Journal of Dairy Research

cambridge.org/dar

Effects of exogenous C18 unsaturated fatty acids on milk lipid synthesis in bovine mammary epithelial cells

Hang Zhang[1,*], Ni Dan[2,*], Changjin Ao[3], Sizhen Wang[2], Khas Erdene[3] and Mohammed Umair Ashraf[3]

[1]College of Animal Science and Technology and; [2]College of Life Science and Food Engineering, Inner Mongolia University for Nationalities, Tong Liao, People's Republic of China and [3]College of Animal Science, Inner Mongolia Agricultural University, Hohhot, People's Republic of Chinaw

Research Article

*These authors contributed equally.

Cite this article: Zhang H, Dan N, Ao C, Wang S, Erdene K and Ashraf MU (2020). Effects of exogenous C18 unsaturated fatty acids on milk lipid synthesis in bovine mammary epithelial cells. *Journal of Dairy Research* **87**, 344–348. https://doi.org/10.1017/S0022029920000722

Received: 8 May 2019
Revised: 26 March 2020
Accepted: 14 April 2020
First published online: 7 September 2020

Keywords:
Bovine mammary epithelial cell; lipogenic gene; milk fat synthesis; unsaturated fatty acid

Author for correspondence:
Changjin Ao, Email: changjinao@aliyun.com

Abstract

We determined the effects of a combination of C18 unsaturated fatty acids **(C18-UFAs)** consisting of oleic, linoleic, and linolenic acids on milk lipogenesis in bovine mammary epithelial cells (**BMECs**). By orthogonal experiments to determine cellular triacylglycerol (**TAG**) accumulation, a combination of 200 µmol/l C18 : 1, 50 µmol/l C18 : 2, and 2 µmol/l C18 : 3 was selected as C18-UFAs combination treatment, and culture in medium containing fatty acid-free bovine serum albumin was used as the control. The expression of genes related to milk lipid synthesis and intracellular FA composition was measured. The results showed that cytosolic TAG formation was higher under C18-UFAs treatment than under control treatment. The mRNA expression of acetyl-CoA carboxylase-α (***ACACA***), fatty acid synthase (***FASN***), and peroxisome proliferator-activated receptor gamma (***PPARG***) did not differ between treatments. The abundance of stearoyl-CoA desaturase (***SCD***) and acyl-CoA synthetase long-chain family member 1 (***ACSL1***) was higher, whereas that of sterol regulatory element binding transcription factor 1 (***SREBF-1***) was lower after C18-UFAs treatment compared to control treatment. The C16 : 0 and SFA content was decreased following C18-UFAs treatment compared to control treatment, while the *cis*-9 C18 : 1 and UFA content was increased. In conclusion, C18-UFAs could stimulate triglyceride accumulation, increase the cellular UFA concentration, and regulate lipogenic genes in BMECs.

Milk fat which contains a high proportion of one or more unsaturated fatty acids (UFAs; examples are oleic and linolenic acids) is a valuable component in the dairy industry as a result of putative health benefits (Massaro *et al*., 1999; Shingfield *et al*., 2008). In contrast, some specific saturated fatty acids (**SFAs**) in milk fat, mainly lauric, myristic and palmitic acids, have been found to have a negative effect on human health when consumed in excess by increasing plasma total and LDL cholesterol concentrations (Williams, 2000). In the mammary gland of ruminants, short- and medium-chain fatty acids (**SMFAs**) and a portion of C16 : 0 fatty acids from acetate and, to a lesser extent, b-hydroxybutyrate must be obtained by *de novo* synthesis, while long-chain fatty acids (**LCFAs**) and the remaining C16 : 0 fatty acids are obtained by the exogenous uptake of preformed fatty acids (**FAs**) from the circulation (Harvatine *et al*., 2009). A gene network analysis study in bovine mammary tissue showed that peroxisome proliferator-activated receptor gamma (***PPARG***), a nuclear receptor transcription factor for which acetyl-coenzyme A carboxylase alpha (***ACACA***), fatty acid synthase (***FASN***) and stearoyl-CoA desaturase (***SCD***) are putative target genes, regulates milk fat synthesis during lactation (Bionaz and Loor, 2008). In addition, lipoprotein lipase (***LPL***) and acyl-CoA synthetase long-chain family member 1 (***ACSL1***), genes involved in FA transport, were reported to be target genes of PPARG (Desvergne *et al*., 2006). The enzymes ACACA and FASN participate in the metabolic *de novo* FA synthesis pathway in mammary tissue, whereas the enzyme LPL is involved in the uptake of plasma FAs. Kadegowda *et al*. (2009) demonstrated the regulatory roles of *PPARG* and exogenous or mammary-synthesized LCFAs in milk fat synthesis in vitro. In addition, Harvatine and Bauman (2006) suggested that sterol regulatory element binding transcription factor 1 (***SREBF-1***) is pivotal in the regulation of milk fat synthesis in cows.

Exogenous supplemental LCFAs are crucial for milk fat synthesis in bovine mammary epithelial cells (**BMECs**). Studies have indicated that LCFAs significantly inhibited *de novo* synthesis of SMFAs (Hansen and Knudsen, 1987; Warntjes *et al*., 2008) as well as the mRNA expression of *ACACA* and *FASN* in BMECs (Bauman *et al*., 2008; Kadegowda *et al*., 2009). In *in vitro* studies, the exogenous LCFAs oleate, linoleate, or linolenic acid increased TAG accumulation and altered the mRNA expression of genes related to FA synthesis in BMECs (Yonezawa *et al*., 2004; Qi *et al*., 2014; Sheng *et al*., 2015). Furthermore, LCFAs are not

Hannah Dairy Research Foundation

CAMBRIDGE UNIVERSITY PRESS

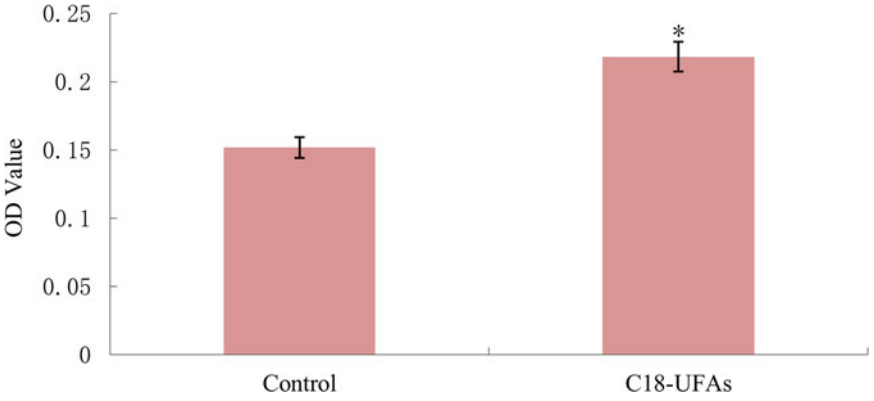

Fig. 1. The effects of exogenous C18 unsaturated fatty acids on triglyceride contents in bovine mammary epithelial cells. The control treatment contained only fatty acid-free BSA without unsaturated fatty acids. *Indicate significant differences from values obtained in control treatment at $P < 0.05$.

only required for milk fat biosynthesis as precursors but also play a role in regulating milk FA composition.

Studies showed that supplementation with a single UFA affected TAG accumulation and lipogenic enzymes in BMECs (Kadegowda *et al.*, 2008; Yonezawa *et al.*, 2008). However, few studies regarding the regulatory effect of combination UFA treatment on milk fat synthesis have been reported. In animals, FAs are not present alone, and FAs in the diet also coexist with endogenous FAs. Therefore, an *in vitro* study was undertaken in BMECs to test the hypothesis that an optimum combination of preformed UFAs consisting of oleic, linoleic, and linolenic acids would regulate cytosolic TAG accumulation and the expression of genes encoding lipogenic enzymes to some extent. To meet this objective, BMECs were cultured with C18-UFAs, following which TAG accumulation, the mRNA expression of lipogenic genes, and the intracellular FA composition were measured.

Materials and methods

Cell culture

BMECs were isolated from the mammary glands of 6 Chinese Holstein dairy cows from a local slaughterhouse during their lactation period. Mammary tissues for cell culture were obtained from the deep layer of the mammary gland and soaked in ice-cold PBS before cell culture. BMECs were cultured according to the methods described by Hu *et al.* (2009) with modifications by the use of type II collagenase (Gibco BRL, Grand Island, NY, USA). Specific detailed procedures are presented in the online Supplementary Materials and Methods. Cells were seeded at 1×10^4 cells/cm^2 in culture flasks and then incubated in basal culture medium for 48 h before stimulation by C18-UFAs treatment and growth in FA-free bovine serum albumin (BSA, Equitech-Bio, Kerrville, TX, USA) medium for an additional 48 h. The FA-free BSA culture medium was prepared as described above for the basal culture medium, except that 10% fetal bovine serum was replaced with 1 g/l FA-free BSA. The control treatment contained only FA-free BSA without UFAs.

Cell proliferation assay

Cell proliferation was determined by MTT assay according to Qi *et al.* (2014) with modifications (online Supplementary Materials and Methods). The results revealed that cell viability was promoted by each LCFA at a low concentration and then inhibited when the concentration increased (online Supplementary Figure).

TAG accumulation

Based on the MTT assay, three different concentrations for which each C18 UFA did not inhibit cell viability were selected and used to determine the synthesis of cellular TAG utilizing L_9 (3^3) orthogonal design to finally confirm the effects of C18 UFAs treatment (online Supplementary Table S1). The selected concentrations were 100, 200 and 400 µmol/l oleic acid; 50, 75 and 100 µmol/l linoleic acid; and 1, 1.5 and 2 µmol/l linolenic acid. Synthesized TAG was determined as reported in the online Supplementary Materials and Methods.

RNA extraction and real-time quantitative PCR

Specific details of these procedures are presented in online Supplementary Materials and Methods and online Supplementary Table S2 (primer sequences). RT-PCR analysis was performed with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001), with the reference genes β-*actin*, *GAPDH* and *RPS9*. The primers were designed according to Sheng *et al.* (2015) and synthesized by Shanghai Sangon Biological Engineering and Technology Service Co., Ltd. (Shanghai, China). Specific gene amplification was confirmed by sequencing.

Measurement of the cellular FA composition

Intracellular FAs were extracted according to a previously described procedure (Zhang *et al.*, 2015) with modifications (online Supplementary Materials and Methods).

Statistical analysis

Treatments in culture wells were replicated at least 3 times, and PCR was performed in triplicate. Data were analyzed using one-way ANOVA with SAS software (SAS Version 9.0; SAS Institute Inc., Cary, NC). RT-PCR data analysis was performed with $2^{-\Delta\Delta Ct}$ values. Differences were considered significant when $P < 0.05$.

Results

Tag content in the cytosol

The results of the orthogonal experiment indicated that 200 µmol/l oleic acid, 50 µmol/l linoleic acid, and 2 µmol/l linolenic acid were the optimal doses for TAG accumulation (online Supplementary Table S3). Compared with the control treatment, C18-UFAs treatment significantly increased the intracellular TAG content in BMECs (Fig. 1).

https://doi.org/10.1017/S0022029920000722 Published online by Cambridge University Press

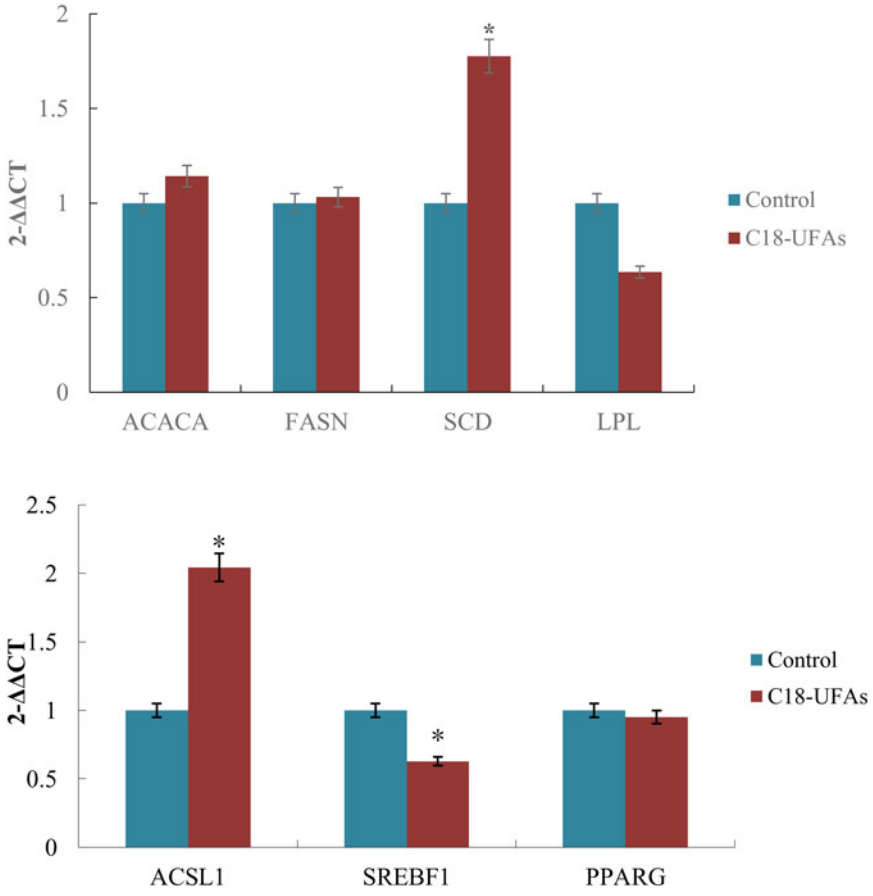

Fig. 2. The effects of exogenous C18 unsaturated fatty acids on lipogenic genes mRNA abundance in cells. FASN, fatty acid synthase; ACACA, acetyl-coenzyme A carboxylase-α; SCD, stearoyl CoA desaturase; LPL, lipoprotein lipase; ACSL1, acyl-CoA synthetase long chain 1; SREBF1, sterol regulatory element binding factor 1; PPARG, peroxisome proliferator activated receptor-γ. *Indicate significant differences from values obtained in control treatment at $P < 0.05$.

Expression of genes related to FA synthesis

There was no significant difference in the mRNA expression of either *ACACA* or *FASN* between the treatments ($P > 0.05$). The abundance of *SCD1* and *ACSL1* was higher after C18-UFAs treatment than after control treatment, but the gene expression of *SREBF1* was reduced ($P < 0.05$). However, treatment did not affect the expression of *PPARG* and *LPL* (Fig. 2).

Intracellular FA composition

The effects of C18-UFAs treatment on FA composition in BMECs are presented in Table 1. The C16 : 0 and SFA content in BMECs was decreased with C18-UFAs treatment compared to the control treatment, whereas the *cis*-9 C18 : 1 and UFA content was significantly increased with C18-UFAs treatment ($P < 0.05$).

Discussion

Several studies have suggested that unsaturated LCFAs play an important role in the proliferation of BMECs (Rose *et al.*, 1993; Yonezawa *et al.*, 2008). In our study, oleic acid, linoleic acid, or linolenic acid at certain concentrations promoted the proliferation of mammary epithelial cells. TAG accumulated in the cytosol of BMECs, and the TAG content directly reflects fat synthesis in BMECs. In this study, TAG accumulation after C18-UFAs combination treatment was higher than that following control treatment. Oleate and linoleate were reported to significantly upregulate TAG synthesis in BMECs, and C18 UFAs at a concentration lower than 100 μM could increase the accumulation of TAG (Yonezawa *et al.*, 2004; Cui *et al.*, 2012). A recent study in Mac-T cells showed similar results and that UFA treatment increased cytosolic TAG accumulation (Vargas-Bello-Pérez *et al.*, 2018). Moreover, Green *et al.* (2010) observed that oleic acid is the preferred substrate for TAG synthesis. Therefore, the availability of preformed FAs is important for cellular TAG formation.

The lipogenic genes *ACACA* and *FASN* are involved in the metabolic pathway of *de novo* FA synthesis to produce SMFAs in bovine mammary tissue, whereas *LPL* is related to the uptake of plasma FAs (Bernard *et al.*, 2008; Shingfield *et al.*, 2010). Previous studies found either a significant decrease or no change in *ACACA* and *FASN* abundance in cells treated with exogenous long-chain UFAs (Jacobs *et al.*, 2013; Li *et al.*, 2018). In the current study, C18-UFAs treatment had no influence on the expression of *ACACA* or *FASN*, and SMFA levels in cells thus remained unchanged, further suggesting that long-chain UFAs do not positively regulate *de novo* FA synthesis in BMECs. In accordance with the results of Sheng *et al.* (2015), no difference in the abundance of *LPL* was observed after C18-UFAs treatment, suggesting that combination UFAs treatment had little effect on the uptake of exogenous FAs. In ruminant milk fat synthesis, *ACACA*, *FASN* and *LPL* are thought to be putative *PPARG* target genes, indicating *PPARG* as a key factor that manipulates lipid biosynthesis in BMECs (Kadegowda *et al.*, 2009). We found that like that of *ACACA*, *FASN*, and *LPL*, the mRNA level of *PPARG* was not altered by exogenous UFA treatment, which again suggests that these genes are at least in part controlled by *PPARG*.

https://doi.org/10.1017/S0022029920000722 Published online by Cambridge University Press

Table 1. Effect of exogenous C18 unsaturated fatty acids on fatty acid composition in bovine mammary epithelial cells

	Treatments[a]			
FA(g/100 g)	Control	C18-UFAs	SEM	*P*-value
C16 : 0	20.67[a]	17.68[b]	3.2	0.05
C16 : 1	2.11	1.72	0.26	0.71
C18 : 0	12.66	13.81	0.65	0.47
C18 : 1c9	6.05[b]	8.22[a]	4.01	0.05
C18 : 2c6	2.82	4.71	2.4	0.65
C18 : 3n6	0.98	0.81	0.02	0.85
<16	31.41	28.94	5.05	0.11
>16	48.07	52.53	14.5	0.18
SFA	77.04[a]	72.32[b]	12.5	0.02
UFA	22.96[b]	27.68[a]	4.7	0.05

[a]Control treatment contained only fatty acid-free BSA without unsaturated fatty acids (0 μM), C 18-UFAs contained 200 μmol/l of oleic acid, 50 μmol/l of linoleic acid, and 3 μmol/l of linolenic acid. SFA, saturated fatty acids; UFA, unsaturated fatty acids.
[a,b]Values in the same row with different superscript letters differ significantly from each other.

Paton and Ntambi (2009) suggested the multiple roles of *SCD* in the regulation of lipid and carbohydrate metabolism. In bovine mammary lipid metabolism, *SCD* is responsible for introducing a cis double bond at the Δ9 position in a wide range of FAs. Decreased *SCD* abundance was observed in some studies in which BMECs were treated with preformed UFAs (Jacobs *et al*., 2013; Li *et al*., 2018). The caprine *SCD* genotype showed suggestive associations on milk FA composition with higher percentages of PUFA (Zidi *et al*., 2010). In our study, the increase in the expression of *SCD* suggested an increase in the desaturation ability of mammary cells following UFA treatment relative to control treatment. Together with the increased intracellular total UFA and C18 : 1 content and decreased total SFA and C16 : 0 content, we propose a positive feedback mechanism that increased the abundance of *SCD* when the cells were supplemented with C18-UFAs and an enhanced degree of unsaturation within cells. However, the reason for the difference in *SCD* expression response to FA between this study and earlier researches is not clear, and further research is needed.

ACSL1 is an isozyme of the LCFA coenzyme A ligase family that plays a key role in lipid biosynthesis and FA degradation. Prior to use in TAG synthesis, FAs must be activated by *ACSL1* (Rudolph *et al*., 2007). In the present study, the significantly higher expression of *ACSL1* in C18-UFAs-treated cells suggested the stimulation of LCFA activation by preformed UFAs, which may further explain the enhanced TAG accumulation following UFAs treatment relative to control treatment.

Investigations have focused on the dominant role of *SREBF1* and verified its function in the regulation of lipid metabolism (Desvergne *et al*., 2006). In the ruminant mammary epithelium, *SREBF1* has been revealed to play a central role in the integrated regulation of lipid biosynthesis through the regulation of key enzymes (Ma and Corl, 2012; Xu *et al*., 2018). In this study, a mixture of oleic acid, linoleic acid, and linoleic acid was used to stimulate BMECs and found to markedly reduce *SREBF1* expression, which suggested that *SREBF1* was negatively regulated by preformed UFAs. This is in line with studies in which BMECs were treated with a single C18 UFA, resulting in a significant reduction in *SREBF1* expression (Kadegowda *et al*., 2009; Jacobs *et al*., 2013; Harvatine *et al*., 2018). These results elucidated that *SREBF1* is a master regulator of bovine mammary lipid synthesis that could be repressed by PUFAs.

In accordance with our study showing that the cellular oleic acid and total UFA concentrations were higher following incubation with a mixture of oleic, linoleic and linolenic acids, Vargas-Bello-Pérez *et al*. (2019) showed that the single addition of oleate increased cytosolic MUFAs and UFAs in Mac-T cells. Together with the present results, these findings suggest that oleic acid serves as a potent regulator of FA unsaturation in BMECs. Although exogenously supplied FAs could be affected by transcriptional and translational processes in BMECs before synthesized FAs in milk, the types and content of the FAs provided may significantly influence milk FA composition. The current experiment revealed that milk FA composition and exogenous supplemented FA composition tended to be consistent, indicating that variation in milk LCFAs could reflect changes in exogenous LCFAs to a certain extent.

In conclusion, a mixture of oleic acid, linoleic acid, and linolenic acid regulated milk lipid synthesis in BMECs by controlling genes encoding enzymes involved in FA desaturation and LCFA activation, the transcription factor SREBF1, and TAG accumulation. Exogenous C18 UFAs supplementation may have a significant benefit for human health by improving milk fat composition.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029920000722.

Acknowledgment. The authors express their gratitude to the postgraduate students of the Animal Science College of Inner Mongolia Agricultural University for their help with collection of the experimental samples. This work was supported by the National Basic Research Program of China (2011CB100803) and Inner Mongolia Natural Science Foundation (2018LH03011).

References

Bauman DE, Perfield JW, Harvatine KJ and Baumgard LH (2008) Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. *Journal of Nutrition* **138**, 403–409.

Bernard L, Leroux C and Chilliard Y (2008) Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. *Advances in Experimental Medicine and Biology* **606**, 67–108.

Bionaz M and Loor JJ (2008) Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics* **9**, 366.

Cui RL, Wang JQ, Bu DP, Wei HY, Nan XM, Hu H and Zhou LY (2012) Effects of 18-carbon fatty acids on cell proliferation and triacylglycerol accumulation in bovine mammary epithelial cells in vitro. *Acta Veterinaria Et Zootechnica Sinica* **43**, 1064–1070.

Desvergne B, Michalik L and Wahli W (2006) Transcriptional regulation of metabolism. *Physiological Review* **86**, 465–514.

Green CD, Ozguden-Akkoc CG, Wang Y, Jump DB and Olson LK (2010) Role of fatty acid elongases in determination of de novo synthesized monounsaturated fatty acid species. *Journal of Lipid Research* **51**, 1871–1877.

Hansen HO and Knudsen J (1987) Effect of exogenous long-chain fatty acids on individual fatty acid synthesis by dispersed ruminant mammary gland cells. *Journal of Dairy Science* **70**, 1350–1354.

Harvatine KJ and Bauman DE (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. *Journal of Nutrition* **136**, 2468–2474.

Harvatine KJ, Boisclair YR and Bauman DE (2009) Recent advances in the regulation of milk fat synthesis. *Animal: An International Journal of Animal Bioscience* **3**, 40–54.

Harvatine KJ, Boisclair YR and Bauman DE (2018) Time-dependent effects of trans-10,cis-12 conjugated linoleic acid on gene expression of lipogenic

enzymes and regulators in mammary tissue of dairy cows. *Journal of Dairy Science* **101**, 7585–7592.

Hu H, Wang J, Bu D, Wei H, Zhou L, Li F and Loor JJ (2009) In vitro culture and characterization of a mammary epithelial cell line from Chinese Holstein dairy cow. *PLoS ONE* **4**, e7636.

Jacobs AAA, Dijkstra J, Liesman JS, Vandehaar MJ, Lock AL, van Vuuren AM, Hendriks WH and van Baal J (2013) Effects of short- and long-chain fatty acids on the expression of stearoyl-CoA desaturase and other lipogenic genes in bovine mammary epithelial cells. *Animal: An International Journal of Animal Bioscience* **7**, 1508–1516.

Kadegowda AK, Piperova LS and Erdman RA (2008) Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. *Journal of Dairy Science* **91**, 749–759.

Kadegowda AKG, Bionaz M, Piperova LS, Erdman RA and Loor JJ (2009) Peroxisome proliferator-activated receptor-γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents. *Journal of Dairy Science* **92**, 4276–4289.

Li D, Xing Y, Li H, Wang W, Hou X and Gao M (2018) Effect of linoleic acid supplementation on triglyceride content and gene expression in milk fat synthesis in two- and three-dimensional cultured bovine mammary epithelial cells. *Italian Journal of Animal Science* **17**, 714–722.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2_{\mathrm{T}}^{-\Delta\Delta\mathrm{C}}$ method. *Methods (San Diego, Calif.)* **25**, 402–408.

Ma L and Corl BA (2012) Transcriptional regulation of lipid synthesis in bovine mammary epithelial cells by sterol regulatory element binding protein-1. *Journal of Dairy Science* **95**, 3743–3755.

Massaro M, Carluccio MA and De Caterina R (1999) Direct vascular antiatherogenic effects of oleic acid: a clue to the cardioprotective effects of the Mediterranean diet. *Cardiologia* **44**, 507–513.

Paton CM and Ntambi JM (2009) Biochemical and physiological function of stearoyl-CoA desaturase. *American Journal of Physiology. Endocrinology and Metabolism* **297**, E28–E37.

Qi L, Yan S, Sheng R, Zhao Y and Guo X (2014) Effects of saturated long-chain fatty acid on mRNA expression of genes associated with milk fat and protein biosynthesis in bovine mammary epithelial cells. *Asian-Australasian Journal of Animal Sciences* **27**, 414–421.

Rose DP, Hatala MA, Connolly JM and Rayburn J (1993) Effect of diets containing different levels of linoleic acid on human breast cancer growth and lung metastasis in nude mice. *Cancer Research* **53**, 4686–4690.

Rudolph MC, McManaman JL, Phang T, Russell T, Kominsky DJ, Serkova NJ, Stein T, Anderson SM and Neville MC (2007) Metabolic regulation in the lactating mammary gland: a lipid synthesizing machine. *Physiological Genomics* **28**, 323–336.

Sheng R, Yan SM, Qi LZ and Zhao YL (2015) Effect of the ratios of unsaturated fatty acids on the expressions of genes related to fat and protein in the bovine mammary epithelial cells. In Vitro *Cellular & Developmental Biology – Animal* **51**, 381–389.

Shingfield KJ, Chilliard Y, Toivonen V, Kairenius P and Givens DI (2008) Trans fatty acids and bioactive lipids in ruminant milk. *Advances in Experimental Medicine and Biology* **606**, 3–65.

Shingfield KJ, Bernard L, Leroux C and Chilliard Y (2010) Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. *Animal: An International Journal of Animal Bioscience* **4**, 1140–1166.

Vargas-Bello-Pérez E, Juan LL and Garnsworthy PC (2019) Effect of different exogenous fatty acids on the cytosolic triacylglycerol content in bovine mammary cells. *Animal Nutrition Journal* **5**, 202–208.

Warntjes JL, Robinson PH, Galo E, DePeters EJ and Howes D (2008) Effects of feeding supplemental palmitic acid (C16 : 0) on performance and milk fatty acid profile of lactating dairy cows under summer heat. *Animal Feed Science and Technology* **140**, 241–257.

Williams CM (2000) Dietary fatty acids and human health. *Annales de Zootechnie* **49**,165–180

Xu H, Luo J, Tian H, Li J, Zhang X, Chen Z, Li M and Loor JJ (2018) Rapid communication: lipid metabolic gene expression and triacylglycerol accumulation in goat mammary epithelial cells are decreased by inhibition of SREBP-1. *Journal of Animal Science* **96**, 2399–2407.

Yonezawa T, Yonekura S, Kobayashi Y, Hagino A, Katoh K and Obara Y (2004) Effects of long-chain fatty acids on cytosolic triacylglycerol accumulation and lipid droplet formation in primary cultured bovine mammary epithelial cells. *Journal of Dairy Science* **87**, 2527–2534.

Yonezawa T, Sanosaka M, Haga S, Kobayashi Y, Katoh K and Obara Y (2008) Regulation of uncoupling protein 2 expression by long-chain fatty acids and hormones in bovine mammary epithelial cells. *Biochemical and Biophysical Research Communications* **375**, 280–285.

Zhang H, Ao CJ, Khas-Erdene, Song LW and Zhang XF (2015) Effects of different model diets on milk composition and expression of genes related to fatty acid synthesis in the mammary gland of lactating dairy goats. *Journal of Dairy Science* **98**, 4619–4628.

Zidi A, Fernández-Cabanás VM, Urrutia B, Carrizosa J, Polvillo O, González-Redondo P, Jordana J, Gallardo D, Amills M and Serradilla JM (2010) Association between the polymorphism of the goat stearoyl-CoA desaturase 1 (SCD1) gene and milk fatty acid composition in Murciano-Granadina goats. *Journal of Dairy Science* **93**, 4332–4339.