserva negli animali che hanno assunto lipidi di origine marina, giacché il calo di questo isomero 18:1 aumenterebbe il punto di fusione, rendendo più difficile la secrezione del grasso del latte. Gli AG n-3 a catena molto lunga, abbondanti nei lipidi marini, sono noti inibitori dell'ultimo passo della bioidrogenazione ruminale, il che provoca una diminuzione della quantità di 18:0 che abbandona il rumine e che rappresenta il substrato necessario per la formazione di *cis*-9 18:1 nella ghiandola mammaria.

Dall'altro lato, basandosi su risultati ottenuti in precedenza in pecore in lattazione la cui dieta era stata arricchita con microalghe marine (MA), è stata proposta l'ipotesi di un adattamento della micropopolazione ruminale all'apporto di lipidi marini che avrebbe permesso un recupero graduale della percentuale di grasso del latte.

Partendo da queste premesse, si è condotta una seconda prova sperimentale (prova II) per studiare la persistenza della risposta di 36 pecore in lattazione al supplemento della dieta con MA. Questa risposta si misurò in termini di rendimento produttivo e composizione del latte, con speciale interesse per il profilo lipidico in generale e in quegli AG collegati alla MFD in particolare. Gli animali furono divisi in 6 gruppi di 6 pecore ciascuno e sottoposti a 2 trattamenti sperimentali: 3 gruppi ricevettero la dieta controllo che consisteva in una razione completa mescolata con un rapporto foraggio:concentrato 40:60 e che conteneva 25 g di olio di girasole/kg di materia secca, e gli altri 3 gruppi ricevettero la stessa dieta, con l'aggiunta di 8 g di MA/kg di materia secca, La produzione, la composizione e il profilo lipidico del latte furono analizzati prima (giorno 0) e dopo 6, 12, 18, 24, 34, 44 e 54 giorni di trattamento.

L'aggiunta di microalghe alla dieta non alterò la produzione di latte, ma ridusse il suo contenuto in grasso, il che risultò evidente a partire dal giorno 18 e raggiunse il –17% alla fine dell'esperimento (giorno 54). Rispetto al gruppo controllo, il consumo di MA causò una riduzione nella concentrazione di 18:0 e del prodotto della sua desaturazione, il *cis*-9 18:1, che perdurò per tutta la durata dello studio. Inoltre, nel latte delle pecore alimentate con MA si osservò un aumento di AG di conformazione *trans*, particolarmente di *trans*-10 18:1 (il quale è stato messo in relazione con la MFD), e di *trans*-11 18:1, *cis*-9, *trans*-11 18:2, *trans*-10, *cis*-12 18:2, e *trans*-9, *cis*-11 18:2, insieme all'aumento di AG n-3 di 20-22C, principalmente il 22:6 n-3.

Nell'insieme, la persistenza della risposta non ha permesso di accettare l'ipotesi iniziale di un adattamento della micropopolazione ruminale all'apporto quotidiano di AG n-3 a catena molto lunga.

D'altro canto, sebbene sia noto che la nutrizione rappresenti il fattore principale che regola la produzione di grasso del latte, sono ancora piuttosto scarse le informazioni disponibili sulla possibile relazione esistente fra la dieta e la regolazione dei geni implicati nel metabolismo lipidico nelle pecore da latte. Questa relazione è stata studiata nel bovino durante la MFD, ma è ancora sconosciuta nell'ovino, soprattutto quando la stessa sindrome è indotta attraverso il consumo di lipidi marini.

Perciò, partendo dagli animali della Prova II, si pianificò uno studio per analizzare i cambi nell'abbondanza di RNAm dei principali geni coinvolti nel metabolismo lipidico del tessuto mammario, di quello adiposo sottocutaneo ed epatico, in risposta alla MFD causata nelle pecore da latte dall'ingestione di MA per 8 settimane (Prova III). A questo scopo, si utilizzarono 11 animali, 5 del trattamento controllo e 6 del trattamento MA, che furono sacrificati alla fine dello studio (giorno 54). Si prelevarono campioni di ghiandola mammaria, tessuto adiposo e fegato che furono analizzati attraverso la tecnica di PCR quantitativa con trascrittasi inversa.

Non si osservò nessuna differenza fra i due trattamenti nell'abbondanza di RNAm dei geni che codificano per le principali proteine di ghiandola mammaria e tessuto adiposo necessarie per la captazione e attivazione degli AG (*ACSS2*, *LPL*), il trasporto intracellulare (*FABP3*, *FABP4*), la sintesi de novo (*ACACA*, *FASN*), l'esterificazione (*DGAT1*, *DGAT2*, *LPIN1*), la desaturazione (*SCD*), l'allungamento della catena carboniosa (*ELOVL6*), la formazione della goccia lipidica (*ADFP*, *BTN1A1*, *XDH*), e la regolazione trascrizionale (*INSIG1*, *MED1*, *PPARG*, *RXRA*, *SCAP*, *SREBF1*, *THRSP*). A livello di tessuto epatico, l'aggiunta di MA non ebbe effetti sull'espressione dei geni responsabili della ß-ossidazione e della sintesi delle lipoproteine (*ACOX1*, *APOB*, *CPT1A*, *PPARA*, *RXRA*), sebbene si osservò un aumento dell'espressione di *HMGCS2*, un gene che controlla la chetogenesi. Da parte loro, la concentrazione plasmatica di ß-idrossibutirrato, AG liberi, glucosio, trigliceridi, ormone della crescita, fattore di crescita insulino-simile 1, insulina, e leptina non risultò alterata in maniera significativa a seguito dell'ingestione di microalghe dopo 54 giorni. In tutti i casi, non si può scartare la possibilità che i cambi a livello di trascrittoma si siano prodotti relativamente presto a partire dal consumo di

MA, come si osserva anche nel profilo lipidico del latte (Prova II), e che fosse più complicato rilevarli a lungo termine.

Nell'insieme, i risultati mostrano la complessità dei fattori determinanti lo sviluppo della sindrome di scarso grasso nel latte e sottolineano l'importanza di continuare le ricerche al riguardo nell'ovino da latte, giacché le incertezze a questo proposito sono ancora numerose.

INTRODUCCIÓN

En la Unión Europea, la producción de leche de oveja se concentra mayoritariamente en los países del área mediterránea, siendo Grecia, España e Italia los principales productores (FAOSTAT, 2013). La leche de oveja se destina, fundamentalmente, a la elaboración de productos como el queso y el yogur (Bocquier y Caja, 2001; Boyazoglu y Morand-Fehr, 2001; de Rancourt y Carrère, 2011), ya que su alto porcentaje de sólidos garantiza un buen rendimiento quesero (Pellegrini et al., 1997; Pulina y Nudda, 2004; Park et al., 2007). Además, la leche de oveja presenta una elevada calidad nutritiva gracias a su contenido, entre otros, de aminoácidos esenciales, minerales, vitaminas, ácidos grasos de cadena corta, ácidos grasos monoinsaturados o ácido linoleico conjugado (Park et al., 2007; Raynal-Ljutovac et al., 2008).

La concentración de grasa en la leche de oveja es mayor que en las de vaca y cabra (Boyazoglu y Morand-Fehr, 2001; Pulina y Nudda, 2004; Park et al., 2007) y confiere, tanto a la leche como al queso, un olor y gusto característicos que se deben principalmente a la alta proporción de ácidos grasos de cadena corta y media (Moio et al., 1996; Barron et al., 2004).

La leche de la especie ovina es también rica en otros ácidos grasos, entre los cuales cabría destacar el ácido linoleico conjugado o CLA (del inglés *conjugated linoleic acid*; Chilliard y Ferlay, 2004; Tsiplakou et al., 2009). El CLA es una mezcla de diversos isómeros geométricos y posicionales del ácido linoleico (Griinari et al., 2000; Palmquist et al., 2005) que ha sido y sigue siendo objeto de numerosos estudios debido a sus efectos potencialmente beneficiosos para la salud de los consumidores (e. g., su acción anticancerígena, antiarteriosclerótica, antidiabética o inmunomoduladora; Parodi, 2004; Dilzer y Park, 2012).

Diversos estudios en rumiantes lecheros han demostrado que la síntesis del CLA tiene un doble origen: por una parte ruminal, mediante la acción de la microbiota local, y por otra endógena en la glándula mamaria, por medio de la enzima Δ^9-desaturasa o estearoil-CoA desaturasa (conocida como SCD por sus siglas en inglés) a partir del ácido vaccénico (Griinari et al., 2000; Corl et al., 2001). Aunque se sabe que la mayor parte del CLA presente en la leche es de origen endógeno (Griinari et al., 2000; Corl et al., 2001), la información en pequeños rumiantes sobre el proceso de síntesis de éste y de otros ácidos grasos de interés (e. g., *cis*-9 18:1) es todavía muy escasa (y lo era especialmente en el momento en que se planteó esta tesis doctoral), aunque existe algún estudio en cabras (Annison et al., 1967; Bickerstaffe y Johnson,

1972; Bernard et al., 2010) o en la especie ovina (Palmquist et al., 2004; Frutos et al., 2014). Sin embargo, el alto contenido de CLA de la leche de oveja y su posible papel en la prevención de enfermedades crónicas, justifican sobradamente la necesidad de conocer en más detalle su síntesis endógena para poder llegar a desarrollar estrategias nutricionales dirigidas a la producción de alimentos potencialmente más saludables para los consumidores.

Tal y como se ha señalado anteriormente, además de la síntesis endógena, la biohidrogenación ruminal constituye la otra fuente de los ácidos grasos de la leche, gracias a la acción de la microbiota local. En este sentido, se ha observado que determinadas dietas y, más concretamente, determinados suplementos de la dieta (por ejemplo, aceites vegetales o lípidos marinos) pueden afectar a la biohidrogenación de los lípidos insaturados de la dieta. A lo largo de ese proceso, llevado a cabo por los microorganismos ruminales, se forma una serie de metabolitos intermedios de los cuales se ha señalado que podrían reducir el contenido de grasa de la leche (Toral et al., 2010a,b, 2012). Por ello, es fundamental conocer su posible papel en el control de la síntesis lipídica en la glándula mamaria de la oveja en lactación.

Los trabajos de Toral et al. (2010c, 2012) demostraron que la variación en la composición de la microbiota ruminal está estrechamente relacionada con los cambios que se producen en el perfil lipídico de la grasa de la leche. Así, determinados metabolitos de origen ruminal, que aparecen básicamente cuando se produce una alteración del proceso de biohidrogenación, parecen tener un papel clave como moduladores no solo de la composición sino también de la producción de grasa láctea. Este hecho resulta especialmente relevante en la investigación sobre la interacción entre la dieta y el desarrollo de determinadas disfunciones, como el denominado "síndrome de baja grasa en la leche" o depresión de la grasa láctea (MFD por las siglas en inglés de *milk fat depression*).

Este síndrome se ha descrito ampliamente en la vaca en lactación cuya dieta es rica en concentrado y pobre en forraje o está suplementada con una fuente de ácidos grasos insaturados (Bauman y Griinari, 2003; Shingfield et al., 2010). La oveja, en cambio, es menos propensa a la depresión de la grasa de la leche con dietas altas en concentrado y enriquecidas con aceites vegetales (Pulina et al., 2006; Gómez-Cortés et al., 2008; Hervás et al., 2008). Sin embargo, al igual que ocurre en las vacas, la suplementación de su dieta con lípidos marinos ejerce un claro efecto inhibidor sobre la producción de grasa (Toral et al., 2010a,b). Esta caída coincide con el aumento de

determinados ácidos grasos de origen ruminal (e. g., el *trans*-10 18:1 o el *trans*-9, *cis*-11 18:2) cuyo papel en la síntesis lipídica en la glándula mamaria de la oveja no ha sido todavía suficientemente estudiado. En este sentido, la evolución que se observa, por ejemplo, en el estudio de Toral et al. (2010b) de la concentración del *trans*-10 18:1 en la leche podría indicar una posible adaptación de la microbiota ruminal a los lípidos de la dieta, tras cuatro semanas de consumo de microalgas marinas, que se traduciría en una recuperación gradual del contenido de grasa láctea. Para esclarecer la posibilidad de dicha adaptación sería necesario un estudio a más largo plazo que permitiera relacionar los cambios del porcentaje de grasa de la leche con la evolución de su perfil lipídico.

Por otra parte, aunque la dieta representa uno de los principales factores reguladores de la producción de grasa de la leche y constituye un instrumento eficaz para modificar su composición de ácidos grasos (Harvatine et al., 2009; Shingfield et al., 2010; Bauman et al., 2011), es aún muy escasa la información disponible sobre la relación que pudiera existir entre esta (i. e., la dieta) y la regulación de los genes implicados en la lipogénesis en el ovino lechero (Hussein et al., 2013).

En el vacuno, la expresión de los principales genes candidatos relacionados con el metabolismo lipídico en la glándula mamaria se ha analizado a lo largo de la lactación (Bionaz y Loor, 2008) y durante el síndrome de baja grasa en la leche (Piperova et al., 2000; Harvatine y Bauman, 2006; Invernizzi et al., 2010). En el caprino, por su parte, hay muy pocos estudios al respecto (Bernard et al., 2012; Toral et al., 2013), de forma similar a lo que sucede en el ovino lechero (Tsiplakou et al., 2009; Hussein et al., 2013; Castro-Carrera et al., 2015). Esta falta de información era especialmente acusada en el momento en que se planteó esta tesis, tal y como puede deducirse a partir de las fechas de las referencias bibliográficas y las de los artículos que aquí se recogen.

Volviendo al síndrome de baja grasa en la leche, la oveja parece situarse en una situación intermedia entre la vaca y la cabra (Lock et al., 2006, Toral et al., 2010b; Oliveira et al., 2012), aunque los mecanismos responsables de su desarrollo permanecen inciertos. Así, por ejemplo, metabolitos como el *trans*-10, *cis*-12 18:2, que en el vacuno lechero se han relacionado claramente con la MFD (Chilliard et al., 2007; Shingfield et al., 2009), no tienen un efecto tan claro en el ovino. Cuando el *trans*-10, *cis*-12 18:2 se administra a ovejas en lactación, se provoca el síndrome de baja grasa en la leche (Lock et al., 2006; Sinclair et al., 2010; Oliveira et al., 2012) y

se altera la expresión de los principales genes estudiados (Hussein et al., 2013). Por el contrario, cuando la depresión de la grasa láctea se induce mediante el consumo de lípidos marinos, el *trans*-10, *cis*-12 18:2 no parece jugar un papel determinante y el efecto inhibidor se suele atribuir a otros metabolitos (e. g., el *trans*-10 18:1 o el *trans*-9, *cis*-11 18:2), o a otros mecanismos diferentes (Toral et al., 2010a,b). Uno de esos posibles mecanismos podría ser la alteración de la fluidez de la grasa debida a la caída del aporte de 18:0 de origen ruminal, y la consecuente reducción del *cis*-9 18:1 sintetizado endógenamente en la glándula mamaria, y al aumento paralelo del contenido de ácidos grasos *trans* frente al de *cis* (Timmen y Patton, 1988; Gama et al., 2008).

Teniendo en consideración todo lo anteriormente expuesto, este trabajo se propuso fundamentalmente con tres objetivos:

1. Profundizar en el estudio de la síntesis endógena de *cis*-9, *trans*-11 CLA y de otros ácidos grasos relevantes (p. ej., *cis*-9 18:1) en la glándula mamaria de la oveja en lactación
2. Estudiar la persistencia de la respuesta de ovejas en lactación a la suplementación de la dieta con fuentes lipídicas que provocan una caída del contenido de grasa de la leche y
3. Esclarecer la relación entre la dieta y la expresión de los principales genes candidatos implicados en el metabolismo lipídico durante el síndrome de baja grasa en la leche.

Para ello, además de este capítulo de Introducción y el de Revisión bibliográfica, la disertación se estructura alrededor de tres apartados que corresponden a los estudios realizados para perseguir cada uno de estos objetivos.

El primer apartado se refiere a un experimento llevado a cabo con ovejas en lactación para cuantificar la síntesis endógena de determinados ácidos grasos mediante la acción de la SCD en la glándula mamaria. Para ello, se administró ácido estercúlico, un inhibidor de esta enzima que, en los rumiantes lecheros, es responsable de la síntesis de los ácidos grasos de cadena larga con doble enlace en posición *cis*-9 que se encuentran en la leche (e. g., el *cis*-9 18:1 a partir de 18:0 y el *cis*-9, *trans*-11 18:2 a partir de *trans*-11 18:1). La inhibición directa de la Δ^9-desaturasa permite determinar la procedencia del CLA de la leche y confirmar si esta es fundamentalmente endógena, como se ha señalado en otras especies (Griinari et al., 2000; Corl et al., 2001; Bernard et al., 2010).

Los otros apartados derivan de un segundo experimento en el que la dieta de las ovejas lecheras se suplementó con aceite de girasol y microalgas marinas. Como se había observado en trabajos previos (Toral et al., 2010b, 2012), la adición de lípidos marinos a una dieta rica en ácido linoleico provoca una caída en el contenido de grasa de la leche posiblemente debida a los cambios que se producen en el proceso de biohidrogenación ruminal. En concreto, las microalgas inhiben el último paso de la hidrogenación del ácido linoleico, disminuyendo así el flujo de 18:0 y aumentando el de otros ácidos grasos que llegan a la glándula mamaria a través de la sangre. Algunos de esos metabolitos podrían ser corresponsables del síndrome de baja grasa. No obstante, como ya se ha mencionado, los resultados de Toral et al. (2010b) parecen sugerir la posibilidad de una adaptación a largo plazo de la microbiota ruminal al aporte de lípidos marinos con la dieta, lo que podría implicar una recuperación gradual del porcentaje de grasa de la leche. Con el objetivo de esclarecer este punto, el segundo apartado se centra en el estudio de la persistencia de la respuesta a la suplementación con microalgas marinas en términos de rendimiento productivo y composición de la leche, con especial interés en el perfil lipídico en general y en ciertos ácidos grasos relacionados con el síndrome de baja grasa en la leche en particular.

Otro aspecto relevante acerca de la MFD son los mecanismos que regulan el metabolismo lipídico a nivel transcripcional, tanto en la glándula mamaria como en otros tejidos periféricos. Para intentar explicar su función durante la depresión de la grasa de la leche, el tercer estudio se llevó a cabo utilizando algunas de las ovejas involucradas en el experimento previo. Para ello, los animales fueron sacrificados al final del ensayo y se tomaron muestras de glándula mamaria, tejido adiposo e hígado para analizar la abundancia de ARNm de genes candidatos relacionados con el metabolismo lipídico.

Finalmente, la última sección de esta disertación es una discusión conjunta que pretende integrar y correlacionar los resultados de los tres estudios.

REFERENCIAS

Annison, E. F., Linzell, J. L., Fazakerley, S., and Nichols, B. W. 1967. The oxidation and utilization of palmitate, stearate, oleate and acetate by the mammary gland of the fed goat in relation to their overall metabolism, and the role of plasma phospholipids and neutral lipids in milk-fat synthesis. Biochem. J. 102:637-647.

Barron, L. J. R., Hernández, I., Bilbao, A., Flanagan, C. E., Nájera, A. I., Virto, M., Pérez-Elortondo, F. J., Albisu, M., and de Renobales, M. 2004. Changes in lipid fractions and sensory properties of Idiazabal cheese induced by lipase addition. J. Dairy Res. 71:372-379.

Bauman, D. E., and Griinari, J. M. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Bauman, D. E., Harvatine, K. J., and Lock, A. L. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 21:299-319.

Bernard, L., Leroux, C., Rouel, J., Bonnet, M., and Chilliard, Y. 2012. Effect of the level and type of starchy concentrate on tissue lipid metabolism, gene expression and milk fatty acid secretion in Alpine goats receiving a diet rich in sunflower-seed oil. Br. J. Nutr. 107:1147-1159.

Bernard, L., Mouriot, J., Rouel, J., Glasser, F., Capitan, P., Pujos-Guillot, E., Chardigny, J. M., and Chilliard, Y. 2010. Effects of fish oil and starch added to a diet containing sunflower-seed oil on dairy goat performance, milk fatty acid composition and in vivo Δ^9-desaturation of [^{13}C]vaccenic acid. Br. J. Nutr. 104:346-354.

Bickerstaffe, R., and Johnson, A. R. 1972. The effect of intravenous infusions of sterculic acid on milk fat synthesis. Br. J. Nutr. 27:561-570.

Bionaz, M., and Loor, J. J. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Bocquier, F., and Caja, G. 2001. Effects of nutrition on ewes' milk quality. Prod. Anim. 14:129-140.

Boyazoglu, J., and Morand-Fehr, P. 2001. Mediterranean dairy sheep and goat products and their quality: a critical review. Small Rumin. Res. 40:1-11.

Castro-Carrera, T., Frutos, P., Leroux, C., Chilliard., Y., Hervás, G., Belenguer, A., Bernard, L., and Toral, P. G. 2015. Dietary sunflower oil modulates milk fatty acid composition without major changes in adipose and mammary tissue fatty acid profile or related gene mRNA abundance in sheep. Animal 9:582-591.

Chilliard, Y., and Ferlay, A. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Dev. 44: 467-492.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828-855.

Corl, B. A., Baumgard, L. H., Dwyer, D. A., Griinari, J. M., Phillips, B. S., and Bauman, D. E. 2001. The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA. J. Nutr. Biochem. 12:622-630.

de Rancourt, M., and Carrère, L. 2011. Milk sheep production systems in Europe: diversity and main trends. Options Méditerr. Serie A 100:107-111.

Dilzer, A., and Park, Y. 2012. Implication of conjugated linoleic acid (CLA) in human health. Crit. Rev. Food Sci. Nutr. 52:488-513.

FAOSTAT 2013. FAOSTAT Domains: Production, Livestock Primary. Selectors: Regions (Southern Europe >; List), Elements (Production; tonnes), Items (Milk, whole fresh sheep), Years (2013). Buscado el 5 de agosto 2015 en la página web http://faostat3.fao.org

Frutos, P., Toral, P. G., Ramos-Morales, E., Shingfield, K. J., Belenguer, A., and Hervás, G. 2014. Oral administration of cobalt acetate alters milk fatty acid composition, consistent with an inhibition of stearoyl-coenzyme A desaturase in lactating ewes. J. Dairy Sci. 97:1036-1046.

Gama, M. A. S., Garnsworthy, P. C., Griinari, J. M., Leme, P. R., Rodrigues, P. H. M., Souza, L. W. O., and Lanna, D. P. D. 2008. Diet-induced milk fat depression: association with changes in milk fatty acid and fluidity of milk fat. Livest. Sci. 115:319-331.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., and Hervás, G. 2008. Addition of olive oil to dairy ewe diets: effect on milk fatty acid profile and animal performance. J. Dairy Sci. 91:3119-3127.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V., and Bauman, D. E. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr. 130:2285-2291.

Harvatine, K. J., and Bauman, D. E. 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr. 136:2468-2474.

Harvatine, K. J., Boisclair, Y. R., and Bauman, D. E. 2009. Recent advances in the regulation of milk fat synthesis. Animal 3:40-54.

Hervás, G., Luna, P., Mantecón, A. R., Castañares, N., de la Fuente M. A., Juárez, M., and Frutos, P. 2008. Effect of diet supplementation with sunflower oil on milk production, fatty acid profile and ruminal fermentation in lactating dairy ewes. J. Dairy Res. 75:399-405.

Hussein, M., Harvatine, K. J., Weerasinghe, W. M. P. B., Sinclair, L. A., and Bauman, D. E. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. J. Dairy Sci. 96:3825-3834.

Invernizzi, G., Thering, B. J., McGuire, M. A., Savoini, G., and Loor, J. J. 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Funct. Integr. Genomics 10:561-575.

Lock, A. L., Teles, B. M., Perfield, J. W., Bauman, D. E., and Sinclair, L. A. 2006. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 reduces milk fat synthesis in lactating sheep. J. Dairy Sci. 89:1525-1532.

Moio, L., Rillo, L., Ledda, A., and Addeo, F. 1996. Odorous constituents of ovine milk in relationship to diet. J. Dairy Sci. 79:1322-1331.

Oliveira, D. E., Gama, M. A., Fernandes, D., Tedeschi, L. O., and Bauman, D. E. 2012. An

unprotected conjugated linoleic acid supplement decreases milk production and secretion of milk components in grazing dairy ewes. J. Dairy Sci. 95:1437-1446.

Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Palmquist, D. L., St-Pierre, N., and McClure, K. E. 2004. Tissue fatty acid profiles can be used to quantify endogenous rumenic acid synthesis in lambs. J. Nutr. 134:2407-2414.

Park, Y. W., Juárez, M., Ramos, M., and Haenlein, G. F. W. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88-113.

Parodi, P.W. 2004. Milk fat in human nutrition. Aust. J. Dairy Technol. 59:3-59.

Pellegrini, O., Remeuf, F., Rivemale, M., and Barillet, F. 1997. Renneting properties of milk from individual ewes: influence of genetic and non-genetic variables, and the relationship with physicochemical characteristics. J. Dairy Res. 64:355-366.

Piperova, L. S., Teter, B. B., Bruckental, I., Sampugna, J., Mills, S. E., Yurawecz, M. P., Fritsche, J., Ku, K., and Erdman, R. A. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568-2574.

Pulina, G., and Nudda A. 2004. Milk production. In: Dairy sheep nutrition. Pulina, G. (ed.). CABI International, Wallingford, UK, pp. 1-12.

Pulina, G., Nudda, A., Battacone, G., and Cannas, A. 2006. Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. Anim. Feed Sci. Technol. 131:255-291.

Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I., Chilliard, Y. 2008. Composition of goat and sheep milk products: an update. Small Rumin. Res. 79:57-72.

Shingfield, K. J., Bernard, L., Leroux, C., and Chilliard, Y. 2010. Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.

Shingfield, K. J., Saebø, A., Saebø, P. C., Toivonen, V., and Griinari, J. M. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Sinclair, L. A., Weerasinghe, W. M., Wilkinson, R. G., de Veth, M. J., and Bauman D. E. 2010. A supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat yield but does not alter organ weight or body fat deposition in lactating ewes. J. Nutr. 140:1949-1955.

Timmen, H., and Patton, S. 1988. Milk fat globules: fatty acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685-689.

Toral, P. G., Belenguer, A., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2012. Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 95:794-806.

Toral, P. G., Bernard, L., Delavaud, C., Gruffat, D., Leroux, C., and Chilliard, Y. 2013. Effects of fish oil and additional starch on tissue fatty acid profile and lipogenic gene mRNA abundance in lactating goats fed a diet containing sunflower-seed oil. Animal 7:948-956.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2010a. Changes in milk fatty acid profile and animal performance in response to fish oil

supplementation, alone or in combination with sunflower oil, in dairy ewes. J. Dairy Sci. 93:1604-1615.

Toral, P. G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., and de la Fuente, M. A. 2010b. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93:1655-1667.

Toral, P. G., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2010c. Effect of fish oil and sunflower oil on rumen fermentation characteristics and fatty acid composition of digesta in ewes fed a high concentrate diet. J. Dairy Sci. 93:4804-4817.

Tsiplakou, E., Flemetakis, E., Kalloniati, C., Papadomichelakis, G., Katinakis, P., and Zervas, G. 2009. Sheep and goats differences in CLA and fatty acids in milk fat content in relation with mRNA stearoyl-CoA desaturase and lipogenic genes expression in their mammary gland. J. Dairy Res. 76:392-401.

REVISIÓN BIBLIOGRÁFICA

1. LA GRASA DE LA LECHE DE LOS RUMIANTES

La leche de oveja, al igual que otras leches, es un complejo de sustancias que se encuentran en solución, suspensión o emulsión en agua: la grasa y las vitaminas liposolubles están en emulsión; las proteínas y los minerales acoplados a las micelas de caseína en suspensión; y los glúcidos (lactosa), los minerales, el nitrógeno no proteico y las vitaminas hidrosolubles en solución (Pulina y Nudda, 2004; McCarthy, 2011).

Es necesario destacar que el contenido de grasa de la leche representa un factor determinante de la calidad nutricional de la misma, siendo el mayor responsable de su contenido energético y de las características físicas y organolépticas de los productos lácteos elaborados a partir de ella (Jensen et al., 1991; Park et al., 2007).

En comparación con las de otras especies de rumiantes, la leche de oveja presenta un mayor contenido de grasa, la cual está organizada en glóbulos de un tamaño medio de 3,3-4 μm, rodeados de una membrana lipoproteica (Pulina y Nudda, 2004; Park et al., 2007).

Además, la leche de oveja es particularmente rica en proteínas, especialmente en caseína, la responsable del proceso de coagulación, y, como ya se ha dicho, en grasa, la fuente principal de aroma y gusto del producto final (Pellegrini et al., 1997; Pulina y Nudda, 2004; Park et al., 2007). Por ello, el destino principal de la leche que se obtiene de las explotaciones de ovino lechero es la producción de quesos.

1.1 Composición

La grasa de la leche de los rumiantes contiene más de 400 AG que se diferencian en la longitud de la cadena carbonada y en el número y orientación de los dobles enlaces (Jensen, 2002). Más del 95% de los AG se encuentran esterificados en triglicéridos, mientras que el 5% restante está constituido por fosfolípidos, ésteres de colesterol, diglicéridos, monoglicéridos y AG libres (Jensen y Newburg, 1995). La mayoría de los AG tienen una longitud de cadena comprendida entre 4 y 18 átomos de carbono.

La grasa de la leche de los rumiantes destaca entre la de otros mamíferos por su alta proporción de AG de cadena corta (4, 6, 8 y 10 carbonos). Estos AG no son detectables en la grasa láctea de otras especies no rumiantes (con la excepción del

conejo) ni en los piensos convencionales o en la grasa corporal de reserva de ningún animal (Bauman et al., 2011b).

Los principales AG de la grasa de la leche ovina son: 10:0, 14:0, 16:0, 18:0 e isómeros del 18:1 (Park et al., 2007). Además, el contenido de los AG de cadena corta y media 6:0, 8:0, 10:0 y 12:0 es significativamente más alto en esta leche (y en la de cabra) que en la de vaca (Park et al., 2007). Algunos trabajos han sugerido que, en comparación con la de otros rumiantes, la leche de oveja sería especialmente rica en *trans*-11 18:1, conocido también como ácido vaccénico (VA) y *cis*-9, *trans*-11 18:2, conocido también como ácido ruménico (RA; Park et al., 2007; Tsiplakou y Zervas, 2008).

Al igual que en otras especies, el perfil lipídico de la leche de oveja varía sensiblemente por efecto de varios factores, entre los que el más importante es la dieta (Tsiplakou et al., 2006; de la Fuente et al., 2009; Gómez-Cortés et al., 2011).

En relación con el consumo de leche y productos lácteos, es preciso señalar que algunos AG constituyen el centro de un importante debate entre nutricionistas por el efecto negativo que pudieran ejercer en la salud humana. Así, el excesivo consumo de determinados AG saturados (12:0, 14:0 y 16:0 en particular) ha sido relacionado con un elevado riesgo de enfermedades cardiovasculares; riesgo que podría resultar todavía mayor con determinados AG de conformación *trans* (Shingfield et al., 2008b; Givens, 2010; Shingfield et al., 2013). No obstante, a menudo se olvida destacar el efecto positivo que otros AG abundantes en la leche (p. ej., 4:0, 15:0 *anteiso*, *cis*-9 18:1, CLA, 18:3 n-3) han demostrado en la prevención de enfermedades crónicas como el cáncer, la diabetes y la arteriosclerosis (Pariza et al., 2001; Parodi, 2005; Shingfield et al., 2008b). En este sentido, cabe destacar el poder anticancerígeno que el CLA, y en particular el RA, ha mostrado en distintas líneas de células tumorales (Pariza et al., 2001). En la nutrición humana, la principal fuente de RA es la leche y los productos de ella derivados, lo cual evidencia la importancia que ese alimento puede tener en términos de salud del consumidor.

Por último, es necesario mencionar que los efectos negativos de los AG *trans* han sido claramente relacionados con el consumo de grasas vegetales parcialmente hidrogenadas de origen industrial, mientras que no está claro todavía si a los AG que se encuentran en los productos de origen animal se les puede atribuir el mismo efecto negativo (Livingstone et al., 2012; Gayet-Boyer et al., 2014).

1.2 Efecto de la dieta y otros factores sobre el perfil lipídico de la leche

La grasa es el componente más variable de la leche de los rumiantes. Su contenido varía entre individuos y, como ya se ha mencionado, se puede ver afectado por muchos factores, tales como la especie, la raza, la dieta, la fase de la lactación, la temperatura ambiental y la condición corporal (Pulina y Nudda, 2004; Tsiplakou et al., 2006; Bauman et al., 2011b).

La dieta es el factor de variación más importante del contenido y la composición de la grasa de la leche (Chilliard et al., 2007; Shingfield et al., 2008b). El perfil lipídico de la grasa de los rumiantes es el resultado de la compleja interacción existente entre los nutrientes y el metabolismo ruminal y mamario (Davis y Brown, 1970; Doreau et al., 1999; Bauman y Griinari, 2001). Así, por ejemplo, al variar la cantidad y el tipo de lípidos aportados con la dieta, se observa una modificación importante del perfil lipídico de la leche, tanto en la oveja como en las otras especies de rumiantes (Chilliard et al., 2003; Pulina et al., 2006; Sanz-Sampelayo et al., 2007). Asimismo, diferentes relaciones forraje:concentrado de la dieta se acompañan de cambios en el perfil lipídico de la leche, los cuales se hacen más evidentes cuando se suplementa la ración con aceites vegetales (Mele et al., 2006; Martini et al., 2010; Gómez-Cortés et al., 2011). Comparada con la utilización de forrajes conservados, la alimentación de ovejas en pastoreo produce una leche con un mayor contenido en VA, RA y ácido linolénico (Cabiddu et al., 2005; Gómez-Cortés et al., 2009). Por otra parte, el empleo de aceites y semillas ricas en AG insaturados se relaciona, principalmente, con una disminución del contenido de los AG saturados de cadena corta y media (p. ej., 6:0 y 8:0). Este hecho parece tener un doble origen: por un lado, un posible efecto inhibidor de los AG insaturados de la dieta sobre la fermentación microbiana podría reducir la disponibilidad de acetato para la síntesis *de novo* en las células mamarias, si bien la contribución real de este mecanismo podría ser poco significativa (Harvatine et al., 2009a; Shingfield et al., 2010); por otro lado, el aumento de la disponibilidad de AG de cadena larga para la glándula mamaria, debido a su mayor absorción en el intestino, podría inhibir la actividad de las enzimas responsables de la síntesis *de novo* (Chilliard y Ferlay, 2004). La suplementación de la dieta con aceites y semillas ricas en AG insaturados también se relaciona con un aumento del contenido de AG monoinsaturados de 18 átomos de carbono, tales como el *cis*-9 18:1, el VA y el *trans*-10 18:1. Aunque la mayor parte de los estudios se han llevado a cabo en el vacuno,

este comportamiento se ha confirmado también en el ovino (e. g., Hervás et al., 2008; Gómez-Cortés et al., 2008a,b; Toral et al., 2010a,b).

Por último, cabe mencionar que, además de la dieta, existen otros factores que pueden modificar el contenido de AG de la leche de oveja. En la literatura aparece la estación del año como uno de esos factores, ya que se ha observado que el contenido de RA y ácido linolénico es más alto durante la primavera-verano que durante el invierno (Addis et al., 2005; de la Fuente et al., 2009). No obstante, este es realmente un efecto de la dieta, ya que no es la estación, sino lo que los animales consumen durante ella, lo que provoca los cambios observados. Además, al aumentar la edad del animal disminuye el contenido de AG mono y poliinsaturados de la leche y aumenta el de AG de cadena corta y media, mientras que la influencia del momento de lactación sobre el contenido de CLA aún no está claro (Tsiplakou et al., 2006; de la Fuente et al., 2009). El rebaño es otro factor que se ha señalado como determinante del perfil lipídico de la leche, aunque en parte este vuelve a ser un reflejo del efecto de la dieta (p. ej., alimentación en pastoreo o en estabulación permanente). Finalmente, también las razas pueden ser responsables de diferencias en la composición de AG de la leche (Tsiplakou et al., 2006; de la Fuente et al., 2009; Sánchez et al., 2010).

2. ORIGEN Y SECRECIÓN DE LOS AG DE LA LECHE

Los AG de la leche tienen un doble origen: por una parte, la síntesis de AG *de novo* en la glándula mamaria y, por otra, la captación del torrente sanguíneo. Los AG de cadena corta y media (de 4 a 14 átomos de carbono) y aproximadamente la mitad de los AG de 16 átomos de carbono proceden de la síntesis *de novo* a partir de acetato y, en menor medida, de ß-hidroxibutirato. En términos de proporciones molares, alrededor de un 60% de todos los AG de la leche se sintetizan *de novo* (Bauman et al., 2011b). La otra mitad de los AG de 16 átomos de carbono y todos aquellos con una cadena más larga (>16 átomos de carbono) son captados de la circulación sanguínea y tienen su origen en la dieta, el proceso de BH ruminal o las reservas adiposas corporales. En condiciones normales, los AG que provienen de la movilización de tejido adiposo representan menos del 10% del total de los AG de la leche, excepto durante periodos de balance energético negativo en los que su proporción aumenta considerablemente (Bauman y Griinari, 2001). Por lo tanto, la fuente mayoritaria de

AG de cadena larga es el aporte dietético (y su metabolismo ruminal, como se explica a continuación).

2.1 Metabolismo ruminal de los lípidos de la dieta

Los lípidos presentes en los alimentos de los rumiantes son metabolizados en el rumen a través de dos procesos principales que se conocen como lipolisis y biohidrogenación ruminal (BH). Tras su ingestión, las moléculas lipídicas son separadas de sus componentes estructurales, hidrolizadas y liberadas en el rumen como AG sin esterificar que, si presentan dobles enlaces, son convertidos finalmente en AG saturados (Harfoot y Hazlewood, 1997; Jenkins et al., 2008). La biohidrogenación ruminal es un mecanismo de destoxificación que la microbiota del rumen adopta para protegerse de los efectos nocivos de los lípidos de la dieta, puesto que los AG libres poseen acción bactericida y bacteriostática, siendo su poder antimicrobiano mayor en las moléculas que presentan uno o más dobles enlaces en comparación con los AG saturados (Harfoot y Hazlewood, 1997; Maia et al., 2007).

Los AG poliinsaturados mayoritarios en la dietas de los rumiantes son el ácido α-linolénico (18:3 n-3), abundante por ejemplo en el pasto fresco y en el aceite de linaza, y el ácido linoleico (18:2 n-6), abundante por ejemplo en el ensilado de maíz, en los cereales y en varias semillas oleaginosas, como el girasol y la soja. En el rumen, ambos AG pueden ser metabolizados finalmente a 18:0 (ácido esteárico). Durante el proceso de biohidrogenación de los ácidos linoleico y linolénico se forman muchos metabolitos intermedios de configuración *trans*, entre los cuales cabe destacar el RA y el VA (Harfoot y Hazlewood, 1997; Jenkins et al., 2008; McKain et al., 2010). Si el aporte de lípidos insaturados con la dieta supera la capacidad de destoxificación de la microbiota ruminal, el proceso pierde en eficiencia y aumenta la concentración de AG con doble enlaces que se escapan del rumen y alcanzan el torrente sanguíneo tras ser absorbidos por las células del epitelio intestinal. En consecuencia, tanto en la oveja como en otros rumiantes lecheros, se observan cambios importantes en el perfil de la grasa de la leche (Hervás et al., 2008; Shingfield et al., 2008a; Bernard et al., 2012).

Además, cuando la dieta se suplementa con una combinación de aceites vegetales y lípidos de origen marino (p. ej., microalgas marinas o aceite de pescado), el flujo de

metabolitos intermedios de la biohidrogenación que llega a la glándula mamaria es todavía mayor (Shingfield et al., 2006; Toral et al., 2010a,b). Este hecho es debido a que los lípidos marinos son ricos en AG de cadena muy larga de la serie n-3, tales como el 20:5 n-3 (ácido eicosapentaenoico o EPA) o el 22:6 n-3 (ácido docosahexaenoico o DHA). El EPA y el DHA actúan inhibiendo el último paso de la biohidrogenación ruminal (AbuGhazaleh y Jenkins, 2004a,b; Toral et al., 2012) y aumentando el flujo de AG mono o poliinsaturados que escapan del rumen.

2.2 Síntesis endógena en la glándula mamaria

El acetato y el ß-hidroxibutirato son la fuente principal de moléculas de carbono para la síntesis de AG *de novo* en la glándula mamaria (Moore y Christie, 1981). El acetato se origina en el rumen a partir de la fermentación de los hidratos de carbono de los alimentos, mientras que el ß-hidroxibutirato procede de la transformación del ácido butírico en las paredes ruminales y en el hígado (Bauman et al., 2011b). Ambas moléculas son captadas de la sangre por las células del epitelio mamario y activadas mediante la unión al coenzima-A para dar lugar a la cadena carbonada de la molécula lipídica (Moore y Christie, 1981). La acción de la enzima acil-CoA sintetasa citoplasmática (codificada por el gen *ACSS2*) parece ser la principal responsable de este proceso de activación (Bionaz y Loor, 2008). El ß-hidroxibutirato solo puede ser utilizado como precursor en la síntesis del ácido graso pero no en la elongación, contribuyendo aprox. en un 8% al total de átomos de carbono en la grasa láctea, mientras que el acetato es la fuente de la gran mayoría de los demás carbonos utilizados para la formación de los AG (Bauman et al., 2011b).

La síntesis *de novo* ocurre en el citoplasma de las células mamarias y está relacionada, además de con la disponibilidad de NADPH, con la actividad de dos enzimas clave: la acetil-CoA carboxilasa alfa y la ácido graso sintasa (codificadas por los genes *ACACA* y *FASN*, respectivamente; Barber et al., 1997; Bernard et al., 2008). La ACACA cataliza la primera etapa de la biosíntesis de los AG (la conversión del acetil-CoA en malonil-CoA), la cual es determinante porque controla la velocidad de todo el proceso. La enzima FASN, por su parte, interviene en la elongación de la cadena lipídica añadiendo moléculas de acetil-CoA a la molécula, y es la responsable de la formación de los AG saturados de cadena corta y media hasta el ácido palmítico

(16:0; Bauman y Davis, 1974). El uso de acetato y ß-hidroxibutirato por parte de la ACACA y de la FASN explica por qué la mayoría de los AG de la leche tienen un número par de átomos de carbono. Sin embargo, en la leche también se pueden encontrar AG de cadena impar cuyo origen, aunque es en gran medida ruminal, también puede ser la síntesis *de novo* en la glándula mamaria a partir de ácido propiónico como precursor (Vlaeminck et al., 2006). Junto con el acetato y el ß-hidroxibutirato, el propionato es un AG volátil que se forma en el rumen pero, a diferencia de los otros dos, su principal destino metabólico es la síntesis de glucosa.

2.3 Captación del torrente sanguíneo

Las células de la glándula mamaria captan los AG de cadena larga a partir de los AG no esterificados de la sangre, transportados por la albúmina, o de las lipoproteínas sanguíneas. Dichas lipoproteínas se adhieren al endotelio de los vasos de la glándula mamaria gracias a la acción de la lipoproteinlipasa (codificada por el gen *LPL*), la cual separa los triglicéridos desde el núcleo de las lipoproteínas y libera AG sin esterificar (Barber et al., 1997; Bionaz y Loor, 2008).

Tanto los AG sin esterificar de la sangre como los obtenidos de las lipoproteínas atraviesan la membrana celular mediante proteínas transportadoras y, en menor medida, por difusión pasiva (Bionaz y Loor, 2008; Bauman et al., 2011b).

Las principales proteínas que parecen estar involucradas en el proceso de captación y transporte de los AG en la célula secretora mamaria son la translocasa de ácidos grasos (codificada por el gen *CD36*), las proteínas transportadoras de ácidos grasos (codificadas por *FABP*) y diversas isoformas de la familia de proteínas transportadoras FATP (codificadas por *SLC27A*), con un papel potencialmente clave en el transporte de los AG de cadena larga y muy larga (Bionaz y Loor, 2008).

Una vez alcanzado el interior de la célula, y antes de participar en cualquier proceso metabólico, los AG de cadena larga son activados mediante la esterificación con la coenzima-A (Bionaz y Loor, 2008). Posteriormente son llevados por transportadores específicos, como la proteína transportadora de AG conocida como FABP3, hasta el núcleo y los distintos orgánulos citoplasmáticos (peroxisomas, mitocondrias, retículo endoplasmático) donde son procesados. Además, la FABP3 podría tener la función de proteger a la célula de los efectos negativos de los AG activados y evitar la inhibición

de las enzimas ACACA y Δ^9-desaturasa o estearoil-CoA desaturasa (SCD), habiéndose especulado también sobre un posible papel suministrando los sustratos necesarios para esta última (Bionaz y Loor, 2008).

2.4 Desaturación

A caballo entre el origen dietético y el de síntesis *de novo* se encontraría el proceso de desaturación, que explicaría el origen endógeno de ciertos AG de la leche.

La SCD es la enzima más importante en la síntesis de AG monoinsaturados, ya que introduce un doble enlace de configuración *cis* en la posición Δ^9 (Ntambi y Miyazaki, 2004).

La actividad de la SCD en la glándula mamaria se considera un mecanismo de elevada importancia para aumentar el grado de insaturación de los AG y disminuir así el punto de fusión de los triglicéridos presentes en la leche (Timmen y Patton, 1988), lo que podría resultar crítico a la hora de mantener la fluidez tanto de la grasa láctea como de las membranas celulares (Bauman et al., 2011b).

Los AG 18:0 y *trans*-11 18:1 parecen ser los sustratos principales para la SCD, siendo la conversión del 18:0 en *cis*-9 18:1 predominante en cultivos de células de bovino (Palmquist et al., 2005; Bernard et al., 2008, 2013). Estudios llevados a cabo en vacuno han calculado que alrededor de la mitad (de media, 54%) del 18:0 disponible para la síntesis de triglicéridos sería destinado al proceso de desaturación (Enjalbert et al., 1998; Glasser et al., 2008). También calculan que entre aproximadamente un 50 y un 80% del ácido oleico (*cis*-9 18:1) es producido por síntesis endógena (Enjalbert et al., 1998; Mosley y McGuire, 2007; Glasser et al., 2008). En la especie ovina, trabajos muy recientes indican que entre un 29 y un 51%, según el perfil lipídico de la dieta ofrecida a los animales, del *cis*-9 18:1 presente en la leche resultaría de la síntesis endógena en la glándula mamaria (Frutos et al., 2014; Toral et al., 2015).

La actividad de la SCD también contribuye a la producción del *cis*-9 14:1 y del *cis*-9 16:1. En la leche bovina esto supone en torno a un 90% en el primer caso y un 50-56% en el segundo (Mosley y McGuire, 2007). Muchos otros AG funcionan también como sustratos para la actividad de esa enzima; entre ellos el 10:0, 12:0, 15:0 y 17:0 (Bauman y Davis, 1974; Shingfield et al., 2008a). Sin embargo, lo más relevante en

términos de salud humana podría ser la conversión del VA en RA (Pariza et al., 2001; Ntambi y Miyazaki, 2004). Continuando con lo publicado en vacuno, en general, más del 60% del RA se produce por síntesis endógena (Griinari et al., 2000b; Mosley et al., 2006; Glasser et al., 2008).

En los rumiantes, han sido identificadas dos isoformas de la SCD: la SCD1 y la SCD5 (Lengi y Corl, 2007). La SCD1 se expresa abundantemente en la glándula mamaria de la vaca en lactación y parece jugar un papel determinante en la síntesis de la grasa láctea (McDonald y Kinsella, 1973; Bionaz y Loor, 2008; Bernard et al., 2013). Por el contrario, la SCD5, identificada más recientemente, no parece mostrar una correlación aparente entre su abundancia en el tejido mamario (mucho menor que el de la SCD1) y los índices de Δ^9 desaturación de los AG de la leche, lo cual sugeriría un papel menos importante en la síntesis de la grasa de la leche (Jacobs et al., 2011, 2013; Toral et al., 2013, 2015).

Para cuantificar la síntesis endógena de AG mediante desaturación en la glándula mamaria existen métodos directos, tales como el empleo de trazadores específicos (p. ej., ^{13}C), y métodos indirectos, tales como la cuantificación del flujo de AG en el duodeno y en la leche o la inhibición del complejo enzimático responsable del proceso de desaturación (p. ej., mediante la administración de ácido estercúlico o cobalto). Distintos estudios han empleado el ácido estercúlico, un AG con un anillo de ciclopropeno que inhibe la actividad de la Δ^9-desaturasa (Griinari et al., 2000b; Corl et al., 2001; Kay et al., 2004). Con dicho método se ha calculado que entre el 64 y el 91% del *cis*-9, *trans*-11 18:2 y hasta el 100% del *trans*-7, *cis*-9 18:2 presente en la grasa láctea del bovino se producirían por desaturación mediante la acción de la SCD (Griinari et al., 2000b; Corl et al., 2001; Kay et al., 2004). De forma similar, estudios que involucraban el uso de *trans*-11 18:1 marcado con ^{13}C indican que la desaturación de ese AG en *cis*-9, *trans*-11 18:2 supone aproximadamente el 83% del RA lácteo en la vaca lechera (Mosley et al., 2006) y el 63-73% en la cabra lechera (Bernard et al., 2010). Los datos relativos a la oveja lechera son aún muy escasos y, obtenidos mediante la administración de Co, parecen indicar que gran parte (51-94%) del RA también se origina por síntesis endógena (Frutos et al., 2014; Toral et al., 2015).

Además de la Δ^9-desaturasa, en la glándula mamaria de los mamíferos pueden encontrarse otras importantes enzimas desaturasas (FADS): la FADS1 y la FADS2, las cuales añaden dobles enlaces en la posición Δ^5 y Δ^6 de los ácidos grasos poliinsaturados de cadena larga (≥18 carbonos; Rodriguez-Cruz et al., 2006). Sin

embargo, la abundancia del ARNm de los genes que las codifican parece ser muy baja en el tejido mamario de los rumiantes (Bionaz y Loor, 2008; Toral et al., 2013) y, por lo tanto, el ácido araquidónico (20:4 n-6), el ácido eicosapentaenoico (20:5 n-3) y el ácido docosahexaenoico (22:6 n-3) son tres ejemplos de moléculas cuya presencia en la leche se explicaría especialmente por su captación del torrente sanguíneo.

2.5 Secreción de la grasa en la glándula mamaria

Los AG sintetizados en la glándula mamaria o directamente capturados de la sangre son, en su mayor parte, organizados en triglicéridos antes de su secreción. Para la síntesis de triglicéridos, cabe mencionar el papel de la enzima diacilglicerol O-aciltransferasa 1 (codificada por el gen *DGAT1*), la cual esterifica los AG (principalmente, el ácido butírico, el oleico y el caproico) en posición sn-3 de los diglicéridos (Jensen, 2002; Bernard et al., 2008).

Debido al carácter hidrofóbico de los AG esterificados, la grasa de la leche es secretada por las células epiteliales de la glándula mamaria en forma de gota rodeada por una membrana lipídica, rica en proteínas, de naturaleza polar (Mather y Keenan, 1998; Ollivier-Bousquet, 2002). Los triglicéridos recién formados son transportados desde el retículo endoplasmático rugoso y liberados en el citoplasma en forma de microgotas lipídicas, con un diámetro de hasta 0,5 μm, revestidas de proteínas y lípidos polares (Figura 1). Algunas de estas microgotas se funden entre ellas en el transporte hacia la membrana apical de la célula, dando lugar a gotas relativamente grandes (aprox. 4 μm de diámetro) rodeadas por una membrana sutil denominada membrana del glóbulo graso, la cual tiene la función de agente emulsionante natural que previene la coalescencia de los glóbulos grasos y su degradación enzimática (Mather y Keenan, 1998; Dewettinck et al., 2008). Dicha membrana está formada por fosfolípidos, esfingolípidos y glicoproteínas. Entre estas últimas, por su papel potencialmente relevante en el proceso de excreción de la gota lipídica, cabe mencionar la translocasa de ácidos grasos (CD36), la butirofilina, subfamilia 1, miembro A1 (BTN1A1), la xantina deshidrogenasa (XDH) y la adipofilina (ADFP o PLIN2; Reinhardt y Lippolis, 2006; McManaman et al., 2007).

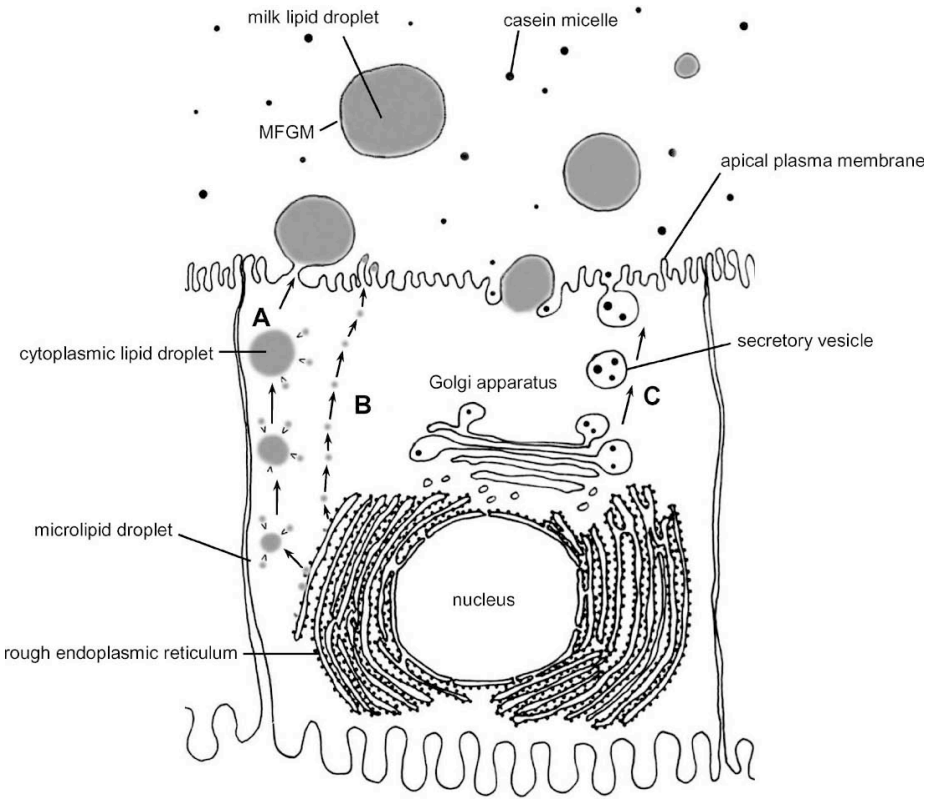

Figura 1. Mecanismo de secreción de las gotas lipídicas en la célula epitelial de la glándula mamaria (Mather y Keenan, 1998). A, glóbulo graso; B, microgotas lipídicas; C, vesículas para la secreción de proteínas; MFGM, membrana del glóbulo graso de la leche.

3. REGULACIÓN DE LA LIPOGÉNESIS EN LA GLÁNDULA MAMARIA

3.1 Principales genes implicados en el metabolismo lipídico en la glándula mamaria

Al comienzo del periodo de lactación, en el núcleo de las células secretoras de la glándula mamaria, se activan todas las enzimas que participan en el proceso de síntesis de lípidos y que están codificadas por sus respectivos genes.

La nutrición, en general, puede ejercer un importante impacto sobre los procesos fisiológicos del animal, incluido el metabolismo lipídico, mediante la modificación de la expresión de los genes relacionados con dicho metabolismo. Los cambios en la expresión génica pueden ser debidos a los propios componentes de la dieta, a los

metabolitos resultantes tras el paso de ésta por el tracto digestivo, o a los cambios hormonales por ellos producidos (Bernard et al., 2008; Bionaz y Loor, 2008; Bauman et al., 2011a).

La respuesta de la expresión génica a los distintos componentes de la dieta implica el control de eventos que pueden ocurrir a nivel transcripcional (i. e., durante la síntesis de ARNm a partir de un molde de ADN), postranscripcional (e. g., estabilidad del ARNm), traduccional (durante la formación de la cadena de aminoácidos en el ribosoma), así como postraduccional (e. g., maduración o degradación de la enzima; Loor y Cohick, 2009; Bernard et al., 2013). Algunas técnicas de biología molecular, como la PCR a tiempo real, permiten la cuantificación relativamente sencilla de la abundancia de ARNm de cada gen, lo que ha posibilitado un avance importante en los estudios al respecto.

En este sentido, se ha observado que la abundancia de ARNm del gen *LPL* aumenta de manera considerable al comienzo de la lactación y se mantiene elevada a lo largo de la misma. Este hecho sugiere que dicho gen, así como la proteína por él codificada, tendría un papel importante en la síntesis lipídica en la glándula mamaria (Bionaz y Loor, 2008; Zhao et al., 2014).

Palin et al. (2014) comprobaron, en vacas lecheras, que la expresión del gen *LPL* es sensible a la composición de la dieta y puede aumentar cuando esta se suplementa con fuentes lipídicas de origen vegetal. Por el contrario, la expresión del gen parece disminuir cuando los suplementos utilizados son aceites de origen marino o cuando la suplementación con aceites vegetales va ligada a una reducción en el porcentaje de grasa láctea (Ahnadi et al., 2002; Peterson et al., 2003; Harvatine y Bauman, 2006).

En relación con los pequeños rumiantes, los datos disponibles son todavía escasos, pero parece ser que la suplementación de su dieta con aceites vegetales no siempre tendría un efecto significativo sobre la abundancia del ARNm del *LPL* (Bernard et al., 2005; Li et al., 2012; Castro-Carrera et al., 2015). No obstante, Hussein et al. (2013) observaron que, en ovejas en lactación, la administración de isómeros específicos del CLA, protegidos de la degradación ruminal, afectó negativamente a su expresión.

Por otra parte, aunque en menor medida que en el gen *LPL*, también se han observado, en bovino lechero y a lo largo de la lactación, mayores abundancias de ARNm de otros genes relacionados con el metabolismo lipídico mamario. Entre ellos, destacan los genes que codifican para proteínas responsables del transporte intracelular (e. g., *FABP3*), de la síntesis *de novo* (*ACACA* y *FASN*) y de la

desaturación de los AG (*SCD*), lo que podría sugerir una interconexión funcional entre las respectivas proteínas codificadas por tales genes (Bionaz y Loor, 2008).

En lo que respecta a la influencia de la dieta sobre la expresión de estos genes, se ha observado que vacas lecheras alimentadas con dietas suplementadas con lípidos marinos presentaron una marcada reducción en la cantidad del ARNm de los genes *ACACA*, *FASN* y, en ciertos casos, *SCD* (Ahnadi et al., 2002; Harvatine y Bauman, 2006; Angulo et al., 2012), mientras que *FABP3* no parece ser un gen excesivamente sensible a la composición de la dieta de la vaca en lactación, aunque los datos al respecto son aún escasos (Peterson et al., 2003; Invernizzi et al., 2010). En los pequeños rumiantes, estos genes son aparentemente menos sensibles al aumento en el aporte de AG de cadena larga con la dieta, cualquiera que sea el origen de dichos AG (Bernard et al., 2005; Toral et al., 2013; Castro-Carrera et al., 2015). Sin embargo, Hussein et al. (2013) sí observaron, en ovejas de leche, una diminución en la expresión de *ACACA*, *FASN* y *SCD* en respuesta al aporte de isómeros específicos del CLA, hecho previamente comprobado también en vacas lecheras (Baumgard et al., 2002).

La correlación detectada entre diferentes genes involucrados en la lipogénesis apunta a la existencia de factores de transcripción que regulen todo el proceso en su conjunto. Así, el *SREBF1* (o factor 1 de unión a elementos reguladores del esterol) se ha propuesto como uno de esos factores (Harvatine y Bauman, 2006). La proteína que codifica (SREBP1) está localizada en la membrana del retículo endoplasmático en forma de precursor inactivo, siendo transportada, antes de entrar en el núcleo celular, al aparato de Golgi para su activación mediante la intervención de la proteína SCAP (o chaperona SREBP), la cual regula sus movimientos (Bernard et al., 2008; Bauman et al., 2011a). A su vez, la proteína SCAP interactúa con la proteína INSIG (codificada por el gen inducido por la insulina) y ambas regulan la activación de SREBP1. La inhibición coordinada de la expresión del gen *SREBF1* junto con la de los otros genes responsables del metabolismo lipídico en la glándula mamaria podría indicar un papel fundamental de este factor de transcripción en el control de la síntesis de los AG de la leche (Harvatine y Bauman, 2006; Bauman et al., 2011a).

Bauman et al. (2011a) señalan que otro factor importante en el proceso de regulación de la síntesis lipídica es el *THRSP* o elemento de respuesta a la hormona tiroidea (previamente conocido como *S14*). Este gen se expresa en todos los tejidos en los que se sintetizan lípidos y aumenta rápidamente en respuesta a señales

lipogénicas, tales como cambios en la concentración de insulina o un elevado aporte de hidratos de carbono con la dieta (Cunningham et al., 1998; Bauman et al., 2011a). Así, al comenzar la lactación, y por tanto la síntesis de los lípidos de la leche, su expresión se incrementa considerablemente (Bionaz y Loor, 2008).

Por último, es importante mencionar también al *PPARG* (o receptor gamma activado por el proliferador de peroxisomas), el cual podría estar implicado en la coordinación de redes de expresión génica relacionadas con el metabolismo lipídico (Schmitt et al., 2011; Shi et al., 2014). El *PPARG* se expresa fundamentalmente en los adipocitos, pero la abundancia de su ARNm aumenta en el tejido mamario en lactación si se compara con los niveles prelactación (Bionaz y Loor, 2008). Al ser activado, se ha sugerido que podría participar en la regulación de la captación de AG, así como quizás en la síntesis de AG *de novo* y la formación de la gota lipídica (Shi et al., 2014), lo que implicaría un papel importante en la regulación de la lipogénesis mamaria.

3.2 Nutrigenómica

La nutrigenómica es una disciplina joven que tiene como objeto el estudio del impacto que los distintos componentes de la dieta pueden provocar sobre los procesos fisiológicos del animal a través de cambios en la expresión de los genes (Minihane, 2009; Bauman et al., 2011a).

En los últimos años se ha podido profundizar en el conocimiento de la relación que existe entre la dieta de los animales de aptitud lechera y sus efectos sobre el metabolismo lipídico en los distintos tejidos gracias a la aplicación de técnicas de biología molecular (Loor y Cohick, 2009; Bauman et al., 2011a; Loor et al., 2013). En este sentido, se ha podido comprobar que dichos efectos en la glándula mamaria, posiblemente mediados en buena parte por cambios en la expresión génica, producen importantes variaciones en la composición de la leche, especialmente en la grasa.

Así, en los rumiantes lecheros, el denominado síndrome de baja grasa en la leche demuestra la interconexión que existe entre algunos componentes bioactivos de la dieta y la regulación de enzimas claves en la síntesis de la leche, constituyendo uno de los ejemplos más interesantes de nutrigenómica.

Dado que en varios apartados previos se ha hablado ya de los principales genes implicados en el metabolismo lipídico en la glándula mamaria, y más adelante se volverá a tratar este tema, en relación directa con la nutrigenómica (e. g., apartado 4.1.3), no se incluirá aquí más información para intentar no repetir ni sobrecargar innecesariamente esta revisión bibliográfica.

4. SÍNDROME DE BAJA GRASA EN LA LECHE

El síndrome de baja grasa o depresión de la grasa en la leche (MFD) ha sido investigado en vacas lecheras durante más de un siglo y, básicamente, consiste en una marcada reducción tanto de la producción de grasa láctea como de su concentración, sin afectar significativamente ni a la producción de lactosa y proteínas de la leche ni a la cantidad total de leche producida (Bauman y Griinari, 2001; Shingfield y Griinari, 2007). Ahora bien, la investigación sobre la MFD en los pequeños rumiantes de aptitud lechera es muy reciente y todavía escasa.

La especie caprina parece ser la menos sujeta a ese tipo de síndrome (Ollier et al., 2009; Bernard et al., 2010; Toral et al., 2013), aunque en la literatura científica existen algunos trabajos que lo describen y caracterizan también en la cabra en respuesta a la administración de *trans*-10, *cis*-12 CLA (Lock et al., 2008; Shingfield et al., 2009) y, más raramente, a la suplementación de la dieta con aceite de pescado (Chilliard et al., 2014). La especie ovina se sitúa en una posición intermedia y, aunque algunos autores la han propuesto como un modelo válido para el estudio de la MFD en vacuno (Lock et al., 2006; Hussein et al., 2013), esto no parece en general recomendable ya que las ovejas son menos sensibles (Papadopoulos et al., 2002; Reynolds et al., 2006; Shingfield et al., 2010) y no muestran MFD cuando su dieta se suplementa con aceites vegetales (Gómez-Cortés et al., 2008b; Hervás et al., 2008).

4.1 Teorías sobre la depresión de la grasa de la leche

La MFD ha protagonizado una parte importante de la investigación científica en nutrición de rumiantes de las últimas décadas, siendo numerosas las hipótesis propuestas para su explicación, tal y como se detalla a continuación.

En la revisión de Bauman y Griinari (2001), se explica que el estudio que por primera vez relacionó la caída en la producción de grasa en la leche con la dieta fue publicado en 1845 por Boussingault (citado por Van Soest, 1994) y utilizaba la remolacha azucarera para la alimentación de vacas lecheras. En dicho estudio, Boussingault señaló que la escasez de grasa en la dieta era la causa de la reducción de la producción de grasa láctea. Sin embargo, rápido se comprobó que el síndrome se producía también con dietas con un alto contenido lipídico. De hecho, en 1894, Sebelien observó que la suplementación de la dieta de los animales con aceite de pescado reducía aún más el contenido de grasa en la leche (trabajo citado por Opstvedt, 1984).

Siguiendo con la información recogida por Bauman y Griinari (2001), en la primera mitad del siglo XX la MFD fue relacionada con dietas suplementadas con aceite de hígado de bacalao (Drummond et al., 1924; Golding et al., 1926), dietas con bajo contenido de fibra (Powell, 1939; Loosli et al., 1945; Balch et al., 1952) y dietas suplementadas con aceites de origen vegetal (Dann et al., 1935; Williams et al., 1939). Además, Powell (1939) comprobó que la concentración de grasa en la leche se veía también afectada por las características físicas de la fibra alimentaria. Desde entonces, muchos trabajos han tratado de dilucidar cuáles son las causas que relacionan determinadas dietas con la MFD con el objetivo de intentar controlar su aparición (Erdman, 1988; Sutton, 1989; Palmquist et al., 1993).

Por otra parte, algunos estudios se centraron en el papel de los AG volátiles producidos en el rumen (Tyznik y Allen, 1951; Balch et al., 1952). Así, una baja relación acetato:propionato, la cual ocurre cuando se emplean dietas ricas en concentrado, ha sido propuesta como una de las posibles causas de la MFD, al producirse una escasez de acetato disponible para la síntesis de AG *de novo* a nivel de glándula mamaria (Harvatine et al., 2009a; Shingfield et al., 2010). Sin embargo, la infusión de acetato a vacas en lactación con MFD no ejerce efectos beneficiosos y, además, la depresión de la grasa de la leche no siempre va ligada a cambios en la

producción ruminal de este AG volátil, siendo esta teoría por tanto descartada (Davis y Brown, 1970; Bauman y Griinari, 2001).

En esta misma línea de investigación, se propuso que si el propionato aumentaba en cantidades importantes podría originar un incremento de los niveles plasmáticos de glucosa, estimulando la secreción de insulina (Bauman y Griinari, 2001; Shingfield et al., 2010). Esta hormona, debido a sus propiedades lipogénicas, favorecería el depósito de AG en el tejido adiposo y disminuiría su movilización, lo cual también podría limitar el aporte de AG de cadena larga a la glándula mamaria (McClymont y Vallance, 1962; Jenny et al., 1974; Annison, 1976). No obstante, cuando se infundieron propionato, glucosa o directamente insulina, a vacas en lactación, solamente se consiguió una ligera reducción del contenido lipídico de la leche (nunca superior al 15%), lo que llevó a descartar también esta hipótesis (Palmquist et al., 1969; Griinari et al., 1997; Hurtaud et al., 1998).

A continuación, otros estudios postularon otro tipo de teoría para tratar de explicar la MFD, atribuyendo la caída en el porcentaje de grasa de la leche a la inhibición directa de una o más etapas de la síntesis lipídica en la glándula mamaria (Bauman y Griinari, 2001). Frobish y Davis (1977) la relacionaron con una disminución en la síntesis de vitamina B12 en el rumen cuando se empleaban dietas con un alto porcentaje de concentrado y bajo de forraje. La vitamina B12 es un componente de la enzima metilmalonil-CoA mutasa que está involucrada en el metabolismo hepático del ácido propiónico. Un déficit de esta vitamina y un exceso de ácido propiónico provocarían una acumulación de ácido metilmalónico en el hígado, el cual podría alcanzar el torrente sanguíneo y llegar hasta la glándula mamaria donde inhibiría la síntesis de AG *de novo*. Sin embargo, esta teoría tampoco se ha aceptado, ya que en diferentes estudios en los que se incluyó vitamina B12 en la dieta de los animales, no se observó ninguna mejoría destacable en el síndrome de MFD (Elliot et al., 1979; Croom et al., 1981a,b).

En la actualidad, la teoría más aceptada es la denominada "teoría de la biohidrogenación", inicialmente denominada "teoría de los AG *trans*" y que fue propuesta en los años 70 (Davis y Brown, 1970; Pennington y Davis, 1975).

4.1.1 La teoría de la biohidrogenación

Esta teoría postula que la causa de la MFD es la formación de ciertos AG con propiedades antilipogénicas durante el proceso de biohidrogenación ruminal (Harfoot y Hazlewood, 1997; Griinari y Bauman, 1999). Cuando los lípidos de la dieta alcanzan el rumen son inmediatamente separados en AG no esterificados mediante el proceso que se conoce como lipolisis. Los AG que presentan en su cadena carbonada dobles enlaces son transformados en AG saturados mediante la biohidrogenación (Jenkins et al., 2008). Como ya se ha señalado previamente, la BH es un mecanismo de protección que las bacterias ruminales ponen en funcionamiento para evitar los efectos tóxicos de los ácidos grasos insaturados (Maia et al., 2007, 2010; Jenkins et al., 2008).

Las dietas que causan la aparición de MFD se caracterizan generalmente por cumplir dos condiciones: la primera, provocar una alteración del ambiente ruminal (pH, composición bacteriana, etc.) y la segunda, estar suplementadas con AG insaturados (Bauman et al., 2011a). Ambas condiciones originan un cambio en la ruta metabólica habitual de los AG, aumentando el flujo de AG insaturados desde el rumen hasta el intestino delgado, donde son absorbidos, concretamente en el duodeno, sin sufrir cambios significativos en su estructura química (Bauman y Griinari, 2001; Shingfield et al., 2010). Algunos de estos AG han demostrado propiedades bioactivas relacionadas con la síntesis de la grasa de la leche y han sido ampliamente estudiados en el ganado bovino lechero (Shingfield y Griinari, 2007).

El primer AG en ser identificado, y el más investigado, es el *trans*-10, *cis*-12 18:2, un isómero del CLA, comprobándose que su infusión abomasal provoca una rápida disminución del contenido graso de la leche (Baumgard et al., 2000a,b; de Veth et al., 2004). Su aumento en la leche se produce mayoritariamente por la alteración, debida a la dieta, de la principal ruta de BH ruminal del ácido linoleico (Griinari y Bauman, 1999; Shingfield et al., 2010). El efecto de la infusión abomasal de *trans*-10, *cis*-12 CLA sobre la secreción de aquellos AG que se originan mediante síntesis *de novo* en la glándula mamaria o por captación del torrente sanguíneo parece depender de la dosis (Baumgard et al., 2000b, 2001; Peterson et al., 2002). En todo caso, a pesar del demostrado efecto inhibitorio de este isómero del CLA sobre la síntesis lipídica en la glándula mamaria, varios estudios han demostrado que el síndrome de baja grasa en la

leche se puede producir también en ausencia de aumentos de su contenido lácteo (Shingfield y Griinari, 2007).

Otros AG *trans* de 18 C (Griinari y Bauman, 1999) han sido objeto de numerosos estudios para comprobar una posible correlación entre su contenido en la dieta y el porcentaje de grasa de la leche (véase, por ejemplo, la revisión de Griinari et al., 1999). No obstante, no todos los AG *trans* inducen MFD. En este sentido, se ha podido constatar, mediante la infusión abomasal de isómeros puros, que los AG *trans*-9 18:1, *trans*-11 18:1 y *trans*-12 18:1 no tienen efecto sobre la producción de grasa de la leche (Rindsig y Schultz, 1974; Griinari et al., 2000b). Por el contario, sí parece haber relación entre la caída del contenido de grasa en la leche y el aumento del isómero *trans*-10 18:1 (Griinari et al., 1998; Newbold et al., 1998, Griinari et al., 2000a; Piperova et al., 2000), aunque su papel es aún controvertido (Shingfield et al., 2010). El *trans*-10 18:1 se puede formar en el proceso de biohidrogenación ruminal, por reducción del AG conjugado *trans*-10, *cis*-12 18:2 (Griinari y Bauman, 1999; McKain et al., 2010), que es el único AG del que se ha demostrado con certeza su carácter antilipogénico (Baumgard et al., 2000a,b; Perfield et al., 2002; Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005). En cambio, no ejercen tales efectos los isómeros *trans*-8, *cis*-10; *trans*-9, *trans*-11; *trans*-10, *trans*-12; *cis*-9, *trans*-11 y *cis*-11, *trans*-13 18:2 (Perfield et al., 2004, 2006, 2007; Saebo et al., 2005), pero sí lo podrían ejercer el *cis*-10, *trans*-12 18:2 (Saebo et al., 2005) y el *trans*-9, *cis*-11 18:2 (Perfield et al., 2007).

Dado lo anteriormente expuesto, la "teoría de los AG *trans*" fue denominada "teoría de la biohidrogenación" y se basa en una alteración del proceso normal de biohidrogenación ruminal de los ácidos grasos de la dieta que da lugar a la producción de ciertos AG capaces de inhibir la síntesis de grasa láctea (Bauman y Griinari, 2001).

En comparación con la especie bovina, en los pequeños rumiantes hay pocos datos disponibles en relación con el empleo de AG puros y sus efectos sobre el contenido graso de la leche. La mayoría de los estudios se limitan a la administración del *trans*-10, *cis*-12 18:2 y aunque el efecto de su administración en ovejas de leche puede ser comparable al observado en vacas (Lock et al., 2006; Sinclair et al., 2007, 2010; Weerasinghe et al., 2012), los resultados en los casos de MFD en la especie ovina sugieren que la depresión de la grasa está ligada al consumo de lípidos marinos, caso en el que este isómero del CLA no parece ejercer un papel relevante (Toral et al., 2010a,b). Por su parte, los resultados obtenidos en la cabra son contradictorios, ya que

algunos señalan que no afecta a la producción de grasa láctea (Erasmus et al., 2004; Schmidely y Morand-Fehr, 2004; de Andrade y Schmidely, 2006) y otros que sí (e. g., Lock et al., 2008; Shingfield et al., 2009).

4.1.2 Regulación de la fluidez de la grasa láctea

Como ya se ha mencionado, la depresión de la grasa de la leche no va siempre ligada a un aumento de *trans*-10, *cis*-12 18:2. De hecho, en la originada por dietas suplementadas con aceite de pescado puede no haber cambios significativos en la concentración de este AG y, sin embargo, la depresión es igualmente marcada (Loor et al., 2005; Shingfield y Griinari, 2007). Las dietas ricas en lípidos de origen marino aumentan el contenido ruminal de otros AG de conformación *trans* y disminuyen la síntesis endógena de ácido oleico, *cis*-9 18:1, en la ubre (Loor et al., 2005; Shingfield et al., 2006).

El papel del *cis*-9 18:1 en la formación de los triglicéridos de la leche se estableció en los años 70 (Kinsella, 1972; Parodi, 1979) e introdujo un concepto importante: el mantenimiento de la fluidez de la leche. La grasa láctea tiene que encontrarse en forma líquida para su secreción, por lo que su punto de fusión no puede superar la temperatura corporal del animal (Timmen y Patton, 1988). Chilliard et al. (2000) indicaron que el aumento de AG de conformación *trans*, junto con la caída de *cis*-9 18:1, podría provocar un incremento del punto de fusión de la grasa láctea que dificultaría su secreción. Por lo tanto, la combinación de altos niveles de AG *trans* 18:1, junto con una reducida disponibilidad de ácido esteárico (18:0) para la síntesis endógena de *cis*-9 18:1, se ha propuesto como posible causa de la MFD en vacas y ovejas de leche cuya dieta se suplementaba con lípidos marinos (Loor et al., 2005; Gama et al., 2008; Toral et al., 2010a,b).

La célula mamaria, como alternativa al ácido oleico, puede incorporar en la posición sn-3 de los triglicéridos otros AG con bajos puntos de fusión, tales como AG de cadena corta y media (4:0-10:0), para intentar mantener la fluidez de la grasa láctea (Timmen y Patton, 1988; Chilliard et al., 2000). Sin embargo, este mecanismo puede no ser suficiente y se ha propuesto que con un porcentaje inferior al 8% de ácido oleico en la composición de los triglicéridos de la grasa láctea, la glándula mamaria no es capaz de mantener la fluidez adecuada a la temperatura corporal de 39-40ºC, lo

que sugiere un papel de dicho AG en el mecanismo de secreción de la gota lipídica (Gama et al., 2008).

No obstante, parece improbable que este mecanismo pueda explicar por sí mismo la caída del contenido graso de la leche durante la MFD, ya que, por ejemplo, la inhibición de la Δ^9-desaturasa no implica necesariamente una disminución de la secreción de grasa láctea (Kay et al., 2004; Frutos et al., 2014). Tampoco la adición de un suplemento de 18:0 a la dieta permitió aliviar la depresión de la grasa (Toral et al., en prensa).

4.1.3 Regulación de la expresión génica

Durante la MFD, en la glándula mamaria se observa una reducción tanto de la síntesis de AG *de novo* como de la captación de AG desde la sangre, lo cual sugiere que debe de existir una coordinación en la regulación de las enzimas involucradas en la lipogénesis. En la especie bovina, la inhibición de la expresión de los genes responsables de la síntesis de los AG de la leche se ha relacionado fundamentalmente con la administración de *trans*-10, *cis*-12 CLA (Baumgard et al., 2002; Gervais et al., 2009; Harvatine et al., 2009b) y con el consumo de dietas que provocan MFD (Piperova et al., 2000; Ahnadi et al., 2002; Peterson et al., 2003; Harvatine y Bauman, 2006).

En ambas situaciones tiene lugar la reducción coordinada en la expresión de un buen número de genes candidatos relacionados con el metabolismo lipídico en la glándula mamaria (y de los cuales se ha hablado ya en varios apartados previos), como por ejemplo *FASN*, *ACACA*, *LPL* y *SCD*, lo cual plantea la existencia de un regulador central de la síntesis lipídica (Harvatine et al., 2009a; Shingfield et al., 2010; Bauman et al., 2011a).

Por ello, una de las hipótesis más recientes para explicar la MFD postula su relación con un regulador de la transcripción de las enzimas involucradas en la lipogénesis que sería sensible al efecto inhibidor de determinados AG con propiedades bioactivas (Harvatine et al., 2009a; Bauman et al., 2011b).

En este sentido, se ha observado que el *SREBF1* disminuye paralelamente y en la misma medida que la producción de grasa de la leche cuando ocurre la MFD, independientemente de las causas que induzcan el síndrome (Bauman et al., 2011a).

Por lo tanto, este factor de transcripción podría ser una de las dianas principales de los AG responsables de la MFD. Esta tesis es respaldada por el hecho de que la expresión del *INSIG1*, un gen responsable de la activación del *SREBF1*, se ve también afectada negativamente durante la MFD (Harvatine y Bauman, 2006). No obstante, aún no están completamente esclarecidos los mecanismos moleculares implicados en la inhibición de la expresión del *SREBF1* por parte del *trans*-10, *cis*-12 18:2 o de otros AG y, por lo tanto, se necesita más información para comprender el papel que desempeñaría este factor de transcripción en el desencadenamiento de la MFD.

Además del *SREBF1*, otro factor de transcripción que se ha sugerido como potencialmente sensible al efecto inhibidor de los AG involucrados en la MFD es el *THRSP* (Harvatine y Bauman, 2006) y el *PPARG* (Bionaz y Loor, 2008; Shingfield et al., 2010).

Estudios realizados con líneas de células tumorales (Donnelly et al., 2009) mostraron que el *THRSP*, sobre el cual ya se ha hablado previamente, se ve afectado por la acción inhibitoria del *trans*-10, *cis*-12 18:2 (Donnelly et al., 2009) por lo que, aunque la información al respecto todavía es insuficiente en los rumiantes, no se puede descartar su papel en la aparición de la MFD (Bauman et al., 2011a).

También el *PPARG* ha sido identificado como posible diana de los efectos inhibidores de los AG responsables de la MFD. Este gen parece tener mayor relevancia en la regulación del metabolismo lipídico en el tejido adiposo que en la glándula mamaria, pero su papel en el desarrollo de la MFD también podría ser importante (Bionaz y Loor, 2008; Shingfield et al., 2010).

En cualquier caso, quizás sea conveniente mencionar, aunque aquí no se insista porque ya se ha hablado de ello, que la aparición y desarrollo de la MFD podría estar ligada no solo a cambios en los factores de transcripción sino también, lógicamente, en los distintos genes implicados en la lipogénesis en el tejido mamario (Shingfield et al., 2010; Bauman et al., 2011a).

En los pequeños rumiantes, la información disponible sobre la regulación de la expresión génica como mecanismo responsable de la MFD es todavía muy escasa. En la cabra, algunos datos preliminares indican que la inhibición que algunas dietas provocan en la expresión de genes involucrados en la síntesis lipídica (como el *SCD* y el *FADS*) no parece estar mediada por la variación en la expresión de los principales factores de transcripción (Toral et al., 2013). En la oveja, Hussein et al. (2013) comprobaron que la suplementación de la dieta con *trans*-10, *cis*-12 CLA indujo

MFD junto con un claro efecto negativo sobre la cantidad de ARNm del *SREBF1* y del *INSIG1*, mientras que la expresión del *THRSP* y el *PPARG* no se vieron afectadas.

4.2 MFD y otros tejidos

Los numerosos cambios que la MFD origina en el metabolismo lipídico de la glándula mamaria sugieren una posible alteración de otros metabolitos relacionados con la lipogénesis. Así, como ya se ha señalado previamente, los primeros estudios sobre el tema proponían la escasez de acetato o el aumento del propionato, glucosa o insulina como factores desencadenantes del síndrome (véase las revisiones de Bauman y Griinari, 2001, 2003).

Sin embargo, estudios posteriores han demostrado que, durante la MFD, los cambios en la concentración plasmática de los principales metabolitos relacionados con la lipogénesis son mínimos; lo cual sugiere que los efectos son específicos sobre la biosíntesis de AG en la glándula mamaria. En este sentido, en la vaca no se ha podido comprobar ninguna alteración relevante en la concentración sanguínea ni de glucosa, ni de AG no esterificados, ni del ß-hidroxibutirato, en casos de MFD inducida por la administración de *trans*-10, *cis*-12 18:2, ni a corto (< de 1 semana) ni a largo plazo (hasta 20 semanas; Baumgard et al., 2000b; Perfield et al., 2002; Castañeda-Gutiérrez et al., 2005; de Veth et al., 2006). Tampoco se han podido observar cambios en los niveles plasmáticos de hormonas (e. g., insulina, factor de crecimiento insulínico tipo 1 y leptina) que pudieran relacionarse con la MFD. En los pequeños rumiantes, si bien la información disponible es todavía muy limitada, en principio esta parece concordar con los resultados obtenidos en la especie bovina (Oliveira et al., 2012).

En relación con el metabolismo lipídico en el tejido hepático, otro punto de relevancia cuando se estudia el metabolismo lipídico, diversos autores (Castañeda-Gutiérrez et al., 2005; Schlegel et al., 2012) constataron que en vacas de leche con MFD provocada por la infusión de *trans*-10, *cis*-12 18:2, la concentración de triglicéridos hepáticos no variaba. Resultados análogos fueron obtenidos en vacas alimentadas con una dieta que contenía aceite de pescado (Schmitt et al., 2011). En la especie ovina, todavía hay poca información relativa a los cambios que diferentes suplementos grasos producen sobre el metabolismo lipídico hepático. El estudio de

Sinclair at al. (2010), en el que se administró *trans*-10, *cis*-12 CLA a ovejas, parece apuntar hacia resultados similares a los del ganado vacuno, ya que no se detectaron modificaciones en el contenido de lípidos totales en el hígado, pero sí en su perfil de AG.

Por otra parte, en vacas en lactación con MFD, tanto inducida por CLA (Harvatine et al, 2009b) como por la adición de aceite de pescado a la dieta (Thering et al., 2009; Schmitt et al., 2011), el aumento en la expresión de genes responsables de la adipogénesis (e. g., *LPL*, *FASN, SCD*, *ADFP*, *DGAT1, LPIN1*, por citar algunos) podría traducirse en un aumento en la deposición de grasa en el tejido adiposo, como energía de reserva (Bauman y Griinari, 2001; Shingfield et al., 2010). Sin embargo, en los pequeños rumiantes (cabras y ovejas de aptitud lechera), la suplementación lipídica de la dieta no parece afectar en gran medida ni a la cantidad de ARNm ni a la actividad de las enzimas codificadas por los genes lipogénicos del tejido adiposo (Bernard et al., 2005, 2009; Toral et al., 2013; Castro-Carrera et al., 2015). No obstante, la información disponible en estas especies es todavía insuficiente y no queda claro si las diferencias se deben realmente a características específicas de cada especie, a las dietas, a sus interacciones o a otros factores.

5. REFERENCIAS

AbuGhazaleh, A. A., and Jenkins, T. C. 2004a. Disappearance of docosahexaenoic and eicosapentaenoic acids from cultures of mixed ruminal microorganisms. J. Dairy Sci. 87:645-651.

AbuGhazaleh, A. A., and Jenkins, T. C. 2004b. Docosahexaenoic acid promotes vaccenic acid accumulation in mixed ruminal cultures when incubated with linoleic acid. J. Dairy Sci. 87:1047-1050.

Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Pirisi, A., and Molle, G. 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. J. Dairy Sci. 88:3443-3454.

Ahnadi, C. E., Beswick, N., Delbecchi, L., Kennelly, J. J., and Lacasse, P. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69:521-531.

Angulo, J., Mahecha, L., Nuernberg, K., Nuernberg, G., Dannenberger, D., Olivera, M., Boutinaud, M., Leroux, C., Albrecht, E., and Bernard, L. 2012. Effects of polyunsaturated fatty acids from plant oils and algae on milk fat yield and composition are associated with mammary lipogenic and *SREBF1* gene expression. Animal 6:1961-1972.

Annison, E. F. 1976. Energy utilization in the body. In: Principles of cattle production. Swan, H., and Broster, W. H. (eds.). Butterworths, London, UK, pp. 169-199.

Balch, C. C., Balch, D. A., Bartlett, S., Cox, C. P., and Rowland, S. J. 1952. Studies of the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. J. Dairy Res. 19:39-50.

Barber, M. C., Clegg, R. A., Travers, M. T., Vernon, R. G. 1997. Lipid metabolism in the lactating mammary gland. Biochim. Biophys. Acta 1347:101-126.

Bauman, D. E., and Davis, C. L. 1974. Biosynthesis of milk fat. In: Lactation: a comprehensive treatise. Larson, B. L., and Smith, V. R. (eds.). Academic Press, New York, USA, Vol. 2, pp. 31-75.

Bauman, D. E., and Griinari, J. M. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15-29.

Bauman, D. E., and Griinari, J. M. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23:203-227.

Bauman, D. E., Harvatine, K. J., and Lock, A. L. 2011a. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Bauman, D. E., McGuire, M.A., and Harvatine, K. J. 2011b. Mammary gland, milk biosynthesis and secretion. In: Encyclopedia of dairy sciences. Fuquay, J. W., Fox, P. F., and McSweeney, P. L. H. (eds.). Elsevier, London, UK, pp. 352-358.

Baumgard, L. H., Corl, B. A., and Bauman, D. E. 2000a. Effect of CLA isomers on fat synthesis during growth and lactation. Proceedings of Cornell Nutrition Conference, pp. 180-190.

Baumgard, L. H., Corl, B. A., Dwyer, D. A., Sæbo, A., and Bauman, D. E. 2000b. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 278:R179-R184.

Baumgard, L. H., Matitashvili, E., Corl, B. A., Dwyer, D. A., and Bauman, D. E. 2002. *Trans*-10, *cis*-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163.

Baumgard, L. H., Sangster, J. K., and Bauman, D. E. 2001. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). J. Nutr. 131:1764-1769.

Bernal-Santos, G., Perfield, J. W., Barbano, D. M., Bauman, D. E., and Overton T. R. 2003. Production responses of dairy cows to dietary supplementation with conjugated linoleic acid (CLA) during the transition period and early lactation. J. Dairy Sci. 86:3218-3228.

Bernard, L., Bonnet, M., Leroux, C., Shingfield, K. J., and Chilliard, Y. 2009. Effect of sunflower-seed oil and linseed oil on tissue lipid metabolism, gene expression, and milk fatty acid secretion in Alpine goats fed maize silage-based diets. J. Dairy Sci. 92:6083-6094.

Bernard, L., Leroux, C., Bonnet, M., Rouel, J., Martin P., and Chilliard, Y. 2005. Expression and nutritional regulation of lipogenic genes in mammary gland and adipose tissues of lactating goats. J. Dairy Res. 72:250-255.

Bernard, L., Leroux, C., and Chilliard, Y. 2008. Expression and nutritional regulation of

lipogenic genes in the ruminant lactating mammary gland. Adv. Exp. Med. Biol. 606:67-108.

Bernard, L., Leroux, C., and Chilliard, Y. 2013. Expression and nutritional regulation of stearoyl-CoA desaturase genes in the ruminant mammary gland: relationship with milk fatty acid composition. In: Stearoyl-CoA desaturase genes in lipid metabolism. Ntambi, J. M. (ed.). Springer Science+Business Media, New York, USA, pp. 161-193.

Bernard, L., Leroux, C., Rouel, J., Bonnet, M., and Chilliard, Y. 2012. Effect of the level and type of starchy concentrate on tissue lipid metabolism, gene expression and milk fatty acid secretion in Alpine goats receiving a diet rich in sunflower-seed oil. Br. J. Nutr. 107:1147-1159.

Bernard, L., Mouriot, J., Rouel, J., Glasser, F., Capitan, P., Pujos-Guillot, E., Chardigny, J. M., and Chilliard, Y. 2010. Effects of fish oil and starch added to a diet containing sunflower-seed oil on dairy goat performance, milk fatty acid composition and in vivo Δ^9-desaturation of [^{13}C]vaccenic acid. Br. J. Nutr. 104:346-354.

Bionaz, M., and Loor, J. J. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Boussingault, J. B. 1845. Rural economy in its relations with chemistry, physics and meteorology. Bailliere, London, UK (citado por Van Soest, 1994).

Cabiddu, A., Decandia, M., Addis, M., Piredda, G., Pirisi, A., and Molle, G. 2005. Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. Small Rumin. Res. 59:169-180.

Castañeda-Gutiérrez, E., Overton, T. R., Butler, W. R., and Bauman, D. E. 2005. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J. Dairy Sci. 88:1078-1089.

Castro-Carrera, T., Frutos, P., Leroux, C., Chilliard., Y., Hervás, G., Belenguer, A., Bernard, L., and Toral, P. G. 2015. Dietary sunflower oil modulates milk fatty acid composition without major changes in adipose and mammary tissue fatty acid profile or related gene mRNA abundance in sheep. Animal 9:582-591.

Chilliard, Y., and Ferlay, A. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Dev. 44:467-492.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., and Doreau, M. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181-205.

Chilliard, Y., Ferlay, A., Rouel, J., and Lamberet, G. 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86:1751-1770.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828-855.

Chilliard, Y., Toral, P. G., Shingfield, K. J., Rouel, J., Leroux, C., and Bernard, L. 2014. Effects of diet and physiological factors on milk fat synthesis, milk fat composition and lipolysis in the goat. A short review. Small Rumin. Res. 122:31-37.

Corl, B. A., Baumgard, L. H., Dwyer, D. A., Griinari, J. M., Philips B. S., and Bauman, D. E. 2001. The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA. J. Nutr. Biochem. 12:622-630.

Croom, W. J., Bauman, D. E., and Davis, C. L. 1981a. Methylmalonic acid in low-fat milk syndrome. J. Dairy Sci. 64:649-654.

Croom, W. J., Rakes, A. H., Linnerud, A. C., Ducharme, G. A., and Elliot, J. M. 1981b. Vitamin B_{12} administration for milk fat synthesis in lactating dairy cows fed low fiber diet. J. Dairy Sci. 64:1555-1560.

Cunningham, B. A., Moncur, J. T., Huntington, J. T., and Kinlaw, W. B. 1998. "Spot 14" protein: a metabolic integrator in normal and neoplastic cells. Thyroid 8:815-825.

Dann, W. J., Moore, T., Booth, R. G., Golding, J., and Kon, S. K. 1935. A new spectroscopic phenomenon in fatty acid metabolism. The conversion of 'pro-absorptive' to 'absorptive' acids in the cow. Biochem. J. 29:138-146.

Davis, C. L., and Brown, R. E. 1970. Low-fat milk syndrome. In: Physiology of digestion and metabolism in the ruminant. Phillipson, A. T. (ed.). Oriel Press, Newcastle upon Tyne, UK, pp. 545-565.

de Andrade, P. V. D., and Schmidely, P. 2006. Effect of duodenal infusion of *trans*-10, *cis*-12 CLA on milk performance and milk fatty acid profile in dairy goats fed high or low concentrate diet in combination with rolled canola seed. Reprod. Nutr. Dev. 46:31-48.

de la Fuente, L. F., Barbosa, E., Carriedo, J. A., Gonzalo, C., Arenas, R., Fresno, J. M., and San Primitivo, F. 2009. Factors influencing variation of fatty acid content in ovine milk. J. Dairy Sci. 92:3791-3799.

de Veth, M. J., Castaneda-Gutierrez, E., Dwyer, D. A., Pfeiffer, A. M., Putnam, D. E., and Bauman, D. E. 2006. Response to conjugated linoleic acid in dairy cows differing in energy and protein status. J. Dairy Sci. 89:4620-4631.

de Veth, M. J., Griinari, J. M., Pfeiffer, A. M., and Bauman, D. E. 2004. Effect of CLA on milk fat synthesis in dairy cows: comparison of inhibition by methyl esters and free fatty acids, and relationships among studies. Lipids 39:365-372.

Dewettinck, K., Rombaut, R., Thienpont, N., Le, T. T., Messens, K., and Van Camp, J. 2008. Review: Nutritional and technological aspects of milk fat globule membrane material. Int. Dairy J. 18:436-457.

Donnelly, C., Olsen, A. M., Lewis, L. D., Eisenberg, B. L., Eastman, A., and Kinlaw, W. B. 2009. Conjugated linoleic acid (CLA) inhibits expression of the Spot 14 (*THRSP*) and fatty acid synthase genes and impairs the growth of human breast cancer and liposarcoma cells. Nutr. Cancer 61:114-122.

Doreau, M., Chilliard, Y., Rulquin, H., and Demeyer, D. I. 1999. Manipulation of milk fat in dairy cows. In: Recent advances in animal nutrition. Garnsworthy, P. C., and Wiseman, J. (eds). Notthingham Univ. Press, Notthingham, UK, pp. 81-109.

Drummond, J. C., Channon, H. J., Coward, K. H., Golding, J., Mackintosh, J., and Zilva, S. S. 1924. The influence of the administration of certain oils on the nutritive value of the butter fat of cows on winter rations. J. Agric. Sci. 14:531-547.

Elliot, J. M., Barton, E. P., and Williams, J. A. 1979. Milk fat as related to vitamin B_{12} status. J. Dairy Sci. 62:642-645.

Enjalbert, F., Nicot, M. C., Bayourthe, C., and Moncoulon, R. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525-1532.

Erasmus, L. J., Bester, Z., Fouric, T., Coertze, R. J., and Hall, L. 2004. Effect of level of rumen protected CLA supplementation on milk yield and composition in Saanen goats. S. Afr. J. Anim. Sci. 34(Suppl. 1):42-45.

Erdman, R. A. 1988. Dietary buffering requirements of the lactating dairy cow: a review. J. Dairy Sci. 71:3246-3266.

Frobish, R. A., and Davis, C. L. 1977. Theory involving propionate and vitamin B_{12} in the low-milk fat syndrome. J. Dairy Sci. 60:268-273.

Frutos, P., Toral, P. G., Ramos-Morales, E., Shingfield, K. J., Belenguer, A., and Hervás, G. 2014. Oral administration of cobalt acetate alters milk fatty acid composition, consistent with an inhibition of stearoyl-coenzyme A desaturase in lactating ewes. J. Dairy Sci. 97:1036-1046.

Gama, M. A. S., Garnsworthy, P. C., Griinari, J. M., Leme, P. R., Rodrigues, P. H. M., Souza, L. W. O., and Lanna, D. P. D. 2008. Diet-induced milk fat depression: association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115:319-331.

Gayet-Boyer, C., Tenenhaus-Aziza, F., Prunet, C., Marmonier, C., Malpuech-Brugère, C., Lamarche, B., and Chardigny, J. M., 2014. Is there a linear relationship between the dose of ruminant *trans*-fatty acids and cardiovascular risk markers in healthy subjects: results from a systematic review and meta-regression of randomised clinical trials. Br. J. Nutr. 112:1914-1922.

Gervais, R., McFadden, J. W., Lengi, A. J., Corl, B. A., and Chouinard, P. Y. 2009. Effects of intravenous infusion of *trans*-10, *cis*-12 18:2 on mammary lipid metabolism in lactating dairy cows. J. Dairy Sci. 92:5167-5177.

Givens, D. I. 2010. Milk and meat in our diet: good or bad for health? Animal 4:1941-1952.

Glasser, F., Ferlay, A., Doreau, M., Schmidely, P., Sauvant, D., and Chilliard, Y. 2008. Long-chain fatty acid metabolism in dairy cows: a meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91:2771-2785.

Golding, J., Soames, K. M., and Zilva, S. S. 1926. The influence of the cow's diet on the fat-soluble vitamins of winter milk. Biochem. J. 20:1306-1319.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., and Hervás, G. 2008a. Addition of olive oil to dairy ewe diets: effect on milk fatty acid profile and animal performance. J. Dairy Sci. 91:3119-3127.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., and Hervás, G. 2008b. Milk production, conjugated linoleic acid content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. J. Dairy Sci. 91:1560-1569.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., and Hervás, G. 2009. Effect of supplementation of grazing dairy ewes with a cereal concentrate on animal performance and milk fatty acid profile. J. Dairy Sci. 92:3964-3972.

Gómez-Cortés, P., de la Fuente, M. A., Toral, P. G., Frutos, P., Juárez, M., and Hervás, G. 2011. Effects of different forage:concentrate ratios in dairy ewe diets supplemented with sunflower oil on animal performance and milk fatty acid profile. J. Dairy Sci. 94:4578-

4588.

Griinari, J. M., and Bauman, D. E. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Advances in conjugated linoleic acid research. Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., and Nelson, G. J. (eds.). AOCS Press, Champaign, IL, USA, Vol. 1, pp. 180-200.

Griinari, J. M., Bauman, D. E., Chilliard, Y., Perajoki, P., and Nurmela, K. V. 2000a. Dietary influences on conjugated linoleic acids (CLA) in bovine milk fat. In: Oils and fats: from basic research to industrial applications. Proceedings of 3rd Meeting of the European Section of AOCS, June 18-21, Helsinki, Finland.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P.Y., Nurmela, K. V., and Bauman, D. E. 2000b. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr. 130:2285-2291.

Griinari, J. M., Dwyer, D. A., McGuire, M. A., Bauman, D. E., Palmquist, D. L., and Nurmela, K. V. V. 1998. *Trans*-octadecenoic acids and milk fat depression in lactating cows. J. Dairy Sci. 81:1251-1261.

Griinari, J. M., McGuire, M. A., Dwyer, D. A., and Bauman, D. E. 1997. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076-1084.

Griinari, J. M., Nurmela, K. V. V., Dwyer, D. A., Barbano, D. M., and Bauman, D. E. 1999. Variation of milk fat concentration of conjugated linoleic acid and milk fat percentage is associated with a change in ruminal biohydrogenation. J. Anim. Sci. 77:117-118.

Harfoot, C. G., and Hazlewood, G. P. 1997. Lipid metabolism in the rumen. In: The rumen microbial ecosystem. Hobson, P. N., and Stewart, C. S. (eds.). Elsevier, Amsterdam, The Netherlands, pp. 382-426.

Harvatine, K. J., and Bauman, D. E. 2006. SREBP1 and thyroid hormone responsive to spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr. 136:2468-2474.

Harvatine, K. J., Boisclair, Y. R., and Bauman, D. E. 2009a. Recent advances in the regulation of milk fat synthesis. Animal 3:40-54.

Harvatine, K. J., Perfield, J. W., and Bauman, D. E. 2009b. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-54

Hervás, G., Luna, P., Mantecón, A. R., Castañares, N., de la Fuente, M. A., Juárez, M., and Frutos, P. 2008. Effect of diet supplementation with sunflower oil on milk production, fatty acid profile and ruminal fermentation in lactating dairy ewes. J. Dairy Res. 75:399-405.

Hurtaud, C., Rulquin, H., and Vérité, R. 1998. Effects of graded duodenal infusions of glucose on yield and composition of milk from dairy cows. 1. Diet based on corn silage. J. Dairy Sci. 81:3239-3247.

Hussein, M., Harvatine, K. J., Weerasinghe, W. M., Sinclair, L. A., and Bauman, D. E. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. J. Dairy Sci. 96:3825-3834.

Invernizzi, G., Thering, B. J., McGuire, M. A., Savoini, G., and Loor, J. J. 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting

changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Funct. Integr. Genomics 10:561-575.

Jacobs, A. A. A., Dijkstra, J., Hendriks, W. H., van Baal, J. M., and van Vuuren, A. M. 2013. Comparison between stearoyl-CoA desaturase expression in milk somatic cells and in mammary tissue of lactating dairy cows. J. Anim. Physiol. Anim. Nutr. 97:353-362.

Jacobs, A. A. A., van Baal, J., Smits, M. A., Taweel, H. Z. H., Hendriks, W. H., van Vuuren, A. M., and Dijkstra, J. 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. J. Dairy Sci. 94:874-887.

Jenkins, T. C., Wallace, R. J., Moate, P. J., and Mosley, E. E. 2008. Board-invited review: Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. J. Anim. Sci. 86:397-412.

Jenny, B. F., Polan, C. E., and Thye, F. W. 1974. Effects of high grain feeding and stage of lactation on serum insulin, glucose and milk fat percentage in lactating cows. J. Nutr. 104:379-385.

Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

Jensen, R. G., Ferris, A. M., and Lammi-Keefe, C. J. 1991. The composition of milk fat. J. Dairy Sci. 74:3228-3243.

Jensen, R. G., and Newburg, D. S. 1995. Bovine milk lipids. In: The handbook of milk composition. Jensen, R. G. (ed.). Academic Press, San Diego, CA, USA, pp. 543-575.

Kay, J. K., Mackle T. R., Auldist, M. J., Thomson, N. A., and Bauman, D. E. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369-378.

Kinsella, J. E. 1972. Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglycerides synthesis. Lipids 7:349-355.

Lengi, A. J., and Corl, B. A. 2007. Identification and characterization of a novel bovine stearoyl-CoA desaturase isoform with homology to human SCD5. Lipids 42:499-508.

Li, X. Z., Yan, C. G., Lee, H. G., Choi, C. W., and Song, M. K., 2012. Influence of dietary plant oils on mammary lipogenic enzymes and the conjugated linoleic acid content of plasma and milk fat of lactating goats. Anim. Feed Sci. Technol. 174:26-35.

Livingstone, K. M., Lovegrove, J. A., and Givens, D. I. 2012. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. Nutr. Res. Rev. 25:193-206.

Lock, A. L., Teles, B. M., Perfield, J. W., Bauman, D. E., and Sinclair, L. A. 2006. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 reduces milk fat synthesis in lactating sheep. J. Dairy Sci. 89:1525-1532.

Lock, A. L., Rovai, M., Gipson, T. A., de Veth, M. J., and Bauman, D. E. 2008. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat synthesis in lactating goats. J. Dairy Sci. 91:3291-3299.

Loor, J. J., Bionaz, M., and Drackley, J. K. 2013. Systems physiology in dairy cattle: nutritional genomics and beyond. Annu. Rev. Anim. Biosci. 1:365-392.

Loor, J. J., and Cohick, W. S. 2009. ASAS centennial paper: Lactation biology for the twenty-first century. J. Anim. Sci. 87:813-824.

Loor, J. J., Doreau, M., Chardigny, J. M., Ollier, A., Sébédio, J. L., and Chilliard, Y. 2005. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of *trans*-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim. Feed Sci. Technol. 119:227-246.

Loosli, J. K., Lucas, H. L., and Maynard, L. A. 1945. The effect of roughage intake upon the fat content of milk. J. Dairy Sci. 28:147-153.

Maia, M. R. G., Chaudhary, L. C., Bestwick, C. S., Richardson, A. J., McKain, N., Larson, T. R., Graham, I. A., and Wallace, R. J. 2010. Toxicity of unsaturated fatty acids to the biohydrogenating ruminal bacterium, *Butyrivibrio fibrisolvens*. BMC Microbiol. 10:52-61.

Maia, M. R., Chaudhary, L. C., Figueres, L., and Wallace, R. J. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Anton. Van Leeuw. 91:303-314.

Martini, M., Liponi, G. B., and Salari, F. 2010. Effect of forage:concentrate ratio on the quality of ewe's milk, especially on milk fat globules characteristics and fatty acid composition. J. Dairy Res. 77:239-244.

Mather, I. H., and Keenan, T. W. 1998. Origin and secretion of milk lipids. J. Mammary Gland Biol. Neoplasia 3:259-273.

McCarthy, O. J. 2011. Physical and physico-chemical properties of milk. In: Encyclopedia of dairy sciences. Fuquay, J. W., Fox, P. F., and McSweeney, P. L. H. (eds.). Academic Press, San Diego, CA, USA, pp. 665-669.

McClymont, G. L., and Vallance, S. 1962. Depression of blood glycerides and milk-fat synthesis by glucose infusion. Proc. Nutr. Soc. 21:41-42.

McDonald, T. M., and Kinsella, J. E. 1973. Stearyl-CoA desaturase of bovine mammary microsomes. Arch. Biochem. Biophys. 156:223-231.

McKain, N., Shingfield, K. J., and Wallace, R. J. 2010. Metabolism of conjugated linoleic acids and 18:1 fatty acids by ruminal bacteria: products and mechanisms. Microbiology 156:579-588.

McManaman, J. L., Russell, T. D., Schaack, J., Orlicky, D. J., and Robenek, H. 2007. Molecular determinants of milk lipid secretion. J. Mammary Gland Biol. Neoplasia 12:259-268.

Mele, M., Buccioni, A., Petacchi, F., Serra, A., Banni, S., Antongiovanni, M., and Secchiari, P. 2006. Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. Anim. Res. 55:273-285.

Minihane, A. M. 2009. Nutrient gene interactions in lipid metabolism. Curr. Opin. Clin. Nutr. Metab. Care 12:357-363.

Moore, J. H., and Christie, W. W. 1981. Lipid metabolism in the mammary gland of ruminant animals. In: Lipid metabolism in ruminant animals. Christie, W. W. (ed.). Pergamon Press Ltd., Oxford, UK, pp. 227-278.

Mosley, E. E., and McGuire, M. A. 2007. Methodology for the in vivo measurement of the Δ^9-desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. Lipids 42:939-945.

Mosley, E. E., Shaffi Dagger, B., Moate, P. J., and McGuire, M. A. 2006. *Cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575.

Newbold, J. R., Robertshaw, K. L., and Morris, H. W. 1998. Association between concentrations of fat and intermediates of ruminal biohydrogenation in milk of dairy cows. Proc. Br. Soc. Anim. Sci. 1998:224.

Ntambi, J. M., and Miyazaki, M. 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res. 43:91-104.

Oliveira, D. E., Gama, M. A. S., Fernandes, D., Tedeschi, L. O., and Bauman, D. E. 2012. An unprotected conjugated linoleic acid supplement decreases milk production and secretion of milk components in grazing dairy ewes. J. Dairy Sci. 95:1437-1446.

Ollier, S., Leroux, C., de la Foye, A., Bernard, L., Rouel, J., and Chilliard, Y. 2009. Whole intact rapeseeds or sunflower oil in high-forage or high-concentrate diets affects milk yield, milk composition, and mammary gene expression profile in goats. J. Dairy Sci. 92:5544-5560.

Ollivier-Bousquet, M. 2002. Milk lipid and protein traffic in mammary epithelial cells: joint and independent pathways. Reprod. Nutr. Dev. 42:149-162.

Opstvedt, J. 1984. Fish fats. In: Fats in animal nutrition. Wiseman, J. (ed.). Butterworths, London, UK, Vol. 37, pp. 53-82.

Palin, M. F., Côrtes, C., Benchaar, C., Lacasse, P., and Petit, H. V. 2014. mRNA expression of lipogenic enzymes in mammary tissue and fatty acid profile in milk of dairy cows fed flax hulls and infused with flax oil in the abomasum. Br. J. Nutr. 111:1011-1020.

Palmquist, D. L., Beaulieu, A. D., and Barbano, D. M. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753-1771.

Palmquist, D. L., Davis, C. L., Brown, R. E., and Sachan, D. S. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-) ß-hydroxybutrate. J. Dairy Sci. 53:633-638.

Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Papadopoulos, G., Goulas, C., Apostolaki, E., and Abril, R. 2002. Effects of dietary supplements of algae, containing polyunsaturated fatty acids, on milk yield and the composition of milk products in dairy ewes. J. Dairy Res. 69:357-365.

Pariza, M. W., Park, Y., and Cook, M. E. 2001. The biologically active isomers of conjugated linoleic acid. Prog. Lipid Res. 40:283-298.

Park, Y. W., Juárez, M., Ramos, M., and Haenlein, G. F. W. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88-113.

Parodi, P. W. 1979. Stereospecific distribution of fatty acids in bovine milk fat triglycerides. J. Dairy Res. 46:75-81.

Parodi, P. W. 2005. Dairy product consumption and the risk of breast cancer. J. Am. Coll. Nutr. 24:556S-568S.

Pellegrini, O., Remeuf, F., Rivemale, M., and Barillet, F. 1997. Renneting properties of milk from individual ewes: influence of genetic and non-genetic variables, and the relationship with physicochemical characteristics. J. Dairy Res. 64:355-366.

Pennington, J. A., and Davis, C. L. 1975. Effects of intraruminal and intra-abomasal additions of cod-liver oil on milk fat production in the cow. J. Dairy Sci. 58:49-55.

Perfield, J. W. Bernal-Santos, G., Overton, T. R., and Bauman, D. E. 2002. Effects of dietary supplementation of rumen-protected conjugated linoleic acid in dairy cows during established lactation. J. Dairy Sci. 85:2609-2617.

Perfield, J. W., Delmonte, P., Lock, A. L., Yurawecz, M. P., and Bauman, D. E. 2006. *Trans*-10, *trans*-12 conjugated linoleic acid does not affect milk fat yield but reduces Δ^9-desaturase index in dairy cows. J. Dairy Sci. 89:2559-2266.

Perfield, J. W., Lock, A. L., Griinari, J. M., Sæbo, A., Delmonte, P., Dwyer, D. A., and Bauman, D. E. 2007. *Trans*-9, *cis*-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90:2211-2218.

Perfield, J. W., Sæbo, A., and Bauman, D. E. 2004. Use of conjugated linoleic acid (CLA) enrichments to examine the effects of *trans*-8, *cis*-10 CLA, and *cis*-11, *trans*-13 CLA on milk-fat synthesis. J. Dairy Sci. 87:1196-1202.

Peterson, D. G., Baumgard, L. H., and Bauman, D. E. 2002. Milk fat response to low doses of *trans*-10, *cis*-12 conjugated linoleic acid (CLA). J. Dairy Sci. 1764-1766.

Peterson, D. G., Matitashvili, E. A., and Bauman, D. E. 2003. Diet-induced milk fat depression in dairy cows results in increased *trans*-10, *cis*-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133:3098-3102.

Piperova, L. S., Teter, B. B., Bruckental, I., Sampugna, J., Mills, S. E., Yurawecz, M. P., Fritsche, J., Ku, K., and Erdman, R. A. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2658-2674.

Powell, E. B. 1939. Some relations of the roughage intake to the composition of milk. J. Dairy Sci. 22:453-454.

Pulina, G., and Nudda A. 2004. Milk production. In: Dairy sheep nutrition. Pulina, G. (ed.). CABI International, Wallingford, UK, pp. 1-12.

Pulina, G., Nudda, A., Battacone, G., and Cannas, A. 2006. Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. Anim. Feed Sci. Technol. 131:255-291.

Reinhardt, T. A., and Lippolis, J. D. 2006. Bovine milk fat globule membrane proteome. J. Dairy Res. 73:406-416.

Reynolds, C. K., Cannon, V. L., and Loerch, S. C. 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131:333-357.

Rindsig, R. B., and Schultz, L. H. 1974. Effects of abomasal infusions of safflower oil or elaidic acid on blood lipids and milk fat in dairy cows. J. Dairy Sci. 57:1459-1466.

Rodriguez-Cruz, M., Tovar, A. R., Palacios-González B., del Prado, M., and Torres, N. 2006. Synthesis of long-chain polyunsaturated fatty acids in lactating mammary gland: role of Δ5 and Δ6 desaturases, SREBP-1, PPARα, and PGC-1. J. Lipid Res. 47:553-560.

Sæbo, A., Sæbo, P. C., Griinari, J. M., and Shingfield, K. J. 2005. Effect of abomasal infusions of geometric isomers of 10,12 conjugated linoleic acid on milk fat synthesis in dairy cows. Lipids 40:823-832.

Sánchez, J. P., San Primitivo, F., Barbosa, E., Varona, L., and de la Fuente, L. F. 2010. Genetic determination of fatty acid composition in Spanish Churra sheep milk. J. Dairy Sci. 93:330-339.

Sanz-Sampelayo, R., Chilliard, Y., Schmidely, P., and Boza, J. 2007. Influence of type of diet on the fat constituents of goat and sheep milk. Small Rumin. Res. 68:42-67.

Schlegel, G., Ringseis, R., Windisch, W., Schwarz, F. J., and Eder, K. 2012. Effects of rumen-protected mixture of conjugated linoleic acids on hepatic expression of genes involved in lipid metabolism in dairy cows. J. Dairy Sci. 95:3905-3918.

Schmidely, P., and Morand-Fehr, P. 2004. Effects of intravenous infusion of *trans*-10, *cis*-12 or *cis*-9, *trans*-11 conjugated linoleic acid (CLA) on milk fat synthesis and composition in dairy goats during mid-lactation. S. Afr. J. Anim. Sci. 34(Suppl.1):195-197.

Schmitt, E., Ballou, M. A., Correa, M. N., DePeters, E. J., Drackley, J. K., and Loor, J. J. 2011. Dietary lipid during the transition period to manipulate subcutaneous adipose tissue peroxisome proliferator-activated receptor-γ co-regulator and target gene expression. J. Dairy Sci. 94:5913-5925.

Sebelien, J. 1894. Some recent methods and apparatus for the examination of the fat content of milk by means of centrifugal force. Norsk Landmandsblad 13, 219-223 (citado por Opstvedt, 1984).

Shi, H. B., Zhao, W. S., Luo, J., Yao, D. W., Sun, Y. T., Li, J., Shi, H. P. and Loor, J. J. 2014. Peroxisome proliferator-activated receptor γ1 and γ2 isoforms alter lipogenic gene networks in goat mammary epithelial cells to different extents. J. Dairy Sci. 97:5437-5447.

Shingfield, K. J., Ahvenjärvi, S., Toivonen, V., Vanhatalo, A., Huhtanen, P., and Griinari, J. M. 2008a. Effect of incremental levels of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. Br. J. Nutr. 99:971-983.

Shingfield, K. J., Bernard, L., Leroux, C., and Chilliard, Y. 2010. Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.

Shingfield, K. J., Bonnet, M., and Scollan, N. D. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7(S1):132-162.

Shingfield, K. J., Chilliard, Y., Toivonen, V., Kairenius, P., and Givens, D. I. 2008b. *Trans* fatty acids and bioactive lipids in ruminant milk. Adv. Exp. Med. Biol. 606:3-65.

Shingfield, K. J., and Griinari, J. M. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Sci. Technol. 109:799-816.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., and Beever, D. E. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., Rouel, J., and Chilliard, Y. 2009. Effect of calcium salts of a mixture of

conjugated linoleic acids containing *trans*-10, *cis*-12 in the diet on milk fat synthesis in goats. Br. J. Nutr. 101:1006-1019.

Sinclair, L. A., Lock, A. L., Early, R., and Bauman, D. E. 2007. Effects of *trans*-10, *cis*-12 conjugated linoleic acid on ovine milk fat synthesis and cheese properties. J. Dairy Sci. 90:3326-3335.

Sinclair, L. A., Weerasinghe, W. M. P. B., Wilkinson, R. G., de Veth, M. J., and Bauman, D. E. 2010. A supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat yield but does not alter organ weight or body fat deposition in lactating ewes. J. Nutr. 140:1949-1955.

Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801-2814.

Thering, B. J., Graugnard, D. E., Piantoni, P., and Loor, J. J. 2009. Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J. Dairy Sci. 92:4290-4300.

Timmen, H., and Patton, S. 1988. Milk fat globules: fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685-689.

Toral, P. G., Belenguer, A., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2012. Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 95:794-806.

Toral, P. G., Bernard, L., Delavaud, C., Gruffat, D., Leroux, C., and Chilliard, Y. 2013. Effects of fish oil and additional starch on tissue fatty acid profile and lipogenic gene mRNA abundance in lactating goats fed a diet containing sunflower-seed oil. Animal 7:948-956.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2010a. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. J. Dairy Sci. 93:1604-1615.

Toral P. G., Hervás, G., Carreño, D., and Frutos, P. May supplemental 18:0 alleviate fish oil-induced milk fat depression in dairy ewes? J. Dairy Sci. (en prensa).

Toral P. G., Hervás, G., and Frutos, P. 2015. Reductions in milk Δ^9-desaturation ratios to oral dosing of cobalt-acetate are accompanied by the downregulation of *SCD1* in lactating ewes. J. Dairy Sci. 98:1961-1971.

Toral, P. G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., and de la Fuente, M. A. 2010b. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93:1655-1667.

Tsiplakou, E., Mountzouris, K. C., and Zervas, G. 2006. The effect of breed, stage of lactation and parity on sheep milk fat CLA content under the same feeding practices. Livest. Sci. 105:162-167.

Tsiplakou, E., and Zervas, G. 2008. Comparative study between sheep and goats on rumenic acid and vaccenic acid in milk fat under the same dietary treatments. Livest. Sci. 119:87-94.

Tyznik, W., and Allen, N. N. 1951. The relation of roughage intake to the fat content of the milk and the level of fatty acids in the rumen. J. Dairy Sci. 34:493.

Van Soest, P. J. (ed.) 1994. Nutritional ecology of the ruminants. Cornell University Press, Ithaca, NY, USA.

Vlaeminck, B., Fievez, V., Tamminga, S., Dewhurst, R. J., van Vuuren, A., De Brabander, D., and Demeyer, D. 2006. Milk odd- and branched-chain fatty acids in relation to the rumen fermentation pattern. J. Dairy Sci. 89:3954-3964.

Williams, N. K., Cannon, C. Y., and Espe, D. 1939. Two methods of feeding soybean fat to cows and their effect on milk and butterfat production and on the nature of the butterfat. J. Dairy Sci. 22:442-443.

Weerasinghe, W. M. P. B., Wilkinson, R. G., Lock, A. L., de Veth, M. J., Bauman, D. E., and Sinclair, L. A. 2012. Effect of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on the performance of dairy ewes fed 2 levels of metabolizable protein and at a restricted energy intake. J. Dairy Sci. 95:109-116.

Zhao, W. S., Hu, S. L., Yu, K., Wang, H., Wang, W., Loor, J. J., and Luo, J. 2014. Lipoprotein lipase, tissue expression and effects on genes related to fatty acid synthesis in goat mammary epithelial cells. Int. J. Mol. 15:22757-22771.

PRUEBA I

INHIBITION OF Δ^9-DESATURASE ACTIVITY WITH STERCULIC ACID: EFFECT ON THE ENDOGENOUS SYNTHESIS OF *cis*-9 18:1 AND *cis*-9, *trans*-11 18:2 IN DAIRY SHEEP

E. Bichi[1], P. G. Toral[1], G. Hervás[1], P. Frutos[1], P. Gómez-Cortés[2], M. Juárez[2], and M. A. de la Fuente[2]

[1]*Instituto de Ganadería de Montaña, CSIC-Universidad de León, Finca Marzanas s/n, 24346 Grulleros, León, España*

[2]*Instituto de Investigación en Ciencias de la Alimentación, CSIC-Universidad Autónoma de Madrid, Nicolás Cabrera, 9. Campus de Cantoblanco, 28049 Madrid, España*

Journal of Dairy Science 95 (2012) 5242-5252

Doi:10.3168/jds.2012-5349

Presentado en parte en el *15th Congress European Society of Veterinary and Comparative Nutrition,* 14-16 de septiembre de 2011, Zaragoza, España
y en parte en el *8th International Symposium on the Nutrition of Herbivores*, 6-9 de septiembre de 2011, Aberystwyth, Reino Unido

INHIBITION OF Δ^9-DESATURASE ACTIVITY WITH STERCULIC ACID: EFFECT ON THE ENDOGENOUS SYNTHESIS OF *cis*-9 18:1 AND *cis*-9, *trans*-11 18:2 IN DAIRY SHEEP

E. Bichi, P. G. Toral, G. Hervás, P. Frutos, P. Gómez-Cortés, M. Juárez, and M. A. de la Fuente

Abstract. This study was conducted in lactating ewes to examine the involvement of Δ^9-desaturase in mammary lipogenesis, especially in the endogenous synthesis of *cis*-9, *trans*-11 18:2 and *cis*-9 18:1, because no information on this matter was available for dairy sheep. With this aim, 6 Assaf ewes were monitored in a 15-d experiment, which included a 5-d pretreatment period, a 5-d treatment period, and a 5-d posttreatment period. During the treatment period, ewes received 0.5 g/d of sterculic acid (a cyclopropene fatty acid that inhibits Δ^9-desaturase), delivered intravenously in 4 equal doses at 6-h intervals. Animals were fed pasture to supply mainly α-linolenic acid and minimize the amount of milk *cis*-9, *trans*-11 18:2 of ruminal origin. Sterculic acid administration was calculated to inhibit Δ^9-desaturase by 70% based on the milk content of *cis*-9 14:1. This inhibition resulted in decreases in the milk content of the enzyme products (e.g., *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1, and *cis*-9, *trans*-11 18:2) and increases in its substrates (e.g., 14:0, 18:0, and *trans*-11 18:1), as well as in reductions in the desaturase indexes. Some other milk fatty acids, further to previously reported products or substrates of Δ^9-desaturase (e.g., *cis*-15 18:1 and *cis*-9, *cis*-15 18:2, or *trans*-11, *trans*-15 18:2, and *cis*-9, *trans*-11, *trans*-15 18:3), were also affected by sterculic acid administration. Endogenous synthesis was the major source of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2, accounting for 63 and 74% of its content in milk fat, respectively. To our knowledge, the present study provides the first estimates of endogenous synthesis of these 2 bioactive fatty acids in ovine milk fat.

Key words: desaturase system, cyclopropene fatty acid, oleic acid, rumenic acid

1. INTRODUCTION

In ruminants, the enzyme Δ^9-desaturase has a key role in the synthesis of some milk FA through the introduction of a *cis* double bond between carbons 9 and 10 (Palmquist et al., 2005). Since Griinari et al. (2000) demonstrated that endogenous synthesis of the potentially health-promoting *cis*-9, *trans*-11 conjugated linoleic acid (CLA) from *trans*-11 18:1 of ruminal origin represents the primary source of CLA in the milk fat of lactating cows, the study of Δ^9-desaturase has had a renewed interest.

However, available information on the role of Δ^9-desaturase in mammary lipogenesis is still scarce in cows and goats and is almost nonexistent in sheep, despite the fact that a putatively higher activity of this enzyme has been suggested in the latter species based on differences in its mRNA abundance (Tsiplakou et al., 2009).

In addition, the number of studies quantifying the activity of Δ^9-desaturase on other substrates besides *cis*-9, *trans*-11 CLA is very limited. This is especially notable in relation to the synthesis of *cis*-9 18:1 (oleic acid) from 18:0 desaturation (Bickerstaffe and Johnson, 1972; Jeffcoat and Pollard, 1977; Mosley and McGuire, 2007), in spite of the relevance of its role in decreasing the milk fat melting point and probably preventing milk fat depression (Shingfield and Griinari, 2007).

Available studies estimating the activity of Δ^9-desaturase in ruminants include the use of direct methods, by means of a tracer (for example, [1-^{13}C]*trans*-11 18:1; Mosley et al., 2006; Bernard et al., 2010), and indirect methods, through quantification of duodenal and milk FA flows (Shingfield et al., 2007; Glasser et al., 2008) or inhibition of the desaturase system (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). The present study was conducted in lactating ewes with the aim of examining the involvement of Δ^9-desaturase in mammary lipogenesis, using a cyclopropene FA (sterculic acid) that inhibits the enzymatic system (Gomez et al., 2003). Special attention was paid to the endogenous synthesis of *cis*-9, *trans*-11 18:2 and *cis*-9 18:1 in the mammary gland.

2. MATERIALS AND METHODS

Animals, Experimental Design, and Management

All experimental procedures were performed in accordance with the Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes.

Six primiparous Assaf ewes (73.3 ± 3.30 kg of BW) in midlactation (94 ± 1.5 DIM at the beginning of the experiment) were used. Sheep were housed in individual tie stalls and fed ad libitum a pasture diet to supply mainly 18:3 n-3 (α-linolenic acid) and minimize the amount of milk *cis*-9, *trans*-11 18:2 of ruminal origin. The pasture was harvested from an irrigated sward of *Lolium perenne*, *Trifolium pratense*, and *Dactylis glomerata* before commencing the trial and kept frozen at −30ºC until the evening before being used. After thawing at approximately 4 to 10ºC, the pasture was offered twice daily at 0900 and 1900 h.

Before the experiment, all animals were allowed to graze pasture (a plot of the same sward described above) for 4 wk, followed by another week of adaptation to indoor conditions. The 15-d experiment consisted of a pretreatment period (d 1 to 5), a treatment period (d 6 to 10), and a posttreatment period (d 11 to 15). During the 5-d treatment period, the ewes received 0.5 g/d of chemically synthesized sterculic acid (Planta Piloto de Química Fina, University of Alcalá, Alcalá de Henares, Spain), suspended in 6 mL of 10% Intralipid (Fresenius Kabi SA, Barcelona, Spain) and 0.5 g of Simulsol 5817 (Seppic, Paris, France) and brought to 7 mL with 0.9% (wt/vol) saline solution (B. Braun Medical SA, Barcelona, Spain). The mixture was sonicated at 100 A for 1 min to ensure thorough mixing. One-fourth of the daily treatment dose was delivered every 6 h by jugular infusion.

Ewes were milked twice daily at approximately 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain) and had continuous access to clean water and a vitamin-mineral supplement (Tegablock, Inatega, León, Spain).

Measurements, Sampling Procedures, and Chemical Analyses

During the experimental period, DMI was recorded daily by weighing the amount of pasture DM offered and refused by each ewe. Samples of the pasture were collected at harvest, freeze-dried, and stored at −30ºC until they were analyzed for DM (ISO 6496:1999; ISO, 1999a), ash (ISO 5984:2002; ISO, 2002a), and CP (ISO 5983-2:2009; ISO, 2009). Neutral and acid detergent fibers were determined as described by Mertens (2002) and AOAC (2006; official method 973.18), respectively, using an Ankom^{2000} Fiber Analyzer (Ankom Technology Corp., Macedon, NY). Neutral detergent fiber was assayed with sodium sulfite and α-amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by Ankom Filter Bag Technology (procedure Am 5-04; AOCS, 2008).

The fat in the pasture was extracted following AOAC (2006) official methods (969.33 and 963.22) and then methylated and analyzed by gas chromatography, under the same conditions as described below for milk FA methyl esters (FAME).

Individual milk yield was recorded daily, and milk samples were collected from each animal and composited according to morning and evening milk yield. One aliquot of milk was treated with natamycin and stored at 4ºC until analyzed for fat, protein, lactose, and TS content by infrared spectrophotometry (ISO 9622:1999; ISO, 1999b), using a MilkoScan 255 A/S N (Foss Electric, Hillerød, Denmark). Milk FA composition was determined in untreated aliquots that were stored at −30ºC until analysis. Milk fat was extracted as described by Luna et al. (2005), and FAME were prepared by base-catalyzed methanolysis of the glycerides (ISO 15884:2002; ISO, 2002b). Analysis of FAME in hexane was performed on a gas chromatograph (Agilent 6890 N Network System; Agilent, Palo Alto, CA) with auto injector and fitted with a flame-ionization detector. The FAME profile was determined by split injection (1:100) onto a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d., 0.20-µm film thickness; Varian, Middelburg, the Netherlands) using a gradient temperature program. The initial oven temperature was 160ºC. After 80 min, it was increased at 10ºC/min to 210ºC and then held for 35 min. Helium was the carrier gas, and the injector and detector temperatures were 250ºC. Quantification of individual FAME was made by reference to a milk fat with a known composition (CRM 164; European Community Bureau of Reference, Brussels, Belgium). Individual CLA

isomers were identified by comparison with standard mixtures distributed by Nu-Chek Prep. Inc. (Elysian, MN). Standard GLC-461 from Nu-Chek Prep Inc. was also used to identify other FA.

Calculations and Statistical Analysis

The content of *cis*-9 14:1 in the pretreatment period and on the last day of sterculic acid administration was used to calculate a correction factor for incomplete inhibition of Δ^9-desaturase, to estimate the endogenous synthesis of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2 (Griinari et al., 2000). Based on previous studies in lactating cows (Mosley and McGuire, 2007), which estimated that approximately 90% of *cis*-9 14:1 was synthesized in the mammary gland via Δ^9-desaturation, it was assumed that the mammary gland was also the major site for conversion of 14:0 to *cis*-9 14:1 in lactating sheep. Desaturase indexes were calculated as follows: product of Δ^9-desaturase/(product of Δ^9-desaturase + substrate of Δ^9-desaturase).

Data were evaluated by repeated measurement analysis using the MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) and assuming a covariance structure on the basis of Schwarz's Bayesian information model fit criterion. The statistical model included the fixed effect of period, animal was considered a random effect, and day, nested in period, appeared in the repeated statement.

Least squares means are reported throughout. Significance differences were declared at $P < 0.05$ and tendencies accepted if $P < 0.10$.

3. RESULTS

Pasture Composition

Chemical composition of the pasture, including its FA profile, is presented in Table 1. α-Linolenic acid was the most abundant FA present in pasture (55.4%), followed by linoleic (14.1%) and palmitic (12.4%) acids. None of the other FA represented more than 5% of total FAME.

Table 1. Chemical composition and FA profile of the pasture (n = 9).

	Pasture	SEM
Chemical composition, g/kg DM		
DM[1]	255	0.7
OM	932	0.2
CP	136	0.4
NDF	460	0.7
ADF	252	0.4
Ether extract	33	0.1
FA profile, % total FA methyl esters		
16:0	12.4	0.16
18:0	1.5	0.09
cis-9 18:1	4.3	0.26
cis-9, *cis*-12 18:2	14.1	0.51
cis-9, *cis*-12, *cis*-15 18:3	55.4	0.82
20:0	1.5	0.11
22:0	3.1	0.12
22:2	4.5	0.28

[1]DM is in g/kg of fresh matter.

Animal Performance and Milk Composition

As shown in Table 2, sterculic acid did not affect DMI during its administration but caused a 7% decrease in the posttreatment period ($P = 0.004$). No changes were found in milk yield. However, a progressive decrease ($P < 0.001$) in milk fat production was observed throughout the trial, in association with reductions in milk fat content during the treatment and posttreatment periods (−5 and −13%, respectively, relative to the mean value of the pretreatment period). Infusion of sterculic acid had only a slight effect on milk protein content (−2.3%; $P < 0.001$), both during and after administration, whereas lactose percentage was minimally increased in the posttreatment period (+1.3%; $P < 0.001$).

Table 2. Dry matter intake, milk yield, and milk composition in dairy ewes before (pretreatment), during (treatment) and after (posttreatment) sterculic acid administration.

	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
DMI, g/d	2,345[a]	2,360[a]	2,188[b]	55.9	0.004
Yield, g/d					
Milk	1,137	1,136	1,135	22.5	0.994
Fat	68.7[a]	64.4[b]	61.2[c]	1.54	0.001
Protein	59.3	58.0	57.9	1.23	0.447
Lactose	55.7	55.6	56.2	1.08	0.855
TS	193.3	189.1	187.4	4.52	0.435
Composition, g/100 g of raw milk					
Fat	6.03[a]	5.71[b]	5.26[c]	0.122	<0.001
Protein	5.23[a]	5.12[b]	5.10[b]	0.029	<0.001
Lactose	4.89[b]	4.88[b]	4.95[a]	0.018	<0.001
TS	16.96[a]	16.62[b]	16.12[c]	0.115	<0.001

[a-c]Means within a row with different superscripts differ significantly ($P < 0.05$).
[1]SED = standard error of the difference.
[2]Probability of significant differences between periods.

Milk FA Composition

Treatment with sterculic acid reduced ($P < 0.001$) the indices of Δ^9-desaturase activity shown in Table 3. The greatest decrease (−71%, always in comparison with the pretreatment period) corresponded to the index of *cis*-9 14:1 and 14:0, although the changes observed for *cis*-9 10:1 and 10:0, *cis*-9 16:1 and 16:0, *cis*-9 18:1 and 18:0, *cis*-9, *trans*-11 18:2 and *trans*-11 18:1, and *cis*-9, *trans*-11, *trans*-15 18:3 and *trans*-11, *trans*-15 18:2 were also substantial (−47, −28, −37, −42, and −46%, respectively). The initial values were not recovered during the posttreatment period ($P < 0.001$).

Table 3. Indices of desaturase activity and major classes of FA of milk in dairy ewes before (pretreatment), during (treatment) and after (posttreatment) sterculic acid administration.

	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
Desaturase index					
cis-9 10:1/(*cis*-9 10:1 + 10:0)	0.040[a]	0.022[c]	0.026[b]	0.0007	<0.001
cis-9 14:1/(*cis*-9 14:1 + 14:0)	0.014[a]	0.005[c]	0.006[b]	0.0005	<0.001
cis-9 16:1/(*cis*-9 16:1 + 16:0)	0.053[a]	0.039[b]	0.041[b]	0.0014	<0.001
cis-9 18:1/(*cis*-9 18:1 + 18:0)	0.636[a]	0.405[c]	0.561[b]	0.0082	<0.001
cis-9, *trans*-11 18:2/(*cis*-9, *trans*-11 18:2 + *trans*-11 18:1)	0.316[a]	0.182[c]	0.221[b]	0.0087	<0.001
cis-9, *trans*-11, *trans*-15 18:3/(*cis*-9, *trans*-11, *trans*-15 18:3 + *trans*-11, *trans*-15 18:2)	0.351[a]	0.193[c]	0.240[b]	0.0083	<0.001
cis-9, *trans*-11, *cis*-15 18:3/(*cis*-9, *trans*-11, *cis*-15 18:3 + *trans*-11, *cis*-15 18:2)	0.400	0.377	0.358	0.0240	0.216
According to degree of saturation, g/100g of total FA methyl esters					
Saturated FA	66.87[c]	74.32[a]	70.87[b]	0.692	<0.001
Monounsaturated FA	24.93[a]	18.00[c]	20.82[b]	0.494	<0.001
Polyunsaturated FA	6.13[b]	5.91[b]	6.42[a]	0.143	0.005
According to origin,[3] g/100g of total FA methyl esters					
Σ 6–14 carbon FA	29.42	29.80	29.49	0.520	0.745
Σ 16 carbon FA	24.64	24.49	24.43	0.214	0.580
Σ ≥18 carbon FA	37.99	37.46	38.11	0.627	0.544

[a-c]Means within a row with different superscripts differ significantly ($P < 0.05$).
[1]SED = standard error difference.
[2]Probability of significant differences between periods.
[3]6–14 carbon FA represent de novo synthesized FA, ≥18 carbon FA represent preformed FA taken up from circulation, and 16 carbon FA represent FA derived from both sources.

Similarly, the reductions ($P < 0.001$) in concentrations of the Δ^9-desaturase products persisted partially into the posttreatment period (see Figure 1). According to the temporal variations, the inhibition of Δ^9-desaturase activity reached its maximum value around the second or third day on treatment (d 7 or 8 of the experiment), but its recovery was slower and the values in the pretreatment period were achieved only for *cis*-9 18:1 and on the last day of the experiment (d 15; Figure 1). Comparable changes (i.e., significant reductions that persisted partially after the administration) were also observed for *cis*-9 10:1, *cis*-9 17:1, *cis*-9, *cis*-15 18:2, *cis*-9, *trans*-12 18:2, and *cis*-9, *trans*-13 18:2 (Figure 2 and Table 4).

The inhibition of Δ^9-desaturase induced an increase ($P \leq 0.001$) in the relative concentration of 14:0 (+6%), 18:0 (+57%), *trans*-11 18:1 (+16%), and *trans*-11, *trans*-15 18:2 (+20%), with temporal patterns of variation that were consistent with the persistency of the response (Figure 1). However, the percentages of 10:0 and 16:0 in milk were not affected (Table 4).

The activity of Δ^9-desaturase was calculated to be reduced by 70% with sterculic acid administration. When the data from the last day of sterculic acid administration were used, the endogenous synthesis of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2 was estimated to account for 63 and 74%, respectively, of their content in milk fat.

Regarding other FA, some increased during the treatment period (e.g., *cis*-15 18:1, *trans*-12 18:1, *cis*-9, *cis*-12 18:2, *cis*-9, *cis*-12, *cis*-15 18:3), whereas others decreased (e.g., *cis*-5, *cis*-8, *cis*-11, *cis*-14 20:4) or showed a tendency to decrease (e.g., *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5). As expected, the proportion of SFA was increased with the administration of sterculic acid, whereas those of MUFA and PUFA were decreased. Linoleic and α-linolenic acids achieved the highest concentration in the posttreatment period (+20 and +32%, respectively), whereas the proportion of FA synthesized de novo or preformed was not affected by treatment ($P > 0.10$; Table 3).

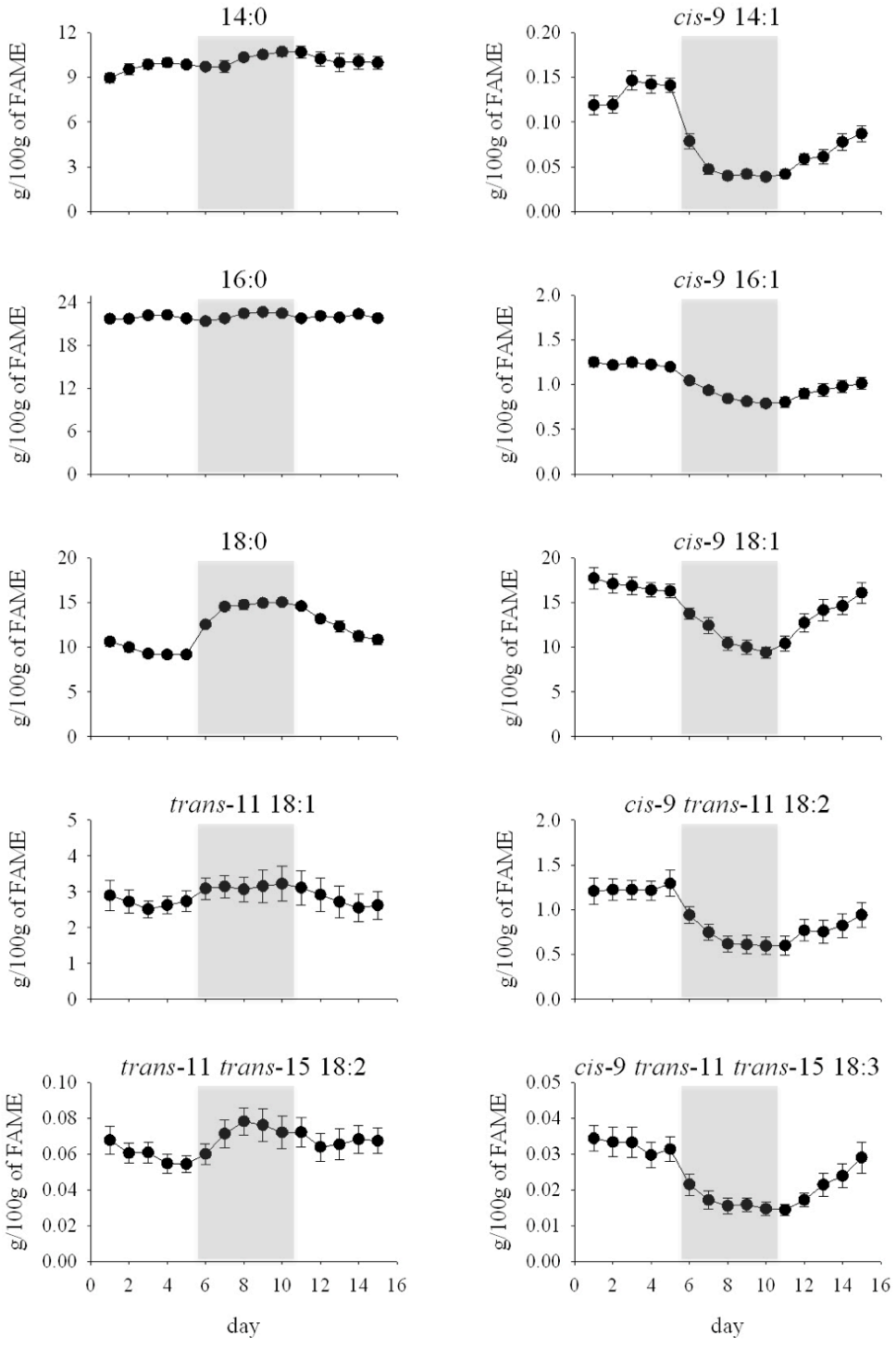

Figure 1. Temporal changes in milk 14:0, *cis*-9 14:1, 16:0, *cis*-9 16:1, 18:0, *cis*-9 18:1, *trans*-11 18:1, *cis*-9, *trans*-11 18:2, *trans*-11, *trans*-15 18:2, and *cis*-9, *trans*-11, *trans*-15 18:3 content [g/100 g of total FA methyl esters (FAME)] in dairy ewes before (d 1 to 5), during (d 6 to 10; grey shadow) and after (d 11 to 15) sterculic acid administration. Vertical bars represent the standard error of the mean (n = 6).

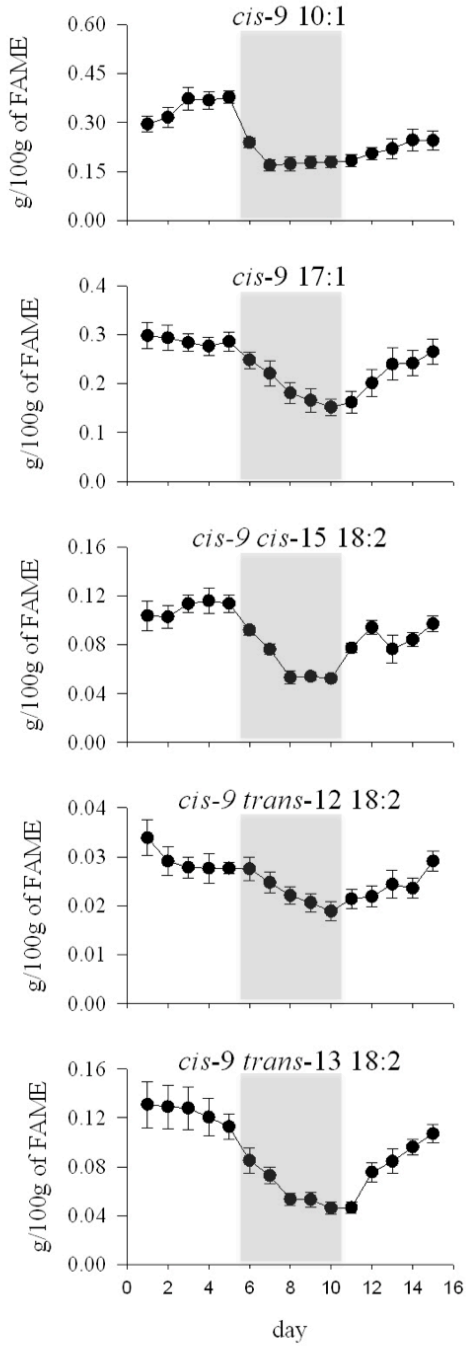

Figure 2. Temporal changes in milk *cis*-9 10:1, *cis*-9 17:1, *cis*-9, *cis*-15 18:2, *cis*-9, *trans*-12 18:2 and *cis*-9, *trans*-13 18:2 content [g/100 g of total FA methyl esters (FAME)] in dairy ewes before (d 1 to 5), during (d 6 to 10; grey shadow) and after (d 11 to 15) sterculic acid administration. Vertical bars represent the standard error of the mean (n = 6).

Table 4. Fatty acid profile of milk in dairy ewes before (pretreatment), during (treatment) and after (posttreatment) sterculic acid administration.

FA, g/100g of total FA methyl esters	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
Saturated FA					
4:0	3.90[b]	4.29[a]	3.97[b]	0.051	<0.001
6:0	3.22	3.11	3.12	0.055	0.121
8:0	2.96	2.96	2.87	0.058	0.201
10:0	8.31	8.60	8.44	0.207	0.228
12:0	4.10[b]	4.34[a]	4.39[a]	0.080	0.003
13:0 *iso*	0.030[b]	0.032[ab]	0.035[a]	0.0014	0.009
13:0 *anteiso*	0.052[a]	0.029[c]	0.033[b]	0.0015	<0.001
13:0	0.20[a]	0.16[b]	0.16[b]	0.005	<0.001
14:0 *iso*	0.19	0.20	0.19	0.005	0.553
14:0	9.63[b]	10.19[a]	10.18[a]	0.163	0.001
15:0 *iso*	0.38[b]	0.43[a]	0.42[a]	0.012	<0.001
15:0 *anteiso*	0.78[a]	0.70[b]	0.69[b]	0.020	<0.001
15:0	1.42[a]	1.34[b]	1.34[b]	0.022	<0.001
16:0 *iso*	0.28[a]	0.27[b]	0.27[b]	0.006	0.018
16:0	21.89	22.11	21.96	0.226	0.602
17:0	0.79[b]	0.91[a]	0.93[a]	0.018	<0.001
18:0 *iso*	0.08[b]	0.08[b]	0.09[a]	0.004	0.003
18:0	9.49[c]	14.91[a]	12.84[b]	0.385	<0.001
19:0	0.06[b]	0.09[a]	0.09[a]	0.002	<0.001
20:0	0.19[b]	0.20[a]	0.21[a]	0.004	<0.001
21:0	0.070	0.071	0.075	0.0021	0.051
22:0	0.12	0.12	0.13	0.003	0.184
23:0	0.08[b]	0.08[b]	0.09[a]	0.003	<0.001
24:0	0.060[b]	0.057[b]	0.064[a]	0.0017	0.001

(continued)

Table 4. *(continued)*

FA, g/100g of total FA methyl esters	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
Monounsaturated FA					
cis-9 10:1	0.35[a]	0.19[c]	0.22[b]	0.011	<0.001
cis-9 14:1	0.14[a]	0.04[c]	0.06[b]	0.005	<0.001
cis-9 15:1	0.059[c]	0.064[b]	0.076[a]	0.0017	<0.001
cis-7 16:1	0.47[b]	0.46[b]	0.51[a]	0.009	<0.001
cis-8 16:1	0.02[b]	0.02[b]	0.19[a]	0.001	<0.001
cis-9 16:1[3]	1.23[a]	0.88[b]	0.93[b]	0.030	<0.001
cis-10 16:1	0.04	0.03	0.04	0.002	0.662
cis-11 16:1	0.03	0.03	0.03	0.001	0.192
cis-12 16:1	0.035[b]	0.036[b]	0.038[a]	0.0011	0.004
cis-13 16:1	0.07[a]	0.02[c]	0.03[b]	0.005	<0.001
trans-9 16:1[4]	0.59[b]	0.62[a]	0.60[ab]	0.008	0.005
Other *trans* 16:1	0.11[b]	0.12[a]	0.12[a]	0.003	<0.001
cis-7 17:1	0.08[b]	0.09[a]	0.09[a]	0.002	<0.001
cis-9 17:1	0.29[a]	0.19[c]	0.22[b]	0.012	<0.001
cis-9 18:1	16.71[a]	9.98[c]	12.85[b]	0.465	<0.001
cis-11 18:1	0.28[c]	0.33[a]	0.30[b]	0.009	<0.001
cis-12 18:1	0.12	0.12	0.12	0.004	0.673
cis-13 18:1	0.05[b]	0.05[b]	0.06[a]	0.002	0.004
cis-15 18:1	0.12[c]	0.13[b]	0.16[a]	0.005	<0.001
cis-16 18:1	0.03[b]	0.04[a]	0.04[a]	0.002	<0.001
trans 6+7+8 18:1	0.21[b]	0.25[a]	0.25[a]	0.005	<0.001
trans-9 18:1	0.20[a]	0.17[b]	0.17[b]	0.004	<0.001
trans-10 18:1	0.27	0.27	0.25	0.011	0.360
trans-11 18:1	2.70[b]	3.13[a]	2.78[b]	0.111	<0.001
trans-12 18:1	0.19[b]	0.24[a]	0.25[a]	0.007	<0.001

(continued)

Table 4. *(continued)*

FA, g/100g of total FA methyl esters	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
trans-15 18:1	0.11[b]	0.18[a]	0.18[a]	0.006	<0.001
trans-16 18:1[5]	0.39[b]	0.40[b]	0.43[a]	0.009	<0.001
cis-11 20:1	0.02[b]	0.03[a]	0.03[a]	0.001	0.007
24:1	0.03[a]	0.03[a]	0.02[b]	0.001	<0.001
Nonconjugated 18:2					
cis-9, *cis*-12 18:2	1.41[c]	1.58[b]	1.69[a]	0.049	<0.001
cis-9, *trans*-12 18:2	0.03[a]	0.02[b]	0.02[b]	0.001	<0.001
cis-9, *cis*-15 18:2	0.11[a]	0.07[c]	0.09[b]	0.004	<0.001
cis-9, *trans*-13 18:2	0.12[a]	0.06[c]	0.08[b]	0.006	<0.001
trans-8, *cis*-13 18:2	0.09[b]	0.14[a]	0.09[b]	0.004	<0.001
trans-9, *cis*-12 18:2	0.04	0.05	0.04	0.001	0.376
trans-9, *trans*-12 18:2	0.06[a]	0.03[c]	0.04[b]	0.003	<0.001
trans-11, *cis*-15 18:2	0.34	0.36	0.35	0.013	0.269
trans-11, *trans*-15 18:2	0.06[b]	0.07[a]	0.07[a]	0.002	<0.001
Conjugated 18:2					
cis-9, *trans*-11 18:2	1.23[a]	0.70[c]	0.78[b]	0.043	<0.001
trans-9, *cis*-11 18:2	0.008[a]	0.005[c]	0.006[b]	0.0004	<0.001
trans-9, *trans*-11 18:2	0.008[a]	0.006[b]	0.006[b]	0.0004	<0.001
trans-10, *cis*-12 18:2	0.003[a]	0.002[b]	0.003[a]	0.0002	<0.001
trans-11, *cis*-13 18:2	0.12	0.12	0.12	0.008	0.794
trans-11, *trans*-13 18:2	0.09[b]	0.11[a]	0.11[a]	0.004	<0.001
trans-12, *trans*-14 18:2	0.01	0.01	0.01	0.001	0.347
trans-13, *trans*-15 18:2	0.01	0.01	0.01	0.001	0.652
Other polyunsaturated FA					
cis-6, *cis*-9, *cis*-12 18:3	0.03[b]	0.03[b]	0.04[a]	0.001	<0.001
cis-9, *cis*-12, *cis*-15 18:3	1.42[c]	1.59[b]	1.87[a]	0.056	<0.001

(continued)

Table 4. *(continued)*

FA, g/100g of total FA methyl esters	Pretreatment	Treatment	Posttreatment	SED[1]	*P*-value[2]
cis-9, *trans*-11, *cis*-15 18:3	0.23	0.22	0.20	0.016	0.131
cis-9, *trans*-11, *trans*-15 18:3	0.032[a]	0.017[c]	0.021[b]	0.0013	<0.001
cis-11, *cis*-14 20:2	0.012[c]	0.014[b]	0.016[a]	0.0006	<0.001
cis-8, *cis*-11, *cis*-14 20:3	0.01[b]	0.01[b]	0.02[a]	0.001	0.001
cis-11, *cis*-14, *cis*-17 20:3	0.014[c]	0.016[b]	0.018[a]	0.0005	<0.001
cis-5, *cis*-8, *cis*-11, *cis*-14 20:4	0.140[a]	0.128[c]	0.137[b]	0.0034	0.002
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5	0.09	0.08	0.09	0.004	0.058
cis-13, *cis*-16 22:2	0.15[a]	0.13[b]	0.12[b]	0.004	<0.001
cis-7, *cis*-10, *cis*-13, *cis*-16 22:4	0.03[a]	0.02[b]	0.03[a]	0.001	0.026
cis-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:5	0.17	0.16	0.16	0.005	0.455
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6	0.06	0.05	0.05	0.002	0.273

[a-c]Means within a row with different superscripts differ significantly ($P < 0.05$).
[1]SED = standard error of the difference.
[2]Probability of significant differences between periods.
[3]Coelutes with 17:0 *anteiso*.
[4]Coelutes with 17:0 *iso*.
[5]Coelutes with *cis*-14 18:1.

4. DISCUSSION

Previous research has shown that endogenous synthesis via Δ^9-desaturation is the major source of *cis*-9, *trans*-11 18:2 in cow and goat milk fat (Griinari et al., 2000; Corl et al., 2001; Bernard et al., 2010). The present study investigates the role of Δ^9-desaturase in mammary lipogenesis in the dairy sheep and provides the first estimates of endogenous synthesis of *cis*-9, *trans*-11 18:2 and *cis*-9 18:1 in ovine milk fat.

Sterculic acid administration did not affect milk yield, which is in agreement with previous studies using sterculic oil in lactating cows (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). However, and also in accordance with results observed in experiments in caprines and bovines (Bickerstaffe and Johnson, 1972; Corl et al., 2001), slight reductions in milk fat content and yield were due to the treatment with sterculic acid. These reductions cannot be explained by changes in the proportion of FA synthesized de novo, but they are likely related to the decrease in the proportion of MUFA. The inhibition of Δ^9-desaturase has been reported to detrimentally affect milk fat synthesis, probably because the reduction in the content of *cis*-9 18:1 (and perhaps other Δ^9-desaturase products) influences the physical properties of milk fat by increasing its melting point (Chilliard et al., 2000). The persistence of enzyme inhibition over time might explain the persistence of the lower milk fat content in the posttreatment period. The DMI was also reduced in this period, but the experimental design did not allow us to attribute this effect to the treatment or to a period.

As expected, sterculic acid administration elicited a decrease in the concentration of Δ^9-desaturase products and a concurrent increase in many of its substrates because of the inhibitory effect of this cyclopropene FA on the activity of the enzyme (Jeffcoat and Pollard, 1977; Gomez et al., 2003). Inhibitory effects of sterculic acid are probably mediated via direct reductions in enzyme activity rather than by lowered Δ^9-desaturase gene expression or protein translation (Gomez et al., 2003; Palmquist et al., 2005). Beginning with *cis*-9 18:1, whose levels in the milk fat of grazing dairy sheep have been reported to range from 13 to up to 41% of total FA (Addis et al., 2005; Gómez-Cortés et al., 2009a; Buccioni et al., 2010), sterculic acid administration allowed us to estimate an endogenous synthesis of 63% in this ruminant species. This value is within the range calculated in cows (e.g., Enjalbert et al., 1998; Griinari et al.,

2000; Shingfield et al., 2008; Taugbøl et al., 2008). One of the highest estimates in cows was calculated by abomasal infusion of sterculic acid in grazing animals (approximately 71%; Kay et al., 2004). In goats, the mean contribution of mammary desaturation to the synthesis of oleic acid was estimated at approximately 82% (Annison et al., 1967).

Regarding *cis*-9, *trans*-11 18:2, which is the most abundant CLA isomer in sheep milk fat and represents between 1.1 and 2.4% of total FA in grazing dairy ewes (Addis et al., 2005; Cruz-Hernandez et al., 2006; Gómez-Cortés et al., 2009a), its endogenous synthesis was estimated at 74%. This value is similar to the estimate calculated by Bernard et al. (2010) in dairy goats (63 to 73%) and within the range of 64 to 97% reported in cows (e.g., Palmquist et al., 2005; Mosley et al., 2006; Glasser et al., 2008). It is interesting that in a study in lactating cows fed fresh pasture and receiving an abomasal infusion of sterculic oil, the estimated value was higher than that observed in this study (91%; Kay et al., 2004). The authors attributed the high endogenous synthesis of milk rumenic acid to the elevated supply of *trans*-11 18:1 coming from ruminal biohydrogenation of α-linolenic acid, the most abundant FA in pasture. In sheep, the results from the present study not only show the expected relationship between rumenic and vaccenic acids (Griinari et al., 2000), but also suggest that mammary desaturation of *trans*-11 18:1 is not as elevated as in the cow. Therefore, given the high contents of rumenic acid in ovine milk (Park et al., 2007; Tsiplakou et al., 2009; Buccioni et al., 2010), it might be hypothesized that a greater portion of rumenic acid is coming from duodenal flow in sheep compared with that in cows. Consistent with this hypothesis, a comparison of data from studies on the FA profile of ruminal digesta showed that the content of rumenic acid is less abundant in cows (approximately 0.1%; AbuGhazaleh et al., 2002; Lock and Garnsworthy, 2002; Loor et al., 2004) than in ewes (approximately 0.2 to 0.3%; Toral et al., 2010, 2012).

Sterculic acid administration caused a decrease in the milk fat content of several Δ^9-desaturase products, such as *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 17:1, *cis*-9, *trans*-12 18:2, and *cis*-9, *trans*-13 18:2, reported to come from Δ^9-desaturation of 10:0, 14:0, 16:0, 17:0, *trans*-12 18:1, and *trans*-13 18:1, respectively (Mahfouz et al., 1980; Mosley and McGuire, 2007; Shingfield et al., 2007). A similar trend was observed for *cis*-9, *cis*-15 18:2 and *cis*-9, *trans*-11, *trans*-15 18:3, but they have not been described previously as Δ^9-desaturase products. However, the concurrent increases in *cis*-15

18:1 and *trans*-11, *trans*-15 18:2 during the treatment with sterculic acid allowed us to speculate that they might be 2 putative products coming from Δ^9-desaturation. To the knowledge of the authors, the isomer *cis*-9, *trans*-11, *trans*-15 18:3 has not been described to date in rumen effluent, plasma, or the products of in vitro rumen metabolism of α-linolenic acid. The hypothesis of mammary Δ^9-desaturation of *trans*-11, *trans*-15 18:2 does not preclude the existence of a rumen biohydrogenation pathway involving *cis*-9, *trans*-11, *trans*-15 18:3, *trans*-11, *trans*-15 18:2, and *trans*-15 18:1, as postulated by Gómez-Cortés et al. (2009b), but it would imply a relevant role of the mammary gland in *cis*-9, *trans*-11, *trans*-15 18:3 synthesis.

In contrast, the ratio of *cis*-9, *trans*-11, *cis*-15 18:3 to *trans*-11, *cis*-15 18:2 plus *cis*-9, *trans*-11, *cis*-15 18:3 (also belonging to the α-linolenic acid biohydrogenation pathways) calculated from our data does not support the view that mammary Δ^9-desaturation of *trans*-11, *cis*-15 18:2 was the main source of *cis*-9, *trans*-11, *cis*-15 18:3 because its ratios in the pretreatment and treatment periods were not significantly different (Table 3). In fact, the group of isomers *cis*-9, *trans*-11, *cis*-15 18:3, *trans*-11, *cis*-15 18:2, and *cis*-15 18:1 has been found in the plasma of cows fed linseed (Akraim et al., 2007), which would indicate that they are predominantly originated in the rumen. Furthermore, Gómez-Cortés et al. (2009b) observed a higher ratio of *cis*-9, *trans*-11, *cis*-15 18:3 to *trans*-11, *cis*-15 18:2 plus *cis*-9, *trans*-11, *cis*-15 18:3 in ewes fed a control diet than in ewes fed an α-linolenic acid-enriched diet (0.57 vs. 0.09), which would also support the view that rumen biohydrogenation of α-linolenic acid was the main source of *cis*-9, *trans*-11, *cis*-15 18:3.

The decrease in *cis*-9 14:1 and *cis*-9 16:1 was expected to be accompanied by a concurrent increase in their corresponding substrates (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004). However, as previously reported by Corl et al. (2001), no change was observed in 16:0 concentration, which would suggest that a low proportion of this FA is subjected to desaturation. According to Corl et al. (2001, 2002), the increase in *trans*-6+7+8 18:1 might reflect the use of *trans*-7 18:1 to synthesize *trans*-7, *cis*-9 18:2.

It was surprising that sterculic acid administration seemed to modify odd- and branched-chain FA. Nevertheless, temporal variations in these FA might be independent of the treatment because no evidence exists in the literature that sterculic acid affects ruminal metabolism, and branched-chain FA are known to be largely

derived from bacteria leaving the rumen (Vlaeminck et al., 2006). On the other hand, part of these FA may be synthesized de novo in the mammary gland in lactating ewes (Vlaeminck et al., 2006); therefore, further research would be necessary to clarify this behavior.

Finally, sterculic acid administration affected other FA that were not correlated with the Δ^9-desaturase system. Thus, the percentage of linoleic and α-linolenic acids increased in the sterculic treatment, in agreement with results observed in cows when Δ^9-desaturase was inhibited with cobalt (Taugbøl et al., 2010). This effect was attributed to cobalt interfering not only with Δ^9-desaturase but also with other Δ-desaturase enzymes (e.g., Δ^5 and Δ^6) that would convert, for example, α-linolenic acid into *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5 (Taugbøl et al., 2010). Even though the mode of action of sterculic acid is not completely understood, Cao et al. (1993) demonstrated that cyclopropene FA inhibit Δ^5- and Δ^6-desaturase systems in rat liver microsomes. Thus, even though some authors found no changes in 18:2 n-6 and 18:3 n-3 when they infused sterculic oil in cows (Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004) and only little evidence of Δ^6-desaturase exists in the mammary gland (Bionaz and Loor, 2008), it should not be ruled out that an inhibition of this enzyme in other tissues might explain, at least in part, their increase in this study. In addition, an inhibition of Δ^5-desaturase would account for the reduction in 20:4 n-6 and the tendency in 20:5 n-3.

5. CONCLUSIONS

Administration of sterculic acid to lactating ewes resulted in a 70% inhibition of Δ^9-desaturase in the mammary gland that persisted partially over time. Similar to previous findings in the cow and goat, endogenous synthesis was the major source of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2, accounting for 63 and 74%, respectively, of its content in ovine milk fat.

6. REFERENCES

AbuGhazaleh, A. A., Schingoethe, D. J., Hippen, A. R., Kalscheur, K. F., and Whitlock, L. A. 2002. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. J. Dairy Sci. 85:2266-2276.

Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Pirisi, A., and Molle, G. 2005. Milk and cheese fatty acid composition in sheep fed mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. J. Dairy Sci. 88:3443-3454.

Akraim, F., Nicot, M. C., Juaneda, P., and Enjalbert, F. 2007. Conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA) and other biohydrogenation intermediates in plasma and milk fat of cows fed raw or extruded linseed. Animal 1:835-843.

Annison, E. F., Linzell, J. L., Fazakerley, S., and Nichols, B. W. 1967. The oxidation and utilization of palmitate, stearate, oleate and acetate by the mammary gland of the fed goat in relation to their overall metabolism, and the role of plasma phospholipids and neutral lipids in milk-fat synthesis. Biochem. J. 102:637-647.

AOAC. 2006. Official Methods of Analysis of the Association of Analytical Communities. 18th ed. 1st rev. AOAC Int., Gaithersburg, MD.

AOCS. 2008. Official Methods and Recommended Practices of the American Oil Chemist's Society. 5th ed. 2nd printing. Am. Oil Chem. Soc., Urbana, IL.

Bernard, L., Mouriot, J., Rouel, J., Glasser, F., Capitan, P., Pujos-Guillot, E., Chardigny, J. M., and Chilliard, Y. 2010. Effects of fish oil and starch added to a diet containing sunflower-seed oil on dairy goat performance, milk fatty acid composition and in vivo Δ^9-desaturation of [^{13}C]vaccenic acid. Br. J. Nutr. 104:346-354.

Bickerstaffe, R., and Johnson, A. R. 1972. The effect of intravenous infusions of sterculic acid on milk fat synthesis. Br. J. Nutr. 27:561-570.

Bionaz, M., and Loor, J. J. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Buccioni, A., Rapaccini, S., Antongiovanni, M., Minieri, S., Conte, G., and Mele, M. 2010. Conjugated linoleic acid and C18:1 isomers content in milk fat of sheep and their transfer to Pecorino Toscano cheese. Int. Dairy J. 20:190-194.

Cao, J., Blond, J., and Bézard, J. 1993. Inhibition of fatty acid Δ^6- and Δ^5-desaturation by cyclopropene fatty acids in rat liver microsomes. Biochim. Biophys. Acta 1210:27-34.

Chilliard, Y., Ferlay, A., Mansbridge, R., and Doreau, M. 2000. Ruminant milk fat plasticity: Nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181-205.

Corl, B. A., Baumgard, L. H., Dwyer, D. A., Griinari, J. M., Phillips, B. S., and Bauman, D. E. 2001. The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA. J. Nutr. Biochem. 12:622-630.

Corl, B. A., Baumgard, L. H., Griinari, J. M., Delmonte, Morehouse, K. M., Yurawecz, M. P., and Bauman, D. E. 2002. *Trans*-7, *cis*-9 CLA is synthesized endogenously by Δ^9-desaturase in dairy cows. Lipids 37:681-688.

Cruz-Hernandez, C., Kramer, J. K. G., Kraft, J., Santercole, M., Or-Rashid, M., Deng, Z., Dugan, M. E. R., Delmonte, P., and Yurawecz, M. P. 2006. Systematic analysis of *trans*

and conjugated linoleic acids in the milk and meat of ruminants. In: Advances in Conjugated Linoleic Acid Research. Yurawecz, M. P., Kramer, J. K. G., Gudmundsen, O., Pariza, M. W., and Banni, S. (eds.). AOCS Press, Champaign, IL, Vol. 3, pp. 45-93.

Enjalbert, F., Nicot, M. C., Bayourthe, C., and Moncoulon, R. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128:1525-1532.

Glasser, F., Ferlay, A., Doreau, M., Schmidely, P., Sauvant, D., and Chilliard, Y. 2008. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91:2771-2785.

Gomez, F. E., Bauman, D. E., Ntambi, J. M., and Fox, B. G. 2003. Effects of sterculic acid on stearoyl-CoA desaturase in differentiating 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 300:316-326.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A, and Hervás, G. 2009a. Effect of supplementation of grazing dairy ewes with a cereal concentrate on animal performance and milk fatty acid profile. J. Dairy Sci. 92:3964–3972.

Gómez-Cortés, P., Tyburczy, C., Brenna, J. T., Juárez, M., and de la Fuente, M. A. 2009b. Characterization by GC-MS and CACI-MS/MS of *cis*-9 *trans*-11 *trans*-15 C18:3 in milk fat. J. Lipid Res. 50:2412-2420.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K. V., and Bauman, D. E. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr. 130:2285-2291.

ISO (International Organization for Standardization). 1999a. Animal feeding stuffs—Determination of moisture and other volatile matter content. ISO 6496:1999. Int. Organ. Stand., Geneva, Switzerland.

ISO (International Organization for Standardization). 1999b. Whole milk—Determination of milk fat, protein and lactose content—Guidance on the operation of mid-infrared instruments. ISO 9622:1999. Int. Organ. Stand., Geneva, Switzerland.

ISO (International Organization for Standardization). 2002a. Animal feeding stuffs—Determination of crude ash. ISO 5984:2002. Int. Organ. Stand., Geneva, Switzerland.

ISO (International Organization for Standardization). 2002b. Milk fat—Preparation of fatty acid methyl esters. ISO 15884:2002. Int. Organ. Stand., Geneva, Switzerland.

ISO (International Organization for Standardization). 2009. Animal feeding stuffs—Determination of nitrogen content and of crude protein content—Part 2: Block digestion and steam distillation method. ISO 5983-2:2009. Int. Organ. Stand., Geneva, Switzerland.

Jeffcoat, R., and Pollard, M. R. 1977. Studies on the inhibition of the desaturases by cyclopropenoid fatty acids. Lipids 12:480-485.

Kay, J. K., Mackle, T. R., Auldist, M. J., Thomson, N. A., and Bauman, D. E. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369-378.

Lock, A., and Garnsworthy, P. 2002. Independent effects of dietary linoleic and linolenic fatty acids on the conjugated linoleic acid content of cows' milk. Anim. Sci. 74:163-176.

Loor, J. J., Ueda, K., Ferlay, A., Chilliard, Y., and Doreau, M. 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of *trans* fatty acids and conjugated linoleic

acids in response to dietary forage:concentrate ratio and linseed oil in dairy cows. J. Dairy Sci. 87:2472-2485.

Luna, P., Juárez, M., and de la Fuente, M. A. 2005. Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. J. Dairy Sci. 88:3377-3381.

Mahfouz, M. M., Valicenti, A. J., and Holman, R. T. 1980. Desaturation of isomeric *trans*-octadecenoic acids by rat-liver microsomes. Biochim. Biophys. Acta 618:1-12.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217-1240.

Mosley, E. E., and McGuire, M. A. 2007. Methodology for the in vivo measurement of the Δ^9-desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. Lipids 42:939-945.

Mosley, E. E., Shafii Dagger, B., Moate, P. J., and McGuire, M. A. 2006. *Cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575.

Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Park, Y. W., Juárez, M., Ramos, M., and Haenlein, G. F. W. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88-113.

Shingfield, K. J., Ahvenjärvi, S., Toivonen, V., Vanhatalo, A., and Huhtanen, P. 2007. Transfer of absorbed *cis*-9, *trans*-11 conjugated linoleic acid into milk is biologically more efficient than endogenous synthesis from absorbed vaccenic acid in lactating cows. J. Nutr. 137:1154-1160.

Shingfield, K. J., Arölä, A., Ahvenjärvi, S., Vanhatalo, A., Toivonen, V., Griinari, J. M., and Huhtanen, P. 2008. Ruminal infusions of cobalt-EDTA reduce mammary Δ^9-desaturase index and alter milk fatty acid composition in lactating cows. J. Nutr. 138:710-717.

Shingfield, K. J., and Griinari, J. M. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Sci. Technol. 109:799-816.

Taugbøl, O., Karlengen, I. J., Bolstad, T., Aastveit, A. H., and Harstad, O. M. 2008. Cobalt supplied per os reduces the mammary Δ^9-desaturase index of bovine milk. J. Anim. Sci. 86:3062-3068.

Taugbøl, O., Karlengen, I. J., Salbu, B., Aastveit, A. H., and Harstad, O. M. 2010. Intravenous injections of cobalt reduce fatty acid desaturation products in milk and blood of lactating cows. J. Anim. Physiol. Anim. Nutr. (Berl.) 94:635-640.

Toral, P. G., Belenguer, A., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2012. Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 95:794-806.

Toral, P. G., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2010. Effect of fish oil and sunflower oil on rumen fermentation characteristics and fatty acid composition of digesta in ewes fed a high concentrate diet. J. Dairy Sci. 93:4804-4817.

Tsiplakou, E., Flemetakis, E., Kalloniati, C., Papadomichelakis G., Katinakis, P. and Zervas, G. 2009. Sheep and goats differences in CLA and fatty acids milk fat content in relation

with mRNA stearoyl-CoA desaturase and lipogenic genes expression in their mammary gland. J. Dairy Res. 76:392-401.

Vlaeminck, B., Fievez, V., Cabrita, A. R. J., Fonseca, A. J. M., and Dewhurst, R. J. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131:389-417.

PRUEBA II

MILK FAT DEPRESSION INDUCED BY DIETARY MARINE ALGAE IN DAIRY EWES: PERSISTENCY OF MILK FATTY ACID COMPOSITION AND ANIMAL PERFORMANCE RESPONSES

E. Bichi[1], G. Hervás[1], P. G. Toral[1], J. J. Loor[2,3], and P. Frutos[1]

[1]*Instituto de Ganadería de Montaña, CSIC-Universidad de León, Finca Marzanas s/n. 24346 Grulleros, León, España*

[2]*Mammalian NutriPhysioGenomics Laboratory, Department of Animal Sciences, and*

[3]*Division of Nutritional Sciences, University of Illinois, Urbana 61801, USA*

Journal of Dairy Science 96 (2013) 524-532

Doi:10.3168/jds.2012-5875

MILK FAT DEPRESSION INDUCED BY DIETARY MARINE ALGAE IN DAIRY EWES: PERSISTENCY OF MILK FATTY ACID COMPOSITION AND ANIMAL PERFORMANCE RESPONSES

E. Bichi, G. Hervás , P. G. Toral, J. J. Loor, and P. Frutos

Abstract. Addition of marine algae (MA) to the diet of dairy ruminants has proven to be an effective strategy to enhance the milk content of some bioactive lipids, but it has also been associated with the syndrome of milk fat depression. Little is known, however, about the persistency of the response to dietary MA in sheep. Based on previous experiments with dairy ewes fed sunflower oil plus MA, it was hypothesized that the response might be mediated by time-dependent adaptations of the rumen microbiota, which could be evaluated indirectly through milk fatty acid (FA) profiles. Animal performance and milk FA composition in response to MA in the diet were studied using 36 Assaf ewes distributed in 6 lots and allocated to 2 treatments (3 lots/ treatment) consisting of a total mixed ration (40:60 forage:concentrate ratio) supplemented with 25 g of sunflower oil (SO)/kg of dry matter plus 0 (SO; control diet) or 8 g of MA/kg of dry matter (SOMA diet). Milk production and composition, including FA profile, were analyzed on d 0, 6, 12, 18, 24, 34, 44, and 54 of treatment. Diet supplementation with MA did not affect milk yield but did decrease milk fat content. Differences in the latter were detected from d 18 onward and reached −17% at the end of the experiment (i.e., on d 54). Compared with the control diet, the SOMA diet caused a reduction in milk 18:0 and its desaturation product (*cis*-9 18:1) that lasted for the whole experimental period. This decrease, together with the progressive increase in some putative fat synthesis inhibitors, especially *trans*-10 18:1, was related to the persistency of milk fat depression in lactating ewes fed MA. Additionally, inclusion of MA in the diet enhanced the milk content of *trans*-11 18:1, *cis*-9, *trans*-11 18:2, and C20–22 n-3 polyunsaturated FA, mainly 22:6 n-3. Overall, the persistency of the responses observed suggests that the ruminal microbiota did not adapt to the dietary supply of very long-chain n-3 polyunsaturated fatty acids.

Key words: conjugated linoleic acid, lipid supplementation, sheep, *trans* fatty acid

1. INTRODUCTION

Addition of marine algae (MA) to the diet of dairy ruminants is an effective strategy to enhance the milk content of bioactive lipids such as n-3 PUFA or conjugated linoleic acid (CLA; Franklin et al., 1999; Reynolds et al., 2006). This approach in cows has also been associated with a severe decline in milk fat content (Franklin et al., 1999; Boeckaert et al., 2008a). Although the syndrome of milk fat depression (MFD) in ewes was not first described in response to MA (Papadopoulos et al., 2002; Reynolds et al., 2006), Toral et al. (2010b) recently observed that dietary supplementation with 2.5% sunflower oil plus incremental amounts of MA (0.8, 1.6, and 2.4%) strongly reduced milk fat content. Based on comparison with the diet supplemented with sunflower oil only, those authors attributed the MFD to the addition of MA as reflected by changes in milk FA profile. Those changes included not only an enrichment in some potentially healthy FA (e.g., *trans*-11 18:1, *cis*-9, *trans*-11 18:2, and 22:6 n-3) but also a marked increase in *trans*-10 18:1, whose effect on human health is uncertain (see review by Gebauer et al., 2011). Although some results seem contradictory (Lock et al., 2007), *trans*-10 18:1 has been proposed as a putative cause of MFD (Griinari et al., 1998; Loor et al., 2005a; Shingfield et al., 2009).

Interestingly, after 28 d on the diet supplemented with sunflower oil plus the lowest dose of MA (0.8%), Toral et al. (2010b) observed a decrease in milk *trans*-10 18:1 concentration as well as in *trans*-9, *cis*-11 18:2. Both FA arise from alternative rumen microbial biohydrogenation pathways and have been related to MFD in dairy cows (Perfield et al., 2007; Shingfield et al., 2009). This occurred together with a slight but significant increase in milk fat content. Analysis of the FA composition of the ruminal digesta (Toral et al., 2012) confirmed a lower content of *trans*-10 18:1 and suggested an adaptation of the rumen bacterial community to the consumption of MA and a potential reestablishment of the main pathway of biohydrogenation. If this is true and the response to marine lipid supplementation varies over time, a longer-term study would be necessary to evaluate time-dependent variations. Therefore, the main objective of this study was to evaluate animal performance and milk FA composition in dairy ewes over a 54-d period, with the aim of studying the persistency of the response to the addition of MA to a diet rich in linoleic acid.

2. MATERIALS AND METHODS

Animals, Experimental Diets, and Management

All experimental procedures were performed in accordance with the Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes. Thirty-six Assaf ewes (84.5 ± 1.68 kg of BW) in mid lactation (82 ± 0.9 DIM at the beginning of the experiment) were stratified according to milk yield, BW, days postpartum, lactation number, and milk fat content, and randomly distributed in 6 lots (6 ewes/lot), which in turn were assigned to 1 of 2 dietary treatments (3 lots/treatment). Diets consisted of a TMR based on alfalfa hay and a concentrate (40:60) supplemented with 25 g of sunflower oil (Carrefour S.A., Madrid, Spain)/kg of DM plus 0 (SO; control diet) or 8 g of marine algae (DHA Gold Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD)/ kg of DM (SOMA diet). 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. Ewes were fed the control diet during 20 d of adaptation before the start of the study. Fresh diets were offered daily ad libitum at 0900 and 1900 h, and clean water was always available. Ewes were milked twice daily at 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain) throughout the 54 d experiment.

Measurements and Sampling

Intake of DM was recorded every 4 d for each experimental lot by weighing the amount of DM offered and refused by each lot. Samples of the diets and refusals were collected with the same frequency, stored at –30°C, and then freeze-dried.

Table 1. Ingredients and chemical composition of the experimental diets[1].

	SO	SOMA	SED[2]	*P*-value
Ingredient, g/kg of fresh matter				
Dehydrated alfalfa hay	392	389		
Whole corn grain	184	183		
Soybean meal	147	146		
Whole barley grain	119	118		
Beet pulp	66	65		
Molasses	48	48		
Feed supplement[3]	23	23		
Sunflower oil[4]	21	21		
Marine algae[5]	0	7		
Chemical composition, g/kg DM				
OM	901	900	4.1	0.807
CP	190	189	6.0	0.901
NDF	267	260	9.5	0.454
ADF	174	166	8.9	0.367
Ether extract	58	57	4.1	0.743
FA profile, g/100 g of total FA				
14:0	0.79	1.66	0.029	<0.001
16:0	11.99	14.06	0.743	0.032
18:0	3.67	3.48	0.134	0.215
cis-9 18:1	22.30	19.94	1.404	0.144
cis-9, *cis*-12 18:2	46.22	42.37	1.511	0.044
cis-9, *cis*-12, *cis*-15 18:3	4.53	4.91	0.538	0.498
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16 22:5	0.00	0.71	0.037	<0.001
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6	0.00	1.87	0.097	<0.001

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]SED = standard error of the difference.
[3]Contained (g/kg): $NaHCO_3$ (458.3), $CaCO_3$ (250.0), NaCl (125.0), minerals and vitamins (104.2), and wheat bran (62.5).
[4]Contained (g/100 g of FA): 16:0 (5.5), 18:0 (4.4), 18:1 n-9 (36.4), and 18:2 n-6 (50.3).
[5]As declared by the supplier (Martek Biosciences Corp., Columbia, MD), marine algae (DHA Gold Animal Feed Ingredient) contained (g/kg of DM): OM (912), CP (167), crude fiber (45), and ether extract (558). Fatty acid composition (% FFA): 14:0 (8.5), 16:0 (23.2), 20:3 n-6 (1.6), 20:5 n-3 (1.9), 22:5 n-6 (17.7), and 22:6 n-3 (42.3).

Individual daily milk yields were recorded on d 0, 6, 12, 18, 24, 34, 44, and 54. Milk samples were collected with the same frequency from each ewe and composited according to morning and evening milk yield. One aliquot was stored at 4°C with natamycin (D&F Control Systems Inc., Dublin, CA) until analyzed for fat, protein, lactose, and TS. Milk FA composition was determined in untreated samples from each experimental lot, which were composited according to individual milk yield and stored at –30°C until analysis.

Chemical Analysis

Samples of TMR were analyzed for DM (ISO 6496; ISO, 1999a), ash (ISO 5984; ISO, 2002), and CP (ISO 5983-2; ISO, 2009). Neutral and acid detergent fiber were determined as described by Mertens (2002) and AOAC International (2006; method 973.18), respectively, using an Ankom^{2000} fiber analyzer (Ankom Technology Corp., Macedon, NY). Neutral detergent fiber was assayed with sodium sulfite and α-amylase and expressed with residual ash (the latter also for ADF). The content of ether extract in the diets was determined by the Ankom filter bag technology (AOCS, 2008; Proce-dure Am 5-04). Milk CP, lactose, fat, and TS contents were determined by infrared spectrophotometry (ISO 9622; ISO, 1999b), using a MilkoScan 255 A/S N (Foss Electric, Hillerød, Denmark).

Fatty acid methyl esters (FAME) in freeze-dried samples of TMR were prepared in a one-step extraction-transesterification procedure using chloroform and 2% (vol/vol) sulfuric acid in methanol (Shingfield et al., 2003), with tridecanoic acid (Sigma-Aldrich, Madrid, Spain) as an internal standard. For milk FA composition analysis, lipids in 1 mL of milk were extracted using diethyl ether:hexane (5:4, vol/vol) and transesterified to FAME using freshly prepared methanolic sodium methoxide, as outlined by Shingfield et al. (2003), with tridecanoic acid as an internal standard. Methyl esters were separated and quantified using a gas chromatograph (7890A GC System, Agilent Technologies, Santa Clara, CA) equipped with a flame-ionization detector and a 100-m fused-silica capillary column (0.25 mm i.d., 0.2 μm film thickness; CP-SIL 88, Chrompack 7489, Varian Ibérica S.A., Madrid, Spain) and He as the carrier gas. Total FAME profile in a 2-μL sample volume at a split ratio of 1:50 was determined using a temperature gradient program (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). Peaks were identified based on retention time comparisons with authentic standards (from Nu-Chek Prep., Elysian, MN; Sigma-Aldrich; and Larodan Fine Chemicals AB, Malmö, Sweden). Identification of FA was verified based on FAME standard mixtures when available, chromatograms reported in the literature (e.g., Shingfield et al., 2003; Kramer et al., 2008), and by comparison with milk samples for which the FA composition was determined based on GC analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivatives.

Statistical Analysis

All data were analyzed by repeated-measures ANOVA for a completely randomized design, using the MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) and assuming a covariance structure based on Schwarz's Bayesian information model fit criteria. The statistical model included the fixed effects of diet (D), time (T), their interaction (D × T), and the initial record measured at 0 d (covariate), as follows:

$$y_{ijk} = \mu + \alpha_i + d_{j(i)} + \delta_k + (\alpha\delta)_{ik} + (b + \varphi_j)\, x_{ij} + e_{ijk},$$

where y_{ijk} is the dependent variable measured at time k on the jth lot assigned to the ith diet, μ the overall mean effect, α_i the ith fixed diet effect, $d_{j(i)}$ the random effect of the jth lot (subjects) within the ith diet, δ_k the fixed kth time effect, $(\alpha\delta)_{ik}$ the fixed interaction effect between diet and time, b the common regression coefficient of initial value of x_{ij}, φ_j the slope deviation of the ith diet from common slope b, x_{ij} the initial record measure of lot j on diet i at the beginning of the experiment, and e_{ijk} the random error associated with the jth lot assigned to the ith diet at time k. Significant differences were declared at $P < 0.05$ and tendencies accepted if $P < 0.10$. Least squares means (adjusted for the covariance) are reported throughout.

3. RESULTS

Animal Performance and Milk Composition

To better illustrate the persistency of the response to MA supplementation, figures depict comparisons not only between treatments at each sampling time, but also among d 0 (control), d 6 (short term), d 24 (medium term), and d 54 (long term).

Supplementation with MA did not affect milk yield but decreased fat content ($P < 0.05$; Table 2). Differences were detected from d 18 onward (see Figure 1) and reached −17% at the end of the experiment (d 54). A negative effect of MA addition was also observed on milk fat yield ($P < 0.05$), whereas DMI and protein, lactose, and TS yields were not affected ($P > 0.10$).

Table 2. Dry matter intake, milk yield, and milk composition in ewes fed the experimental diets[1].

	Treatment			*P*-value[3]		
	SO	SOMA	SED[2]	D	T	D × T
DMI, g/d	2,651	2,651	103.9	0.999	<0.001	0.049
Yield, g/d						
Milk	2,081	2,069	155.1	0.942	<0.001	0.862
Fat	132.0	118.5	4.00	0.043	<0.001	0.011
Protein	101.9	102.0	3.21	0.982	<0.001	0.386
Lactose	103.7	104.8	2.33	0.656	0.061	0.988
TS	353.6	345.8	11.67	0.548	<0.001	0.660
Composition, g/100 g of raw milk						
Fat	6.29	5.61	0.221	0.037	<0.001	0.008
Protein	4.97	4.88	0.151	0.579	0.134	0.191
Lactose	4.98	4.96	0.089	0.830	<0.001	0.601
TS	17.16	16.35	0.323	0.067	<0.001	0.029

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]SED = standard error of the difference.
[3]Probability of significant effect of experimental diet (D), time on diet (T), and their interaction (D × T).

Milk FA Profile

Dietary supplementation with SOMA resulted in marked variation of milk FA composition relative to the control (SO) diet (see Table 3 and Supplemental Table S1, available at http://www.journalofdairyscience.org/). For most FA, the response to the addition of MA varied with time on diet, whereas the FA profile of milk from control ewes remained relatively constant throughout the trial, as shown in Figure 1.

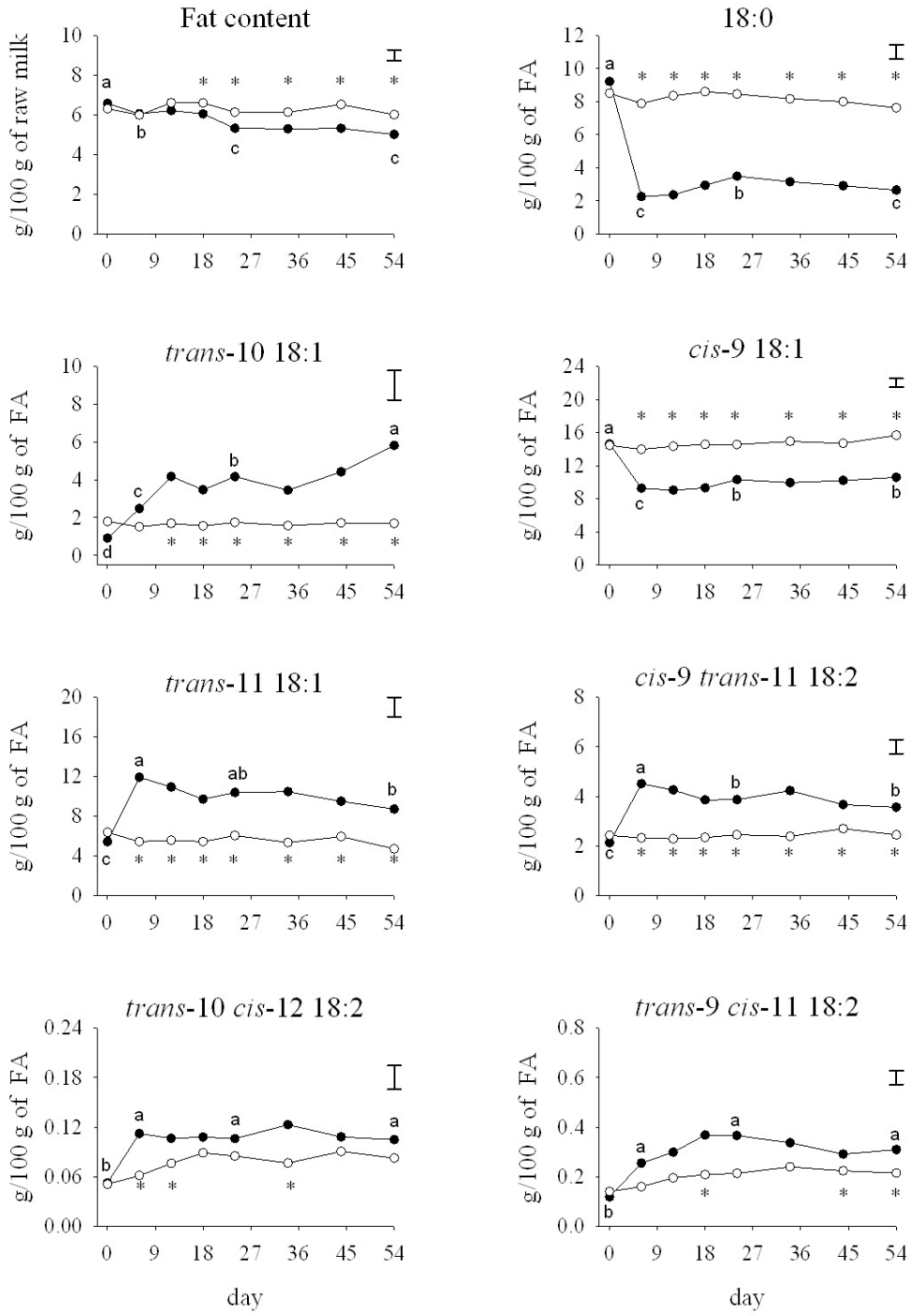

Figure 1. Temporal changes in milk fat content (g/100 g of raw milk) and 18:0, *trans*-10 18:1, *cis*-9 18:1, *trans*-11 18:1, *cis*-9, *trans*-11 18:2, *trans*-10, *cis*-12 18:2, and *trans*-9, *cis*-11 18:2 content (g/100 g of total FA) in ewes fed a TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet; ○) or 8 g of marine algae (SOMA; ●)/kg of DM. Values are the mean from 3 lots of 6 animals per lot; vertical bars represent the SE of the difference. *Differences ($P < 0.05$) between SO and SOMA treatments. $^{a\text{-}c}$Differences ($P < 0.05$) in SOMA treatment among d 0, 6, 24, and 54.

Inclusion of MA had no significant effect on the molar proportions of FA with fewer or more than 16 carbon atoms, but it did cause a reduction in the daily molar yield of FA that was comparable between those synthesized de novo or from plasma uptake (on average −15% vs. −12%, respectively; Table 3). Addition of MA decreased the concentration (g/100 g of total FA) of SFA ($P < 0.001$), mainly due to the substantial reduction of 18:0 (–56%). This response was evident from the first measurement (d 6) and persisted at a relatively constant level during the experimental period (Figure 1). The SOMA treatment affected the proportion of several odd- and branched-chain FA and increased that of saturated oxo-FA, especially 10-O-18:0 ($P < 0.001$).

Supplementation with MA reduced the content of *cis*-9 18:1 ($P < 0.001$), the changes with time on diet being very similar to that explained above for 18:0, although Δ^9-desaturase indices were not significantly affected (data not shown). In contrast, MA addition enhanced total *trans* MUFA ($P = 0.001$), especially *trans*-10 and *trans*-11 18:1 isomers but also several others (e.g., *trans*-4, *trans*-5, *trans*-6+7+8, *trans*-9, and *trans*-12 18:1). The content of *trans*-10 18:1 in the SOMA treatment increased continuously throughout the experiment and reached the greatest difference compared with the control on d 54 (244%). The isomer *trans*-11 18:1 increased quickly and reached the highest level of enrichment on d 6, but then declined until d 24 and remained stable thereafter. For both isomers, no differences among days were detected in animals fed the SO diet.

Supplementation with MA increased total CLA concentration ($P < 0.001$), without affecting significantly that of total nonconjugated 18:2. The content of *cis*-9, *trans*-11 18:2 increased rapidly in animals on the SOMA treatment, showing the maximum difference compared with SO on d 6 (94%) and decreasing gradually to 46% on d 54. Its milk content did not vary over time in ewes fed the control diet ($P > 0.10$). Two other CLA isomers, *trans*-10, *cis*-12 and *trans*-9, *cis*-11 18:2, also increased with the SOMA diet, and their response persisted throughout the trial. However, comparisons with the SO diet were only significant at some individual sampling times, probably because of the large variation within treatments.

Table 3. FA composition of milk from ewes fed the experimental diets[1] (for a complete profile, please see Suppl. Table S1, available at http://journalofdairyscience.org).

	Treatment			*P*-value[3]		
FA, g/100 g of total FA	SO	SOMA	SED[2]	D	T	D × T
Saturated FA						
4:0	3.35	3.27	0.065	0.298	0.001	0.169
6:0	2.61	2.49	0.039	0.020	<0.001	0.327
8:0	2.54	2.40	0.058	0.071	<0.001	0.259
10:0	7.60	7.42	0.228	0.467	<0.001	0.354
12:0	4.14	4.14	0.114	0.985	0.004	0.797
14:0	9.84	10.72	0.209	0.014	0.002	0.174
16:0	21.69	21.82	0.220	0.574	0.001	0.584
18:0	8.20	3.63	0.276	<0.001	<0.001	<0.001
10-oxo-18:0	0.05	0.41	0.027	<0.001	0.001	0.001
20:0	0.17	0.16	0.003	0.005	<0.001	0.195
22:0	0.12	0.13	0.004	0.027	0.262	0.084
Monounsaturated FA						
cis-9 14:1	0.18	0.20	0.021	0.483	<0.001	0.570
cis-9 16:1	0.60	0.57	0.028	0.356	<0.001	0.613
cis-9 18:1[4]	14.63	10.39	0.350	<0.001	<0.001	<0.001
cis-11 18:1	0.42	0.51	0.018	0.007	<0.001	0.001
cis-12 18:1	0.52	0.24	0.050	0.005	0.001	0.003
trans-9 18:1	0.51	0.72	0.024	<0.001	0.045	0.345
trans-10 18:1	1.66	3.61	0.557	0.016	<0.001	0.001
trans-11 18:1	5.49	10.23	0.514	0.003	0.032	0.014
trans-12 18:1	0.68	1.10	0.031	<0.001	0.092	0.630
Polyunsaturated FA						
cis-9, *cis*-12 18:2[5]	2.90	2.53	0.056	<0.001	<0.001	0.543
trans-11, *cis*-15 18:2	0.13	0.23	0.011	<0.001	<0.001	<0.001
cis-9, *trans*-11 18:2[6]	2.42	3.99	0.129	<0.001	0.522	0.064
trans-9, *cis*-11 18:2	0.20	0.29	0.018	0.001	<0.001	0.027
trans-10, *cis*-12 18:2	0.08	0.10	0.006	0.015	<0.001	0.348
cis-9, *cis*-12, *cis*-15 18:3[7]	0.49	0.48	0.010	0.327	0.005	0.307
Very long-chain PUFA						
20:4 n-3	0.002	0.034	0.0029	<0.001	<0.001	<0.001
20:5 n-3	0.04	0.06	0.003	<0.001	0.193	0.053
22:5 n-6	0.04	0.13	0.007	<0.001	0.095	0.065
22:5 n-3[8]	0.10	0.12	0.005	0.001	0.002	0.623
22:6 n-3	0.05	0.38	0.011	<0.001	<0.001	<0.001

(continued)

Table 3. (*continued*)

FA, g/100 g of total FA	Treatment		SED[2]	*P*-value[3]		
	SO	SOMA		D	T	D × T
Summary						
∑ Saturated FA	63.58	60.45	0.478	<0.001	0.005	0.002
∑ Monounsaturated FA	28.57	30.25	0.333	0.001	0.007	0.019
∑ Nonconjugated 18:2	3.82	3.71	0.058	0.101	0.158	0.991
∑ Conjugated 18:2	2.76	4.51	0.143	<0.001	0.583	0.058
∑ C20-22 n-3 PUFA	0.20	0.61	0.017	<0.001	<0.001	<0.001
∑ C20-22 n-6 PUFA	0.30	0.38	0.012	<0.001	0.123	0.178
Molar production (mol/d)						
<16 carbon FA	0.269	0.228	0.0058	0.001	0.023	0.518
16 carbon FA	0.120	0.109	0.0043	0.099	<0.001	0.009
>16 carbon FA	0.207	0.182	0.0254	0.098	<0.001	0.142

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]SED = standard error of the difference.
[3]Probability of significant effect of experimental diet (D), time on diet (T), and their interaction (D × T).
[4]Coelutes with *trans*-13+14 18:1.
[5]Coelutes with 9,15 18:2.
[6]Coelutes with *trans*-7, *cis*-9 18:2 + *cis*-14 20:1.
[7]Coelutes with *cis*-11 20:1.
[8]Coelutes with 26:0.

Most nonconjugated 18:2 tended to be enhanced by the addition of MA, although linoleic acid was always greater in ewes on SO than on SOMA diets ($P < 0.001$), and its temporal pattern was similar with both treatments.

Supplementation of the diet with MA caused a significant increase ($P < 0.001$) in long-chain n-3 PUFA (e.g., 20:3, 20:4, 20:5, 22:5, and 22:6 n-3) and a smaller but still significant increment in n-6 ($P < 0.01$), although the effect on particular FA within this latter group was variable. Inclusion of MA caused a large enrichment in the milk content of docosahexaenoic acid (DHA; $P < 0.001$), which was gradual during the first 3 wk and stable thereafter. At the end of the experiment (d 54), the concentration of DHA was 12-fold greater in the milk from ewes fed the SOMA diet than in milk from ewes on the SO diet, despite the limited transfer efficiency from diet to milk (about 15%).

4. DISCUSSION

Long-term effects of dietary marine n-3 PUFA on milk FA composition have been investigated in dairy cows (AbuGhazaleh and Holmes, 2007; Mohammed et al., 2011). However, reports on the persistency of the response to diet addition of marine lipids are still limited for dairy sheep.

Supplementation with marine lipids often decreases DMI in sheep (Papadopoulos et al., 2002; Reynolds et al., 2006; Toral et al., 2010a), although the low amount of MA used in the present study did not affect this parameter, and the literature provides evidence of no changes with higher doses of MA or fish oil (Capper et al., 2007; Toral et al., 2010b). Milk fat depression induced by marine lipids has only been reported when ewes are fed high-concentrate diets (Capper et al., 2007; Toral et al., 2010a,b), but information on its persistency is limited to just 4 wk. The results of the present study show that the MFD not only persisted but also increased progressively over the 8-wk experiment (see Figure 1).

As expected, diet supplementation with MA resulted in significant alterations in milk FA composition (Franklin et al., 1999; Reynolds et al., 2006; Boeckaert et al., 2008a). The discussion of these changes and their temporal profiles will be focused mainly on those FA potentially modified by altered rumen microbial biohydrogenation and previously related to MFD. Starting with 18:0 and *cis*-9 18:1, the marked decline in their milk concentration was consistent with changes in the FA profile observed not only in milk (Loor et al., 2005b; Gama et al., 2008; Toral et al., 2010b) but also in rumen digesta from cows and ewes fed marine lipids (Boeckaert et al., 2008b; Or-Rashid et al., 2008; Toral et al., 2012), because marine PUFA inhibit the complete biohydrogenation of unsaturated FA and result in a decrease in the ruminal outflow of 18:0. A lower availability of this FA for endogenous synthesis, via Δ^9-desaturation, of *cis*-9 18:1 may play a key role in MFD in animals consuming marine lipids (Loor et al., 2005b; Shingfield and Griinari, 2007). Oleic acid, in fact, has been indicated as one of the principal FA responsible for the maintenance of fluidity of milk fat globules in the mammary gland and, consequently, for their secretion (Timmen and Patton, 1988; Gama et al., 2008).

The lower content of 18:0 and *cis*-9 18:1 persisted until the end of the trial, which is in line with a previous study in cows fed MA for 20 d (Boeckaert et al., 2008a), but disagrees with the transient changes reported in cows fed fish oil and SO (Shingfield

et al., 2006). Variations in the amount and form of the lipid supplement and in the basal diet composition, as well as inter-species differences, might explain that different behavior over an extended period.

Results also showed an increase in milk *trans* 18:1 isomers, especially in *trans*-10 18:1, which arises from alternative pathways of biohydrogenation due to changes in rumen bacterial populations (Boeckaert et al., 2008b; Toral et al., 2012) and increased gradually throughout the whole experiment. This pattern was unexpected given the decrease observed by Toral et al. (2010b) and would refute the hypothesis of an adaptation of the rumen microbiota to the consumption of MA.

Although some controversy still exists in the literature (Lock et al., 2007; Shingfield et al., 2009), *trans*-10 18:1 has been related not only to MFD but also to a long-term persistency in cows fed fish oil (Shingfield et al., 2006; AbuGhazaleh and Holmes, 2007; Mohammed et al., 2011). In the present study, the evolution of this biohydrogenation intermediate followed an opposite trend to that observed for the concentration of milk fat (see Figure 1). However, previous studies in dairy sheep showed greater levels of *trans*-10 18:1 without concomitant reductions in milk fat synthesis (Reynolds et al., 2006; Gómez-Cortés et al., 2008), which would rule out this FA as the principal factor responsible for the observed MFD and suggest that other biohydrogenation intermediates should also contribute to the reduction in milk fat.

Evaluation of several studies indicates that 2 CLA isomers, *trans*-10, *cis*-12 18:2 and *trans*-9, *cis*-11 18:2, exert MFD effects in dairy cows and sheep (Lock et al., 2006; Shingfield and Griinari, 2007; Sinclair et al., 2010), although the concentration of the former showed no change in dairy ewes suffering from marine lipid-induced MFD (Toral et al., 2010a,b). In the present experiment, both CLA isomers increased with treatment and their response persisted until the end of the trial, although they were not strongly correlated with the content of milk fat in ewes on the SOMA diet.

As expected, inclusion of MA also induced an enrichment of the milk in some bioactive components, such as *trans*-11 18:1, *cis*-9, *trans*-11 18:2, and DHA (Papadopoulos et al., 2002; Reynolds et al., 2006; Toral et al., 2010b). With respect to very long-chain PUFA, the content of DHA reached a maximum by the end of the third week of MA addition and then remained relatively constant, which is consistent with previous findings in sheep and cows (Papadopoulos et al., 2002; Reynolds et al., 2006; Boeckaert et al., 2008a). The slight but significant increase observed in n-6

PUFA was due to the higher content of 22:5 n-6, attributable to its supply with MA (Mohammed et al., 2011).

Several milk odd- and branched chain-FA, which are largely derived from bacteria leaving the rumen and are therefore thought to reflect rumen function (Vlaeminck et al., 2006), were affected by dietary MA. Although this is in line with variations observed in the rumen digesta of lactating ewes fed SOMA (Toral et al., 2012), most changes (e.g., the increase in odd-chain *iso* FA, such as 17:0 *iso*) were only moderate compared with those reported in dairy cows (Shingfield et al., 2003, 2006; Boeckaert et al., 2008a). The increment in saturated oxo-FA was also consistent with changes in rumen bacterial populations in response to the presence of marine lipids and with the consequent shifts in biohydrogenation pathways (Toral et al., 2010c, 2012). The fact that most of these differences persisted throughout the experimental period supports that the rumen microbiota did not adapt to the consumption of MA, thus challenging the initial hypothesis.

5. CONCLUSIONS

The gradual and persistent increase in *trans* 18:1 isomers and the reduced availability of 18:0 for endogenous synthesis of *cis*-9 18:1 seem to play a key role in the persistency of MFD in lactating ewes fed marine algae. Further studies, however, are required to elucidate the mechanisms underlying this response in dairy sheep. The fact that the persistency lasted until the end of the monitored period (54 d) suggests that the rumen microbiota failed to adapt to dietary supply of very long-chain n-3 PUFA, as hypothesized.

6. REFERENCES

AbuGhazaleh, A. A., and Holmes, L. D. 2007. Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. J. Dairy Sci. 90:2897-2904.

AOAC International. 2006. Official Methods of Analysis of AOAC International. 18th ed. 1st rev. AOAC Int., Gaithersburg, MD.

AOCS (American Oil Chemists' Society). 2008. Official Methods and Recommended Practices of the American Oil Chemists' Society. 5th ed. 2nd printing. AOCS, Urbana, IL.

Boeckaert, C., Vlaeminck, B., Dijkstra, J., Issa-Zacharia, A., Van Nespen, T., Van Straalen, W., and Fievez, V. 2008a. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91:4714-4727.

Boeckaert, C., Vlaeminck, B., Fievez, V., Maignien, L., Dijkstra, J., and Boon, N. 2008b. Accumulation of *trans* C18:1 fatty acids in the rumen after dietary algal supplementation is associated with changes in the *Butyrivibrio* community. Appl. Environ. Microbiol. 74:6923-6930.

Capper, J. L., Wilkinson, R. G., Mackenzie, A. M., and Sinclair, L. A. 2007. The effect of fish oil supplementation of pregnant and lactating ewes on milk production and lamb performance. Animal 1:889-898.

Franklin, S., Martin, K., Baer, R., Schingoethe, D., and Hippen, A. 1999. Dietary marine algae (*Schizochytrium* sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J. Nutr. 129:2048-2054.

Gama, M. A. S., Garnsworthy, P. C., Griinari, J. M., Leme, P. R., Rodrigues, P. H. M., Souza, L. W. O., and Lanna, D. P. D. 2008. Diet-induced milk fat depression: Association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115:319-331.

Gebauer, S. K., Chardigny, J. M., Jakobsen, M. U., Lamarche, B., Lock, A. L., Proctor, S. D., and Baer, D. J. 2011. Effects of ruminant *trans* fatty acids on cardiovascular disease and cancer: A comprehensive review of epidemiological, clinical, and mechanistic studies. Adv. Nutr. 2:332-354.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A. and Hervás, G. 2008. Milk production, conjugated linoleic acid content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. J. Dairy Sci. 91:1560-1569.

Griinari, J. M., Dwyer, D. A., McGuire, M. A., Bauman, D. E., Palmquist, D. L., and Nurmela, K. V. V. 1998. *Trans*-octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251-1261.

ISO (International Organization for Standardization). 1999a. Animal feeding stuffs—Determination of moisture and other volatile matter content. ISO 6496:1999. ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 1999b. Whole milk—Determination of milk fat, protein and lactose content—Guidance on the operation of mid-infrared instruments. ISO 9622:1999. ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2002. Animal feeding stuffs—Determination of crude ash. ISO 5984:2002. ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2009. Animal feeding stuffs—Determination of nitrogen content and calculation of crude protein content—Part 2: Block digestion and steam distillation method. ISO 5983-2:2009. ISO, Geneva, Switzerland.

Kramer, J. K. G., Hernandez, M., Cruz-Hernandez, C., Kraft, J., and Dugan, M. E. R. 2008. Combining results of two GC separations partly achieves determination of all *cis* and *trans* 16:1, 18:1, 18:2 and 18:3 except CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. Lipids 43:259-273.

Lock, A. L., Teles, B. M., Perfield II, J. W., Bauman, D. E., and Sinclair, L. 2006. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 reduces milk fat synthesis in lactating sheep. J. Dairy Sci. 89:1525-1532.

Lock, A. L., Tyburczy, C., Dwyer, D. A., Harvatine, K. J., Destaillats, F., Mouloungui, Z., Candy, L., and Bauman, D. E. 2007. *Trans*-10 octadecenoic acid does not reduce milk fat synthesis in dairy cows. J. Nutr. 137:71-76.

Loor, J., Doreau, M., Chardigny, J., Ollier, A., Sébédio, J., and Chilliard, Y. 2005b. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of *trans*-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim. Feed Sci. Technol. 119:227-246.

Loor, J. J., Ferlay, A., Ollier, A., Doreau, M., and Chilliard, Y. 2005a. Relationship among *trans* and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. J. Dairy Sci. 88:726-740.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217-1240.

Mohammed, R., Khorasani, R. G., Goonewardene, L. A., Kramer, J. K. G., and Kennelly, J. J. 2011. Persistency of milk *trans*-18:1 isomers and rumenic acid in Holstein cows over a full lactation. Can. J. Anim. Sci. 91:147-167.

Or-Rashid, M. M., Kramer, J. K. G., Wood, M. A., and McBride, B. W. 2008. Supplemental algal meal alters the ruminal *trans*-18:1 fatty acid and conjugated linoleic acid composition in cattle. J. Anim. Sci. 86:187-196.

Papadopoulos, G., Goulas, C., Apostolaki, E., and Abril, R. 2002. Effects of dietary supplements of algae, containing polyunsaturated fatty acids, on milk yield and the composition of milk products in dairy ewes. J. Dairy Res. 69:357-365.

Perfield II, J. W., Lock, A. L., Griinari, J. M., Sæbø, A., Delmonte, P., Dwyer, D. A., and Bauman, D. E. 2007. *Trans*-9, *cis*-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90:2211-2218.

Reynolds, C. K., Cannon, V. L., and Loerch, S. C. 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131:333-357.

Shingfield, K. J., Ahvenjärvi, S., Toivonen, V., Äröla, A., Nurmela, K. V. V., Huhtanen, P., and Griinari, J. M. 2003. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim. Sci. 77:165-179.

Shingfield, K. J., and Griinari, J. M. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Sci. Technol. 109:799-816.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., and Beever, D. E. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., Sæbø, A., Sæbø, P. C., Toivonen, V., and Griinari, J. M. 2009. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Sinclair, L. A., Weerasinghe, W. M. P. B., Wilkinson, R. C., de Veth, M. J., and Bauman, D. E. 2010. A supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk

fat yield but does not alter organ weight or body fat deposition in lactating ewes. J. Nutr. 140:1949-1955.

Timmen, H., and Patton, S. 1988. Milk fat globules: Fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685-689.

Toral, P. G., Belenguer, A., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2012. Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 95:794-806.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2010a. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. J. Dairy Sci. 93:1604-1615.

Toral, P. G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., and de la Fuente, M. A. 2010b. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93:1655-1667.

Toral, P. G., Shingfield, K. J., Hervás, G., Toivonen, V., and Frutos, P. 2010c. Effect of fish oil and sunflower oil on rumen fermentation characteristics and fatty acid composition of digesta in ewes fed a high concentrate diet. J. Dairy Sci. 93:4804-4817.

Vlaeminck, B., Fievez, V., Cabrita, A. R. J., Fonseca, A. J. M., and Dewhurst, R. J. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131:389-417.

7. SUPPLEMENTARY MATERIAL

Table S1. Other FA of milk from ewes fed the experimental diets[1] (to complete the FA profile shown in Table 3).

	Treatment			*P*-value[3]		
FA, g/100 g of total FA	SO	SOMA	SED[2]	D	T	D × T
Saturated FA						
5:0	0.01	0.02	0.001	0.227	0.125	0.571
7:0	0.02	0.02	0.002	0.701	<0.001	0.297
9:0	0.04	0.04	0.002	0.774	<0.001	0.338
11:0	0.07	0.07	0.006	0.787	0.004	0.789
13:0 *iso*	0.017	0.021	0.0011	0.023	0.088	0.785
13:0 *anteiso*	0.01	0.01	0.001	0.483	0.134	0.584
4,8,12 trimethyl-13:0	0.15	0.16	0.008	0.545	<0.001	0.809
14:0 *iso*	0.08	0.07	0.004	0.368	0.280	0.505
15:0 *iso*	0.17	0.17	0.012	0.968	0.037	0.750
15:0 *anteiso*	0.31	0.32	0.022	0.798	0.013	0.652
15:0	0.72	0.74	0.029	0.534	0.190	0.321
16:0 *iso*	0.25	0.21	0.011	0.037	0.006	0.142
8-oxo-16:0	0.01	0.05	0.005	0.002	0.017	<0.001
17:0 *iso*	0.37	0.42	0.019	0.042	0.042	0.123
17:0 *anteiso*	0.47	0.46	0.006	0.031	<0.001	0.460
17:0	0.46	0.44	0.004	<0.001	0.147	0.735
18:0 *iso*	0.027	0.031	0.0012	0.057	0.696	0.506
13-oxo-18:0	0.05	0.04	0.005	0.089	0.057	0.105
19:0	0.21	0.15	0.005	<0.001	0.004	0.002
21:0	0.07	0.07	0.006	0.843	0.001	0.239
23:0	0.04	0.04	0.003	0.376	0.010	0.003
24:0	0.04	0.04	0.003	0.893	0.719	0.344
Monounsaturated FA						
cis-9 10:1	0.23	0.21	0.015	0.267	0.021	0.033
cis-9 12:1	0.06	0.05	0.007	0.311	0.064	0.127
trans-9 12:1	0.04	0.04	0.004	0.728	0.017	0.547
cis-12 14:1	0.04	0.04	0.005	0.514	0.010	0.046
trans-5+6 14:1	0.038	0.044	0.0011	<0.001	<0.001	0.040
trans-5 15:1	0.11	0.12	0.005	0.334	<0.001	0.757
trans-6+7 15:1	0.027	0.032	0.0011	0.008	0.003	0.645
cis-7 16:1	0.37	0.38	0.007	0.166	0.049	0.208
cis-14 16:1	0.07	0.06	0.008	0.479	0.002	0.114
trans-5+6+8 16:1	0.12	0.18	0.005	<0.001	0.031	0.091
trans-9 16:1	0.43	0.63	0.020	<0.001	<0.001	<0.001
cis-9 17:1	0.14	0.11	0.009	0.050	0.089	0.007
trans-10 17:1	0.04	0.04	0.002	0.374	0.002	0.567
cis-13 18:1	0.09	0.08	0.006	0.423	0.417	0.619
cis-15 18:1	0.10	0.12	0.008	0.051	0.024	0.077
cis-16 18:1	0.11	0.11	0.008	0.990	0.411	0.303

(continued)

Table S1. (*continued*)

FA, g/100 g of total FA	Treatment		SED[2]	*P*-value[3]		
	SO	SOMA		D	T	D × T
trans-4 18:1	0.03	0.06	0.002	<0.001	<0.001	0.001
trans-5 18:1	0.03	0.07	0.002	<0.001	0.028	0.025
trans-6+7+8 18:1	0.59	0.77	0.015	0.001	0.394	0.332
trans-15 18:1[4]	0.31	0.30	0.012	0.582	0.121	0.541
trans-16 18:1[5]	0.35	0.31	0.030	0.259	0.162	0.320
cis-13 22:1	0.01	0.02	0.001	<0.001	0.169	0.033
Polyunsaturated FA						
cis-9, *trans*-12 18:2	0.20	0.21	0.014	0.346	0.164	0.598
trans-9, *cis*-12 18:2	0.06	0.11	0.003	<0.001	0.539	0.898
trans-9, *trans*-12 18:2	0.03	0.05	0.002	<0.001	<0.001	0.938
trans-11, *trans*-15 18:2	0.066	0.072	0.0020	0.049	0.001	0.007
9,13 18:2	0.29	0.32	0.015	0.083	0.363	0.668
10,14 18:2	0.14	0.16	0.007	0.028	0.052	0.652
trans-9, *trans*-11 18:2[6]	0.06	0.07	0.003	0.175	0.037	0.515
trans-11, *trans*-13 18:2	0.01	0.01	0.001	0.378	0.001	0.615
cis-6, *cis*-9, *cis*-12 18:3	0.05	0.03	0.004	0.021	0.003	0.056
Very long-chain PUFA						
cis-11, *cis*-14 20:2	0.042	0.036	0.003	0.070	<0.001	0.672
cis-8, *cis*-11, *cis*-14 20:3	0.03	0.04	0.001	<0.001	0.809	0.024
cis-11, *cis*-14, *cis*-17 20:3	0.01	0.02	0.002	0.010	<0.001	0.006
cis-5, *cis*-8, *cis*-11, *cis*-14 20:4	0.14	0.13	0.005	0.185	<0.001	0.064
cis-7, *cis*-10, *cis*-13, *cis*-16 22:4	0.05	0.04	0.002	0.053	0.322	0.852

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]SED = standard error of the difference.
[3]Probability of significant effect of experimental diet (D), time on diet (T), and their interaction (D × T).
[4]Coelutes with *cis*-10 18:1.
[5]Coelutes with *cis*-14 18:1.
[6]Coelutes with *trans*-10, *trans*-12 18:2 + *trans*-8, *trans*-10 18:2.

PRUEBA III

DIETARY MARINE ALGAE AND ITS INFLUENCE ON TISSUE GENE NETWORK EXPRESSION DURING MILK FAT DEPRESSION IN DAIRY EWES

E. Bichi[1,2], P. Frutos[1], P.G. Toral[1], D. Keisler[3], G. Hervás[1], and J.J. Loor[2]

[1]*Instituto de Ganadería de Montaña, CSIC-Universidad de León, Finca Marzanas s/n, 24346 Grulleros, León, España*

[2]*Mammalian NutriPhysioGenomics, Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana 61801, USA*

[3]*Department of Animal Science, University of Missouri, Columbia, MO 65211, USA*

Animal Feed Science and Technology 186 (2013) 36-44

Doi:10.1016/j.anifeedsci.2013.09.010

DIETARY MARINE ALGAE AND ITS INFLUENCE ON TISSUE GENE NETWORK EXPRESSION DURING MILK FAT DEPRESSION IN DAIRY EWES

E. Bichi, P. Frutos, P.G. Toral, D. Keisler, G. Hervás, and J.J. Loor

Abstract. Supplementation of a linoleic acid-rich diet with marine algae reduces milk fat content while impacting milk fatty acid profile in dairy sheep. Unlike other ruminant species, in ovine there are limited data on the molecular mechanisms that may regulate adipose, liver, and mammary responses to dietary marine lipids. This study was conducted to investigate changes in mRNA expression and relative mRNA abundance of key enzymes involved in lipid metabolism in mammary, subcutaneous adipose and liver tissue in response to long-term milk fat depression induced by marine algae. Eleven Assaf ewes were randomly assigned to 2 experimental diets consisting of a TMR based on alfalfa hay and concentrate (40:60) supplemented with 25 g of sunflower oil/kg DM plus 0 (SO; control diet) or 8 g of marine algae/kg of DM (SOMA diet) for 54 d. Quantitative reverse transcription-PCR was used to study expression of target genes in tissues harvested at slaughter at the end of the feeding period (54 d). There was no effect of SOMA on mammary and adipose tissue expression of genes encoding proteins required for fatty acid uptake and activation (*ACSS2*, *LPL*), intracellular fatty acid transport (*FABP3*, *FABP4*), de novo fatty acid synthesis (*ACACA*, *FASN*), esterification (*DGAT1*, *DGAT2*, *LPIN1*), desaturation (*SCD*), elongation (*ELOVL6*), transcriptional regulation (*INSIG1*, *MED1*, *PPARG*, *RXRA*, *SCAP*, *SREBF1*, *THRSP*) and lipid droplet formation (*ADFP*, *BTN1A1*, *XDH*). Abundance of *PPARG* (0.04%) and *INSIG1* (2.22%) in mammary tissue was markedly greater than that of *SREBF1* (0.002%), suggesting that they may play a more important role in milk fat synthesis regulation. Addition of marine algae did not affect the expression of ß-oxidation- and lipoprotein-related genes (*ACOX1*, *APOB*, *CPT1A*, *PPARA*, *RXRA*) in hepatic tissue. However, feeding SOMA up-regulated hepatic *HMGCS2*, which controls ketogenesis. Concentration of plasma ß-hydroxybutyrate, NEFA, glucose, triacylglycerol, growth hormone, insulin-like growth factor 1, insulin, and leptin was not different between groups at d 54. Taken together with the milk fat responses and previous data from bovine fed similar diets, results suggest that transcriptional control mechanisms regulating fat synthesis in mammary secretory tissue were likely established during earlier stages of the feeding period.

Keywords: marine lipids, gene expression, lipid metabolism, sheep

1. INTRODUCTION

Inclusion of marine lipids in the diet of dairy sheep represents an effective nutritional strategy for altering milk fat composition (Papadopoulos et al., 2002; Reynolds et al., 2006; Capper et al., 2007). However, dietary marine algae (MA) have recently been associated with milk fat depression (MFD) in dairy ewes (Toral et al., 2010a; Bichi et al., 2013). Milk fat synthesis is known to involve the coordinated expression of several transcription regulators and their target genes (Bionaz and Loor, 2008). The role of altered lipogenic gene expression has been examined in lactating dairy cows fed a MFD diet (Piperova et al., 2000; Ahnadi et al., 2002; Harvatine and Bauman, 2006), and the transcriptomic adaptations outlined not only in mammary (Invernizzi et al., 2010) but also in adipose tissue (Thering et al., 2009).

Some long-chain fatty acids (LCFA) including 16:0, 18:0, *trans*-10 18:1, and *trans*-10, *cis*-12 18:2 alter bovine mammary cell lipogenic gene networks and in turn lipid droplet synthesis (Kadegowda et al., 2009). In particular, the *trans*-10 LCFA intermediates arising from ruminal metabolism of dietary unsaturated FA (Bauman et al., 2011) are potent inhibitors of lipogenesis via their negative effect on transcriptional control mechanisms regulating target genes (Kadegowda et al., 2009).

Data on the nutritional regulation of expression of lipogenic enzymes in small ruminants are limited (Agazzi et al., 2010; Shingfield et al., 2013; Hussein et al., 2013). Previous studies with goats evaluated lipogenic gene expression in adipose and mammary tissue in response to dietary vegetable and fish oil (Bernard et al., 2009a,b; Li et al., 2012; Toral et al., 2013) but direct comparisons between the sheep and the goat have identified species-specific differences in mRNA levels of mammary lipogenic genes (Tsiplakou et al., 2009). Furthermore, the goat appears to be less responsive to MFD than the ewe (Bernard et al., 2012; Shingfield et al., 2013). In lactating ewes, exogenous *trans*-10, *cis*-12 18:2 induced MFD (Lock et al., 2006; Sinclair et al., 2010; Oliveira et al., 2012; Hussein et al., 2013), but this isomer does not seem to play a relevant role in diet-induced MFD (Toral et al., 2010a,b; Bichi et al., 2013). To our knowledge, there is only limited information about the molecular adaptations in ovine tissues in response to diets that induce MFD (Hussein et al., 2013).

The objective of this study was to use adipose, liver, and mammary tissue from sheep fed a linoleic acid-rich TMR supplemented with MA that resulted in MFD

(Bichi et al., 2013) to evaluate mRNA expression of transcription regulators and target genes to better understand the role of transcriptional mechanisms in the long-term nutritional regulation of diet-induced MFD.

2. MATERIALS AND METHODS

Animals, Experimental Diets and Management

All experimental procedures were performed in accordance with the Spanish Royal Decree 1201/2005 for the protection of animals used for experimental and other scientific purposes. Details of the experimental procedures have been described previously (Bichi et al., 2013). Briefly, eleven fat-tailed Assaf ewes (82.4 ± 3.26 kg BW) in mid-lactation (84 ± 2.3 DIM at the beginning of the experiment) were randomly assigned to two experimental diets consisting of a TMR based on alfalfa hay and a concentrate (40:60) supplemented with 25 g of sunflower oil (Carrefour S.A., Madrid, Spain)/kg of DM plus 0 (SO; control diet, n = 5) or 8 g of marine algae (DHA Gold Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD)/kg of DM (SOMA diet, n = 6). Ingredients, chemical composition and FA profile of the experimental diets have been reported previously (Bichi et al., 2013). Fresh diets were offered daily ad libitum at 0900 and 1900 h and clean water was always available. Ewes were milked twice daily at approximately 0830 and 1830 h in a 1 × 10 stall milking parlor (DeLaval, Madrid, Spain) throughout the 54 d experiment.

Measurements, Sampling Procedures and Chemical Analysis

Blood samples from the jugular vein were individually collected in 10-mL Vacutainer tubes (BD Vacutainer, Plymouth, UK) containing lithium heparin, before the morning milking on d 54. Plasma obtained after centrifugation (3000 rpm, 10 min, 4°C) was stored at −30°C. Concentrations of glucose, triacylglycerol (TAG), BHBA, and NEFA were analyzed at the Veterinary Diagnostics Laboratory, College of Veterinary Medicine, University of Illinois. Concentration of insulin was analyzed using a commercial kit from Mercodia (Cat#10-1201-01). Leptin, growth hormone (GH), and IGF-1 were measured via Radioimmunoassay (RIA).

At the end of the experiment, the ewes were slaughtered humanely via intravenous

injection of an euthanasia solution (T-61, Intervet, Salamanca, Spain). Then, mammary, subcutaneous adipose from the tail-head region, and liver tissue samples were collected from each animal under sterile conditions. Tissue samples were immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction.

RNA Extraction, Quantitative Reverse Transcription-PCR, Normalization, and Percentage Relative mRNA Abundance

Complete details of these procedures can be found in the Supplemental material. Briefly, the total RNA of mammary, adipose and liver tissue was extracted using ice-cold Trizol (Invitrogen Corp., Carlsbad, CA) as described by Loor et al. (2005). Genomic DNA was removed using RNeasy Mini Kit columns (Qiagen, Hilden, Germany). The purity and concentration of the RNA from each sample were measured using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative reverse transcription-PCR was carried out as described by Graugnard et al. (2010). The final data were normalized using the geometric mean of 4 internal control genes (*UXT*, *EIF3K*, *TUBB*, and *YWHAZ*). The relative % mRNA abundance for each gene was calculated using the median ΔCt values, corrected for the efficiency of PCR amplification (Bionaz and Loor, 2008).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (Version 9.2; SAS Institute Inc., Cary, NC). The statistical model included the fixed effect of experimental treatment and the random effect of animal, nested within the diet to contrast the effect of the algae supplementation. Before statistical analysis, data of gene expression were log-transformed ($\log_2$ scale). The UNIVARIATE procedure of SAS was used to identify and remove outliers from the data set. Significant differences were declared at $P < 0.05$ and tendencies at $P < 0.10$.

3. RESULTS

Plasma Metabolite Concentrations

No differences between diets were observed for the plasma concentration of glucose, BHBA, NEFA, TAG, GH, IGF-1, insulin, and leptin concentration ($P > 0.05$; Table 1).

Table 1. Concentration of plasma metabolites in dairy ewes after 54 days on the experimental diets.[1]

	Treatment			
Item	SO	SOMA	SEM	*P*-value[2]
BHBA, mmol/L	0.59	0.49	0.061	0.23
Glucose, mg/dL	49.7	58.8	4.07	0.13
NEFA, mmol/L	0.16	0.12	0.018	0.19
Triacylglycerol, mg/dL	34.5	29.4	4.15	0.38
Growth hormone, ng/mL	1.20	0.87	0.305	0.43
IGF-1, ng/mL	219.7	195.9	14.62	0.26
Insulin, μg/L	0.59	0.76	0.172	0.49
Leptin, ng/mL	17.9	16.6	2.062	0.66
NEFA:Insulin	0.32	0.21	0.067	0.24
Glucose:Insulin	113.2	82.6	16.49	0.20
IGF-1:GH	242.9	260.1	89.72	0.89

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]Probability of significant differences between diets.

mRNA Expression in Mammary, Adipose and Liver Tissue

In mammary and adipose tissue, feeding MA had no effect ($P > 0.05$; Table 2) on the expression of mRNA encoding for genes associated with LCFA uptake (*LPL*), intracellular acetate activation (*ACSS2*), intracellular LCFA transport (*FABP3*), desaturation (*SCD*), elongation (*ELOVL6*), esterification (*DGAT1*, *DGAT2*, *LPIN*), and de novo FA synthesis (*ACACA*, *FASN*). Furthermore, MA did not affect ($P > 0.05$) the expression of transcription regulators (*INSIG1*, *MED1*, *PPARG*, *RXRA*, *SCAP*, *SREBF1*, *THRSP*). Expression of milk fat globule membrane proteins (*BTN1A1*, *XDH*) in mammary tissue did not differ ($P > 0.05$) due to MA.

In the liver, there were no differences ($P > 0.05$) between treatments in mRNA encoding genes associated with lipoprotein metabolism (*APOB*), LCFA oxidation

(*ACOX1*, *CPT1A*) and regulation of transcription (*PPARA*, *RXRA*); whereas mRNA expression of *HMGCS2*, a gene associated with ketogenesis, was greater ($P < 0.05$) in response to SOMA (Table 2).

Table 2. Gene expression (Log_2-transformed) in mammary, adipose and liver tissue in dairy ewes after 54 days on the experimental diets.[1]

	Treatment			
	SO	SOMA	SEM	*P*-value[2]
Mammary				
ACACA	-1.03	-1.26	0.210	0.43
ACSS2	1.75	1.36	0.325	0.38
BTN1A1	1.51	1.20	0.342	0.49
DGAT1	0.27	0.11	0.317	0.71
ELOVL6	-5.13	-5.20	0.389	0.89
FABP3	1.40	1.05	0.620	0.67
FASN	-1.28	-1.66	0.470	0.54
INSIG1	0.77	0.28	0.325	0.27
LPIN1	0.77	0.59	0.464	0.78
LPL	1.08	1.06	0.237	0.96
MED1	0.03	-0.06	0.077	0.39
PPARG	-1.34	-1.79	0.350	0.35
RXRA	-0.24	-0.31	0.052	0.35
SCAP	0.55	0.50	0.102	0.72
SCD	0.82	0.54	0.241	0.41
SREBF1	-0.03	-0.33	0.323	0.50
THRSP	-10.59	-10.50	0.693	0.93
XDH	1.41	0.97	0.245	0.21
Adipose				
ACACA	0.61	1.13	0.411	0.37
ACSS2	-0.35	-0.48	0.229	0.69
ADFP	-4.26	-4.05	0.307	0.63
DGAT2	-0.72	-0.19	0.675	0.58
ELOVL6	0.44	1.16	0.525	0.33
FABP4	0.93	0.98	0.194	0.86
FASN	-2.79	-2.88	0.717	0.93
INSIG1	-1.89	-1.88	0.419	0.99
LPIN1	0.12	0.19	0.240	0.83
LPL	-0.26	-0.02	0.302	0.56
MED1	-0.18	-0.34	0.112	0.31
PPARG	2.48	2.90	0.232	0.22
RXRA	0.96	0.58	0.165	0.12
SCAP	0.55	0.64	0.235	0.80
SCD	-0.00	0.81	0.570	0.32
SREBF1	0.71	0.63	0.373	0.87
THRSP	0.30	0.66	0.976	0.79

(continued)

Table 2. *(continued)*

	Treatment			
	SO	SOMA	SEM	*P*-value[b]
Liver				
ACOX1	-1.37	-1.12	0.207	0.40
APOB	-0.58	-0.36	0.195	0.42
CPT1A	-0.83	-0.75	0.110	0.58
HMGCS2	-0.96	-0.26	0.204	0.03
PPARA	-0.60	-0.36	0.118	0.17
RXRA	0.03	0.17	0.148	0.51

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]Probability of significant differences between diets.

Percentage Relative mRNA Abundance among Measured Genes

Analysis of percentage relative mRNA is presented in Figure 1. Results revealed that in mammary gland tissue the most-abundant genes, accounting for >20% of total measured mRNAs, were *BTN1A1* (27%), *SCD* (24%), *LPL* (24%), and *XDH* (21%). The abundance of *PPARG* and *INSIG1* was 0.04% and 2.2% compared with *SREBF1* and *THRSP* which averaged 0.002% and 0.001%. All other genes had relative abundance <1.0%.

In the adipose tissue the highest relative % mRNA abundance was observed for *FABP4* (47%) followed by *SCD* (31%) and *LPL* (13%). The abundance of *PPARG* was ∼1% and that of *THRSP* and *SREBF1* (the least-abundant gene) 1.4% and 0.003%. Expression of *INSIG1* averaged 0.45% and, except for *ACACA* (1.8%) and *ELOVL6* (2.5%), most other lipogenic and esterification enzymes had relative abundance <1.0%.

In the liver the highest relative % mRNA abundance was observed for *APOB* (85%). The abundance of *PPARA* was markedly lower (0.25%) compared with *RXRA* (6.3%) and the ketogenic enzyme *HMGCS2* (7.3%). The lowest abundance was observed for *CPT1A1* (0.13%).

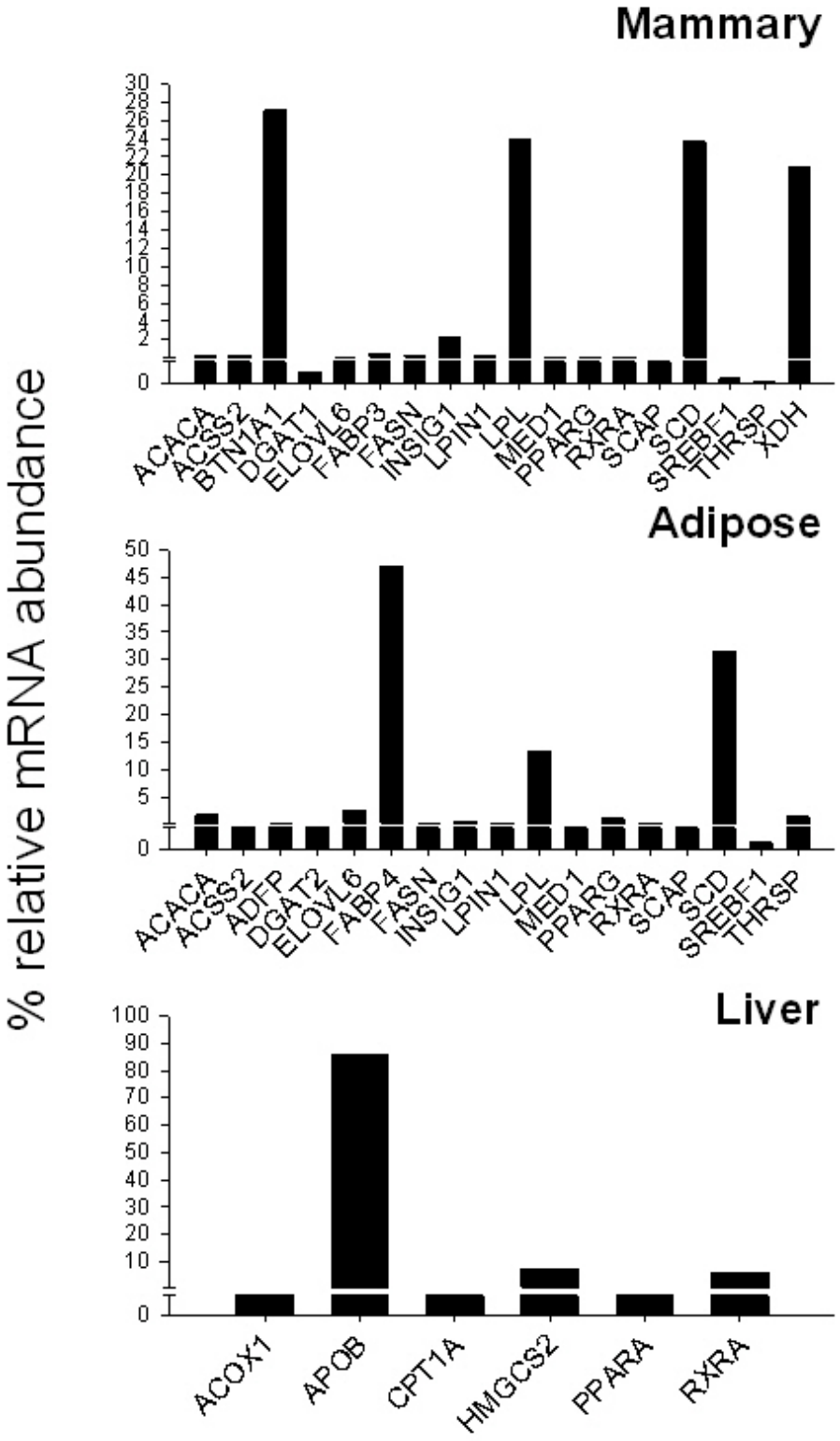

Figure 1. Percentage relative mRNA abundance among measured genes in mammary, adipose and liver tissue. Values below the break line were <0.04%, <0.10%, and <0.40% of total measured mRNA in mammary, adipose, and liver tissue, respectively.

4. DISCUSSION

As pointed out in the companion study (Bichi et al., 2013), it was evident that the addition of MA to a linoleic acid-rich diet in dairy sheep not only led to a lower milk fat yield and content, but also to an important change in milk FA profile, with a strong decrease in the concentration of 18:0 and *cis*-9 18:1 and an increase in *trans*-10 18:1, as well as *cis*-9, *trans*-11 18:2 and n-3 PUFA (Table 3).

Table 3. Milk and fat yield, fat content, and fatty acid (FA) profile in dairy ewes after 54 days on the experimental diets.[1]

	Treatment			
Item	SO	SOMA	SEM	*P*-value[2]
Milk yield, g/d	1,274	1,608	204.6	0.26
Fat yield, g/d	80.9	75.1	13.19	0.75
Fat content, g/100 g of raw milk	6.57	4.80	0.323	0.003
FA, g/100g of total FA				
4:0	3.60	3.50	0.146	0.65
6:0	2.65	2.15	0.240	0.16
8:0	2.46	1.85	0.278	0.14
10:0	7.01	5.44	0.837	0.20
12:0	3.83	3.48	0.342	0.46
14:0	9.89	11.16	0.490	0.07
cis-9 14:1	0.25	0.28	0.042	0.55
15:0	0.72	0.73	0.026	0.72
16:0	20.88	23.36	0.860	0.06
cis-9 16:1	0.76	0.87	0.093	0.41
17:0	0.44	0.42	0.028	0.64
18:0	8.26	2.04	0.553	<0.001
cis-9 18:1[3]	16.80	10.33	1.115	0.002
Other *cis*-18:1	1.27	1.21	0.120	0.70
trans-10 18:1	0.77	6.91	1.465	0.01
trans-11 18:1	5.89	8.85	1.182	0.10
Other *trans*-18:1	2.49	3.04	0.200	0.07
cis-9, *cis*-12 18:2[4]	2.57	2.01	0.213	0.08
cis-9, *trans*-11 18:2[5]	2.93	3.52	0.670	0.53
trans-9, *cis*-11 18:2	0.14	0.26	0.038	0.04
trans-10, *cis*-12 18:2	0.04	0.07	0.006	0.01
cis-9, *cis*-12, *cis*-15 18:3	0.41	0.31	0.041	0.10
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5	0.03	0.06	0.006	<0.001
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6	0.06	0.49	0.033	<0.001
Other FA	5.85	7.64	0.231	<0.001

[1]Refers to TMR containing 25 g of sunflower oil/kg of DM and supplemented with 0 (SO; control diet) or 8 g of marine algae (SOMA diet)/kg of DM.
[2]Probability of significant differences between diets.
[3]Coelutes with *trans*-13+14 18:1.
[4]Coelutes with 9,15 18:2.
[5]Coelutes with *trans*-7, *cis*-9 18:2 + *cis*-14 20:1.

Therefore, the objective of the present study was to investigate if the MFD induced by MA and the related changes in milk FA profile are associated with a coordinated down-regulation in mRNA expression of genes with key functions in the overall process of milk fat synthesis as recently observed in CLA-induced MFD (Hussein et al., 2013).

Mammary Lipid Metabolism

The changes in milk FA profile and the MFD due to SOMA were not associated with statistical differences in mRNA expression of mammary genes at the end of the 54 d feeding period. This contrasts recent findings by Hussein et al. (2013) during CLA-induced MFD in ewes where the remarkable increase in milk *trans*-10, *cis*-12 18:2 concentration was correlated with the coordinated down-regulation in transcript abundance of lipogenic enzymes involved in mammary lipid synthesis. Even though a slight but significant increase of *trans*-10, *cis*-12 18:2 concentration also was evident in the present study (Table 3), our previous study partly ruled out an involvement of this CLA in causing and maintaining MFD due to feeding SOMA because of its low content and the lack of correlation with milk fat content (Bichi et al., 2013). The lack of difference in mRNA expression of mammary genes in our study is consistent with data from cows fed fish oil and experiencing MFD demonstrated that changes in the mammary transcriptome, as well as in milk FA profile, occur relatively quickly after initiation of treatments (Invernizzi et al., 2010) such that once milk fat concentration was set and remained nearly unchanged, there were no differences in gene expression despite marked MFD.

The absence of differences in mammary mRNA expression of genes involved in the de novo synthesis (*ACACA*, *FASN*), as well as uptake, transport and trafficking of FA in the cells (*LPL*, *FABP3*) or coding for the major proteins of the milk fat globule membrane (*BTN1A1*, *XDH*), was similar to previous observations in goats fed plant oils (Ollier et al., 2009; Bernard et al., 2012). In relative terms our data revealed that sheep mammary tissue expresses similar amounts of *BTN1A1* and *XDH*, while in cows *XDH* is more abundant (Bionaz and Loor, 2008). Conversely, marine lipid in cows has sometimes been associated with a down-regulation of mammary *LPL* (Ahnadi et al., 2002; Harvatine and Bauman, 2006). The marked abundance of *LPL* relative to both *ACACA* and *FASN* in our study contrasts with data from cows in

which both *LPL* and *FASN* were among the most-abundant genes during lactation (Bionaz and Loor, 2008). Such differences, however, might be attributable to the fact that we supplemented oil and therefore more *LPL* was probably necessary to handle an increase in circulating chylomicrons and very low density lipoproteins (Thering et al., 2009).

In accordance with the present study, Bernard et al. (2010) reported no changes in *SCD* expression in goats despite a putative decreased supply of 18:0 from the rumen. Desaturation of 18:0 in *cis*-9 18:1 by mammary Δ^9-desaturase is considered the predominant mechanism that maintains and regulates milk fluidity (Shingfield et al., 2010). The response of *SCD* in cows fed a blend of soybean and fish oil for 4-d was non-significant in the study of Harvatine and Bauman (2006); whereas, Invernizzi et al. (2010) reported a gradual up-regulation of *SCD* over time as a consequence of the reduction in 18:0 supply from the rumen induced by dietary fish oil feeding for 3 week. The analysis of percentage relative mRNA abundance among the genes measured in the present study revealed that, despite the lack of difference in expression between treatments, *SCD* was one of the most abundant genes involved in mammary lipid metabolism (Figure 1). Its relative abundance was similar to that observed in bovine mammary tissue during lactation (Bionaz and Loor, 2008). Thus, just as in cows fed fish oil (Invernizzi et al., 2010), it appears that when the diet is supplemented with marine lipids, milk fat synthesis in sheep also is dependent on other factors such as endogenous synthesis of oleic acid from rumen-derived 18:0.

A recent in vitro study with bovine kidney cells demonstrated that dietary 16:0, 20:5 and 22:6 n-3 LCFA, whose content was significantly greater in SOMA than SO (Bichi et al., 2013), are able to modulate lipid metabolism by increasing the expression of some key transcription factors or their co-activators (Bionaz et al., 2012). The fact that MA did not affect transcription regulators of milk fat synthesis in the present study partly differs from data in the bovine during MFD (Invernizzi et al., 2010; Harvatine and Bauman, 2006; Bauman et al., 2011). Species-specific differences among ruminants in the response of milk fat secretion and composition cannot be discarded to explain these results. This point is particularly relevant because our analyses revealed that expression of *PPARG* and *INSIG1* is markedly greater relative to *SREBF1*, which is opposite to cows (Bionaz and Loor, 2008). This suggests a different adaptive response to the reduction in milk fat synthesis between lactating dairy cows and ewes. Even though the present study analyzed only one time point, our

results underscore the importance of *PPARG* in the control of milk fat synthesis in sheep.

To further clarify mechanisms, an investigation of the temporal adaptation of the transcriptome in sheep mammary tissue is needed. Particularly to better understand the role of molecular mechanisms in this species as it relates to the supply of dietary and/or ruminally-derived long-chain FA.

Adaptations of Peripheral Tissues to Changes in Milk FA Secretion

Despite the high percentage of mRNA abundance of some key lipid-related genes such as *FABP4*, *SCD*, and *LPL* (see Figure 1), on d 54 there was no difference between treatments on mRNA expression of genes encoding for lipid metabolism-related networks in subcutaneous adipose and liver tissue. A similar lack of response was observed in the expression pattern of *LPL*, *ACACA*, *FASN*, and *SCD* in adipose tissue of goats receiving a diet rich in n-3 PUFA (i.e., linseed oil) for 21 d (Bernard et al., 2009a,b). These results contrast the observed up-regulation at 21 d of feeding of several lipid-related genes (particularly, *LPL* and *SCD*) in adipose tissue of cows with MFD in response to supplemental fish oil (Thering et al., 2009), suggesting that the energy spared from reduced milk fat synthesis might be partitioned toward non-mammary tissues, specifically adipose (Harvatine et al., 2009). However, based on mammary and adipose data from studies evaluating long-term nutritional regulation of milk fat synthesis during MFD (Thering et al., 2009; Invernizzi et al., 2010) it is not possible to conclude that in the ovine MA did not affect the lipogenic network in subcutaneous adipose tissue. It is likely that control mechanisms regulating fat synthesis were established before the time of sampling in the present study. Support for this is the fact that in the parent study involving all animals (Bichi et al., 2013) there was a clear temporal adaptation in the profile of FA in milk, as shown previously in cows (Shingfield et al., 2006).

The relative abundance of mammary *FABP3* (0.52% of total measured genes) and adipose *FABP4* (47% of total measured genes), along with low *DGAT1* (0.005%) in mammary and higher *DGAT2* (0.05%) in adipose, are suggestive of active utilization of LCFA in adipose likely for esterification into triacylglycerol (Graugnard et al., 2009). However, this hypothesis is contrasted by the absence of differences in concentrations of NEFA, insulin and glucose, which are known to be involved in the

regulation of adipose lipogenesis (Vernon, 1980). Similar to our study, no effect on plasma concentration of metabolites, including glucose, NEFA, BHBA, or metabolic hormones, such as leptin, insulin and IGF-1, were observed during short- (Baumgard et al., 2000, 2002) and longer-term MFD (Thering et al., 2009; Oliveira et al., 2012).

The lack of change in liver mRNA expression of genes encoding proteins associated with cholesterol and lipoprotein metabolism (*APOB*), FA oxidation (*ACOX1*, *CPT1A*) and with regulation of oxidation and ketogenesis (*PPARA*, *RXRA*) is in agreement with recent findings in dairy cows with MFD induced by supplemental CLA (Schlegel et al., 2012). There was evidence, indeed, that 14 week of CLA supplementation persistently decreased the milk fat content without apparently influencing hepatic lipid metabolism and plasma concentration of TAG (Schlegel et al., 2012).

Regression analysis revealed a weak negative correlation between *HMGCS2* mRNA expression and plasma BHBA ($r = -0.49$, $P < 0.01$) or NEFA ($r = -0.50$, $P < 0.01$) concentrations. The lack of a strict interdependence between *HMGCS2*, a key enzyme controlling hepatic ketogenesis, and BHBA agrees with the study of van Dorland et al. (2009), in which the increase in BHBA in cows after parturition was not associated with any increase in *HMGCS2* expression. However, the up-regulation of this gene was in line with the numerically, albeit not statistically significant, greater *PPARA* and *RXRA* suggesting a mechanistic response in liver to handle greater flux of LCFA as a result of MFD. Recently, an in vitro study provided support for several of the major LCFA found in SOMA as being *PPARA* agonists (Bionaz et al., 2012) and the present results seem to corroborate the suggestion that, once activated, *PPARA* is able to up-regulate target genes associated with ketogenesis (Loor et al., 2005). Consistent also with previous researches in dairy goats (Bernard et al., 2009a,b), the present results would indicate that hepatic lipid metabolism had a low relevance in nutritional mechanisms altering milk FA composition.

5. CONCLUSIONS

Altogether the present results highlight several differences among ruminant species in the mechanisms regulating the response to an MFD diet not only at the mammary level but also in adipose depots and liver. Transcriptional control mechanisms regulating mammary fat synthesis during long-term MA-induced MFD in sheep likely were established during earlier stages of the feeding period. Further studies are

necessary to clarify the role of the transcriptome on the regulation of the onset of MFD in dairy ewes.

6. REFERENCES

Agazzi, A., Invernizzi, G., Campagnoli, A., Ferroni, M., Fanelli, A., Cattaneo, D., Galmozzi, A., Crestani, M., Dell'Orto, V., and Savoini, G. 2010. Effect of different dietary fats on hepatic gene expression in transition dairy goats. Small Rumin. Res. 93:31-40.

Ahnadi, C., Beswick, N., Delbecchi, L., Kennelly, J., and Lacasse, P. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69:521-531.

Bauman, D. E., Harvatine, K. J., and Lock, A. L. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Baumgard, L. H., Corl, B. A., Dwyer, D. A., Saebø, A., and Bauman, D. E. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278:R179-R184.

Baumgard, L. H., Corl, B. A., Dwyer, D. A., and Bauman, D. E. 2002. Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows. J. Anim. Sci. 80:1285-1293.

Bernard, L., Bonnet, M., Leroux, C., Shingfield, K. J., and Chilliard, Y. 2009a. Effect of sunflower-seed oil and linseed oil on tissue lipid metabolism, gene expression, and milk fatty acid secretion in alpine goats fed maize silage-based diets. J. Dairy Sci. 92:6083-6094.

Bernard, L., Leroux, C., Faulconnier, Y., Durand, D., Shingfield, K. J., and Chilliard, Y. 2009b. Effect of sunflower-seed oil or linseed oil on milk fatty acid secretion and lipogenic gene expression in goats fed hay-based diets. J. Dairy Res. 76:241-248.

Bernard, L., Mouriot, J., Rouel, J., Glasser, F., Capitan, P., Pujos-Guillot, E., Chardigny, J. M., and Chilliard, Y. 2010. Effects of fish oil and starch added to a diet containing sunflower-seed oil on dairy goat performance, milk fatty acid composition and in vivo Δ^9-desaturation of [^{13} C]vaccenic acid. Br. J. Nutr. 104:346-354.

Bernard, L., Leroux, C., Rouel, J., Bonnet, M., and Chilliard, Y. 2012. Effect of level and type of starchy concentrate on tissue lipid metabolism, gene expression and milk fatty acid secretion in alpine goats receiving a diet rich in sunflower-seed oil. Br. J. Nutr. 107:1147-1159.

Bichi, E., Hervás, G., Toral, P. G., Loor, J. J., and Frutos, P. 2013. Milk fat depression induced by dietary marine algae in dairy ewes: persistency of milk fatty acid composition and animal performance responses. J. Dairy Sci. 96: 524-532.

Bionaz, M., and Loor, J. J. 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Bionaz, M., Thering, B. J., and Loor, J. J. 2012. Fine metabolic regulation in ruminants via nutrient-gene interactions: saturated long-chain fatty acids increase expression of genes involved in lipid metabolism and immune response partly through PPAR-alpha activation.

Br. J. Nutr. 107:179-191.

Capper, J. L., Wilkinson, R. G., Mackenzie, A. M., and Sinclair, L. A. 2007. The effect of fish oil supplementation of pregnant and lactating ewes on milk production and lamb performance. Animal 1:889-898.

Graugnard, D. E., Piantoni, P., Bionaz, M., Berger, L. L., Faulkner, D. B., and Loor, J. J. 2009. Adipogenic and energy metabolism gene networks in longissimus lumborum during rapid post-weaning growth in Angus and Angus × Simmental cattle fed high-starch or low-starch diets. BMC Genomics 10:142.

Graugnard, D. E., Berger, L. L., Faulkner, D. B., and Loor, J. J. 2010. High-starch diets induce precocious adipogenic gene network up-regulation in longissimus lumborum of early-weaned angus cattle. Br. J. Nutr. 103:953-963.

Harvatine, K. J., and Bauman, D. E. 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr. 136:2468-2474.

Harvatine, K. J., Perfield II., J. W., and Bauman, D. E. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849–854.

Hussein, M., Harvatine, K. J., Weerasinghe, W. M. P. B., Sinclair, L. A., and Bauman, D. E. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. J. Dairy Sci. 96:3825-3834.

Invernizzi, G., Thering, B. J., McGuire, M. A., Savoini, G., and Loor, J. J. 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Funct. Integr. Genomics 10:561-575.

Kadegowda, A. K. G., Bionaz, M., Piperova, L. S., Erdman, R. A., and Loor, J. J. 2009. Peroxisome proliferator-activated receptor-gamma activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. J. Dairy Sci. 92:4276-4289.

Li, X. Z., Yan, C. G., Lee, H. G., Choi, C. W., and Song, M. K. 2012. Influence of dietary plant oils on mammary lipogenic enzymes and the conjugated linoleic acid content of plasma and milk fat of lactating goats. Anim. Feed Sci. Technol. 174:26-35.

Lock, A. L., Teles, B. M., Perfield II., J. W., Bauman, D. E., and Sinclair, L. A. 2006. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 reduces milk fat synthesis in lactating sheep. J. Dairy Sci. 89:1525-1532.

Loor, J. J., Dann, H. M., Everts, R. E., Oliveira, R., Green, C. A., Janovick Guretzky, N. A., Rodriguez-Zas, S. L., Lewin, H. A., and Drackley, J. K. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217-226.

Oliveira, D. E., Gama, M. A. S., Fernandes, D., Tedeschi, L. O., and Bauman, D .E. 2012. An unprotected conjugated linoleic acid supplement decreases milk production and secretion of milk components in grazing dairy ewes. J. Dairy Sci. 95:1437-1446.

Ollier, S., Leroux, C., de la Foye, A., Bernard, L., Rouel, J., and Chilliard, Y. 2009. Whole intact rapeseeds or sunflower oil in high-forage or high-concentrate diets affects milk

yield, milk composition, and mammary gene expression profile in goats. J. Dairy Sci. 92:5544-5560.

Papadopoulos, G., Goulas, C., Apostolaki, E., and Abril, R. 2002. Effects of dietary supplements of algae, containing polyunsaturated fatty acids, on milk yield and the composition of milk products in dairy ewes. J. Dairy Res. 69:357-365.

Piperova, L., Teter, B., Bruckental, I., Sampugna, J., Mills, S., Yurawecz, M., Fritsche, J., Ku, K., and Erdman, R. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568-2574.

Reynolds, C. K., Cannon, V. L., and Loerch, S. C. 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131:333-357.

Schlegel, G., Ringseis, R., Windisch, W., Schwarz, F. J., and Eder, K. 2012. Effects of rumen-protected mixture of conjugated linoleic acids on hepatic expression of genes involved in lipid metabolism in dairy cows. J. Dairy Sci. 95:3905-3918.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., and Beever, D. E. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., Bernard, L., Leroux, C., and Chilliard, Y. 2010. Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.

Shingfield, K. J., Bonnet, M., and Scollan, N. D. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7 (S1):132-162.

Sinclair, L. A., Weerasinghe, W. M. P. B., Wilkinson, R. C., de Veth, M. J., Bauman, and D. E. 2010. A supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat yield but does not alter organ weight or body fat deposition in lactating ewes. J. Nutr. 140:1949-1955.

Thering, B. J., Graugnard, D. E., Piantoni, P., and Loor, J. J. 2009. Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J. Dairy Sci. 92:4290-4300.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2010b. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. J. Dairy Sci. 93:1604-1615.

Toral, P. G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., and de la Fuente, M. A. 2010a. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93, 1655–1667.

Toral, P. G., Bernard, L., Delavaud, C., Gruffaut, D., Leroux, C., and Chilliard, Y. 2013. Effects of fish oil and additional starch on tissue fatty acid profile and lipogenic gene mRNA abundance in lactating goats fed a diet containing sunflower-seed oil. Animal 7:948-956.

Tsiplakou, E., Flemetakis, E., Kalloniati, C., Papadomichelakis, G., Katinakis, P., and Zervas, G. 2009. Sheep and goats differences in CLA and fatty acids milk fat content in relation with mRNA stearoyl-CoA desaturase and lipogenic genes expression in their mammary

gland. J. Dairy Res. 76:392-401.

van Dorland, H. A., Richter, S., Morel, I., Doherr, M. G., Castro, N., and Bruckmaier, R. M. 2009. Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. J. Dairy Sci. 92:1924-1940.

Vernon, R.G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19:23-106.

7. SUPPLEMENTARY MATERIAL

RNA Extraction

RNA samples were extracted using established protocols (Loor et al., 2005). Briefly, tissue was weighed (~0.3 g for mammary gland, ~1 g for adipose tissue, ~0.1 g for liver) and placed straightway inside a 15 mL centrifuge tube (Cat. No. 430052, Corning Inc.®) with 1 μl of Linear Acrylamide (Ambion® Cat. No. 9520, Austin, TX) as a co-precipitant, and 5 mL ice-cold Trizol reagent (Invitrogen Corp., Carlsbad, CA). Tissue was then homogenized. Genomic DNA was removed from RNA with DNase using RNeasy Mini Kit columns (Qiagen, Hilden, Germany). RNA concentration was measured using a Nano-Drop ND-1000 spectrophotometer (Nano-Drop Technologies). The purity of RNA (A_{260}/A_{280}) for all samples was above 1.81. Also, RNA quality was evaluated using the Agilent Bioanalyzer system (Agilent Technologies). The average RIN number of the samples used was > 8.0.

qPCR Analysis

For qPCR analysis, cDNA was synthesized using 100 ng RNA, 1 μL dT18 (Operon Biotechnologies, Huntsville, AL), 1 μL 10 mmol/L dNTP mix (Invitrogen Corp., CA), 1 μL random primer $p(dN)_6$ (Roche Cat. No 11034731001, Indianapolis, IN), and 7 μL DNase/RNase free water. The mixture was incubated at 65°C for 5 min and kept on ice for 3 min. A total of 9 μL of master mix composed of 4.5 μL 5X First-Strand Buffer, 1 μL 0.1 M DTT, 0.25 μL (50 U) of SuperScript™ III RT (Invitrogen Corp., Carlsbad, CA), 0.25 μL of RNase Inhibitor (10 U, Promega, Madison, WI) and 3 μL DNase/RNase free water was added. The reaction was performed in an Eppendorf Mastercycler® Gradient (Eppendorf, Hauppauge, NY) using the following temperature program: 25°C for 5 min, 50°C for 60 min and 70°C for 15 min. cDNA was then diluted 1:4 (v:v) with DNase/RNase free water.

Quantitative PCR (qPCR) was performed using 4 μL diluted cDNA (dilution 1:4) combined with 6 μL of a mixture composed of 5 μL 1x SYBR Green master mix (Applied Biosystems, Foster City, CA), 0.4 μL each of 10 μM forward and reverse primers, and 0.2 μL DNase/RNase free water in a MicroAmp™ Optical 384-Well

Reaction Plate (Applied Biosystems, Foster City, CA). Each sample was run in triplicate and a 7 point relative standard curve plus the non-template control (NTC) were used (User Bulletin #2, Applied Biosystems, Foster City, CA). The reactions were performed in an ABI Prism 7900 HT SDS instrument (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C (denaturation) and 1 min at 60°C (annealing + extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95°C for 15 s plus 65°C for 15 s. Data were calculated with the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, Foster City, CA).

Selection of Internal Control Genes

The final data were normalized using the geometric mean of 4 selected ICG (Table S1): ubiquitously expressed transcript (*UXT*), eukaryotic translation initiation factor 3, subunit K (*EIF3K*), which were identified as suitable internal controls in bovine adipose and mammary tissues (Thering et al., 2009; Invernizzi et al., 2010), tyrosine 3-monooxygenase (*YWHAZ*), and tubulin beta, subunit 2A (*TUBB*), which were identified as suitable internal controls in ovine tissues (Zang et al., 2011).

Primer Design and Testing

Primers were designed using Primer Express 3.0 with minimum amplicon size of 100 bp and limited 3' G+C (Applied Biosystems, CA). When possible, primers were designed to fall across exon–exon junctions. Primers were aligned against publicly available databases using BLASTN at NCBI and UCSC's Sheep (*Ovis aries*) Genome Browser Gateway (Table S3). Prior to qPCR, primers were tested in a 20 µL PCR reaction using the same protocol described for qPCR except for the final dissociation protocol. For primer testing we used a pool of cDNA samples (mixture from 3 different ovine tissues) to ensure identification of desired genes. Five µL of the PCR product were run in a 2% agarose gel stained with ethidium bromide (2 µL). The remaining 15 µL were cleaned using QIAquick® PCR Purification Kit (QIAGEN) and sequenced at the Core DNA Sequencing Facility of the Roy J. Carver Biotechnology Center at the University of Illinois, Urbana-Champaign. Only those primers that did

not present primer-dimer, a single band at the expected size in the gel, and had the right amplification product (verified by sequencing) were used for qPCR. The accuracy of a primer pair also was evaluated by the presence of a unique peak during the dissociation step at the end of qPCR. Sequencing results for all genes are reported in Table S2 and in previous publication (Loor et al., 2007; Bionaz and Loor, 2008a,b; Kadegowda et al., 2009; Schmitt et al., 2011; Naeem et al., 2012; Ji et al., 2012).

Genes selected for transcript profiling in the present study were grouped as follows: FA uptake from blood, lipoprotein lipase (*LPL*); intracellular FA trafficking, fatty acid binding protein 3, muscle and heart (*FABP3*) and 4, adipocyte (*FABP4*); intracellular activation of VFA, acyl-CoA synthetase short-chain family member 2 (*ACSS2*); de novo FA synthesis, acetyl-coenzyme A carboxylase α (*ACACA*), fatty acid synthase (*FASN*); desaturation, stearoyl-CoA desaturase (*SCD*); triacylglycerol synthesis, diacylglycerol acyltransferase 1 and 2 (*DGAT1, DGAT2*), lipin 1 (*LPIN1*); long-chain FA elongation, ELOVL family member 6 (*ELOVL6*); lipid droplet formation, adipose differentiation related protein (*ADFP*), butyrophilin, subfamily 1, member A1 (*BTN1A1*), xanthine dehydrogenase (*XDH*); transcription regulation, insulin induced gene 1 (*INSIG1*), mediator of RNA polymerase II transcription subunit 1 (*MED1*), SREBP cleavage activating protein (*SCAP*), sterol regulatory element-binding transcription factor 1 (*SREBF1*), thyroid hormone responsive SPOT14 (*THRSP*), peroxisome proliferator activated receptor gamma (*PPARG*); cholesterol transport and lipoprotein synthesis, apolipoprotein B (*APOB*); ketogenesis, 3-hydroxy-3-methylglutaryl-CoA synthase 12 (*HMGCS2*); long-chain fatty acid oxidation, acyl-CoA oxidase 1, palmitoyl (*ACOX1*), carnitine palmitoyl-transferase 1A (*CPT1A*); transcriptional regulation of fatty acid oxidation, retinoid X receptor, alpha (*RXRA*), peroxisome proliferator-activated receptor alpha (*PPARA*).

Relative mRNA Abundance among Transcripts

Efficiency of PCR amplification for each gene was calculated using the standard curve method ($E = 10^{(-1 / Slope)}$). Relative mRNA abundance among measured genes was calculated as previously reported (Bionaz and Loor, 2007), using the inverse of PCR efficiency raised to ΔCt (gene abundance = $1/E^{\Delta Ct}$, where ΔCt = Ct sample - geometric mean Ct of 4 internal control genes). Overall mRNA abundance for each

gene among all samples measured was calculated using the median ΔCt. Use of this technique for estimating relative mRNA abundance among genes was necessary because relative mRNA quantification was performed using a standard curve (made from a mixture of RNA from different ovine tissues, which precluded a direct comparison among genes). Together, use of Ct values corrected for the efficiency of amplification plus internal control genes as baseline overcome this limitation.

Table S1. Description of 4 internal control genes.

Symbol	**Entrez Gene Name**
EIF3K	Eukaryotic translation initiation factor 3, subunit K
TUBB	Tubulin beta, subunit 2A
UXT	Ubiquitously expressed transcript
YWHAZ	Tyrosine 3-monooxygenase

Table S2. Sequencing results of PCR products from primers of genes designed for this experiment. Best hits using BLASTN (http://www.ncbi.nlm.nih.gov) are shown.

Gene	Sequence
ACACA	GGGGCCCTGTCAACGGTGACGTCGGATAGCATCTCCAACTTCCTTCACTCCTTAGAGAGGGGGGGTCAAGTCCTCTCTG CTCATACACTTCTAA
ACSS2	CGCATCAGTCTCGGGTGCCTCACAGGGACAGACAACAAGGGTCCCACATGGGGGAATCTGGATGTTTCTCTTGAGCAGG AGATGGGAC
ADFP	GCTCCTCTCTCCGTGATTGGACTGTGCCGGGAGATGGTGGCATAATGGCCAACCAGAAGATTCACGATGGCTCAA
DGAT1	ACCGGTTTGTCGGATTGGGACGGCTTCCCTCCTCCCTCCCCCACTTTCATCTTAGAGACTGGCTGCATATCCGGGTCAGT GTGGTTGTAA
FABP3	CCCTTCCTCTCGTGAGTCCTGTGCCTAAAATAACCTTGCTCCTGAATGGACCCAAAAACCTAGAGGAATGGGAATGAGA A
FABP4	AGAGACTTCACACGTGGGATGGAATCACCCCATAAAGAGAAAACTTGTTGGATGATAAGCCTGGTGCTGGAATGTACC CAGCAGCACCTTCATCTAAGGTTTAA
LPL	GACTTCGACGTCTCGTTCTCTCTTATTGACTCTCTGTTGAATGAAGAAAATCCAAGGTAAGGGCCTACCGGG
PPARG	CAGATCTCGGGCTTGTTGACCCTTGACTGGAATGACCAAGGTAACTCTCCTAAAATACGGGCGTGCACGAGATAA
SCD	GGGAGAAAGGCAAGGCCCAGCCTTGAGGTATGTTTGGAGAAACATCATCCTCATGGGTCTGTTACACTTGGGAA
SREBF1	CACCAACGTCCGGCCCGTCAGACCCTGGTGAAGTGGCGGGGCCATCCCTGGCCACTGTGTCCACTGGTAGTGGACACCT GTACAAGCTGCCAA
TUBB	CAGAGCGTCCGAACAGGACACGGCCGGCCTACCTTCGTGGGAATGGGATCCCCAACAACGTCAAGGACAGCTGGTTTG GCGACATCCAA
XDH	AGGTAGATGACTCTTCCGGGCTAGATGCCCTGCCACCCCGGAGGAAGATCCGCAATGCCTGTGTGGACAAGGTTTCAAA
YWHAZ	GTTGTACTCTCCTTTTTGCAAGACGGAAGGGTGTCTTGAGAAAACAGCAGATGTGGCTTCGAGAATTACAGAGAGAAA

Table S3. Sequencing results of genes using BLASTN from NCBI against nucleotide collection (nr / nt) with total score.

Gene	Best hits	Score
ACACA	*Ovis aries* acetyl-CoA carboxylase alpha (*ACACA*), mRNA >emb|X80045.1| *O. aries* mRNA for acetyl-CoA carboxylase	105
ACSS2	*Ovis aries* acetyl-CoA synthetase 2 (*ACAS2*) mRNA, partial cds	93.3
ADFP	*Ovis aries* perilipin 2 (*PLIN2*), mRNA >gb|EF660332.1| *Ovis aries* adipose differentiation-related protein (*ADFP*) mRNA, complete cds	53.6
DGAT1	*Ovis aries* diacylglycerol acyltransferase 1 (*DGAT1*) gene, complete cds	114
FABP3	*Ovis aries* heart fatty acid binding protein (*H-FABP*) gene, exons 2 through 4 and partial cds	62.6
FABP4	*Ovis aries* fatty acid binding protein 4, adipocyte (*FABP4*), mRNA >gb|EU301804.1| *Ovis aries* adipocyte fatty acid-binding protein 4 (*FABP4*) mRNA, complete cds	73.4
LPL	*Ovis aries* lipoprotein lipase (*LPL*), mRNA	77.0
PPARG	*Ovis aries* proliferator-activated receptor gamma mRNA, partial cds	84.2
SCD	*Ovis aries* stearoyl-CoA desaturase (*SCD*) gene, complete cds	93.3
SREBF1	*Ovis aries* sterol regulatory element-binding transcription factor 1 gene, exons 6, 7 and partial cds	95.1
TUBB	*Ovis aries* beta-tubulin mRNA, partial cds	59.0
XDH	*Ovis aries* xanthine dehydrogenase (*XDH*) mRNA, partial cds	87.8
YWHAZ	*Ovis aries* tyrosine 3-monooxygenase (*YWHAZ*) mRNA, partial cds	46.4

Table S4. Gene ID, GenBank accession number, hybridization position, sequence and amplicon size of primers for *Ovis aries* used to analyze gene expression by qPCR.

Gene ID	Accession #	Gene	Primers[1]	Primers (5'-3')	bp[2]
443186	NM_001009256.1	*ACACA*	F.2201	ACCATGCTGGGAGTTGTCTGT	118
			R.2319	AGAAGTGTATGAGCAGAGAGGACTTG	
780456	DQ272257.1	*ACSS2*	F.197	CGAAGCCATAAAGATCTGTCCAT	106
			R.303	CCATCTCCTGCTCAAGAGAAACA	
100125354	NM_001104932.1	*ADFP*	F.746	AAGAGGCCAGGAGACCATTTC	101
			R.847	TGAGCATCGTGAATCTTCTGGTT	
100126245	EU178818.1	*DGAT1*	F.2763	AGGGATCTGGAAAAGCTTGAATAA	114
			R.2877	ACAACCACACTGACCGGATATG	
2828237	AY157617.1	*FABP3*	F.1954	AGGGCAAGAACCCCAATTAAA	100
			R.2054	CTCATTCCCATTCCTCTAGTTTTTG	
100137067	NM_001114667.1	*FABP4*	F.255	AAACTTAGATGAAGGTGCTCTGGTACA	99
			R.354	ACATTCCAGCACCAGCTTATCA	
443408	NM_001009394	*LPL*	F.942	TGGAGATGTGGACCAGCTAGTG	99
			R.1041	CCGGTAGGCCTTACTTGGATT	
443513	FJ200441.1	*PPARG*	F.64	CCGTGCAGGAGATCACAGAGT	99
			R.163	ATCTCGTGCACGCCGTATTT	
443185	FJ513370	*SCD*	F.1100	GATGACATCTATGACCCAACTTACCA	100
			R.1200	CCCAAGTGTAACAGACCCATGA	
100329218	GU206528	*SREBF1*	F.152	GGGACAAGGTTTGCTCACATG	115
			R.267	GGCAGCTTGTCAGTGTCCACTA	
100303606	AF035420.1	*TUBB*	F.181	GAAGGAGGTAGATGAGCAGATGCT	99
			R.280	GATGTCGCAAACAGCTGTCTTG	
780499	EF529448.1	*XDH*	F.631	GCTCGAGCTCAGCACACAGA	99
			R.730	TGAACTTGTCCACACAGGCATT	
780452	AY970970.1	*YWHAZ*	F.	TGTAGGAGCCCGTAGGTCATCT	102
			R.	TTCTCTCTGTATTCTCGAGCCATCT	

[1]Primer direction (F – forward; R – reverse) and hybridization position on the sequence.
[2]Amplicon size in base pair (bp).

Table S5. Quantitative PCR performance among the genes measured in mammary, adipose and liver tissue.

Gene	Median Ct[1]	Median ΔCt[2]	Slope[3]	(R^2)[4]	Efficiency[5]
Mammary gland					
ACACA	23.77	0.63	-3.16	0.996	2.07
ACSS2	23.74	0.60	-2.97	0.990	2.17
BTN1A1	17.69	-5.59	-3.28	0.998	2.02
DGAT1	30.31	7.18	-3.58	0.982	1.90
ELOVL6	27.01	3.73	-3.41	0.988	1.96
FABP3	23.04	0.05	-2.96	0.992	2.18
FASN	23.71	0.52	-2.94	0.979	2.19
INSIG1	20.70	-2.02	-3.29	0.995	2.01
LPIN1	24.15	0.88	-3.14	0.990	2.08
LPL	17.27	-4.96	-3.01	0.991	2.15
MED1	25.03	3.36	-3.59	0.994	1.90
PPARG	25.64	3.30	-2.96	0.983	2.18
RXRA	23.75	1.97	-3.07	0.993	2.12
SCAP	27.27	4.18	-3.03	0.981	2.14
SCD	17.25	-5.10	-3.11	0.993	2.10
SREBF1	28.30	6.51	-2.66	0.981	2.38
THRSP	30.56	7.58	-2.66	0.984	2.38
XDH	18.10	-5.04	-3.17	0.998	2.07
Adipose tissue					
ACACA	21.01	-1.53	-3.16	0.996	2.07
ACSS2	25.33	2.44	-2.97	0.990	2.17
ADFP	24.99	2.28	-3.12	0.997	2.09
DGAT2	25.95	3.17	-2.94	0.968	2.19
ELOVL6	19.94	-2.25	-3.41	0.988	1.96
FABP4	16.42	-6.30	-3.30	0.997	2.01
FASN	24.44	1.82	-2.94	0.979	2.19
INSIG1	22.92	0.34	-3.29	0.995	2.01
LPIN1	24.05	1.31	-3.14	0.990	2.08
LPL	19.05	-4.10	-3.01	0.991	2.15
MED1	25.48	3.77	-3.59	0.994	1.90
PPARG	22.67	-0.68	-2.96	0.983	2.18
RXRA	22.89	1.14	-3.07	0.993	2.12
SCAP	26.67	4.09	-3.03	0.981	2.14
SCD	17.69	-5.39	-3.11	0.993	2.10
SREBF1	27.77	5.94	-2.66	0.981	2.38
THRSP	21.25	-1.06	-2.66	0.984	2.38

(continued)

Table S5 *(continued)*

Gene	Median Ct[1]	Median ΔCt[2]	Slope[3]	(R^2)[4]	Efficiency[5]
Liver					
ACOX1	27.79	2.95	-3.12	0.988	2.09
APOB	20.03	-4.60	-3.28	0.997	2.02
CPT1A	28.44	3.82	-2.68	0.988	2.36
HMGCS2	23.92	-0.98	-2.92	0.997	2.20
PPARA	28.09	3.19	-2.83	0.989	2.25

[1]The median is calculated considering all ewes.
[2]The median of ΔCt is calculated as [Ct gene – geometrical mean of Ct internal controls] for each ewe.
[3]Slope of the standard curve.
[4]R^2 stands for the coefficient of determination of the standard curve.
[5]Efficiency is calculated as [$10^{(-1 / Slope)}$]

References

Bionaz, M., and Loor, J. J. 2007. Identification of reference genes for quantitative real-time PCR in the bovine mammary gland during the lactation cycle. Physiol Genomics 29:312-9.

Bionaz, M., and Loor, J. J. 2008a. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366.

Bionaz, M., and Loor, J. J. 2008b. ACSL1, AGPAT6, FABP3, LPIN1, and SLC27A6 are the most abundant isoforms in bovine mammary tissue and their expression is affected by stage of lactation. J. Nutr. 138:1019-1024.

Invernizzi, G., Thering, B. J., McGuire, M. A., Savoini, G., and Loor, J. J. 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Funct. Integr. Genomics 10:561-575.

Ji, P., Osorio, J. S., Drackley, J. K., and Loor, J. J. 2012. Overfeeding a moderate energy diet prepartum does not impair bovine subcutaneous adipose tissue insulin signal transduction and induces marked changes in peripartal gene network expression. J. Dairy Sci. 95:4333-4351.

Kadegowda, A. K. G., Bionaz, M., Thering, B., Piperova, L. S., Erdman, R. A., and Loor, J. J. 2009. Identification of internal control genes for quantitative polymerase chain reaction in mammary tissue of lactating cows receiving lipid supplements. J. Dairy Sci. 92:2007-2019.

Loor, J. J., Dann, H. M., Everts, R. E., Oliveira, R., Green, C., Guretzky, N., Rodriguez-Zas, S. L., Lewin, H., and Drackley, J. K. 2005. Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. Physiol. Genomics 23:217-226.

Loor, J. J., Everts, R. E., Bionaz, M., Dann, H. M., Morin, D. E., Oliveira, R., Rodriguez-Zas, S. L., Drackley, J. K., and Lewin, H. A. 2007. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. Physiol. Genomics 32:105-116.

Naeem, A., Drackley, J. K., Stamey, J., and Loor, J. J. 2012. Role of metabolic and cellular proliferation genes in ruminal development in response to enhanced plane of nutrition in neonatal Holstein calves. J. Dairy Sci. 95:1807-1820.

Schmitt, E., Ballou, M. A., Correa, M. N., DePeters, E. J., Drackley, J. K., and Loor, J. J. 2011. Dietary lipid during the transition period to manipulate subcutaneous adipose tissue peroxisome proliferator-activated receptor-γ co-regulator and target gene expression. J. Dairy Sci. 94:5913-25.

Thering, B. J., Graugnard, D. E., Piantoni, P., and Loor, J. J. 2009. Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J. Dairy Sci. 92:4290-4300.

Zang, R., Bai, J., Xu, H., Zhang, L., Yang, J., Yang, L., Lu, J., and Wu, J. 2011. Selection of suitable reference genes for real-time quantitative PCR studies in Lanzhou fat-tailed sheep (*Ovis aries*). Asian J. Anim. Vet. Adv. 6:789-804.

DISCUSIÓN GENERAL

El síndrome de baja grasa en la leche ha sido objeto de estudio desde hace más de cien años porque está relacionado negativamente con la economía de la producción lechera puesto que, históricamente, el contenido de grasa láctea ha determinado el precio de la leche. Tras numerosos trabajos en este campo, se han propuesto distintas teorías para intentar explicar la reducción del contenido de grasa que caracteriza dicho síndrome. Actualmente parece evidente que la MFD supone una alteración de la relación existente entre la composición de la dieta, las rutas metabólicas ruminales y el metabolismo lipídico en la glándula mamaria (Bauman y Griinari, 2001).

Ha sido en la especie bovina, y debido a su importancia económica a nivel mundial, en la que más se ha estudiado la MFD, mientras que todavía es escasa la información relativa a los pequeños rumiantes de aptitud lechera. Esto podría ser explicado, al menos en parte, por el hecho de que algunos trabajos llevados a cabo en caprino apuntaban inicialmente a que esta especie era resistente a la MFD (Chilliard et al., 2007; Bernard et al., 2009; Shingfield et al., 2010), lo cual, a su vez, fue extrapolado al ovino y se asumió que la situación era parecida. Sin embargo, publicaciones más actuales demuestran que tanto la cabra (Lock et al., 2008; Shingfield et al., 2009a; Chilliard et al., 2014) como especialmente la oveja (Toral et al., 2010a,b; Weerasinghe et al., 2012; Hussein et al., 2013) presentan ese síndrome y, por lo tanto, es necesario su estudio en estas especies.

En los apartados previos de esta tesis se describen tres experimentos que versan sobre la lipogénesis en la glándula mamaria de la oveja en lactación (Prueba I) y su relación con los cambios inducidos por la dieta, durante la MFD, tanto en el proceso de BH ruminal, como en la expresión de una serie de genes candidatos ligados al metabolismo lipídico (Pruebas II y III).

En el primer estudio se mostró cómo, al igual que en la vaca lechera, la inhibición de la enzima Δ^9-desaturasa, conseguida mediante la administración de ácido estercúlico, permite cuantificar la síntesis endógena de ciertos AG con un doble enlace en posición *cis*-9 en la glándula mamaria de la oveja.

En el segundo ensayo se indujo la MFD mediante la suplementación de la dieta con microalgas marinas durante 8 semanas y se observó, junto con la disminución del contenido de la grasa de la leche, una reducción a lo largo de todo el periodo experimental del contenido de 18:0 y *cis*-9 18:1 y un aumento del de algunos AG de conformación *trans*.

En el tercer experimento se estudió la expresión de un grupo de genes candidatos que codifican las principales enzimas del metabolismo lipídico y se comparó la de los animales con MFD con la de aquellos del grupo control, no siendo posible evidenciar diferencias significativas en la abundancia del ARNm de los mismos.

Síntesis endógena de AG en la glándula mamaria

La glándula mamaria de los rumiantes lecheros es el principal órgano donde ocurre la síntesis endógena de AG que poseen un doble enlace en la posición *cis*-9 (Kinsella, 1972; Mosley et al., 2006; Bernard et al., 2013). La responsable de esta síntesis es fundamentalmente la enzima Δ^9-desaturasa o SCD, que inserta un doble enlace en un amplio número de AG (Ntambi y Miyazaki, 2004). El sustrato principal de la SCD es el 18:0, que es convertido en *cis*-9 18:1 (Ntambi y Miyazaki, 2004; Palmquist et al., 2005).

La grasa de la leche de los rumiantes contiene una gran variedad de AG organizados en triglicéridos (TG) y, a la temperatura corporal del animal, es fluida, lo que permite que las células mamarias la secreten (Timmen y Patton, 1988). No obstante, dicha fluidez depende de la estructura de los AG que componen sus TG. Así, los AG de cadena corta y los insaturados *cis* de cadena larga aumentan la fluidez, mientras que los AG saturados (≥C12) y los *trans* 18:1 aumentan la temperatura de fusión de la grasa favoreciendo que la consistencia sea más sólida (Timmen y Patton, 1988; Taylor y McGibbon, 2011). Entre los AG insaturados de cadena larga, cabe destacar el *cis*-9 18:1 por una doble razón: por un lado, este AG parece jugar un papel clave en el mantenimiento de la fluidez de la grasa láctea (Timmen y Patton, 1988; Chilliard et al., 2000; Gama et al., 2008) y por otro, despierta un gran interés en la comunidad científica por su efecto beneficioso sobre la salud humana (Lou-Bonafonte et al., 2012; Silva et al., 2015).

Otra función especialmente relevante de la SCD es la síntesis endógena del ácido ruménico (*cis*-9, *trans*-11 18:2), cuyos efectos potencialmente beneficiosos para la salud de los consumidores siguen siendo de incuestionable interés (Parodi, 2003; Palmquist et al., 2005).

Los datos disponibles en las especies bovina y caprina indican que aprox. entre el 50 y 80% del ácido oleico presente en la grasa de la leche tiene su origen en la síntesis endógena en la glándula mamaria a partir de la Δ^9-desaturación del ácido esteárico

(Mosley y McGuire 2007; Glasser et al., 2008; Bernard et al., 2013). Paralelamente, los estudios en los que se calculó la cantidad de *cis*-9, *trans*-11 18:2 producida de forma endógena indican que esta representa un amplio porcentaje del total (aprox. entre el 64 y el 91% dependiendo de la dieta ofrecida a los animales; Palmquist et al., 2005; Glasser et al., 2008; Shingfield et al., 2008). Por su parte, en el momento de realizar el primer experimento de esta tesis (i. e., el publicado en Bichi et al., 2012) no existía ninguna información al respecto en el ganado ovino.

En ese primer experimento, la inhibición de la Δ^9-desaturasa se logró administrando ácido estercúlico, un potente y específico inhibidor de la enzima, a un grupo de 6 ovejas, y nos permitió cuantificar el porcentaje de los ácidos oleico y ruménico sintetizados endógenamente. Nuestros resultados confirmaron que la SCD es responsable de la mayor parte de la producción tanto del *cis*-9 18:1 (63%) como del *cis*-9, *trans*-11 18:2 (74%). Estos valores son ligeramente diferentes de los aportados en dos estudios recientes (Frutos et al., 2014; Toral et al., 2015b), en los que se usó acetato de cobalto para inhibir la Δ^9-desaturasa. No obstante, más que a diferencias en la metodología utilizada, las variaciones en estos resultados podrían atribuirse, al menos parcialmente, a la distinta composición de la dieta consumida por los animales (pasto fresco en el presente estudio *versus* una ración completa mezclada, suplementada o no con una fuente rica en 18:3 n-3, en el caso de los otros dos trabajos). Estas distintas dietas suponen una diferente disponibilidad de ciertos AG, especialmente vaccénico y esteárico, procedentes de la BH ruminal, para la actividad de la SCD en la glándula mamaria. En relación con los mecanismos de acción, el ácido estercúlico formaría un complejo inactivo entre el anillo ciclopropénico de su molécula y la estructura proteica de la SCD, sin que la expresión del gen *SCD* se viera afectada (Gomez et al., 2003). Por otra parte, aunque el mecanismo de acción del cobalto es aún incierto, algunos trabajos sugieren que su efecto inhibidor podría estar relacionado con su capacidad para sustituir el hierro en el complejo enzimático que constituye la SCD (Shingfield et al., 2008; Taugbøl et al., 2010), pudiendo existir también un efecto negativo sobre la expresión del *SCD* en el tejido mamario (Toral et. al., 2015b). En todo caso, a pesar de que los respectivos mecanismos de acción no están completamente esclarecidos, ambos métodos consiguen inactivar el sistema enzimático de la Δ^9-desaturasa y disminuir el proceso normal de desaturación de los AG (Gomez et al., 2003; Taugbøl et al., 2010; Frutos et al., 2014), lo cual permite estimar su síntesis endógena.

Síndrome de baja grasa en la oveja lechera

Un hallazgo interesante de la Prueba I fue que al inhibir la SCD se produjo una reducción del contenido de grasa de la leche. Este hecho podría relacionarse con la teoría que asocia la aparición de la MFD con la deficiencia de 18:0 para la síntesis de *cis*-9 18:1 en la glándula mamaria, ya que esta afectaría a las propiedades físicas de la grasa láctea, aumentando su punto de fusión y dificultando su secreción (Chilliard et al., 2000; Loor et al., 2005; Shingfield y Griinari, 2007). Este mecanismo se ha postulado frecuentemente como explicación de la MFD inducida por lípidos de origen marino (Gama et al., 2008; Toral et al., 2010a, 2015a). Los AG n-3 de cadena muy larga, abundantes en los lípidos marinos, son conocidos inhibidores del último paso de la BH ruminal, lo cual provoca una disminución de la cantidad de 18:0 que abandona el rumen (Or-Rashid et al., 2008) y que representa el sustrato necesario para la síntesis endógena de *cis*-9 18:1 en la glándula mamaria. Esto coincide con los resultados señalados por Toral et al. (2010b) en ovejas en lactación cuya dieta se suplementó con MA y también con los de la Prueba II. En este último caso, la MFD inducida por las algas persistió a lo largo de todo el tratamiento experimental (8 semanas) y se acompañó de bajos contenidos en la grasa láctea tanto de 18:0 como de *cis*-9 18:1 a partir de la primera semana. Esos datos apuntan a la alteración del proceso de BH ruminal como principal causa de la disminución del contenido de grasa en la leche, más que a una inhibición de la Δ^9-desaturasa en las células del epitelio mamario. Ahora bien, diversos estudios recientes parecen cuestionar el papel clave de la escasez de 18:0 para la síntesis endógena de *cis*-9 18:1 como mecanismo responsable de la MFD inducida por lípidos marinos, ya que, por ejemplo, la adición de un suplemento de 18:0 a la dieta no permite aliviar la depresión de la grasa (Toral et al., en prensa-b).

Por otro lado, es importante mencionar que la inhibición de la Δ^9-desaturasa no siempre va ligada a la caída del contenido de grasa de la leche (Kay et al., 2004; Frutos et al., 2014; Toral et al., 2015b). Por ejemplo, en un trabajo reciente, Toral et al. (2015b) observaron que la disminución de la desaturación de ácido esteárico a ácido oleico por efecto de la inhibición de la SCD mediante el acetato de cobalto, no se acompañó de MFD. En el experimento llevado a cabo por estos últimos autores, la suplementación de la dieta con aceite de linaza habría aumentado los AG poliinstaurados de cadena larga y disminuido los saturados de cadena media, lo que posiblemente contribuyera a evitar problemas relacionados con el punto de fusión de

la grasa y su secreción. Estas observaciones apuntarían de nuevo a que los mecanismos implicados en la MFD son claramente dependientes de la dieta consumida por los animales.

Por otra parte, diversos investigadores (e. g., Capper et al., 2007; Toral et al., 2010a, en prensa-b) han observado también un aumento de AG de conformación *trans* en la leche de ovejas con MFD inducida por lípidos marinos, lo cual podría contribuir a empeorar la capacidad del epitelio mamario para mantener el punto de fusión de la grasa por debajo de la temperatura corporal, y coincide con los resultados de la Prueba II. No obstante, el carácter antilipogénico de algunos AG *trans* va mucho más allá de la alteración del punto de fusión de la grasa láctea.

En este sentido, en la Prueba II cabe destacar la evolución contraria, a lo largo del tiempo, de los contenidos de *trans*-10 18:1 y de grasa láctea. Aunque en diversos ensayos llevados a cabo en ovejas lecheras se han detectado niveles muy elevados de ese AG sin que se produzca MFD (e. g., Reynolds et al., 2006; Gómez-Cortés et al., 2008), este isómero 18:1 se ha relacionado en el vacuno con la aparición del síndrome de baja grasa en la leche (Shingfield et al., 2006; Kadegowda et al., 2008; Shingfield et al., 2009b) y su intervención o no en este proceso es aún materia de estudio y de debate. Otros dos AG con características antilipogénicas que también aumentaron en la Prueba II en respuesta al consumo de MA fueron dos isómeros del CLA (i. e., el *trans*-10, *cis*-12 18:2 y el *trans*-9, *cis*-11 18:2; Perfield et al., 2007; Shingfield y Griinari, 2007; Harvatine et al., 2009). Su concentración permaneció más elevada en las ovejas que recibieron las MA a lo largo de todo el periodo experimental aunque, también en este caso, su implicación en la aparición de la MFD en el ganado ovino no resulta evidente y, en el caso concreto del *trans*-10, *cis*-12 18:2, parece poco probable (Toral et al., 2010a,b y 2014). Así, aunque la suplementación directa de la dieta de ovejas lecheras con *trans*-10, *cis*-12 18:2 provoca una caída del contenido graso de la leche, su papel en la MFD inducida por lípidos marinos se puede considerar irrelevante (Toral et al., en prensa-a). En este último artículo, los autores sugieren que debería investigarse la implicación en este tipo de MFD de ciertos AG con capacidad antilipogénica menos conocida (como por ejemplo, el *trans*-10, *cis*-15 18:2 o el 10-oxo-18:0).

Por último, es interesante mencionar que los aumentos en la concentración de AG antilipogénicos detectados en la Prueba II no pudieron relacionarse con menores abundancias de ARNm de los genes que codifican las principales enzimas implicadas

en el metabolismo lipídico de la glándula mamaria (Prueba III), en contra de lo esperable según los trabajos previos en vacas lecheras alimentadas con aceites marinos (Ahnadi et al., 2002; Harvatine at al., 2009; Invernizzi et al., 2010).

La diferencia entre los resultados descritos en dicha Prueba III y los datos disponibles en el ganado vacuno podría tener varias explicaciones: por un lado, no se puede descartar que los cambios en el transcriptoma se produjeran relativamente pronto tras la ingestión de MA, al igual que ocurre con el perfil lipídico de la leche de las mismas ovejas (Prueba II) y fuera más complicado detectarlos a largo plazo. Esto coincidiría con lo señalado por Invernizzi et al. (2010) en vacas lecheras con MFD inducida por el consumo de lípidos marinos. Sin embargo, en el ovino, Carreño et al. (2015) comprobaron que la suplementación de la dieta con aceite de pescado causaba un síndrome de baja grasa que se acompañaba no solo de aumentos en la concentración de diversos AG con efecto antilipogénico (i. e., *trans*-10 18:1, *trans*-10, *cis*-12 18:2 y *trans*-9, *cis*-11 18:2) sino también de una disminución de la abundancia de ARNm de algunos genes claves en el metabolismo lipídico mamario, incluidos factores de transcripción (e. g., *ACACA, FASN*, *SCD1* y *SREBF1*). La mayor parte de estos cambios fueron detectables en el primer muestreo (es decir, tras solo 7 días de tratamiento) y persistieron hasta el final del experimento.

Ahora bien, no se puede descartar que, como señalan Bernard et al. (2013), la regulación postranscripcional pudiera tener un mayor peso en la MFD que la regulación transcripcional. Este aspecto se plantea también en el trabajo de Castro-Carrera et al. (2015), en el que la modificación del perfil lipídico de la leche debido a la suplementación de la dieta de ovejas lecheras con aceite de girasol no se vio acompañada por cambios significativos en la abundancia del ARNm de los genes candidatos asociados con la lipogénesis que fueron investigados.

Por otro lado, el hecho de que la dieta base de todos los animales utilizados en las Pruebas II y III (incluidos los controles) fuera rica en ácido linoleico, el cual inhibe la síntesis de AG *de novo* en la glándula mamaria (Hervás et al., 2008; Mele et al., 2008; Gómez-Cortés et al., 2011), podría haber dificultado la detección de ciertos cambios en la expresión génica ligados al consumo de MA.

Por último, es fundamental continuar investigando antes de extraer conclusiones acerca de la posible diferencia entre especies de rumiantes en la respuesta a nivel de regulación transcripcional en el tejido mamario. En este momento, parece que en el caprino no hay aún evidencia de una relación clara entre los cambios en el perfil de

AG de la leche y la expresión de los genes candidatos implicados en su síntesis, mientras que en el bovino la relación es más evidente (Ahnadi et al., 2002; Shingfield et al., 2013). En la especie ovina, los datos al respecto son aún muy escasos e inconsistentes (e. g., Hussein et al., 2013; Castro-Carrera et al., 2015) pero los trabajos más recientes (e. g., Carreño et al., 2015) no permiten descartar que la MFD inducida por el consumo de lípidos marinos (es decir, la estudiada en las Pruebas II y III) esté mediada por mecanismos transcripcionales.

En todo caso, y en conjunto, los resultados muestran la complejidad de los factores que pueden determinar el desarrollo de la MFD y subrayan la importancia de continuar la investigación en el ovino lechero, ya que en esta especie las incertidumbres sobre este síndrome son aún numerosas.

REFERENCIAS

Ahnadi, C., Beswick, N., Delbecchi, L., Kennelly, J., and Lacasse, P. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69:521-531.

Bauman, D. E., and Griinari, J. M. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15-29.

Bernard, L., Leroux, C., and Chilliard, Y. 2013. Expression and nutritional regulation of stearoyl-CoA desaturase genes in the ruminant mammary gland: relationship with milk fatty acid composition. In: Stearoyl-CoA desaturase genes in lipid metabolism. Ntambi, J. M. (ed.). Springer Science+Business Media, New York, USA, pp. 161-193.

Bernard, L., Shingfield, K. J., Rouel, J., Ferlay, A., and Chilliard, Y. 2009. Effect of plant oils in the diet on performance and milk fatty acid composition in goats fed diets based on grass hay or maize silage. Br. J. Nutr. 101:213-224.

Bichi, E., Toral, P. G., Hervás, G., Frutos, P., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2012. Inhibition of Δ^9-desaturase activity with sterculic acid: Effect on the endogenous synthesis of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2 in dairy sheep. J. Dairy Sci. 95:5242-5252.

Capper, J. L., Wilkinson, R. G., Mackenzie, A. M., and Sinclair, L. A. 2007. The effect of fish oil supplementation of pregnant and lactating ewes on milk production and lamb performance. Animal 1:889-898.

Carreño, D., Hervás, G., Toral, P.G., Castro-Carrera, T., Fernández, M., y Frutos, P. 2015. Efecto de la suplementación de la dieta con aceite de pescado sobre la abundancia de ARNm de genes implicados en la lipogénesis mamaria en ovejas lecheras. En: XVI Jornadas sobre Producción Animal AIDA. 19-20 de mayo de 2015. Zaragoza. J. Álvarez, B. Panea, J.H. Calvo, M. Blanco, A. Abecia, D. Villalba y M.A. Latorre (eds.). AIDA, Zaragoza, España, pp. 152-154.

Castro-Carrera, T., Frutos, P., Leroux, C., Chilliard., Y., Hervás, G., Belenguer, A., Bernard, L., and Toral, P. G. 2015. Dietary sunflower oil modulates milk fatty acid composition without major changes in adipose and mammary tissue fatty acid profile or related gene mRNA abundance in sheep. Animal 9:582-591.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., and Doreau, M. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181-205.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828-855.

Chilliard, Y., Toral, P. G., Shingfield, K. J., Rouel, J., Leroux, C., and Bernard, L. 2014. Effects of diet and physiological factors on milk fat synthesis, milk fat composition and lipolysis in the goat. A short review. Small Rumin. Res. 122:31-37.

Frutos, P. Toral, P. G., Ramos-Morales, E., Shingfield, K. J., Belenguer, A., and Hervás, G. 2014. Oral administration of cobalt acetate alters milk fatty acid composition, consistent with an inhibition of stearoyl-coenzyme A desaturase in lactating ewes. J. Dairy Sci. 97:1036-1046.

Gama, M. A. S., Garnsworthy P. C., Griinari, J. M., Leme, P. R., Rodrigues, P. H. M., Souza, L. W. O., and Lanna, D. P. D. 2008. Diet-induced milk fat depression: association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115:319-331.

Glasser, F., Ferlay, A., Doreau, M., Schmidely, P., Sauvant, D., and Chilliard, Y. 2008. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91:2771-2785.

Gomez, F. E., Bauman, D. E., Ntambi, J. M., and Fox, B. G. 2003. Effects of sterculic acid on stearoyl-CoA desaturase in differentiating 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 300:316-326.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., and Hervás, G. 2008. Milk production, conjugated linoleic acid content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. J. Dairy Sci. 91:1560-1569.

Gómez-Cortés, P., de la Fuente, M. A., Toral, P. G., Frutos, P., Juárez, M., and Hervás, G. 2011. Effects of different forage:concentrate ratios in dairy ewe diets supplemented with sunflower oil on animal performance and milk fatty acid profile. J. Dairy Sci. 94:4578-4588.

Harvatine, K. J., Boisclair, Y. R., and Bauman, D. E. 2009. Recent advances in the regulation of milk fat synthesis. Animal 3:40-54.

Hervás, G., Luna, P., Mantecón, A. R., Castañares, N., de la Fuente, M. A., Juárez, M., and Frutos, P. 2008. Effect of diet supplementation with sunflower oil on milk production, fatty acid profile and ruminal fermentation in lactating dairy ewes. J. Dairy Res. 75:399-405.

Hussein, M., Harvatine, K. J., Weerasinghe, W. M., Sinclair, L. A., and Bauman, D. E. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. J. Dairy Sci. 96:3825-3834.

Invernizzi, G., Thering, B. J., McGuire, M. A., Savoini, G., and Loor, J. J. 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Funct. Integr. Genomics 10:561-575.

Kay, J. K., Mackle, T. R., Auldist, M. J., Thomson, N. A., and Bauman, D. E. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369-378.

Kadegowda, A. K., Piperova, L. S., and Erdman, R. A. 2008. Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. J. Dairy Sci. 91:749-759.

Kinsella, J. E. 1972. Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglycerides synthesis. Lipids 7:349-355.

Lock, A. L., Rovai, M., Gipson, T. A., de Veth, M. J., and Bauman, D. E. 2008. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat synthesis in lactating goats. J. Dairy Sci. 91:3291-3299.

Loor, J. J., Doreau, M., Chardigny, J. M., Ollier, A., Sébédio, J. L., Chilliard, Y. 2005. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of *trans*-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim. Feed Sci. Technol. 119:227-246.

Lou-Bonafonte, J. M., Arnal, C., Navarro, M. A., and Osada, J. 2012. Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development. Mol. Nutr. Food Res. 56:1043-1057.

Mele, M., Sarra, A., Buccioni, A., Conte, G., Pollicardo, A., and Secchiari, P. 2008. Effect of soybean oil supplementation on milk fatty acid composition from Saanen goats fed diets with different forage:concentrate ratios. Ital. J. Anim. Sci. 7:297-311.

Mosley, E. E., and McGuire, M. A. 2007. Methodology for the in vivo measurement of the Δ^9-desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. Lipids 42:939-945.

Mosley, E. E., Shaffi Dagger, B., Moate, P. J., and McGuire, M. A. 2006. *Cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575.

Ntambi, J. M., and Miyazaki, M. 2004. Regulation of stearoyl-CoA desaturase and role in metabolism. Prog. Lipid Res. 43:91-104.

Or-Rashid, M. M., Kramer, J. K. G., Wood, M. A., and McBride, B. W. 2008. Supplemental algal meal alters the ruminal *trans*-18:1 fatty acid and conjugated linoleic acid composition in cattle. J. Anim. Sci. 86:187-196.

Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Parodi, P. W. 2003. Conjugated linoleic acid in food. In: Advances in conjugated linoleic acid research. Sébédio, J. L., Christie, W. W. and Adlof, R. (eds.). AOCS Press, Champaign, IL, USA, pp.101-122.

Perfield, J. W., Lock, A. L., Griinari, J. M., Sæbø, A., Delmonte, P., Dwyer, D. A., and Bauman, D. E. 2007. *Trans*-9, *cis*-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90:2211-2218.

Reynolds, C. K., Cannon, V. L., and Loerch, S. C. 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131:333-357.

Shingfield, K. J., Ärölä, A., Ahvenjärvi, S., Vanhatalo, A., Toivonen, V., Griinari, J. M., and Huhtanen, P. 2008. Ruminal infusions of cobalt-EDTA reduce mammary Δ^9-desaturase index and alter milk fatty acid composition in lactating cows. J. Nutr. 138:710-717.

Shingfield, K. J., Bernard, L., Leroux, C., and Chilliard, Y. 2010. Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.

Shingfield, K. J., Bonnet, M., and Scollan, N. D. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7:s1:132-162.

Shingfield, K. J., and Griinari, J. M. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Technol. 109:799-816.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., and Beever, D. E. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., Rouel, J., and Chilliard, Y. 2009a. Effect of calcium salts of a mixture of conjugated linoleic acids containing *trans*-10, *cis*-12 in the diet on milk fat synthesis in goats. Br. J. Nutr. 101:1006-1019.

Shingfield, K. J., Sæbø, A., Sæbø, P. C., Toivonen, V., and Griinari, J. M. 2009b. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Silva, S., Combet, E, Figueira, M. E., Koeck, T., Mullen, W., and Bronze, M. R. 2015. New perspectives on bioactivity of olive oil: evidence from animal models, human interventions and the use of urinary proteomic biomarkers. Proc. Nutr. Soc. 74:268-281.

Taugbøl, O., Karlengen, I. J., Salbu, B., Aastveit, A. H., and Harstad, O. M. 2010. Intravenous injections of cobalt reduce fatty acid desaturation products in milk and blood of lactating cows. J. Anim. Physiol. Anim. Nutr. 94: 635-640.

Taylor, M. W., and MacGibbon, A. K. H. 2011. Triacylglycerols. In: Fuquay, F. W. (ed.), Encyclopedia of dairy sciences, 2nd edition, Academic Press, San Diego, USA, pp. 665-669.

Timmen, H., and Patton, S. 1988. Milk fat globules: fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685-689.

Toral, P. G., Chilliard, Y., Rouel, J., Leskinen, H., Shingfield, K. J., and Bernard, L. 2015a. Comparison of the nutritional regulation of milk fat secretion and composition in cows and goats. J. Dairy Sci. 98:7277-7297.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2010a. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. J. Dairy Sci. 93:1604-1615.

Toral P. G., Hervás, G., Belenguer, A., Castro-Carrera, T., Carreño, D., and Frutos, P. 2014.

Principal component analysis of diet-induced changes in milk fat content and fatty acid composition in dairy ewes. Proc. Aust. Soc. Anim. Prod. 30:156.

Toral P. G., Hervás, G., Carreño, D., Belenguer, A., and Frutos, P. Comparison of milk fatty acid responses during fish oil- and *trans*-10 *cis*-12 18:2-induced milk fat depression in dairy ewes. Anim. Feed Sci. Technol. (en prensa-a).

Toral P. G., Hervás, G., Carreño, D., and Frutos, P. May supplemental 18:0 alleviate fish oil-induced milk fat depression in dairy ewes? J. Dairy Sci. (en prensa-b).

Toral P. G., Hervás, G., Frutos, P. 2015b. Reductions in milk Δ^9-desaturation ratios to oral dosing of cobalt-acetate are accompanied by the downregulation of *SCD1* in lactating ewes. J. Dairy Sci. 98:1961-1971.

Toral, P. G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., and de la Fuente, M. A. 2010b. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93:1655-1667.

Weerasinghe, W. M. P. B., Wilkinson, R. G., Lock, A. L., de Veth, M. J., Bauman, D. E., and Sinclair, L. A. 2012. Effect of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on the performance of dairy ewes fed 2 levels of metabolizable protein and at a restricted energy intake. J. Dairy Sci. 95:109-116.

DISCUSSIONE GENERALE

La sindrome di scarso grasso nel latte (MFD) è stata oggetto di studio da più di cent'anni perché si relaziona negativamente con l'economia della produzione di latte giacché, storicamente, il contenuto grasso del latte ne ha determinato il prezzo. A seguito di numerosi lavori in questo campo, sono state proposte molte teorie per cercare di spiegare la riduzione del contenuto in grasso che caratterizza detta sindrome. Attualmente sembra evidente che la MFD supponga un'alterazione della relazione esistente fra la composizione della dieta, le vie metaboliche ruminali e il metabolismo lipidico nella ghiandola mammaria (Bauman e Griinari, 2001).

E' quella bovina, dovuto alla sua importanza economica a livello mondiale, la specie in cui più si è studiato la MFD, mentre è ancora scarsa l'informazione relativa ai piccoli ruminanti da latte. Ciò potrebbe essere spiegato, al meno in parte, per il fatto che alcuni lavori realizzati nel caprino indicavano inizialmente questa specie come resistente alla MFD (Chilliard et al., 2007; Bernard et al., 2009; Shingfield et al., 2010), il che, a sua volta, fu riferito all'ovino, assumendo una situazione similare. Tuttavia, pubblicazioni più recenti dimostrano che sia la capra (Lock et al., 2008; Shingfield et al., 2009a; Chilliard et al., 2014), sia soprattutto la pecora (Toral et al., 2010a,b; Weerasinghe et al., 2012; Hussein et al., 2013) presentano questa sindrome che, per tanto, diventa necessario studiare anche in queste specie.

Nei capitoli precedenti di questa tesi si descrivono tre esperimenti che riguardano la lipogenesi nella ghiandola mammaria della pecora in lattazione (Prova I) e la sua relazione con i cambi provocati dalla dieta, durante la MFD, sia nel processo di bioidrogenazione (BH) ruminale, sia nell'espressione di una serie di geni candidati legati al metabolismo lipidico (Prova II e III).

Nel primo studio si dimostrò che, come avviene nella vacca da latte, l'inibizione della Δ^9-desaturasi, ottenuta mediante la somministrazione di acido sterculico, permette di quantificare la sintesi endogena di determinati acidi grassi (AG) con un doppio legame in posizione *cis*-9 nella ghiandola mammaria della pecora.

Nel secondo esperimento si provocò la MFD attraverso il supplemento della dieta con microalghe marine (MA) per 8 settimane e si osservò, oltre alla diminuzione del contenuto grasso del latte, una riduzione lungo tutto il periodo sperimentale del contenuto di 18:0 e *cis*-9 18:1 e un aumento di quello di alcuni AG di conformazione *trans*.

Nel terzo esperimento si studiò l'espressione di un gruppo di geni candidati che codificano per i principali enzimi del metabolismo lipidico, mettendo a confronto

quella degli animali con MFD con quella degli animali del gruppo controllo, senza riuscire a evidenziare differenze significative nella abbondanza di RNAm dei geni in questione.

Sintesi endogena di AG nella ghiandola mammaria

La ghiandola mammaria dei ruminanti da latte è l'organo principale dove avviene la sintesi endogena di AG che possiedono un doppio legame in posizione *cis*-9 (Kinsella, 1972; Mosley et al., 2006; Bernard et al., 2013). Il responsabile di questa sintesi è fondamentalmente l'enzima Δ^9-desaturasi o SCD, il quale inserisce un doppio legame in un ampio numero di AG (Ntambi e Miyazaki, 2004). Il substrato principale dell'SCD è il 18:0, il quale viene convertito in *cis*-9 18:1 (Ntambi e Miyazaki, 2004; Palmquist et al., 2005).

Il grasso del latte dei ruminanti contiene una grande varietà di AG organizzati in trigliceridi (TG) e, alla temperatura corporea dell'animale, si presenta fluido, il che permette alle cellule mammarie la sua secrezione (Timmen e Patton, 1988). Tuttavia, questa fluidità dipende dalla struttura degli AG che compongono i TG. Gli AG a corta catena e quelli *cis*-insaturi a lunga catena ne aumentano la fluidità, mentre gli AG saturi (≥12C) e i *trans* 18:1 aumentano la temperatura di fusione del grasso favorendo una consistenza più solida (Timmen e Patton, 1988; Taylor e McGibbon, 2011). Fra gli AG insaturi a lunga catena, risalta il *cis*-9 18:1 per una doppia ragione: da un lato, questo AG sembra giocare un ruolo chiave nel mantenimento della fluidità del grasso del latte (Timmen e Patton, 1988; Chilliard et al., 2000; Gama et al., 2008), dall'altro suscita un grande interesse nella comunità scientifica per i suoi effetti benefici sulla salute umana (Lou-Bonafonte et al., 2012; Silva et al., 2015).

Un'altra funzione particolarmente rilevante dell'SCD è la sintesi endogena di acido rumenico (*cis*-9, *trans*-11 18:2), i cui effetti potenzialmente benefici per la salute del consumatore sono ancora causa di grande interesse (Parodi, 2003; Palmquist et al., 2005).

I dati disponibili nella specie bovina e caprina indicano che una quota compresa fra il 50 e l'80% circa del *cis*-9 18:1 (o acido oleico) presente nel grasso del latte ha origine dalla sintesi endogena nella ghiandola mammaria per Δ^9-desaturazione del 18:0 (o acido stearico; Mosley e McGuire, 2007; Glasser et al., 2008; Bernard et al., 2013). Parallelamente, gli studi in cui si calcolò la quantità di *cis*-9, *trans*-11 18:2

prodotta in forma endogena indicano che quest'ultima rappresenta un ampia percentuale del totale (fra il 64 e il 91%, circa, a seconda della dieta offerta agli animali; Palmquist et al., 2005; Glasser et al., 2008; Shingfield et al., 2008). D'altro canto, al momento di realizzare il primo esperimento di questa tesi (quello pubblicato in Bichi et al., 2012) non esisteva nessuna informazione al riguardo nell'allevamento ovino.

In questo primo esperimento, l'inibizione della Δ^9-desaturasi si ottenne somministrando acido sterculico, un potente e specifico inibitore dell'enzima, a un gruppo di 6 pecore, il che permise di quantificare la percentuale di acido oleico e rumenico sintetizzati endogenamente. I nostri risultati confermarono che l'SCD è responsabile della maggior parte della produzione sia di *cis*-9 18:1 (63%), sia di *cis*-9, *trans*-11 18:2 (74%). Tali valori sono leggermente diversi da quelli riportati in due recenti studi (Frutos et al., 2014; Toral et al., 2015b), in cui si usò acetato di cobalto per inibire la Δ^9-desaturasi. Tuttavia, più che alla differenza nella metodologia usata, le variazioni in questi risultati potrebbero attribuirsi, al meno parzialmente, alla distinta composizione della dieta consumata dagli animali (foraggio fresco nel presente studio *versus* una razione completa mescolata, supplementata o meno con una fonte ricca di 18:3 n-3, nel caso degli altri due lavori). Queste diverse diete suppongono una differente disponibilità di certi AG, soprattutto vaccenico e rumenico, derivanti dalla BH ruminale, per l'attività dell'SCD nella ghiandola mammaria.

In relazione ai meccanismi d'azione, l'acido sterculico formerebbe un complesso inattivo fra l'anello ciclopropenico della sua molecola e la struttura proteica dell'SCD, senza che l'espressione del gene *SCD* si veda alterata (Gomez et al., 2003). D'altra parte, sebbene il meccanismo d'azione del cobalto sia ancora poco chiaro, alcuni lavori suggeriscono che il suo effetto inibitore potrebbe essere collegato alla sua capacità di sostituire il ferro nel complesso enzimatico che costituisce l'SCD (Shingfield et al., 2008; Taugbøl et al., 2010), essendo anche possibile un effetto negativo sull'espressione dell'*SCD* nel tessuto mammario (Toral et al., 2015b). In tutti i casi, anche se i rispettivi meccanismi d'azione non sono completamente chiari, entrambi i metodi riescono a inattivare il sistema enzimatico della Δ^9-desaturasi e a diminuire il processo normale di desaturazione degli AG (Gomez et al., 2003; Taugbøl et al., 2010; Frutos et al., 2014), il che permette calcolarne la sintesi endogena.

Sindrome di scarso grasso nella pecora da latte

Un dettaglio interessante della Prova I fu che, accanto all'inibizione dell'SCD, si ottenne anche una riduzione del contenuto grasso del latte. Questo fatto potrebbe relazionarsi con la teoria che associa l'apparizione della MFD con la carenza di 18:0 per la sintesi di *cis*-9 18:1 nella ghiandola mammaria, giacché tutto ciò altererebbe le proprietà fisiche del grasso del latte, aumentandone il punto di fusione e rendendone più complicata la secrezione (Chilliard et al., 2000; Loor et al., 2005; Shingfield e Griinari, 2007). Questo meccanismo è stato proposto frequentemente come spiegazione della MFD indotta attraverso lipidi di origine marina (Gama et al., 2008; Toral et al., 2010a, 2015a). Gli AG n-3 a catena molto lunga, abbondanti nei lipidi marini, sono noti inibitori dell'ultimo passo della BH ruminale, il che provoca una diminuzione della quantità di 18:0 che abbandona il rumine (Or-Rashid et al., 2008) e che rappresenta il substrato necessario per la sintesi endogena di *cis*-9 18:1 nella ghiandola mammaria. Ciò coincide con i risultati segnalati da Toral et al. (2010b) nelle pecore in lattazione la cui dieta era stata supplementata con MA e anche con i risultati della Prova II. In quest'ultimo caso, la MFD provocata dalle alghe perdurò per tutta la durata del trattamento sperimentale (8 settimane) e si vide accompagnata da bassi contenuti nel grasso del latte sia di 18:0, sia di *cis*-9 18:1 a partire dalla prima settimana. Questi dati indicano l'alterazione del processo di BH ruminale come principale causa della diminuzione del contenuto grasso del latte, piuttosto che a l'inibizione della Δ^9-desaturasi nelle cellule dell'epitelio mammario. Ciò nonostante, diversi studi recenti sembrano mettere in discussione il ruolo chiave della scarsità di 18:0 per la sintesi endogena di *cis*-9 18:1 come meccanismo responsabile della MFD provocata dai lipidi marini, giacché, per esempio, l'aggiunta di un supplemento di 18:0 alla dieta non permette di attenuare la diminuzione del contenuto grasso del latte (Toral et al., in stampa-b).

D'altro lato, è importante menzionare che l'inibizione della Δ^9-desaturasi non sempre è accompagnata dalla caduta del contenuto grasso del latte (Kay et al., 2004; Frutos et al., 2014; Toral et al., 2015b). Per esempio, in un lavoro recente, Toral et al. (2015b) osservarono che la diminuzione della desaturazione di acido stearico ad acido oleico per effetto dell'inibizione dell'SCD mediante l'acetato di cobalto, non fu accompagnata da MFD. Nell'esperimento condotto dagli stessi autori, il supplemento della dieta con olio di lino avrebbe aumentato gli AG poli-insaturati a lunga catena e

diminuito quelli saturati a media catena, il che probabilmente contribuì a evitare problemi relativi al punto di fusione del grasso e alla sua secrezione. Queste osservazioni indicherebbero ancora una volta che i meccanismi implicati nell'insorgere della MFD sono chiaramente dipendenti dalla dieta assunta dagli animali.

D'altro lato, molti studiosi (Capper et al., 2007; Toral et al., 2010a, in stampa-b) hanno osservato anche un aumento di AG di conformazione *trans* nel latte di pecore con MFD provocata dai lipidi marini, il che potrebbe contribuire a peggiorare la capacità dell'epitelio mammario nel mantenere il punto di fusione del grasso al di sotto della temperatura corporea, e coincide con i risultati della Prova II. Tuttavia, il carattere antilipogenico di alcuni AG *trans* va molto oltre l'alterazione del punto di fusione del grasso del latte.

In questo senso, nella Prova II è importante evidenziare la relazione inversa nel tempo dei contenuti di *trans*-10 18:1 e i livelli del grasso del latte. Sebbene in diversi esperimenti condotti nelle pecore da latte si sono rilevati livelli molto elevati di questo AG senza MFD (ex., Reynolds et al., 2006; Gómez-Cortés et al., 2008), questo isomero 18:1 è stato associato nel bovino con l'apparizione della sindrome di scarso grasso nel latte (Shingfield et al., 2006; Kadegowda et al., 2008; Shingfield et al., 2009b) e il suo possibile intervento in questo processo è ancora materia di studio e dibattito. Altri due AG con caratteristiche antilipogeniche che, a loro volta, aumentarono nella Prova II in risposta al consumo di MA furono due isomeri del acido linoleico coniugato (CLA; il *trans*-10, *cis*-12 18:2 e il *trans*-9, *cis*-11 18:2; Perfield et al., 2007; Shingfield e Griinari, 2007; Harvatine et al., 2009). La loro concentrazione rimase più elevata nelle pecore che ricevettero MA durante tutto il periodo sperimentale sebbene, anche in questo caso, la loro implicazione nell'apparizione della MFD nell'ovino non risulti evidente e, nel caso specifico del *trans*-10, *cis*-12 18:2, appaia poco probabile (Toral et al., 2010a,b e 2014). Pertanto, sebbene il supplemento diretto della dieta di pecore da latte con *trans*-10, *cis*-12 18:2 provochi un calo del contenuto in grasso del latte, il suo ruolo nella MFD provocata da lipidi marini si può considerare irrilevante (Toral et al., in stampa-a). In quest'ultimo articolo, gli autori suggeriscono che si dovrebbe studiare l'implicazione in questo tipo di MFD di certi AG con capacità antilipogenica meno nota (come, per esempio, il *trans*-10, *cis*-15 18:2 o il 10-oxo-18:0).

Per ultimo, è interessante menzionare come gli aumenti nelle concentrazioni di AG antilipogenici rilevati nella Prova II non si siano potuti relazionare con una minore abbondanza di RNAm di quei geni che codificano per i principali enzimi implicati nel metabolismo lipidico della ghiandola mammaria (Prova III), al contrario di quanto ci fosse da aspettarsi in accordo con lavori precedenti in vacche da latte alimentate con oli di origine marina (Ahnadi et al., 2002; Harvatine et al., 2009; Invernizzi et al., 2010).

La differenza fra i risultati descritti nella Prova III e i dati disponibili nel bovino potrebbe avere varie spiegazioni: da un lato, non si può scartare la possibilità che i cambi a livello di trascrittoma si siano prodotti relativamente presto dopo l'ingestione di MA, in maniera similare a ciò che si verifica nel profilo lipidico del latte delle stesse pecore (Prova II), e che fosse più complicato rilevarli nel lungo periodo. Ciò coinciderebbe con quanto segnalato da Invernizzi et al. (2010) in vacche da latte con MFD provocata dal consumo di lipidi marini. Tuttavia, nell'ovino Carreño et al. (2015) dimostrarono che il supplemento della dieta con olio di pesce causava la sindrome di scarso grasso che si accompagnava non solo con un aumento nella concentrazione di diversi AG con effetto antilipogenico (*trans*-10 18:1, *trans*-10, *cis*.12 18:2 e *trans*-9, *cis*-11 18:2), ma anche da una diminuzione nell'abbondanza di RNAm di alcuni geni chiave nel metabolismo lipidico mammario, incluso i fattori di trascrizione (*ACACA*, *FASN*, *SCD1* e *SREBF1*). La maggior parte di questi cambi risultarono evidenti già al primo campionamento (ovvero, dopo solo 7 giorni di trattamento) e perdurarono fino alla fine dell'esperimento.

Non si può inoltre scartare l'ipotesi che, come segnalano Bernard et al. (2013), la regolazione post-trascrizionale possa avere un peso maggiore nella MFD di quella trascrizionale. Quest'aspetto si evidenzia anche nel lavoro di Castro-Carrera et al. (2015), nel quale l'alterazione del profilo lipidico del latte dovuto al supplemento della dieta di pecore da latte con olio di girasole non si vide accompagnato da cambi significativi nell'abbondanza di RNAm dei geni selezionati associati alla lipogenesi che furono oggetto di studio.

D'altro lato, il fatto che la dieta basale di tutti gli animali utilizzati nelle Prove II e III (incluso i gruppi controllo) fosse ricca di acido linoleico, il quale inibisce la sintesi di AG *de novo* nella ghiandola mammaria (Hervás et al., 2008; Mele et al., 2008; Gómez-Cortés et al., 2011), potrebbe aver reso più difficile il rilevamento di determinati cambi nell'espressione dei geni legati al consumo di MA.

Infine, è fondamentale continuare nello studio prima di trarre conclusioni circa la possibile differenza fra le specie di ruminanti nella risposta a livello di regolazione trascrizionale nel tessuto mammario. Attualmente, sembra che nel caprino non ci sia ancora evidenza di una chiara relazione tra i cambi nel profilo di AG nel latte e l'espressione dei geni candidati implicati nella loro sintesi, mentre nel bovino tale relazione è più evidente (Ahnadi et al., 2002; Shingfield et al., 2013). Nella specie ovina, i dati sull'argomento sono ancora molto scarsi e inconsistenti (Hussein et al., 2013, Castro-Carrera et al., 2015), anche se i lavori più recenti (per esempio, Carreño et al., 2015) non permettono di scartare la possibilità che la MFD provocata dal consumo di lipidi marini (ovvero, quella studiata nella Prova II e III) sia regolata da meccanismi trascrizionali.

In tutti i casi e nel suo insieme, questi risultati mostrano la complessità dei fattori che possono determinare lo sviluppo della MFD e sottolineano l'importanza di continuare la ricerca nell'ovino da latte, giacché in questa specie le incertezze sule cause di questa sindrome sono ancora numerose.

REFERENZE

Ahnadi, C., Beswick, N., Delbecchi, L., Kennelly, J., and Lacasse, P. 2002. Addition of fish oil to diets for dairy cows. II. Effects on milk fat and gene expression of mammary lipogenic enzymes. J. Dairy Res. 69:521-531.

Bauman, D. E., and Griinari, J. M. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15-29.

Bernard, L., Leroux, C., and Chilliard, Y. 2013. Expression and nutritional regulation of stearoyl-CoA desaturase genes in the ruminant mammary gland: relationship with milk fatty acid composition. In: Stearoyl-CoA desaturase genes in lipid metabolism. Ntambi, J. M. (ed.). Springer Science+Business Media, New York, USA, pp. 161-193.

Bernard, L., Shingfield, K. J., Rouel, J., Ferlay, A., and Chilliard, Y. 2009. Effect of plant oils in the diet on performance and milk fatty acid composition in goats fed diets based on grass hay or maize silage. Br. J. Nutr. 101:213-224.

Bichi, E., Toral, P. G., Hervás, G., Frutos, P., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A. 2012. Inhibition of Δ^9-desaturase activity with sterculic acid: Effect on the endogenous synthesis of *cis*-9 18:1 and *cis*-9, *trans*-11 18:2 in dairy sheep. J. Dairy Sci. 95:5242-5252.

Capper, J. L., Wilkinson, R. G., Mackenzie, A. M., and Sinclair, L. A. 2007. The effect of fish oil supplementation of pregnant and lactating ewes on milk production and lamb performance. Animal 1:889-898.

Carreño, D., Hervás, G., Toral, P.G., Castro-Carrera, T., Fernández, M., y Frutos, P. 2015. Efecto de la suplementación de la dieta con aceite de pescado sobre la abundancia de ARNm de genes implicados en la lipogénesis mamaria en ovejas lecheras. En: XVI Jornadas sobre Producción Animal AIDA. 19-20 de mayo de 2015. Zaragoza. J. Álvarez, B. Panea, J.H. Calvo, M. Blanco, A. Abecia, D. Villalba y M.A. Latorre (eds.). AIDA, Zaragoza, España, pp. 152-154.

Castro-Carrera, T., Frutos, P., Leroux, C., Chilliard., Y., Hervás, G., Belenguer, A., Bernard, L., and Toral, P. G. 2015. Dietary sunflower oil modulates milk fatty acid composition without major changes in adipose and mammary tissue fatty acid profile or related gene mRNA abundance in sheep. Animal 9:582-591.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., and Doreau, M. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181-205.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828-855.

Chilliard, Y., Toral, P. G., Shingfield, K. J., Rouel, J., Leroux, C., and Bernard, L. 2014. Effects of diet and physiological factors on milk fat synthesis, milk fat composition and lipolysis in the goat. A short review. Small Rumin. Res. 122:31-37.

Frutos, P. Toral, P. G., Ramos-Morales, E., Shingfield, K. J., Belenguer, A., and Hervás, G. 2014. Oral administration of cobalt acetate alters milk fatty acid composition, consistent with an inhibition of stearoyl-coenzyme A desaturase in lactating ewes. J. Dairy Sci. 97:1036-1046.

Gama, M. A. S., Garnsworthy P. C., Griinari, J. M., Leme, P. R., Rodrigues, P. H. M., Souza, L. W. O., and Lanna, D. P. D. 2008. Diet-induced milk fat depression: association with changes in milk fatty acid composition and fluidity of milk fat. Livest. Sci. 115:319-331.

Glasser, F., Ferlay, A., Doreau, M., Schmidely, P., Sauvant, D., and Chilliard, Y. 2008. Long-chain fatty acid metabolism in dairy cows: A meta-analysis of milk fatty acid yield in relation to duodenal flows and de novo synthesis. J. Dairy Sci. 91:2771-2785.

Gomez, F. E., Bauman, D. E., Ntambi, J. M., and Fox, B. G. 2003. Effects of sterculic acid on stearoyl-CoA desaturase in differentiating 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 300:316-326.

Gómez-Cortés, P., Frutos, P., Mantecón, A. R., Juárez, M., de la Fuente, M. A., and Hervás, G. 2008. Milk production, conjugated linoleic acid content, and in vitro ruminal fermentation in response to high levels of soybean oil in dairy ewe diet. J. Dairy Sci. 91:1560-1569.

Gómez-Cortés, P., de la Fuente, M. A., Toral, P. G., Frutos, P., Juárez, M., and Hervás, G. 2011. Effects of different forage:concentrate ratios in dairy ewe diets supplemented with sunflower oil on animal performance and milk fatty acid profile. J. Dairy Sci. 94:4578-4588.

Harvatine, K. J., Boisclair, Y. R., and Bauman, D. E. 2009. Recent advances in the regulation of milk fat synthesis. Animal 3:40-54.

Hervás, G., Luna, P., Mantecón, A. R., Castañares, N., de la Fuente, M. A., Juárez, M., and Frutos, P. 2008. Effect of diet supplementation with sunflower oil on milk production,

fatty acid profile and ruminal fermentation in lactating dairy ewes. J. Dairy Res. 75:399-405.

Hussein, M., Harvatine, K. J., Weerasinghe, W. M., Sinclair, L. A., and Bauman, D. E. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. J. Dairy Sci. 96:3825-3834.

Invernizzi, G., Thering, B. J., McGuire, M. A., Savoini, G., and Loor, J. J. 2010. Sustained upregulation of stearoyl-CoA desaturase in bovine mammary tissue with contrasting changes in milk fat synthesis and lipogenic gene networks caused by lipid supplements. Funct. Integr. Genomics 10:561-575.

Kay, J. K., Mackle, T. R., Auldist, M. J., Thomson, N. A., and Bauman, D. E. 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369-378.

Kadegowda, A. K., Piperova, L. S., and Erdman, R. A. 2008. Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. J. Dairy Sci. 91:749-759.

Kinsella, J. E. 1972. Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglycerides synthesis. Lipids 7:349-355.

Lock, A. L., Rovai, M., Gipson, T. A., de Veth, M. J., and Bauman, D. E. 2008. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 conjugated linoleic acid reduces milk fat synthesis in lactating goats. J. Dairy Sci. 91:3291-3299.

Loor, J. J., Doreau, M., Chardigny, J. M., Ollier, A., Sébédio, J. L., Chilliard, Y. 2005. Effects of ruminal or duodenal supply of fish oil on milk fat secretion and profiles of *trans*-fatty acids and conjugated linoleic acid isomers in dairy cows fed maize silage. Anim. Feed Sci. Technol. 119:227-246.

Lou-Bonafonte, J. M., Arnal, C., Navarro, M. A., and Osada, J. 2012. Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development. Mol. Nutr. Food Res. 56:1043-1057.

Mele, M., Sarra, A., Buccioni, A., Conte, G., Pollicardo, A., and Secchiari, P. 2008. Effect of soybean oil supplementation on milk fatty acid composition from Saanen goats fed diets with different forage:concentrate ratios. Ital. J. Anim. Sci. 7:297-311.

Mosley, E. E., and McGuire, M. A. 2007. Methodology for the in vivo measurement of the Δ^9-desaturation of myristic, palmitic, and stearic acids in lactating dairy cattle. Lipids 42:939-945.

Mosley, E. E., Shaffi Dagger, B., Moate, P. J., and McGuire, M. A. 2006. *Cis*-9, *trans*-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575.

Ntambi, J. M., and Miyazaki, M. 2004. Regulation of stearoyl-CoA desaturase and role in metabolism. Prog. Lipid Res. 43:91-104.

Or-Rashid, M. M., Kramer, J. K. G., Wood, M. A., and McBride, B. W. 2008. Supplemental algal meal alters the ruminal *trans*-18:1 fatty acid and conjugated linoleic acid composition in cattle. J. Anim. Sci. 86:187-196.

Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. 2005. Biosynthesis of

conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res. 50:179-217.

Parodi, P. W. 2003. Conjugated linoleic acid in food. In: Advances in conjugated linoleic acid research. Sébédio, J. L., Christie, W. W. and Adlof, R. (eds.). AOCS Press, Champaign, IL, USA, pp.101-122.

Perfield, J. W., Lock, A. L., Griinari, J. M., Sæbø, A., Delmonte, P., Dwyer, D. A., and Bauman, D. E. 2007. *Trans*-9, *cis*-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. J. Dairy Sci. 90:2211-2218.

Reynolds, C. K., Cannon, V. L., and Loerch, S. C. 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131:333-357.

Shingfield, K. J., Ärölä, A., Ahvenjärvi, S., Vanhatalo, A., Toivonen, V., Griinari, J. M., and Huhtanen, P. 2008. Ruminal infusions of cobalt-EDTA reduce mammary Δ^9-desaturase index and alter milk fatty acid composition in lactating cows. J. Nutr. 138:710-717.

Shingfield, K. J., Bernard, L., Leroux, C., and Chilliard, Y. 2010. Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4:1140-1166.

Shingfield, K. J., Bonnet, M., and Scollan, N. D. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7:s1:132-162.

Shingfield, K. J., and Griinari, J. M. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Technol. 109:799-816.

Shingfield, K. J., Reynolds, C. K., Hervás, G., Griinari, J. M., Grandison, A. S., and Beever, D. E. 2006. Examination of the persistency of milk fatty acid composition responses to fish oil and sunflower oil in the diet of dairy cows. J. Dairy Sci. 89:714-732.

Shingfield, K. J., Rouel, J., and Chilliard, Y. 2009a. Effect of calcium salts of a mixture of conjugated linoleic acids containing *trans*-10, *cis*-12 in the diet on milk fat synthesis in goats. Br. J. Nutr. 101:1006-1019.

Shingfield, K. J., Sæbø, A., Sæbø, P. C., Toivonen, V., and Griinari, J. M. 2009b. Effect of abomasal infusions of a mixture of octadecenoic acids on milk fat synthesis in lactating cows. J. Dairy Sci. 92:4317-4329.

Silva, S., Combet, E, Figueira, M. E., Koeck, T., Mullen, W., and Bronze, M. R. 2015. New perspectives on bioactivity of olive oil: evidence from animal models, human interventions and the use of urinary proteomic biomarkers. Proc. Nutr. Soc. 74:268-281.

Taugbøl, O., Karlengen, I. J., Salbu, B., Aastveit, A. H., and Harstad, O. M. 2010. Intravenous injections of cobalt reduce fatty acid desaturation products in milk and blood of lactating cows. J. Anim. Physiol. Anim. Nutr. 94: 635-640.

Taylor, M. W., and MacGibbon, A. K. H. 2011. Triacylglycerols. In: Fuquay, F. W. (ed.), Encyclopedia of dairy sciences, 2nd edition, Academic Press, San Diego, USA, pp. 665-669.

Timmen, H., and Patton, S. 1988. Milk fat globules: fatty-acid composition, size and in vivo regulation of fat liquidity. Lipids 23:685-689.

Toral, P. G., Chilliard, Y., Rouel, J., Leskinen, H., Shingfield, K. J., and Bernard, L. 2015a. Comparison of the nutritional regulation of milk fat secretion and composition in cows and goats. J. Dairy Sci. 98:7277-7297.

Toral, P. G., Frutos, P., Hervás, G., Gómez-Cortés, P., Juárez, M., and de la Fuente, M. A.

2010a. Changes in milk fatty acid profile and animal performance in response to fish oil supplementation, alone or in combination with sunflower oil, in dairy ewes. J. Dairy Sci. 93:1604-1615.

Toral P. G., Hervás, G., Belenguer, A., Castro-Carrera, T., Carreño, D., and Frutos, P. 2014. Principal component analysis of diet-induced changes in milk fat content and fatty acid composition in dairy ewes. Proc. Aust. Soc. Anim. Prod. 30:156.

Toral P. G., Hervás, G., Carreño, D., Belenguer, A., and Frutos, P. Comparison of milk fatty acid responses during fish oil- and *trans*-10 *cis*-12 18:2-induced milk fat depression in dairy ewes. Anim. Feed Sci. Technol. (in stampa-a).

Toral P. G., Hervás, G., Carreño, D., and Frutos, P. May supplemental 18:0 alleviate fish oil-induced milk fat depression in dairy ewes? J. Dairy Sci. (in stampa-b).

Toral P. G., Hervás, G., Frutos, P. 2015b. Reductions in milk Δ^9-desaturation ratios to oral dosing of cobalt-acetate are accompanied by the downregulation of *SCD1* in lactating ewes. J. Dairy Sci. 98:1961-1971.

Toral, P. G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., and de la Fuente, M. A. 2010b. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93:1655-1667.

Weerasinghe, W. M. P. B., Wilkinson, R. G., Lock, A. L., de Veth, M. J., Bauman, D. E., and Sinclair, L. A. 2012. Effect of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on the performance of dairy ewes fed 2 levels of metabolizable protein and at a restricted energy intake. J. Dairy Sci. 95:109-116.

CONCLUSIONES

Primera. La administración intravenosa de ácido estercúlico a ovejas modifica el perfil lipídico de la leche en concordancia con una inhibición de la enzima Δ^9-desaturasa o estearoil-CoA desaturasa (SCD). Así, el contenido de ácidos grasos obtenidos por Δ^9-desaturación (por ejemplo, *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 y *cis*-9, *trans*-11 18:2) se redujo y aumentó el de los sustratos de dicha enzima (por ejemplo, 14:0, 18:0 y *trans*-11 18:1). La disminución de la concentración de *cis*-9, *cis*-15 18:2 y *cis*-9, *trans*-11, *cis*-15 18:3, junto con el incremento paralelo de *cis*-15 18:1 y *trans*-11, *cis*-15 18:2 sugiere que dichos ácidos grasos podrían ser sintetizados endógenamente mediante la acción de la enzima SCD.

Segunda. La administración intravenosa de ácido estercúlico a ovejas en lactación, alimentadas con pasto (es decir, con una dieta rica en ácido linolénico), permitió estimar que el 63% del *cis*-9 18:1 y el 74% del *cis*-9, *trans*-11 18:2 que aparecen en la leche proceden de su síntesis endógena en la glándula mamaria mediante la acción de la estearoil-CoA desaturasa.

Tercera. La suplementación de una dieta rica en ácido linoleico con microalgas marinas (0,8%) induce una caída en el contenido de grasa láctea en las ovejas lecheras que persiste al menos durante las 8 semanas de duración del ensayo in vivo. Entre las causas de este síndrome de baja grasa en la leche se apunta la menor disponibilidad de 18:0 para la síntesis endógena de *cis*-9 18:1 y el gradual y persistente aumento de isómeros *trans* de los ácidos grasos de 18 C. No parece que exista una adaptación de la microbiota ruminal al consumo de lípidos marinos que permita revertir la depresión de la grasa láctea en ovejas.

Cuarta. En las condiciones del experimento en el que se indujo un síndrome de baja grasa en la leche de oveja mediante el consumo de una dieta suplementada con microalgas marinas, no se detectan cambios significativos, tras 8 semanas de tratamiento, en la abundancia de ARNm de los genes candidatos que codifican las principales proteínas involucradas en el metabolismo lipídico de la glándula mamaria (por ejemplo, *ACACA, ACSS2, ELOVL6, FABP3, FASN, INSIG1, LPL, PPARG, SCD, SREBF1*). Esto coincide con la ausencia de variaciones en la abundancia de ARNm en los genes candidatos implicados en el metabolismo lipídico que se estudiaron en el hígado y en el tejido adiposo.

CONCLUSIONS

First. Intravenous administration of sterculic acid to dairy ewes modifies milk fatty acid profile, consistent with an inhibition of the enzyme Δ^9-desaturase or stearoyl-CoA desaturase (SCD). Thus, there was a decrease in the milk content of fatty acids deriving from Δ^9-desaturation (e.g., *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 and *cis*-9, *trans*-11 18:2) and an increase in the enzyme substrates (e.g., 14:0, 18:0 and *trans*-11 18:1). The decrease in the concentration of *cis*-9, *cis*-15 18:2 and *cis*-9, *trans*-11, *cis*-15 18:3, together with the concurrent increase in *cis*-15 18:1 and *trans*-11, *cis*-15 18:2, suggests that those fatty acids might be endogenously synthetized by Δ^9-desaturation.

Second. Intravenous administration of sterculic acid to lactating ewes fed pasture (i.e., a linolenic acid-rich diet) allowed to estimate that 63% of *cis*-9 18:1 and 74% of *cis*-9, *trans*-11 18:2 present in milk fat arise from endogenous synthesis in the mammary gland through the action of stearoyl-CoA desaturase.

Third. Supplementation of a diet rich in linoleic acid with marine algae (0.8%) induces a drop in the milk fat content of dairy ewes that persists for at least the 8 week in vivo trial. The reduction in 18:0 availability for endogenous synthesis of *cis*-9 18:1, together with the gradual and persistent increase in *trans* isomers of C 18 fatty acids are pointed out among the main causes of this milk fat depression. It seems not to exist an adaptation of the ruminal microbiota to marine lipids consumption allowing to reverse milk fat depression in the ewes.

Fourth. Under the experimental conditions in which milk fat depression was induced in dairy ewes by the consumption of a diet supplemented with marine algae, no significant changes were detected, after 8 weeks of treatment, in the mRNA abundance of the candidate genes encoding key proteins involved in mammary lipid metabolism (e.g., *ACACA*, *ACSS2*, *ELOVL6*, *FABP3*, *FASN*, *INSIG1*, *LPL*, *PPARG*, *SCD*, *SREBF1*). This is consistent with the absence of changes in the mRNA abundance of the candidate genes involved in lipid metabolism that were studied in the liver and adipose tissue.

CONCLUSIONI

Prima. La somministrazione endovenosa di acido sterculico nelle pecore modifica il profilo lipidico del latte, a indicazione dell'inibizione dell'enzima Δ^9-desaturasi o stearoil-CoA desaturasi (SCD). Di conseguenza, il contenuto degli acidi grassi ottenuti per Δ^9-desaturazione (per esempio, *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 e *cis*-9, *trans*-11 18:2) si ridusse, mentre aumentò quello degli acidi grassi che costituiscono il substrato dell'enzima (per esempio, *cis*-9 10:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 18:1 e *cis*-9, *trans*-11 18:2). Inoltre, la diminuzione della concentrazione di *cis*-9, *cis*-15 18:2 e *cis*-9, *trans*-11, *cis*-15 18:3, parallelamente all'aumento di *cis*-15 18:1 e *trans*-11, *cis*-15 18:2, suggerisce che entrambi questi acidi grassi potrebbero essere sintetizzati per via endogena attraverso l'azione della SCD.

Seconda. La somministrazione endovenosa di acido sterculico nelle pecore in lattazione, alimentate con foraggio (vale a dire con una dieta ricca in acido linolenico), ha permesso di calcolare che il 63% del *cis*-9 18:1 e il 74% del *cis*-9, *trans*-11 18:2 che compaiono nel latte derivano dalla sintesi endogena nella ghiandola mammaria mediante l'azione della stearoil-CoA desaturasi.

Terza. Il supplemento di una dieta ricca in acido linoleico con microalghe marine (0,8%) provoca una diminuzione del contenuto in grasso del latte nelle pecore, che si protrae per lo meno durante le 8 settimane di durata dello studio in vivo. Fra le cause di questa sindrome di scarso grasso nel latte si indicano la minore disponibilità di 18:0 per la sintesi endogena di *cis*-9 18:1 e il graduale e persistente aumento degli isomeri *trans* degli acidi grassi di 18C. Non sembra, quindi, instaurarsi un adattamento della popolazione batterica ruminale al consumo di lipidi di origine marina che permetta di ristabilire il contenuto in grasso del latte nelle pecore.

Quarta. Nelle condizioni dell'esperimento in cui si provocò la sindrome di scarso grasso nel latte di pecora attraverso il consumo di una dieta arricchita con alghe marine, non si rilevano cambi significativi, dopo 8 settimane di trattamento, nell'abbondanza del RNAm dei geni selezionati che codificano per le principali proteine coinvolte nel metabolismo lipidico della ghiandola mammaria (per esempio, *ACACA, ACSS2, ELOVL6, FABP3, FASN, INSIG1, LPL, PPARG, SCD, SREBF1*). Ciò coincide con l'assenza di variazioni nell'abbondanza di RNAm dei geni selezionati implicati nel metabolismo lipidico che furono analizzati nel fegato e nel tessuto adiposo.