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Nutritional regulation of mammary lipogenesis and milk fat in ruminant: contribution to sustainable milk production

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Summary

Researches in animal science focus on contributing to the development of sustainable livestock production systems in agreement with societal expectations. In particular, researches in dairy sciences aimed to improve the understanding of the biology of herbivores and developed strategies to modulate the performances of dairy ruminants and the quality of their products. As nutrition constitutes a rapid and efficient means to modulate milk fat synthesis, it may be used to control both the animal energy balance and the nutritional quality of its milk for human consumers. In this context, studies were performed to enhance the understanding of the nutritional regulation of milk fat synthesis. This short review presents the current knowledge on the nutritional regulation of lipid metabolism in the ruminant mammary gland in relation with milk fat yield and fatty acids (FA) composition and the underlying mechanisms, with particular emphasis on lipogenic genes expression. In cows, diet-induced milk fat depression (MFD) is an extreme model of regulation of milk fat synthesis that has the potential to influence productive functions via modulation of energy partitioning that may affect tissue mobilisation and reproductive performances. Use of molecular tools have demonstrated decreased mammary abundance of transcripts encoding the major lipogenic genes (in particular those involved in de novo FA synthesis) and of sterol responsive element binding protein 1 (SREBP1) during MFD in the bovine. However, feeding similar diets do not induce MFD in the caprine and did not substantially alter mammary lipogenic gene expression. Due to the implication of the stearoyl-CoA desaturase (SCD) enzyme in mammary gland physiology and in milk technological and nutritional qualities, studies focused specifically in the SCD gene expression, activity and metabolic pathway. Further knowledge on the regulation of cellular and molecular processes that modulate milk fat synthesis and composition will promote further improvement of metabolic and physiological efficiency of lactating ruminants and quality of dairy products.

Key words: *enzyme activity, gene expression, lactation, mammary gland, milk fatty acid profile, milk fat synthesis.*

Resumen

La investigación en zootecnia se ha enfocado en el desarrollo de sistemas agropecuarios sostenibles que cumplan las expectativas de la sociedad. En el caso específico del sector lácteo, un conocimiento más profundo de la biología de los rumiantes permitiría desarrollar estrategias que ayuden a controlar su rendimiento productivo y la calidad de los productos. En este sentido, la nutrición permite modificar de forma rápida y eficaz la síntesis de grasa láctea, lo que podría mejorar el balance energético del animal y el valor nutricional de su leche, de ahí que se hayan realizado estudios dirigidos a profundizar en el conocimiento de la regulación de la síntesis de grasa láctea en la glándula mamaria. Esta revisión breve recoge la información

disponible sobre la regulación nutricional del metabolismo lipídico, particularmente sobre la expresión de los genes de la lipogénesis, en la glándula mamaria de los rumiantes, en relación con la síntesis de grasa y la composición de los ácidos grasos (AG) de la leche, así como los mecanismos subyacentes. En el vacuno, el síndrome de baja grasa en la leche inducido por la dieta constituye un modelo extremo de regulación de la síntesis de grasa, que puede influir sobre la producción mediante cambios en la partición de la energía, lo que podría afectar a la movilización de reservas, así como al rendimiento reproductivo. El uso de herramientas de biología molecular ha mostrado que, durante dicho síndrome, se reduce la abundancia de los transcritos que codifican los principales genes de la lipogénesis (en particular los implicados en la síntesis de AG de novo) y la proteína de unión a elementos reguladores del esterol 1 (SREBP1) en el tejido mamario del bovino. En el caprino, sin embargo, dietas similares no reducen la síntesis de grasa ni alteran de forma considerable la expresión de los genes de la lipogénesis mamaria. Dada la implicación de la enzima estearoil-CoA desaturasa (SCD) en la fisiología mamaria y en la calidad nutricional y tecnológica de la leche, también se han llevado a cabo ensayos dirigidos específicamente a estudiar la expresión del gen que la codifica, su actividad y las rutas metabólicas en las que está implicada. Sería necesaria, no obstante, más investigación sobre la regulación de los procesos celulares y moleculares que determinan la síntesis y composición de la grasa láctea, con el fin de mejorar la eficiencia metabólica y fisiológica de los rumiantes lecheros y la calidad de sus productos.

Palabras clave: *actividad enzimática, expresión génica, glándula mamaria, lactación, perfil de ácidos grasos de la leche, síntesis de grasa láctea.*

Resumo

A pesquisa em ciência animal, de acordo com as expectativas da sociedade, tem vindo a contribuir para o desenvolvimento de sistemas de produção pecuária sustentável. A investigação em ciência de produção de leite, tem tido como objetivos melhorar a compreensão biológica dos herbívoros a fim de desenvolver estratégias para modular o desempenho de ruminantes leiteiros e a qualidade de seus produtos. A alimentação constitui um meio rápido e eficaz para modular a síntese de gordura no leite, e esta pode ser usada para controlar tanto o balanço energético do animal quanto a qualidade nutricional do leite para consumo humano. Neste contexto, foram realizados alguns estudos com o objetivo de melhorar a compreensão da regulação da síntese de gordura no leite pela glândula mamária. Esta breve revisão apresenta o conhecimento atual sobre a regulação nutricional do metabolismo lipídico na glândula mamária de ruminantes, em relação com a produção de gordura e composição dos ácidos graxos do leite e os mecanismos subjacentes, com especial ênfase, sobre a expressão gênica de enzimas lipogênicas e de enzimas envolvidas nas vias lipogênicas. A depressão de gordura no leite induzida pela dieta (MFD) nas vacas leiteiras constitui um modelo extremo de regulação da síntese de gordura no leite, e isto tem o potencial de influenciar as funções produtivas através da modulação da repartição de energia, podendo afetar a mobilização dos tecidos e o desempenho reprodutivo. O uso de métodos de biologia molecular tem mostrado que, na glândula mamária, a abundância de transcritos que codificam os principais genes lipogênicos diminui (em particular aqueles envolvidos na neossíntese de ácidos graxos) e de "sterol responsive element binding protein 1" (SREBP1) durante o MFD na espécie bovina. No entanto, as rações de composição similar, não provocam o MFD na espécie caprina e não alteram substancialmente a expressão de genes lipogênicos da glândula mamária. Muitos estudos são focados especificamente na expressão, atividade e vias metabólicas da "stearoyl-CoA desaturase" (SCD), devido à sua implicação na fisiologia da glândula mamária e a seus efeitos na produção de leite, alem das propriedades tecnológicas e nutricionais. O desenvolvimento do conhecimento sobre a regulação dos processos celulares e moleculares que regulam a síntese de gordura e a composição no leite vai continuar a promover a eficiência metabólica e fisiológica de ruminantes em lactação, e aumentar deste jeito, a qualidade nutricional dos produtos lácteos.

Palavras chave: *atividade enzimática, expressão gênica, lactação, glândula mamária, síntese de gordura no leite, perfil de ácidos graxos do leite*

Introduction

Today, a major mission of the researches trained in animal science is to contribute to the development of sustainable livestock production systems in agreement with societal expectations, namely taking into account socio-economic viability, environment protection, product quality and animal welfare. In this context, researches in dairy sciences focuse on the development of strategies to control the performances of dairy ruminants and the quality of their products. To achieve this goal, researchers have various levels of approaches (production system, herd, animals, tissues) and different biology tools (biochemistry, molecular biology, genomics) at their disposal to decipher the mechanisms underlying the regulation of animal lactation function. In milk, fat is the major energy constituent and represents a significant proportion of the total energy requirements for lactation in ruminants. Moreover, fat is an important component contributing to the processing, organoleptic and nutritional properties of milk (Palmquist *et al*., 1993; Chilliard and Ferlay, 2004). In addition, nutrition is the major environmental factor that regulates milk fat secretion and composition and constitutes a rapid, reversible and efficient means to modulate milk fat synthesis (Chilliard *et al*., 2007). A better understanding of milk fat synthesis regulation within the mammary gland is central to the development of nutritional strategies to both limit the milk-energy secretion and improve the energy balance of lactating ruminants and to enhance the nutritional value of milk for human consumers. Fat synthesis is under the control of a large number of genes whose regulation is still poorly documented.

Methods to study these complex processes benefit from technological advances of the past few years. Indeed, molecular biology allowed to target a few genes chosen for their biological function and genomics allowed studying a large number of genes simultaneously. In ruminants, these tools based on the increasing knowledge of the mRNA and/or gDNA sequences of several genes were used to study the expression of several lipogenic genes (mRNA quantification by qRT-PCR) and/or the activity of the corresponding enzymes. More recently, transcriptomic studies became a complement to this candidate gene approach proving data on the expression of thousands of genes and a larger understanding of different mammary functions including milk lipid synthesis and secretion.

Then, few studies were performed to relate the effect of diet on milk fatty acids (FA) output to responses of mammary lipid metabolism (considering the major lipogenic genes) to explain the modification of milk fat content and FA composition by dietary manipulation. Moreover, the development and use of in vivo metabolic flux approaches using chemical tracer molecules as stable labeled FA allowed investigating specific metabolic pathway of lipogenesis such as the mammary Δ9-desaturation metabolic activity. Finally, few studies using different in vitro models studied the effect of specific FA on mammary tissue or mammary epithelial cells lipogenesis.

This chapter reviews the present knowledge on the nutritional regulation of lipid metabolism in the ruminant mammary gland gained using these different approaches, in relation with milk fat yield and FA composition. These studies aimed to better control both the animal energy balance and the nutritional quality of ruminant milk for human consumers, thus having the potential to contribute to sustainable milk production.

Nutritional regulation of milk fat synthesis and composition

Dietary manipulation induced large variation of milk FA composition in the dairy ruminants (Chilliard *et al*., 2007), whereas milk fat secretion responded differently according to species. Indeed, in bovine, whereas in some dietary conditions milk fat depression (MFD) was observed, in small ruminants the occurrence of MFD is not common (Shingfield *et al*., 2010). In cows the diets that induce a MFD belong to 3 types**:** a) diets rich in starch without addition of lipid supplements (e.g. high grain/low forage diets), but containing a minimal amount of PUFA in dietary feedstuffs; b) diets with low level of fibre associated with supplemental PUFA of plant origin; c) diets

associated with dietary supplements of marine oils (fish oils, fish meals, oils from marine mammals and/or algae) which induce MFD whatever the level of starch or fibre in the diet. The observed decreases in milk fat output during diet-induced MFD typically occur rapidly (within a few days), and milk fat yield can be lowered by more than 50%, with little or no change in the yields of milk, milk protein or lactose (Shingfield *et al*., 2010).

In these dietary conditions that induced MFD, the secretion of all FA was decreased but the reductions in the output of FA synthesized de novo were much greater (Bauman and Griinari, 2003; Shingfield *et al.*, 2010). After developing and investigating different theories, the decreases in milk fat synthesis during diet-induced MFD have been attributed to direct inhibition of mammary lipogenesis by trans-FA formed during the biohydrogenation of dietary unsaturated FA in the rumen with the trans-10-18**:**1 and trans-10,cis-12-18**:**2 being the major candidates (Bauman and Griinari, 2001). Several studies have also evidenced that decreases in milk fat content which alter nutrient partitioning in favour of non-mammary tissues, adipose in particular (Bauman and Griinari, 2003; Griinari and Bauman, 2006), may improve energy balance and the efficiency of nutrient utilization in cows during early lactation (Moore *et al*., 2004; Shingfield *et al*., 2004; Kay *et al*., 2006) or periods of limited nutrient availability (Kay *et al*., 2007) situation that could be beneficial for reproduction and next lactation. These studies suggest that diet-induced MFD may be used as a means to control bovine energy balance, adaptation and reproduction which could be a major issue in the context of sustainability of livestock systems.

In small ruminants, when goats (Chilliard *et al*., 2003, 2007; Gagliostro *et al*., 2006) and sheep (Pulina *et al*., 2006) are fed with starch rich diets containing plant oils, MFD did not occur, even for goats fed with marine oils (Chilliard *et al*., 2007). However, a recent study with dairy ewes fed with marine oils reported an increase in milk trans-10-18**:**1 and a MFD (Toral *et al*., 2010).

Otherwise, nutrition constitutes a natural and economical way for farmers to markedly and rapidly modulate the milk FA composition. Because ruminant dairy products contain about 70% of saturated FA, they were pointed out to criticism due to the negative effects of excessive consumption of saturated FA on human health. In addition, dairy products contain some trans unsaturated FAs for which the impact on human health need to be clarified. In contrast, dairy products contain other FA which have potentially beneficial effects (anteiso-15**:**0, cis-9-18**:**1, 18**:**3n-3, conjugated linoleic acid (CLA)) on human health with putative anti-cancer activity and/or anti-atherogenic effects (Shingfield *et al*., 2008b). Thus, quality control of dairy products by modulating their FA composition is of major interest and constitutes one of the pillars of animal livestock sustainability, and the nutrition is a strategy used to improve the milk quality.

Thus, to formulate diets and/or supplements to manipulate changes in milk fat content and composition it is necessary to better understand the underlying mechanisms regulating mammary lipogenesis in ruminants. Whereas, numerous reviews have considered the effect of nutrition on milk fat synthesis in ruminants demonstrating large changes in milk FA composition by changing the forages of the diets, particularly pasture, or by adding plant or marine lipid supplements to the diet (Bauman and Griinari, 2003; Chilliard *et al*., 2007; Shingfield and Griinari, 2007), only few studies have considered possible molecular mechanisms underlying diet-induced changes in mammary lipogenesis and fat secretion (Bernard *et al*., 2008). In the following sections, after an overview of the main digestive (within the rumen) and metabolic (within the mammary gland) pathways involved in milk fat secretion processes, the role of dietary factors implicated in the regulation of mammary lipid metabolism and milk fat secretion and composition is examined.

Rumen biohydrogenation of dietary unsaturated fatty acids

Ruminant diets typically contain between 20 and 40 g lipid/kg dry matter (DM) that mainly come from pasture, forages, cereals, and oil seeds. Whereas, these dietary components contain mainly C18 polyunsaturated FA (PUFA)

(18:2n-6 and 18:3n-3), some oil seeds are rich in monounsaturated FA (mainly cis-9-18:1) and marine products (fish oil, algae, etc.) are rich in very long-chain PUFA (mainly 20:5n-3 and 22:6n-3). These dietary FA are extensively metabolized and biohydrogenated by the microbes in the rumen, resulting not only in the production of 18:0 but also in a wide and diverse range of isomers of PUFA and monounsaturated FA, especially trans and conjugated FA (Shingfield *et al*., 2010). Finally, FA that flow from the rumen are constituted of small amount of dietary PUFA (Doreau *et al*., 2007), of 18:0 from dietary origin and from biohydrogenation processes of dietary FA, and of rumen biohydrogenation intermediates produced in the rumen. These intermediates of ruminal biohydrogenation (RBH) are then absorbed in the gut and either directly secreted into milk, or transformed by body tissues, especially by the mammary gland (Figure 1) where the SCD may add a cis-9-double bond on different FA, which partly reverses the effect of RBH and decreases the saturation level and the melting point of milk fat (Chilliard *et al*., 2000; 2007). In addition, RBH intermediates act as regulators of mammary lipogenesis, which may result in changes in the amount of secreted milk fat and also in milk FA composition (Shingfield *et al*., 2010).

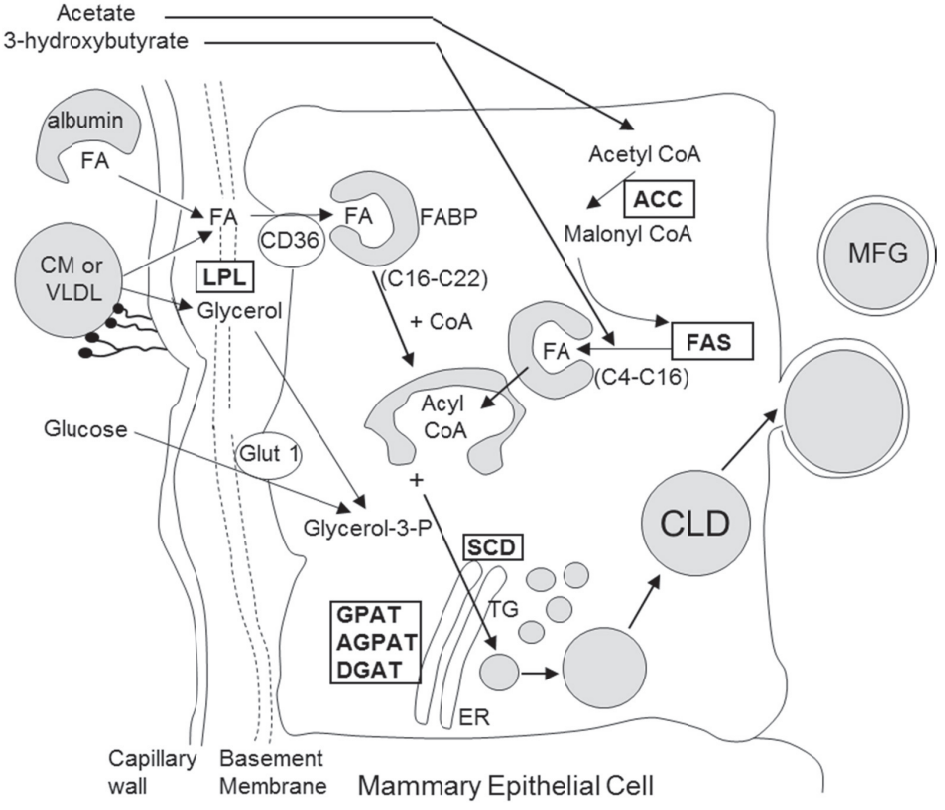

Figure 1. Milk fat synthesis in the ruminant mammary epithelial cell. (From Bernard *et al*., 2008). Abbrevations used: ACC = Acetyl-CoA carboxylase, AGPAT = Acyl glycerol phosphate acyl transferase, CD36 = Cluster of Differentiation 36 or fatty acid translocase; CLD = Cytoplasmic lipid droplet; CoA = Coenzyme A; CM = Chylomicron, DGAT = Diacyl glycerol acyl transferase, ER = Endoplasmic reticulum; FA = Fatty acid, FABP = Fatty Acid Binding Protein; FAS = Fatty acid synthase, Glut 1 = Glucose transporter1, GPAT = Glycerol-3 phosphate acyl transferase, LPL = Lipoprotein lipase, MFG = Milk fat globule, SCD = Stearoyl-CoA desaturase, TG = triglyceride, VLDL = Very low density-lipoprotein.

Mammary lipogenesis

Milk fat is composed of ca. 98% of triglycerides and small amounts of 1,2-diacylyglycerides and monoacylglycerides (0.02%), free FA (0.22%) and retinol esters (Jensen, 2002). Milk triglycerides are thought to contain more than 400 individual FA, but quantitatively saturated FA from C4 to C18, cis-9-16:1, cis-9-18:1, trans-18:1 and linoleic acid

(cis-9,cis-12-18:2) are the most abundant (Jensen, 2002). The FA from milk triglycerides have a dual origin: a) they are either de novo synthesized in the mammary gland via two cytosolic complex enzymatic proteins, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), from circulating acetate and 3-hydroxybutyrate [produced by ruminal fermentation of carbohydrates and by rumen epithelium from absorbed butyrate, respectively], thus resulting in short and medium chain FA (C4:0 to C16:0) that represent 40-50% of the FA secreted in milk, or b) they are imported from the plasma, where they are either released by the enzyme lipoprotein lipase (LPL) (Barber *et al*., 1997) from triglycerides circulating in chylomicra or very low density lipoprotein (VLDL), or derived from the plasma non-esterified FA (NEFA) that circulate bound to albumin, for long-chain FA (≥ C18), as well as ca. one half of the 16:0 and very low amount of 14:0 depending on the diet composition (Figure 1). These FA may be further desaturated by SCD, but not elongated, in the secretory mammary epithelial cells (MEC) (Chilliard *et al*., 2000). The SCD, located in the endoplasmic reticulum, introduces a cis-9 double bond on the fatty acyl-CoA substrates, mainly from C14 to C19, and this reaction requires NADH, oxygen, and an electron transport sequence comprising NADH-cytochrome b5 reductase, cytochrome b5 (CYB5). The preferred substrate of SCD is stearic acid (18:0) leading to the synthesis of oleic acid (cis-9-18:1), the major unsaturated FA found in milk triacylglycerol. In bovine, a number of other saturated acyl CoA also serve as substrates for SCD including 10:0, 12:0, 14:0, 15:0 and 17:0 (Shingfield *et al*., 2008a) as well as trans-FA such as trans-11, trans-7- and trans-12-18:1 (Shingfield *et al*., 2010), thus leading to cis-9,trans-11-18:2 (the major conjugated linoleic acid (CLA) isomer found in dairy products), trans-7,cis-9-18:2 (CLA) and cis-9,trans-12-18:2.

Nutritional regulation of mammary lipogenic gene expression

In order to relate the effect of diet on milk fat content and FA profile with mammary lipid metabolism, few studies were performed by considering the major lipogenic genes. However, in most of the studies, it is difficult to conclude on the level of regulation (transcriptional, post-transcriptional, translational or post-translational) involved since measurements of lipogenic genes mRNA, the enzyme protein content or activity are not studied simultaneously. In ruminants few studies have investigated the role of diet in the regulation of mammary lipogenesis by first focusing on gene expression and/or the activity of the corresponding protein of the major proteins involved in the following pathways: uptake and transport of FA, de novo FA synthesis, FA desaturation and esterification, and the transcription factors involved in their regulation (Figure 1 and Table 1). More recently, thanks to the development of new genomic tools, transcriptomic analysis in ruminant mammary tissues made possible to analyze the nutritional regulation of thousand of genes at once.

Table 1. Effect of dietary lipid supplements on mammary lipogenic genes expression and/or enzyme activity in ruminants. (Adapted from Shingfield *et al*., 2013).

Species	Nature of lipid supplement	Inclusion rate [g/kg dry matter]	Response of milk fat yield, %[3]	Biochemical pathway[1]	Transcript/ protein[2]	Response %[3]	Reference
				Lipogenesis			
Bovine	Rapeseed	33	NS	SCD1/ACC	mRNA	NS	Delbecchi *et al*.,2001
	Fish oil	37	-44	SCD1/ACC/FAS	mRNA	(-29)/-46/-64	Ahnadi *et al*., 2002
	Soyabean oil + fish oil[4]	30 + 15	-38	SCD1/FAS	mRNA	(-25)/-45	Harvatine and Bauman, 2006
	Sunflower oil + marine algae[5]	27 + 4.0	-30		mRNA	-34.5/-31	Angulo *et al*., 2012
	Linseed oil + marine algae[5]	27 + 4.0	-31		mRNA	-45.5/-33	Angulo *et al*., 2012
	Safflower seed	135	NR	SCD1/ACC/FAS	mRNA	(+39/+38/+36)	Murrieta *et al*., 2006
	Sunflower oil[4]	10	-27		mRNA	(-35)/-28/-41	Peterson *et al*., 2003
	Soyabean oil[4]	50	-43	ACC	mRNA	-68	Piperova *et al*., 2000
	Soyabean oil[4]	50	-43		Activity	-61/-44	Piperova *et al*., 2000

Continues

Table 1. Continued

Species	Nature of lipid supplement	Inclusion rate [g/kg dry matter]	Response of milk fat yield, %[3]	Biochemical pathway[1]	Transcript/ protein[2]	Response %[3]	Reference
Caprine	Oleic sunflower oil	35	+16	FAS	mRNA	(-21)	Bernard *et al*., 2005
	Formaldehyde-linseed	112	+8		mRNA	(-1)	Bernard *et al*., 2005
	Oleic sunflower oil	35	+16	SCD1	mRNA	-43	Bernard *et al*., 2005
	Formaldehyde-linseed	112	+8		mRNA	-54	Bernard *et al*., 2005
	Rapeseed	146	(+5)	SCD1/ACC/FAS	mRNA	(+15/+19/+12)	Ollier *et al*., 2009
	Safflower oil	50	+26		mRNA	+166/+72/+74	Li *et al*., 2012
	Sunflower oil	61	(+7)		mRNA	(-7/-8/+5)	Bernard *et al*., 2009a
	Sunflower oil	44	+20		mRNA	(+10/+15/+6)	Ollier *et al*., 2009
	Sunflower oil	55	+17		mRNA	(+17/+10/+9)	Bernard *et al*., 2009b
	Linseed oil	55	+15		mRNA	(+22/+2/+10)	Bernard *et al*., 2009b
	Linseed oil	62	+14		mRNA	(-16/+4/-4)	Bernard *et al*., 2009a
	Linseed oil	50	+34		mRNA	+121/-26/+52	Li *et al*., 2012
	Linseed oil	62	+14		Activity	(+11/-3/+64)	Bernard *et al*., 2009a
	Sunflower oil	61	(+7)	FAS	Activity	+68	Bernard *et al*., 2009a
	Formaldehyde-linseed	112	+8		Activity	(-31)	Bernard *et al*., 2005
	Sunflower oil	55	+17		Activity	(+27)	Bernard *et al*., 2009b
	Oleic sunflower oil	35	+16		Activity	(0)	Bernard *et al*., 2005
	Linseed oil	55	+15		Activity	(+30)	Bernard *et al*., 2009b
	Sunflower oil	55	+17	SCD1	Activity	-30	Bernard *et al*., 2009b
	Oleic sunflower oil	35	+16		Activity	-22	Bernard *et al*., 2005
	Linseed oil	55	+15		Activity	-27	Bernard *et al*., 2009b
	Formaldehyde-linseed	112	+8		Activity	-35	Bernard *et al*., 2005
	Sunflower oil	61	(-3)	SCD1/ACC/FAS	Activity	(-17/+12)/+81	Bernard *et al*., 2009a
				Fatty acid uptake/processing			
Bovine	Safflower seed	135	NR	LPL	mRNA	+50	Murrieta *et al*., 2006
	Soyabean oil + fish oil[4]	30	-38		mRNA	-61	Harvatine and Bauman, 2006
	Sunflower oil[4]	10	-27		mRNA	-30	Peterson *et al*., 2003
	Sunflower oil + marine algae[5]	27 + 4.0	-30		mRNA	-34	Angulo *et al*., 2012
	Linseed oil + marine algae[5]	27 + 4.0	-31		mRNA	-45	Angulo *et al*., 2012
Caprine	Rapeseed	146	(+5)		mRNA	(-0.03)	Ollier *et al*., 2009
	Safflower oil	50	+26		mRNA	+43	Li *et al*., 2012
	Sunflower oil	61	(+7)		mRNA	(+17)	Bernard *et al*., 2009a
	Sunflower oil	44	+20		mRNA	(+23)	Ollier *et al*., 2009
	Sunflower oil	55	+17		mRNA	(+1)	Bernard *et al*., 2009b
	Oleic sunflower oil	35	+16		mRNA	+51	Bernard *et al*., 2005
	Linseed oil	55	+15		mRNA	(-1)	Bernard *et al*., 2009b
	Formaldehyde-linseed	112	+8		mRNA	(+26)	Bernard *et al*., 2005
	Linseed oil	62	+14		mRNA	(+13)	Bernard *et al*., 2009a
	Linseed oil	50	+34		mRNA	+54	Li *et al*., 2012
	Sunflower oil	61	(+7)		Activity	(+42)	Bernard *et al*., 2009a
	Sunflower oil	55	+17		Activity	(+35)	Bernard *et al*., 2009b
	Oleic sunflower oil	35	+16		Activity	(+13)	Bernard *et al*., 2005
	Linseed oil	55	+15		Activity	(+21)	Bernard *et al*., 2009b
	Linseed oil	62	+14		Activity	(-17)	Bernard *et al*., 2009a
				Esterification			
Bovine	Sunflower oil[4]	10	-27	AGPAT/GPAT	mRNA	-27/-52	Peterson *et al*., 2003
	Sunflower oil + marine algae	27 + 4.0	-30	GPAM	mRNA	-10	Angulo *et al*., 2012
	Linseed oil + marine algae[5]	27 + 4.0	-31		mRNA	-26	Angulo *et al*., 2012
				FA transport			
Bovine	Sunflower oil[4]	10	-27	FABP	mRNA	(-25)	Peterson *et al*., 2003
Caprine	Sunflower oil	44	+20	FABP3/FABP4	mRNA	(+11)/+36	Ollier *et al*., 2009
	Rapeseed	146	NS		mRNA	(+21)/(+11)	Ollier *et al*., 2009

Continues

Table 1. Continued

Species	Nature of lipid supplement	Inclusion rate [g/kg dry matter]	Response of milk fat yield, %[3]	Biochemical pathway[1]	Transcript/ protein[2]	Response %[3]	Reference
				Transcription factor			
Bovine	Soyabean oil + fish oil[4]	30 + 15	-38	SREBF1 / S14	mRNA	-45/-60	Harvatine and Bauman, 2006
	Sunflower oil + marine algae[5]	27 + 4.0	-30	SREBF1	mRNA	-30	Angulo *et al*., 2012
	Linseed oil + marine algae[5]	27 + 4.0	-31		mRNA	-31	Angulo *et al*., 2012
	Soybean oil + fish oil	25 + 10	-50	SREBF1/PPARG	mRNA	NS/NS	Invernizzi *et al*., 2010

[1] ACC, acetyl CoA carboxylase; AGPAT, acylglycerol phosphate acyl transferase; FAS, fatty acid synthase; FABP, fatty acid binding protein; FACL, fatty acyl CoA ligase; GPAM, glycerol-3-phosphate acyltransferase 1; GPAT, glycerol phosphate acyl transferase; LPL, lipoprotein lipase; SCD, stearoyl-CoA desaturase; S14, thyroid hormone responsive spot 14; SREBF1, sterol response element binding protein.

[2] Measurement of tissue transcript abundance (mRNA) or protein activity (Activity).

[3] Response reported when treatment effects were significant ($p<0.10$) and calculated as [(Treatment-Control)/Control)X100]. Values in parenthesis are not significant at $p<0.05$. NS, values not reported because unsignificant.

[4] Lipid supplementation also accompanied by decreases in dietary forage:concentrate ratio.

[5] Response compared to a diet supplemented with rumen-stable fractionated palm fat (rich in saturated FA).

NR, not reported.

The in vivo trials reporting the nutritional regulation of few mammary lipogenic genes expression have been carried out in mid-lactation cows and goats and mainly with lipid supplements (Table 1). In cows, studies have been primarily performed with the 3 types of diets that induce milk fat depression (MFD) and with post-ruminal infusion studies of trans-10,cis-12-CLA because of its strong anti-lipogenic effect (Bauman and Griinari, 2001). In cows under these nutritional (Ahnadi *et al*., 2002; Harvatine and Bauman, 2006; Peterson *et al*., 2003) or trans-10,cis-12-CLA infusion (Harvatine and Bauman, 2006; Gervais *et al*., 2009) conditions, it has been reported a decrease of milk fat output (by about 50% in more severe cases), mainly of 4:0 to 16:0 FA (by 30% to 59%), which was associated with a coordinate decrease in mammary mRNA abundance of the major lipogenic genes (except for only a tendency to decrease for SCD1): those involved in de novo FA synthesis (ACACA and FASN) and FA uptake (LPL), transport and esterification, and/or the activity of the transcribed proteins (Table 1). Only in two of these studies (Baumgard *et al*., 2002, Angulo *et al*., 2012) a decrease in mRNA abundance of SCD1 simultaneously to decreases in lipogenic genes was reported. In cows, with these diets, the milk FA desaturation ratios (that represent a proxy for mammary SCD activity) were not largely modified, thus contributing to the maintenance of milk fat fluidity.

Otherwise, in goats, the effect of dietary factors in the regulation of mammary lipogenesis was mainly studied with different lipid supplements (plant oils and oilseeds) and basal diets (hay, silage, different F/C ratios). Under these conditions, no decrease in milk fat secretion was observed even with starch-rich diets (Bernard *et al*., 2008). Moreover, diets causing decreases in the output of FA synthesized de novo in milk (by 18 to 32%) do not induce significant changes in mammary ACACA and FASN mRNA abundance and/or activity of the corresponding enzyme (Bernard *et al*., 2005; 2009a, b). Generally, the addition of plant oils to the diet in goats induced a) large increases in long-chain FA which are not accompanied by elevated mammary LPL mRNA abundance and/or activity, suggesting that the substrate availability could be more limiting than the LPL activity in the uptake of long-chain FA or the implication of other proteins involved in the FA uptake (e.g. fatty acid translocase, CD36) and; b) decreases in the milk desaturation ratios and variable effects on mammary SCD gene expression and/or activity depending on the basal diet and composition of the lipid supplement (Table 1; Bernard *et al.,* 2008; 2010).

So far, only few in vivo trials report the nutritional regulation of mammary transcriptome in ruminants. These studies in cows fed diets-inducing MFD (high-concentrate or silage-based diets with plant and/or fish oils (Loor *et al*., 2005; Invernizzi *et al*., 2009)) have shown that the decreases in

milk fat synthesis were associated with changes in the expression of several genes involved in lipid metabolism, molecular transport and carbohydrate metabolism, and have also related the changes in mammary lipogenesis to the effects on mammary gene networks mediated via different transcription factors/nuclear receptors.

Regarding the transcription factors involved in the regulation of the expression of lipogenic genes in lactating cows, dietary (Angulo *et al*., 2012; Bionaz and Loor, 2008; Loor *et al*., 2005) or intravenous infusions of trans-10,cis-12-CLA (Harvatine and Bauman, 2006; Gervais *et al*., 2009) studies that induced MFD and changes in mammary lipogenic gene expression have shown the implication of SREBP1 and/or PPAR. For SREBF1, this central role was confirmed by in vitro studies (Peterson *et al*., 2004; Ma and Corl, 2012) whereas the role of PPAR still needs to be unraveled (Kadegowda *et al*., 2009). The regulation of SCD1, for which in cows the transcription of the gene varies less or less rapidly in response to the diet than the other lipogenic genes, probably involves other lipogenesis-related transcription factors (e.g. PPARs, LXR, SP1,…) also expressed in the ruminant mammary gland (Bionaz and Loor, 2008; Toral *et al*., 2013) or other regulatory pathway (e.g. miRNA).

Mammary bioconversion of LCFA and milk quality

As discussed earlier, both saturated (mainly 14:0; 16:0, 18:0) and some absorbed RBH intermediates (mainly trans-11-18:1) may be transformed by the mammary gland via the SCD which contribute to a decrease in the melting point of milk fat (Chilliard *et al*., 2007) and to the synthesis of FA that positively impact the milk nutritional quality (by decreasing the saturated FA and increasing FA that may have positive impact on human health such as cis-9-18:1 and cis-9,trans-11-CLA). Different methods have been used in ruminants to determine the metabolic activity of the SCD enzyme *in vivo*, in particular to estimate the conversion of stearic to oleic acid or vaccenic to rumenic acid in the mammary gland. These methods include direct methods, using tracer molecules ([1-13C]trans-11 18:1; Mosley *et al*., 2006; Bernard *et al*., 2010), and indirect methods, using quantification of duodenal and milk FA flows (Shingfield *et al*., 2007; Glasser *et al*., 2008) or inhibition of the SCD using sterculic acid (Griinari *et al*., 2000; Corl *et al*., 2001; Kay *et al*., 2004; Bichi *et al*., 2012). Overall, these studies provided clear evidence that the endogenous desaturation of stearic and vaccenic acids are the main sources of milk oleic and rumenic acids, respectively, with 63-84% of oleic acid coming from stearic acid and 63-97% of rumenic acid coming from vaccenic acid, using different methodology and animal species.

Conclusions

During the last decade, progress has been made in the characterisation of the effects of nutrition on milk fat synthesis and composition in ruminants. Application of molecular approaches has allowed a better understanding of the molecular mechanisms underlying milk fat secretion and composition and evidenced that the effect of the diets, in particular those that induced a MFD in cows, are mediated via alterations in mammary lipogenic gene expression. However, these alterations occur in a lesser extent in caprine species for which MFD is less common. Increasing knowledge on the determinant of milk fat synthesis and composition in particular the nutritional regulation of gene expression could help improve the metabolic and physiological efficiency of lactating ruminants in the future. Elucidating the mechanisms underlying milk fat synthesis will also contribute to improve the dairy product quality by controlling the FA composition.

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