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Advances in molecular regulation of goat lipid metabolism and FAS structure and function regulation

Jiahe GUO[2,#]; Xiaoguang JI[3,#]; Yongjiang MAO[2]; Zhangping YANG[2]; Zhi CHEN[2]; Yuan YUAN[1,*]

[1] School of Nursing, Yangzhou University, Yangzhou, 225009, China.
[2] College of Animal Science and Technology, Yangzhou University, Yangzhou, 225009, China.
[3] Hubei Blue Valley Microbial Technology Co., Ltd., Yichang, 443100, China

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Abstract: Goat milk is widely recognized for its nutritional value. Fatty acid synthase (FAS) is the crucial enzyme of fatty acid *de novo* synthesis. It plays an important role in the formation of goat milk fat. In this paper, we first introduced the molecular regulation process of goat milk fat metabolism based on the structure research of FAS. Secondly, we reviewed some key factors in FAS transcription and post-transcriptional regulation of the goat mammary gland and preliminarily constructed the expression network of the goat mammary gland FAS gene. The purpose of this paper is to systematically introduce the role of FAS in goat milk fat metabolism and to provide a reference for future studies on the mechanism of goat milk fat metabolism.

List of abbreviations

SCFA = short chain fatty acid
ACC = Acetyl-CoA carboxylase
FAS = fatty acid synthase
KS = β-ketoacyl synthase
MAT = malonyl-CoA transacylases
DH = Dehydratase
ER = Enoyl reductase
KR = Ketoacyl reductase
ACP = Acyl carrier protein
TE = Thioesterase
ACSL1 = acyl-CoA synthetase long-chain family member 1
ACSS2 = acetyl-CoA synthetase 2
FABP3 = Fat Acid Binding Protein3
SCD = stearoyl-ACP desaturase
CLA = Conjugated Linoleic Acid
sn = Stereos- pecifically Numbering,
GPAT = Glyceroltriphosphate acyl transferase
DGAT = Diacylglycerol acyltransferase
ADFP = Adipose Differentiation-Related Protein
TIP47 = Tail-interacting protein 47
PLIN1 = Perilipin1
XDH = xanthine dehydrogenase
BTN1A1 = Recombinant Butyrophilin Sub family1, Member A1
PMSF = Phenylmethanesulfonyl fluoride
SREBP1 = Sterol-regulatory element binding proteins1
PPARG = Peroxisome Proliferator Activated Receptor Gamma
THRSP = Thyroid hormone responsive spot
INSIG1 = insulin induced gene
PANK = Panthenic acid kinase

Introduction

Goat's milk is rich in fatty acids, proteins, and 8 essential amino acids needed by the human body. The volume of fat particles in goat's milk is only one-third that of cow's milk, making it easy to digest and absorb. The fatty acids in goat's milk, namely, dodecylic acid, myristic acid, stearic acid, and palmitic acid, are the main energy substances in the human body, accounting for approximately 5, 10, 8, and 27% of the total fatty acids, respectively, while the unsaturated fatty acids are mainly oleic acid and linoleic acid, accounting for 23% and 4% of the total fatty acids, respectively (Garard, 1939). The total dry matter content of goat's milk is 11.4% higher than that of milk and contains 3.28% milk fat and 8.13% nonfat milk solid (4.29% lactose, 3.20% milk protein and 0.64% ash). The fat in goat's milk is in the form of fat

*Address correspondence to: Yuan Yuan, 006707@yzu.edu.cn
#These authors contributed equally to this work.
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globules, the average diameter of which is approximately 3 μm, which is smaller than that of milk (approximately 6 μm). Goat's milk is rich in short- and medium-chain fatty acids and unsaturated fatty acids (Luo *et al.*, 2005). Goat's milk contains more caproic acid (C6:0), caprylic acid (C8:0), and decanoic acid (C10:0) than milk. The hydrolysis of triglycerides by lipase releases fatty acids, which enhance the flavor (and odor) of dairy products (Gelais *et al.*, 2005).

Goat's milk is rich in short- and medium-chain fatty acids, which can be used to prevent and treat some metabolic diseases in humans (Williams, 2000).

These fatty acids play important roles in improving nutritional absorption disorder syndrome and intestinal dysfunction, inhibiting and reducing blood cholesterol content (Dostalova, 1992; Haenlein, 2001; Kusunoki *et al.*, 2007), lowering blood pressure, preventing and treating atherosclerosis (Luna *et al.*, 2008), improving hyperlipidemia (Galina *et al.*, 1999; Bhattacharya *et al.*, 2006) and inhibiting tumor growth (Beppu *et al.*, 2006). Short-chain fatty acids (SCFAs) are important energy substrates in the body, as well as the most basic functional substances in the body of ruminants, and they are involved in the absorption of water and sodium. In addition, SCFAs play important roles in maintaining normal intestinal blood circulation and gastrointestinal hormone secretion. Since SCFAs can improve intestinal microcirculation and promote intestinal cell proliferation and mucosal growth, they are widely used in intestinal surgery (Miller, 2004; Jing *et al.*, 2019). In conclusion, the difference between goat's milk and other milk, especially milk, is largely due to the difference in fatty acid composition, which directly reflects the unique nutritional value of goat's milk. The study of the mechanism of fatty acid metabolism at the molecular level to increase the composition of beneficial fatty acids in goat's milk is an important aspect of goat breeding and thus of great significance to the development of the dairy goat industry.

Fatty acid synthase (FAS) is the crucial enzyme of fatty acid *de novo* synthesis. It plays an important role in the formation of goat milk fat. It is of great significance to study the structure, function, and gene regulation network of fatty acid synthase.

Molecular regulation of milk fat formation

Goat fatty acids are synthesized in the liver, kidney, brain, lung, mammary gland, fat, and other tissues and cells, but the synthesis of fatty acids in different parts of goats in different ages and nutritional states will have certain differences.

No matter where in the body, the main expressions of FAS in tissues were 16:0, 18:0, and C9-18:1. PUFA levels of n-6 and n-3 are low in AT but higher in the secretory tissues of the mammary gland. PUFA concentrations of 10:01, 12:01, 14:0, and C9, T11-18:2 were the highest in mammary gland tissue, but all ultra-long chain PUFAs were much more abundant in the liver (Toral *et al.*, 2013). The following content will focus on the synthesis of fatty acids in the goat mammary gland.

De novo synthesis of goat fatty acids

Goat mammary gland fatty acids have two sources, short-chain, medium-chain fatty acids and one-half of the total palmitic acid, which is obtained from the *de novo* synthesis of mammary epithelial cells, and long-chain fatty acids and the other half of the total palmitic acid pool are obtained from blood (Bernard *et al.*, 2005b). SCFAs, also known as volatile fatty acids (VFAs), are organic fatty acids with 1–6 carbon atoms mainly including acetic acid, propionic acid, butyric acid, isobutyric acid, pentanoic acid, caproic acid or hexanoic acid (Miller, 2004). The acetic acid, propionic acid, and butyric acid levels are the highest, accounting for approximately 90–95% of the short-chain fatty acids, and acetic acid is the main fatty acid (Schmitt Jr. *et al.*, 1977). The synthesis of fats in goat is accomplished by a series of enzymatic reactions that require two substrates as precursors: α-glycerophosphate and aliphatic CoA (or fatty acids). Alpha-phosphate glycerol is mainly derived from dihydroxyacetone phosphate and glycerol phosphate. Acyl-CoA (or fatty acid) is synthesized by acetyl-CoA carboxylase (ACACA) and FAS. Since α-glycerophosphate is mainly derived from glucose metabolism, ACAC A and FAS in goat play rate-limiting and decisive roles in fat synthesis (Zhu *et al.*, 2014).

Lactation has been an important process throughout biological evolution, characterized by breast milk, which provides complete nutrition for offspring. Lactation is a microcosm of the basic biological process of an organism, including all the processes of cell proliferation, differentiation, growth, and apoptosis, which determine the nutritional composition of the milk (Lemay *et al.*, 2007; Bionaz and Loor, 2008). In goat (including their milk), there are two sources of fatty acids: exogenous intake from the diet and *de novo* synthesis. The process of biosynthesis *in vivo* is driven mainly via (FAS, which acts as a catalyst (Menendez *et al.*, 2009). As a key enzyme for the *de novo* synthesis of fatty acids in cells, fatty acid synthase plays a core regulatory role in cell lipid metabolism (Chirala *et al.*, 1997). Currently, it is known that FAS mainly produces long-chain fatty acids in cells, and long-chain fatty acids are the synthetic substrates of ultralong-chain fatty acids. FAS can catalyze all the steps of *de novo* synthesis of a long-chain stearic acid (such as palmitate) with acetyl-CoA and malonyl-CoA as substrates (Jenni *et al.*, 2007; Livore *et al.*, 2007) (Fig. 1). The decrease of FAS expression also resulted in a significant decrease of relative contents of decanoic acid (C10:0) and lauric acid (C12:0) in GMEC (Zhu *et al.*, 2014). Sun *et al.* (2016) treat goat mammary gland epithelial cells (GMECs) with acetate, propionate, or butyrate and find that messenger RNA (mRNA) expression of FAS and LXRα was not affected by propionate but reduced by butyrate.

Goat mammary gland fatty acid synthesis occurs in the cytoplasm of mammary epithelial cells and consists of two connected phases (Glass *et al.*, 1969). In the first step, acetyl-CoA is carboxylated to malonyl-CoA under the action of ACACA. The second step is catalyzed by fatty acid synthase complexes. The whole process of synthesis begins with the deacylation of malonyl-CoA transacylases (MAT).

$$CH_3COS\text{-}CoA+7HOOCCH_2COS\text{-}CoA+14NADPH+14H^+ \rightarrow CH_3(CH_2CH_2)_7COOH+14NADP^++8CoA\text{-}SH+7CO_2+6H_2O$$

FIGURE 1. Formula of palmitic synthesis.

First, MAT transfers acetyl groups (i.e., initial substrates) to acyl carrier protein (ACP), and they are transferred from ACP to β-ketoacyl synthase (KS). At the same time, MAT catalyzes the deacylation of malonyl-CoA to transfer malonic acid monoacyl (prolonging substrates) to the pan-acyl-triethylamine arm of ACP. Then, by KS catalysis, a carbon unit is integrated into acetyl-ACP compounds and by Ketoacyl reductase (KR) catalytic Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-dependent beta-carbon reduction. Upon the hydrolysis of Dehydratase (DH), the acyl compounds are transferred to the alpha- or beta-olefin fatty acyl intermediate. With the participation of NADPH, Enoyl reductase (ER) catalyzes the decrease in the olefin fatty acyl intermediate and the generation of four-carbon acyl chains, which enter into the next cycle. When the acyl chain is extended to 16–18 carbons, Thioesterase (TE) catalyzes the release of the acyl chain from ACP to form the final long-chain fatty acid (Asturias, 2006) (Fig. 2).

Studies have shown that, in ruminants, acetyl-CoA and malonyl-CoA are tra nsferred into the fatty acid synthesis cycle through the activity of acetyl/malonyl transferases. Knudsen *et al.* (Knudsen *et al.*, 1976; Libertini and Smith, 1978; Mikkelsen *et al.*, 1985) found that short-chain fatty acids are formed by transferase activity. Both butyryl-CoA and caproyl-CoA are sensitive substrates of fatty acid synthase, and they complete the loading process of the fatty acid synthesis cycle through the activity of acetyl/malonic acid monoacyl transferases. However, FAS of nonruminants can only effectively transfer acetyl-CoA and butyryl-CoA (Bloch and Vance, 1977; Knudsen and Grunnet, 1980), and ruminants can also transfer C12 fatty acids (Smith, 2009). Competitive inhibition experiments showed that acetyl/malonic acid monoacyl transferases had not only acetyl/malonic acid monoacyl transferase activity but also butyryl and acyltransferase activity, and the activity of goat mammary acid synthase was higher than that of acetyl, butyryl, or diacyl transfer (Mikkelsen *et al.*, 1985). This result is consistent with that of Carey and Dils (1970; 1972). Sequencing results and active site analysis showed that the sequence and site of the transferase region binding to the acyl group were consistent with those of the acetyl/malonic acid monoacyl transferase region. This finding indicated that acetyl/malonic acid monoacyl transferase acted to transfer acyl groups. In addition, only one active site of an acyltransferase was found on goat fatty acid synthase. These results also illustrate that short-chain fatty acid synthesis in ruminants and nonruminant animals is dependent on b/single acyltransferase activity and malonic acid. The formation of chain fatty acids in nonruminant animals is undertaken by sulfur esterase II. Ruminant acetyltransferase/propylene acid in the basic form can also form a chain of fatty acids.

Exogenous fatty acid uptake

In 1998, Fielding and Frayn (1999) reported that the ruminant mammary gland is capable of absorbing free and bound fatty acids from the blood. Triglycerides in the blood are mainly in the form of high-density lipoprotein, low-density lipoprotein, and chylomicron. Under the catalyzed action of lipoprotein lipase (LPL), low-density lipoprotein and chylomicron can be hydrolyzed to release free long-chain fatty acids that are absorbed by mammary epithelial cells. CD36 is an important protein in the body that is involved in the uptake of Long Chain Fatty Acids (LCFAs) by fat and muscle cells. In 1997, Sfeir *et al.* (1997) conducted adipocyte transport studies and found CD36 gene expression and fatty acid uptake during adipocyte induction and differentiation. In 1998, Watanabe *et al.* (1998) reported that the transfer of the CD36 gene into cells without endogenous CD36 expression enhanced the binding ability of LCFAs, and this binding effect was reduced after the application of relevant protein inhibitors. CD36 in tissue lipoprotein and lipid metabolism. However, mammary cells absorb foreign fatty acids in a complicated process for which the exact mechanism is still unclear.

Transportation and desaturation of fatty acids

Massimo and Loor (2008) reported that long-chain fatty acids play a role in mammary epithelial cells in mammary gland

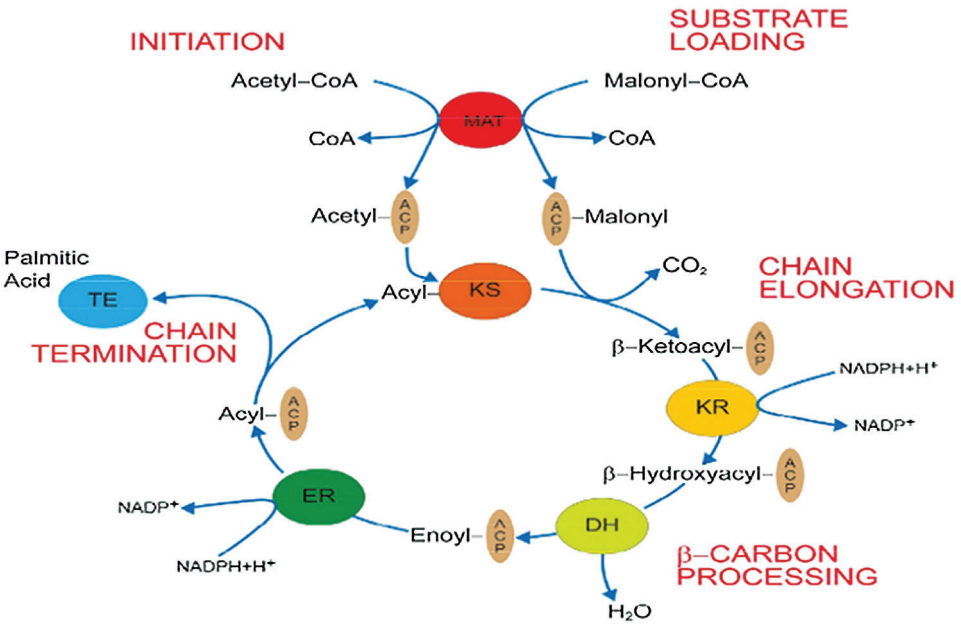

FIGURE 2. *De novo* fatty acid synthesis catalyzed by FAS (Asturias, 2006).

tissue. The initial step is the transfer of the long-chain fatty acids from plasma into cells and their activation. Previous studies have suggested that CD36 plays an extremely important role in the uptake of long-chain fatty acids into mammary epithelial cells. Mashek *et al.* (2006) reported that acyl-CoA synthetase long-chain family member 1 (ACSL1) and acetyl-CoA synthetase 2 (ACSS2) catalyze the intracellular long-chain and short-chain fatty acid conversion into their active forms, respectively. McArthur *et al.* (1999) reported that Fat Acid Binding Protein3 (FABP3) and FABP4 are fatty acid-binding proteins critical mainly for the transport of fatty acids in cells. Yonezawa *et al.* (2004) found that oleic acid and linoleic acid can upregulate the expression of CD36, FABP4, and ACSL1. Kadegowda *et al.* (2009b) found that stearic acid has a greater up-regulatory effect on CD36 and FABP4 than do other unsaturated fatty acids. Massimo and Loor (2008) found that the expression of FABP3 was inhibited by the addition of long-chain fatty acids. They believed that FABP3 mainly provided fatty acids for SCD and catalyzed the *de novo* synthesis of fatty acids. After adding linolenic acid, the expression of CD36 was upregulated, while the expression of ACSL1 and FABP3 was downregulated. It has been reported that long-chain fatty acids can inhibit the *de novo* synthesis of short-chain fatty acids (Bauman *et al.*, 2008). In the study of Kadegowda *et al.* (2009a), unsaturated fatty acids inhibited the expression ofACSS2 to a greater extent than did saturated fatty acids.

The desaturation of fatty acid chains is catalyzed by the product of stearoyl-ACP desaturase (*SCD* (*in vivo*)) genes, which introduces a cis double bond at delta 9 to generate a delta 9 unsaturated fatty acid. Active fatty acids mainly target C14–C19. In contrast to monogastric animals, ruminants have only one *SCD* gene, which encodes a 5 kb transcript in goats (Bernard *et al.*, 2005a). In goats, the *SCD* gene is highly expressed in mammary gland tissue and subcutaneous adipose tissue (Corl *et al.*, 2001). In cow mammary glands, most cis-9, trans-11 (Griinari *et al.*, 2000; Loor *et al.*, 2005; Shingfield *et al.*, 2016) and trans-7, cis-9 (Corl *et al.*, 2002). Conjugated Linoleic Acid (CLAs) are catalyzed by *SCD*. The transcription enhancer element (STE) in the promoter region of *SCD* plays an important role in the desaturation effect of *SCD*. STE can block the generation of trans-10 and cis-12 fatty acids by *SCD* and inhibit the production of cis-9 and trans-11 CLAs (Corl *et al.*, 2001; Bernard *et al.*, 2008).

Synthesis of triglycerides and the secretion of lipid droplets

Fat is an important part of milk and plays an important role in energy supply and improving milk quality. Milk fat is composed of 98% triglycerides, a small amount of cholesterol, diglycerides, glycerides, and free fatty acids (Dils, 1986). Triglycerides constitute the main part of milk fat, which is mainly composed of 95% fatty acids and 1% phospholipids combined with glycerol (Bernard *et al.*, 2008). Triglycerides are selective in the utilization of fatty acids, which determines the function and nutritional value of different triglycerides (German *et al.*, 1997). Of the fatty acids, 56% to 62% are mainly bound at the sn-1 and sn-2 position of triglyceride, respectively, and are mainly composed of medium- and long-chain saturated fatty acids (C10–C18), of which C16:0 is evenly distributed at sn-1 and sn-2, while C8:0, C10:0, C12:0, and C14:0 are mainly bound to sn-2 and C18:0 is mainly bound to sn-1. In addition, C18:1 accounts for 24% of sn-1 fatty acid sources. Short-chain fatty acids (44%) C4:0, C6:0, C8:0, and oleic acid (27%) bind to Stereo-specifically Numbering (sn)-3. When hydrolyzed, the sn-1 and sn-3 fatty acids are specifically released under the action of an esterase, while the sn-2 fatty acids are preferentially absorbed and utilized in the form of glycerol monoester. The synthesis of triglycerides begins with the preferential esterification at the sn-1 site of glycerol 3-phosphate. This process is catalyzed by Glyceroltriphosphate acyltransferase (GPAT). GPAT in mammals can be divided into two subtypes according to its different location; namely, GPAT1 is located in the endoplasmic reticulum, and GPAT2 is located in mitochondria. GPAT1 is sensitive to ethyl maleimide (NEM), while GPAT2 is resistant to NEM. Both subtypes of GPAT catalyze the cooperation of mouse triglycerides (Coleman, 2004). AGPAT/LPAAT catalyzes the esterification of sn-2 fatty acids. AGPAT has a higher affinity for saturated medium- and long-chain fatty acids, and the esterification sequence is C16 > C14 > C12 > C10 > C8 (Marshall and Knudsen, 1977; Mistry and Medrano, 2002). C16:0 accounts for 43% of the sn-2 chains. However, the concentration of the substrate can also change the fatty acid composition at sn-2. The third step of triglyceride synthesis is catalyzed by the Diacylglycerol acyltransferase (*DGAT*) gene. The DGAT protein is located on the endoplasmic reticulum and is the only triglyceride synthesis function-specific protein (Mayorek *et al.*, 1989). DGAT plays an important role in the synthesis of milk fat (Harris *et al.*, 2011). There are two forms of DGAT: DGAT1 and DGAT2. These two forms can catalyze the synthesis of triglycerides. DGAT1 is highly expressed in the small intestine, where it plays an important role in the absorption of TG. DGAT1 also accounting for more than 90% of the DGAT activity in the small intestine (Cases *et al.*, 2001; Buhman *et al.*, 2002). The triglyceride content was significantly reduced in dgat1-deficient mice (Smith *et al.*, 2000). When the activity of DGAT1 decreases, DGAT2 can compensate and exert the activity of the DGAT1 enzyme, thus ensuring the normal synthesis of triglycerides in the small intestine. However, DGAT2 is mainly found in the liver and white adipose tissue (Smith *et al.*, 2000). Loss of DGAT2 in adipose tissue can significantly reduce triglyceride content (Meegalla *et al.*, 2002) (Fig. 3). In addition, there are functional differences between the two DGAT subtypes. Studies have shown that DGAT1 is mainly expressed in response to exogenous fatty acids, while DGAT2 is expressed in response to endogenous fatty acid synthesis (Smith *et al.*, 2000). From lactating mammary gland tissue, triglycerides are mainly secreted in the form of milk fat globules. The formation of lipid globules begins with the formation of lipid droplets. The formation of fat droplets is mainly controlled by PAT family genes, including the Adipose Differentiation-Related Protein (*ADFP*), Tail-interacting protein 47(*TIP47*), and Perilipin1 (*PLIN1*) genes.

Lipid droplets (less than 0.5 μm in diameter) released from the endoplasmic reticulum are released and then coated with proteins and polar lipids. Some of these droplets undergo a series of enzymatic fusion events that lead to the formation of

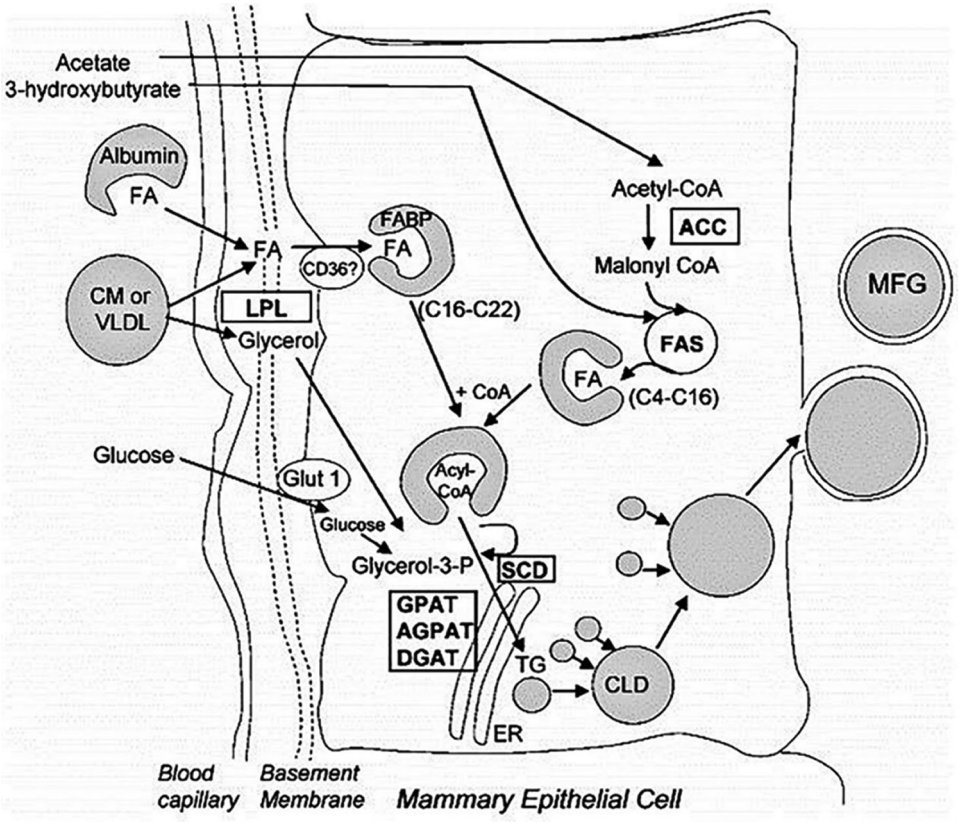

FIGURE 3. Triglyceride synthesis and milk fat secretion (Meegalla *et al.*, 2002).

large droplets in the cytoplasm that are transported to the cell membrane for secretion. However, many lipid droplets do not fuse and are secreted at their initial size (Bauman *et al.*, 2006). While the average droplet diameter is close to 4 μm, more than 80% have a diameter of less than 1 μm.

The secretion of lipid droplets is mainly facilitated by three proteins. First, the newly formed lipid droplets are aggregated upon binding to ADFP and fused with other lipid droplets and then transported to the cell membrane. Under the action of Recombinant Butyrophilin Sub family1, Member A1 (BTN1A1) and xanthine dehydrogenase (XDH) are released through the cell membrane by exocytosis. Among these proteins, ADFP plays the role of coordinator in the whole process by binding to BTN1A1, which mediates the binding of ADFP to lipid droplets. The recruitment of XDH under the action of BTN1A1 promotes the formation of a three-step process, which leads to the release of lipid droplets (Chong *et al.*, 2011) (Fig. 4).

FAS structure and function regulation

The distribution of fatty acid synthase gene expression in goat organs was significantly different. The mRNA expression of FAS was the lowest in the liver, which was significantly lower than that in mesenteric fat and muscle fat ($P < 0.05$). The mRNA expression level of FAS was the highest in mammary gland tissues and was significantly higher than that in mesenteric adipose tissues ($P < 0.05$). This phenomenon indicates that endogenous fatty acid synthesis is less in the liver and more in mammary gland tissues. The following content will mainly introduce the FAS structure and function regulation of goats.

The structure of goat FAS

Naturally occurring FAS is categorized into two types: FASI and FASII. In animals, FASI is the main form (Smith *et al.*, 2003). Mammalian fatty acid synthase must be in the form of a dimer to render its catalytic activity for fatty acid

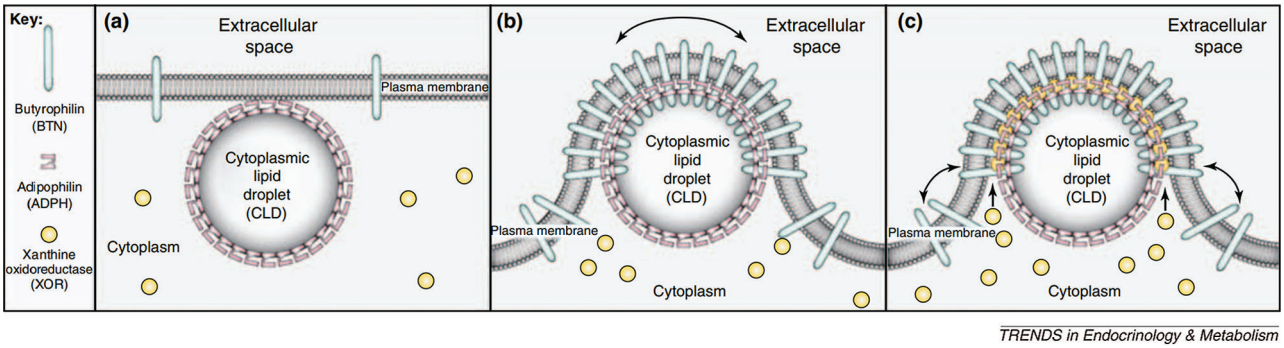

FIGURE 4. Milk droplet model of milk lipid secretion (Chong *et al.*, 2011).

synthesis. The monomer of fatty acid synthase does not have full catalytic ability; but, studies have shown that some of the functional domains in the monomer have catalytic activity, primarily to transfer the substrate in advance to the ACP functional areas and interact with ACP functional domains (Pappenberger *et al.*, 2010).

Two models have been proposed to modify the structure of FAS in mammals (Asturias *et al.*, 2005; Pappenberger *et al.*, 2010): the conventional model and the revised model. In the conventional model, the two monomers functionally interact in inversely parallel forms, with clear boundaries between the monomers (Fig. 5). The revised model is based on the interaction between two monomers connected at the N-terminus and C-terminus (Asturias *et al.*, 2005). Cryo-electron microscopy also revealed that the FAS monomers are arranged in a coiled manner (Fig. 6c). The crystallographic density map of FAS reveals the structural interface of a central region formed by the combination of the two monomers, with DH and ER, and MAT located on both sides of KS (Smith, 2009) (Fig. 6b). Grunnet and Knudsen (1979) confirmed for the first time in goats that MAT has not only transferase activity but also thioesterase activity. The systematic study on the overall spatial structure of goat FAS has not been reported yet, but based on cDNA sequence-related studies, the FAS structure of goat is different from that of other mammals.

In Xinong dairy goats, the FAS gene is located in chromosome 19 and encodes the homodimeric multifunctional enzyme. It has 8217 bp comprised of 42 exons, an ORF of 7545 bp, and 5'- and 3'-UTR regions of 88 bp and 584 bp, respectively, with the start codon located in the 2nd exon, and the stop codon is in the 42nd exon. It encompasses seven active functional domains, which participate in all the processes of fatty acid synthesis. It encodes 3514 amino acids with an approximate molecular weight (MW) of 273.8 kDa (Zhu *et al.*, 2014). The similarity of the FAS gene exon 9–15 in the cells from the mammary gland of Saanen goats was 95, 85.7, 82.7, 73.2, 92.9, 77.7, 82.3, and

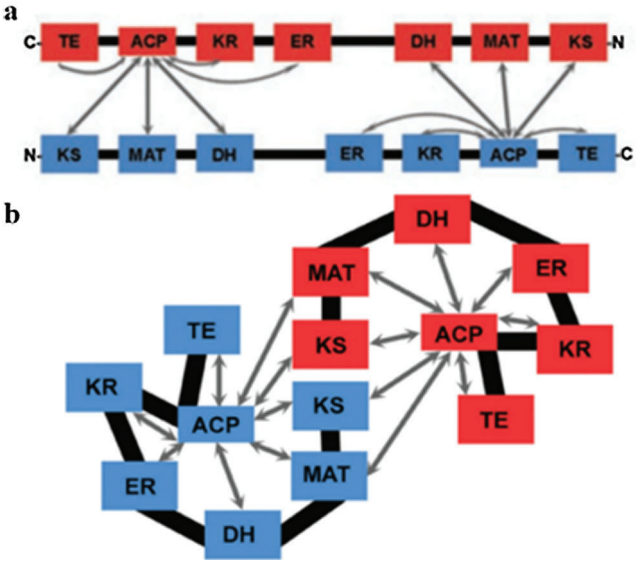

FIGURE 5. Structure model of fatty acid synthase. (a) Conventional model. (b) Revised model (Asturias *et al.*, 2005).

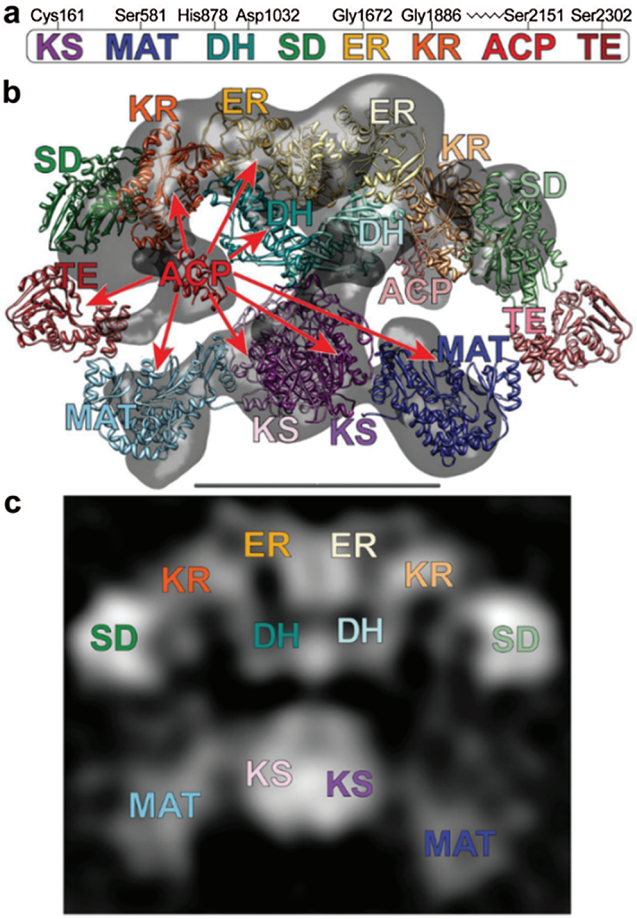

FIGURE 6. (a) The domains of FAS. (b) Crystal and cryo-EM structures capture different conformations of FAS. (c) Images of FAS molecules (Smith, 2009).

64.7%, respectively, with that of cow (NM_001012669), human (NM_004104), rat (NM_017332) and chicken (NM_205155). Its catalytic structure, starting at the N-terminus, has the following order (Fig. 7): β-ketoacyl synthase (KS), acetyl-CoA and malonyl-CoA transacylase (MAT), dehydratase (DH), enoyl reductase (ER), and thioesterase (TE). FAS can be divided into three regions: regional I contains KS, MAT, and DH, regional II contains ER, KR, and ACP, and regional III consists of TE (Chirala and Wakil, 2004). The active sites in the AT/MT region were highly conserved serine residues among all species, but exon 10 in the Saanen goat had one less amino acid than the other species, which may have an important impact on the spatial conformation and physiological functions of the AT/MT region.

Regulation of Milk fat metabolism by goat FAS

Wang *et al.* (2010) used RNA interference technology to interfere with the expression of the FAS gene in goats and found that, after FAS gene silencing, the content of C10:0, C12:0, C16:0, and C18:0 in the cells decreased by 42, 42.37, 18.51, and 29.3%, respectively. The C14:0 content increased by 29.82%. Quantitative real-time PCR results showed that

FIGURE 7. The linear map of fatty acid synthase domains of Saanen goat (Zhu *et al.*, 2014).

the expression levels of the leptin receptor (LEPR) gene and liver X receptor (LXRα) gene were 1.71- and 1.36-fold that of the control group, respectively. The expression levels of the fatty acid-binding protein (AFABP) gene and lipoprotein lipase (LPL) gene were decreased by 32 and 25%, respectively. These results indicate that the FAS gene plays an important role in regulating fatty acids, according to chain length, in mammary gland epithelial cells.

The termination mechanism of medium-chain fatty acids in ruminants is quite different from that in nonruminants. Medium-chain fatty acids in ruminants are terminated by the activity of a transferase. Knudsen and Grunnet (1982) found that purified mammary fatty acid synthase from lactating swine and rat cannot produce fatty acid when incubated with goat microsomes, and only goat and cattle fatty acid synthase can prompt the synthesis of C10 fatty acids. Furthermore, research shows that these fatty acids are not present in the ruminant mammary gland independent of TE II. These findings indicate that the activity of the medium-chain fatty acid synthase is terminated in goat (Grunnet and Knudsen, 1978). Goat FAS can terminate the synthesis of long-chain fatty acids and may terminate fatty acid chain synthesis when treated with the proteasome inhibitor Phenylmethanesulfonyl fluoride (PMSF), for which the synthase of long-chain fatty acid termination activity was significantly affected but not sensitive. The authors speculated that goat fatty acid synthase might contain two hydrolase sites or that two hydrolysis activities are undertaken. Kundsen and Grunnet (1982) found that the ratio of C10 to C12 was significantly higher in the presence of 5.2 mg/mL albumin, and the content of C10 was 4-fold higher when 10.4 mg/mL albumin was used. The addition of monoacyl-CoA of malonate inhibits the effect of albumin and promotes the formation of C12. However, changing the concentration of monoacyl-CoA of malonate cannot fundamentally change the chain length of fatty acids. The addition of globulin also had the same effect, but only at a higher concentration and only in ruminants.

Shi *et al.* (2015) used GO and KOGG enrichment analysis to obtain genes related to milk fat metabolism in goat mammary tissue and established a regulatory network model of these genes. They found that genes encoding FABP3, FAS, SCD, PLIN2, whey proteins, and caseins at 100 and 310 days postpartum increased significantly compared with the non-lactating period (Fig. 8).

In 2014, Zhao *et al.* (2014), using goat mammary epithelial cells (GMEC), knockdown of LPL reduced the expression of mRNA sterol-regulatory element-binding proteins-1 (SREBP-1), FAS, and Peroxisome Proliferator-Activated Receptor Gamma (PPARG), results show that LPL is the key gene for FAS regulation. The prolactin receptor (PRLR) gene is the same as LPL, both in the upstream of FAS gene regulation. PRLR, as a transmembrane receptor, results in a large variety of physiological processes (Bole-Feysot *et al.*, 1998). PRLR signaling during lactation triggers the copious synthesis of triglycerides meanwhile controls these substance's secretions (Rudolph *et al.*, 2011). Treatment of goat mammary epithelial cells (GMECs) with prolactin decreased the expression of PPARG, SREBP1, FAS, and ACACA, which relative to the control group averaged 76, 55, 52, and 68%. Some scholars have also studied the relationship between SREBP-1, PPARG, and FAS

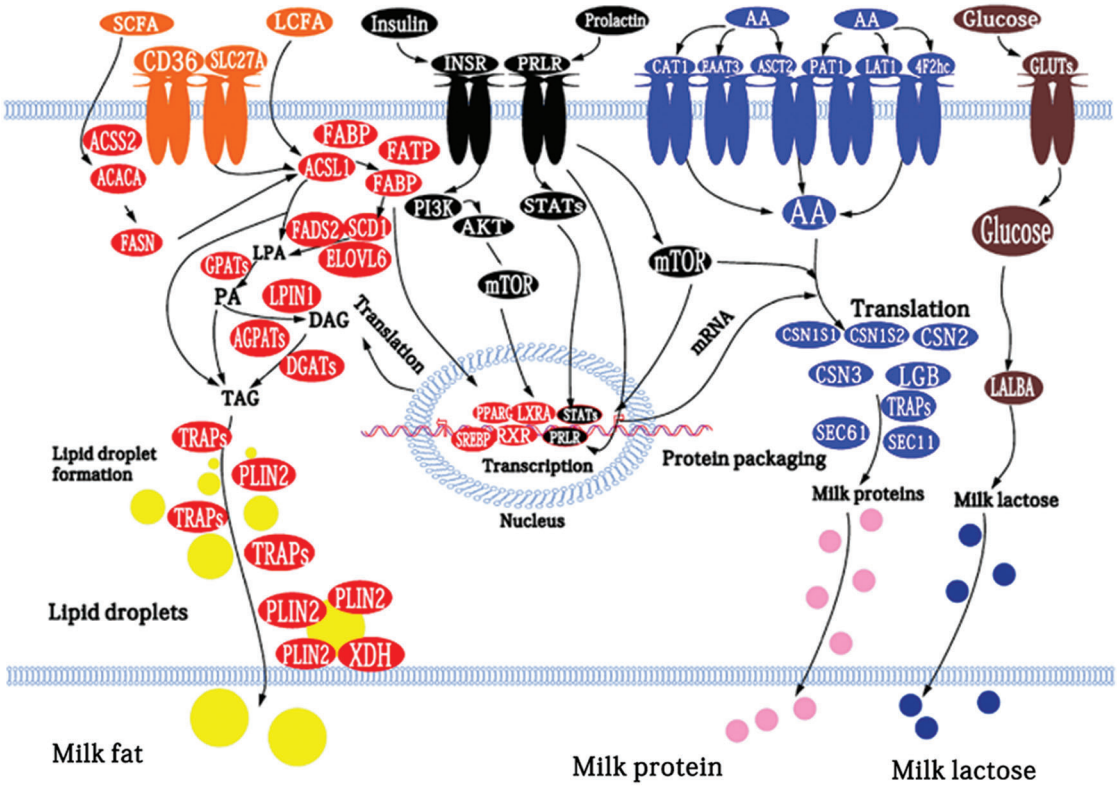

FIGURE 8. Model of the networks of factors (proteins, hormones, and enzymes) potentially involved in the regulation of milk fat, protein, and lactose biosynthesis in goat mammary tissue (Shi *et al.*, 2015).

(Shi *et al.*, 2016). Cholesterol regulatory element-binding protein 1 (SREBP1) is a membrane-bound transcription factor involved in many roles of lipid homeostasis. SREBP1 is central in the transcription regulation of many genes related to milk fat synthesis and secretion in dairy cattle (Li *et al.*, 2014). Li *et al.* (2015) found that SREBP-1 overexpression and knockdown by small interference RNA in goat mammary epithelial cells influenced the abundance of endogenous FAS. SREBP-1 regulates FAS expression at the transcriptional level. Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors that belong to the nuclear receptor superfamily and include 3 closely related members: PPAR alpha (PPARA), gamma (PPARG), and delta (PPARD). ALL of them have been proved to affect the expression of the FAS gene in goats.

Goat PPARG has two isoforms which are PPARG1 and PPARG2. PPARG1 upregulates the transcription regulators SREBF1 and FAS, ACACA, and SCD; these data suggest that PPARG1 is the isoform controlling lipogenesis in goat mammary cells (Shi *et al.*, 2014). PPARD is associated primarily with the catabolism of fatty acids in goats. Previous studies have found that PPARD expresses higher levels of PPARG in the bovine mammary gland (Bionaz *et al.*, 2013). Shi *et al.* (2017b) found that the expression of fatty acid synthesis (FAS) gene downregulated significantly after knockdown of PPARD in goat mammary gland cells incubated with GW. The involvement of PPARA in fatty acid metabolism was also confirmed this year. Activation of PPARA up-regulated FAS, SCD1, ACSL1, DGAT1, FABP4, and CD36 in goat mammary gland epithelial cells, but down-regulated DGAT2 and PGC1A abundance (Tian *et al.*, 2020).

The researchers found that knockdown of THRSP reduced the incorporation of breast cancer cytoplasmic acetate into lipids (Martel *et al.*, 2006). Yao *et al.* (2016) found overexpression of THRSP upregulated the expression of fatty acid synthase (FAS) in goat mammary epithelial cells. In contrast, overexpression of THRSP led to downregulation of thrombospondin receptor CD36. Thyroid hormone responsive (THRSP) is a crucial protein for cellular *de novo* lipogenesis in goat. Tian *et al.* (2018) used CRISPR/Cas9 knockout SCD1gene in GMEC. The deletion of SCD1 decreased the expression of other genes involved in *de novo* fatty acid synthesis, including SREBF1 and FAS. SCD1 is regulated by Peroxisome proliferator-activated receptor-(PPARG). Shi *et al.* (2013) observed a positive correlation between PPARG and SCD expression in the goat mammary gland at peak lactation. Overexpression of PPARG leads to increased SCD gene expression.

LXR is an important nuclear receptor that regulates lipid and cholesterol homeostasis (Ulven *et al.*, 2005). Overexpression of LXRB dramatically upregulated SREBP1c and FAS to levels higher than overexpression of LXRA. These results highlight an important role for LXRB in the transcriptional regulation of SREBP1c and FAS in goat mammary epithelial cells (Shi *et al.*, 2017a).

Posttranscriptional regulation of goat FAS gene

Post-transcriptional control refers to the regulation of gene expression at the post-transcriptional level (RNA), including a series of modifications and processing of the transcription products of eukaryotic genes (Liu *et al.*, 2020; Yang and Lu, 2020; Zheng *et al.*, 2020). MicroRNA (miRNA) is a small non-coding RNA molecule that regulates mRNA expression at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of the target mRNA (Bartel, 2009). In recent years, several microRNAs have been reported to modify cellular lipid metabolism by regulating the expression of lipid-related genes. For instance, miR-221 regulates lipid metabolism in mammary epithelial cells (MECs) and is expressed differentially at various stages in mice (Chu *et al.*, 2018a).

Synergistic regulation among microRNAs (miRNAs) is important to understand the mechanisms underlying the complex molecular regulatory networks in goats. Goat milk fat synthesis is driven by a gene network that involves many biological processes in the mammary gland. These biological processes are affected by several miRNAs rather than a single miRNA. Lin *et al.* (2013b) found that the expression of 11 miRNAs that have the potential to regulate milk fat synthesis in the goat mammary gland. They also found that prolactin promotes the expression of four miRNAs (miR-23a, miR-27b, miR-103, and miR-200a).

Functional verification of these microRNAs has also been widely reported. Downregulation of miR-26a/b and their host genes in goat mammary gland cells decreased the expression of genes relate to fatty acid synthesis (*PPARG*, *LXRA*, *SREBF1*, *FAS*, *ACACA*, *GPAM*, *LPIN1*, *DGAT1*, and *SCD1* [*in vitro*]). Luciferase reporter assays confirmed INSIG1 as a direct target of miR-26a/b. This suggests that FAS and other lactate metabolism genes regulate insulin-induced gene1 (INSIG1 [*in vitro*]) in the goat nucleus (Wang *et al.*, 2016).

It was found that miR-103 expression level was significantly higher in the mid-lactation period than in the dry period. The miR-103 family has three members, miR-103-1, miR-103-2, and miR-107, which reside in the sense oriented intron 5 of three members of the pantothenic acid kinase (PANK [*in vitro*]) gene family members across species: PANK3, PANK2, and PANK1, respectively (Rock *et al.*, 2000). Overexpression of miR-103 in goat mammary gland epithelial cells resulted in increased expression of FAS, ACACA, and other fat metabolic-related genes (Lin *et al.*, 2013a) (Fig. 9). miR-103 plays an important role in the molecular regulation of milk fat metabolism.

MiR-145 expression level in goat mammary gland was significantly higher in mid-lactation period than in dry period. Overexpression of miR-145 increased transcription of genes associated with milk fat synthesis. In contrast, the silencing of miR-145 impaired fatty acid synthesis. Inhibition of miR-145 increased methylation levels of fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1), peroxisome proliferator-activated receptor gamma (PPARG) (Wang *et al.*, 2017). The role of Mir-15b in mammary fatty acid metabolism in goats has been studied. Mir-15b down-regulates lipid metabolism in goat mammary gland epithelial cells and its expression was lower during lactation. Overexpression of miR-15b reduced lipid content in mammary epithelial cells with decreasing levels of FAS (Chu *et al.*, 2018b). Some miRNAs do not directly regulate FAS expression but can indirectly affect FAS gene expression by affecting the expression levels of other milk fat metabolism

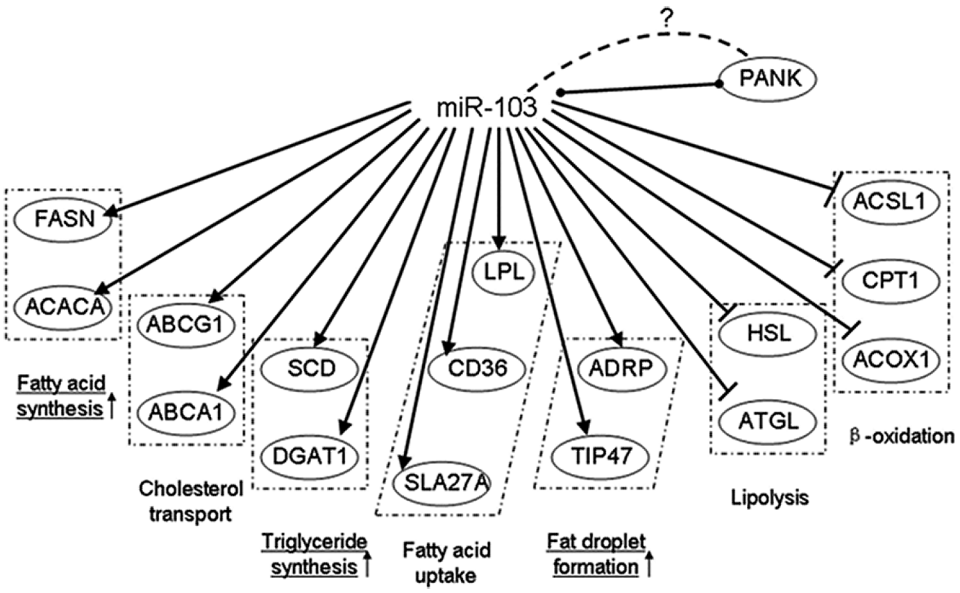

FIGURE 9. Gene networks modulated by miR-103 (Lin *et al.*, 2013a).

genes like miR-148a and miR-17-5p. They regulate milk TAG synthesis via PPARGC1A and PPARA in goat mammary epithelial cells, which affect the whole milk fat metabolism network (Chen *et al.*, 2017). In summary, miRNA plays a very important role in the post-transcriptional regulation of FAS genes in goats.

Conclusion

FAS is a key enzyme in the *de novo* synthesis of fatty acids and is considered to play an important role in the formation of goat milk fat. Genes that regulate FAS in goat mammary epithelial cells are LPL, CD36, ACACA, SCD, SERBP-1, PPARG, PPARD, PPARA, PRLR, LXRB, THRSP. Some genes regulate FAS directly, and some regulate FAS indirectly. MicroRNA is an important factor in FAS post-transcriptional regulation which contained miR-145, miR-26a/b, miR-103, Mir-15b.

Currently, there are few studies on the gene expression profile of milk goats at different lactation stages and that address how FAS regulates milk fat formation. The systematic study of the role of FAS in milk fat formation in milk goats is of great significance for discovering the molecular regulatory mechanism of milk fat formation.

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