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Candidate genes expression affect intramuscular fat content and fatty acid composition in Tan sheep

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ABSTRACT. Ningxia Tan sheep meat is tender, has no strong smell, a delicious taste, and even fat distribution. Intramuscular fat (IMF) content and the composition of fatty acid (FA) both are important factors associated with meat quality, and are also important for meat tenderness and flavor assessment. We examined the correlation of candidate genes expression with these factors in four muscle tissues, including longissimus dorsi, triceps, biceps and supraspinatus from Tan sheep. The IMF and FA composition in the different muscle tissues were measured and associated with mRNA expression of related genes (*PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC*). IMF content in longissimus dorsi muscle was 45.4% higher than that in triceps brachii ($P < 0.05$), 48.4% higher than that in biceps femoris, and 20.1% higher than that in supraspinatus. *PPARγ*, *C/EBPα*, *FAS* and *LPL* mRNA levels in supraspinatus were significantly different among longissimus dorsi muscle, biceps femoris and triceps brachii. There was significant positive correlation between IMF content and the relative mRNA expression of *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4* and *ACC* in longissimus dorsi muscle. The ratio of polyunsaturated fatty acids to saturated fatty acid was positively and significantly correlated with *C/EBPα* and *ACC* mRNA levels, and negatively and significantly correlated with *FAS* and *FABP4* mRNA levels. Omega-6 fatty acid (ω6)/omega-3 fatty acid (ω3) ratio was positively and significantly correlated with *C/EBPα*, *FAS* and *LPL* mRNA levels, but negatively and significantly correlated with *FAS*

and *PPARγ* mRNA levels. These correlations indicate that *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* are key candidate genes for fatty deposits and the components and contents of FA in sheep, and the evaluation and functional verification of these genes may be useful for improving the meat quality of Tan sheep.

Key words: FA; IMF; Muscles tissues; Candidate genes; Tan sheep

INTRODUCTION

Tan sheep, a sheep breed with several unique characteristics, are the main livestock species reared in the Ningxia Hui Autonomous Region of China. Tan sheep provide many livestock resources (e.g., meat, sheepskin and wool). Tan mutton is the main income source for local farmers (Kang et al., 2013). As the quality of life around the world improves, people are gradually expecting meat to be high quality and healthy. Intramuscular fat (IMF) and intramuscular fatty acid (FA) composition play a crucial role in meat quality (Gerbens et al., 2001; Hausman et al., 2009). Meat flavor, tenderness and juiciness can be enhanced by boosting the IMF levels and FA profile (Fernandez et al., 1999). The level of intramuscular fat deposition in Tan sheep is lower, but the fat content in the tail is higher compared with lean-tailed sheep (e.g. Shanbei Fine Wool sheep) (Xu et al., 2015). Many studies have shown that IMF and FA levels are affected by age, variety, gender and diet (Teye et al., 2006; Zhang et al., 2007; Bosch, 2012). Lipid accumulation and decomposition and FA transport lead to IMF deposition, and the deposition of IMF and FA levels are mainly affected by genetic and nutritional factors (Maltin, 2003; De Smet, 2004). The IMF content of Tan sheep is quite different from other varieties, which indicates that it is affected by genetic in addition to non-genetic factors. Supplying mutton with good IMF and FA levels remains a huge challenge in modern sheep breeding practice. The main problem in improving the quality of mutton is that the genetic correlation between IMF content and key candidate genes is not clear (Serão et al., 2011). IMF is consisted of fat droplets stored in the myofibers cytoplasm and adipocytes located between fiber bundles. Therefore, the content of IMF is closely associated with the number of adipocytes in muscle (Shi-Zheng, 2009). FA is the major flavor precursor of meat and FA composition is partially determined by genetic factors (Wang et al., 2013). With the development of biotechnology, molecular breeding has provided an effective way to improve IMF content and FA levels in Tan sheep.

Reports have shown that the IMF and FA levels of the longissimus dorsi muscle are related to the relative mRNA levels of key candidate genes (He, 2013). The peroxisome proliferator activated receptor (*PPAR*) signalling pathway is significant for meat quality in mammals among this candidate pathway (Adoligbe, 2015). The CCAAT/enhancer-binding protein α (*C/EBPα*) plays a major role in 3T3-L1 cells differentiation and gene expression regulation. It's key to study adipose tissue regulation in vitro by C/EBPα gene and the importance of IMF in food-producing animals (Munoz et al., 2003). Fatty acid synthase (FAS) is a critical multifunctional enzyme that contributes to fatty acid biosynthesis. The mRNA level of *FAS* shows a positive correlation with fat content in animals (Munoz et al., 2003). Several important factors are participated in three lipid pathways, including FA synthesis and FA transport genes, for instance, acetyl-CoA carboxylase (*ACC*) and fatty acid synthase (*FAS*), and lipoprotein lipase (*LPL*) in adipose tissues (Lee, 2002; Peterson,

2003; Tara et al., 2005). Fatty acid binding protein 4 (*FABP4*) is an essential candidate gene in fat deposition and lipid metabolism, joining in the transport and metabolism of intracellular free FA. Although many genes are known to be associated with FA composition, high-throughput screening methods are needed to identify major genes or genes with novel functions that might be responsible for FA profiles (Kuriyama et al., 2005; Elmasri et al., 2012).

The influence of different sets of genes on IMF deposits, FA composition and contents in various muscles of the Tan sheep are unknown. The candidate genes we described before might be related to IMF deposition and FA levels and could improve deposition of intramuscular adipocytes in Tan sheep. Therefore, the purpose of this study was to study the relationship between intramuscular fat content, fatty acid composition and content and the relative mRNA expression levels of candidate genes *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4* and *ACC* in muscle tissues of Tan sheep.

MATERIAL AND METHODS

Animals and sample collection

All animal experiments were performed based on the animal slaughter procedures published by the Ministry of Agriculture of China. They were approved by the Animal Protection and Use Committee of North Minzu University. Twenty-four 9-month old castrated male Tan sheep with similar body weight (21.4 ± 1.9 kg) were applied on a single-factor completely random experimental design. Samples were collected from the longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus of Tan sheep. All instruments for collecting muscle tissues were sterilized beforehand. A portion of the sample was washed with a 0.9% NaCl solution and another portion was placed in a storage bag at -20℃ for routine nutrient and fatty acid composition determination. The third part of the sample was placed in liquid nitrogen and then stored at -80℃ for total RNA extraction later.

Measurement of IMF content

The IMF contents in the longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus were detected by the Soxhlet petroleum-ether extraction method based on Chinese National Standards GB/T 5009.6.2004. The IMF content was expressed as a percentage by weight of dry muscle tissue.

FA analyses

FA compositions and contents were detected following the way described by Bhuiyan et al. (2018) (Bhuiyan et al., 2018). The gas chromatography (Agilent/GC7890, USA), flame ionization detector (FID), automatic sample injection and capillary column (30 m length; 0.32 mm internal diameter; 0.25 μm film thickness) were used for detection.

The temperatures of syringe and detector were 225℃ and 215℃, respectively. As the developing solvent system, the gas carrier (nitrogen) flow velocity was 22 mL/min, chloroform: methanol: water (45:35:10, v:v:v). The SFA, monounsaturated fatty acid

(MUFA), PUFA, ω3 and ω6 as well as the ratios of PUFA/SFA and ω6/ω3, were calculated. Selected abbreviations are SFA without any double bonds (C12:0-C20:0); MUFA all fatty acids with a single bond (C14:1-C20:1); and PUFA all fatty acids with 2 or more double bonds (including C18:2n6c, C20:3n3, C20:4n6, C20:5n3 and C22:6n3); eicosapentaenoic acid (EPA); docosahexaenoic (DHA); the sum of the parts of C18:3n3, C20:3n3, C20:5n3 and C20:6n3 (ω3); the sum of the parts of C18:2n6c and C20:4n6 (ω6). The composition and content of fatty acids are expressed in mg/100g. The main statistical and analytical data are shown in Table 1.

Table 1. Fatty acid composition in longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus of Tan sheep.

Fatty acid (mg/100g)	Longissimus dorsi muscle	Triceps brachii	Biceps femoris	Supraspinatus
C12:0 Laurate acid	0.29^{b}	2.74^{a}	2.54^{a}	0.24^{b}
C14:0 Myristic acid	0.90^{a}	0.67^{ab}	0.55^{bc}	0.31^{c}
C14:1 Myristoleic acid	42.59	49.81	54.97	52.64
C15:0 Pentadecanoic acid	2.96^{bc}	6.53^{b}	11.99^{a}	2.25^{c}
C16:0 Palmitic acid	1.71^{b}	1.69^{b}	3.54^{a}	0.57^{c}
C16:1 Palmitoleic acid	238.62^{c}	273.19^{bc}	249.08^{c}	333.06^{a}
C17:0 Margaric acid	40.09	46.92	51.23	42.54
C17:1 Heptadecanoic acid	20.88	20.90	24.10	20.94
C18:0 Stearic acid	48.36^{a}	63.28^{a}	68.17^{a}	24.24^{b}
C18:1n9t Elaidic acid	369.37^{b}	391.96^{b}	484.77^{a}	379.40^{b}
C18:1n9c Oleic acid	54.55^{b}	64.60^{b}	75.08^{ab}	125.85^{a}
C18:2n6c Linolenic acid	6.06^{b}	11.95^{a}	2.46^{c}	0.56^{d}
C18:3n3 Linolenic acid	4.06^{b}	7.23^{b}	24.58^{a}	2.41^{b}
C20:0 Arachidic acid	332.27^{ab}	398.72^{a}	359.27^{ab}	265.72^{b}
C20:1 Eicosenoic acid	15.24^{b}	39.98^{ab}	49.98^{a}	31.05^{ab}
C20:3n3 Eicosatrienoic acid	6.41^{b}	17.48^{a}	4.71^{b}	6.99^{b}
C20:4n6 Arachidonic acid	85.96^{c}	180.72^{a}	152.68^{b}	62.53^{c}
C20:5n3 EPA	5.56^{c}	12.09^{b}	32.65^{a}	9.14^{bc}
C22:6n3 DHA	2.13	6.28	1.70	3.93
∑SFA	426.57^{ab}	520.54^{a}	497.29^{a}	335.87^{b}
∑MUFA	741.25^{b}	840.44^{ab}	937.99^{a}	942.93^{a}
∑PUFA	110.19^{b}	235.74^{a}	218.79^{a}	85.56^{b}
∑ω-3	18.16^{c}	43.08^{b}	63.65^{a}	22.47^{c}
∑ω-6	92.03^{c}	192.66^{a}	155.14^{b}	63.09^{d}
PUFA/SFA ratio	0.26^{b}	0.46^{a}	0.44^{a}	0.25^{b}
ω6/ω3 ratio	5.06^{a}	4.47^{b}	2.44^{c}	2.82^{c}

Note: ^{a,b,c} Means in a row with different small letter superscripts differ significantly ($p < 0.05$). IMF, intramuscular fat; ΣSFA, saturated fatty acid (without any double bonds, C12:0~C20:0); ΣMUFA, monounsaturated fatty acid, all fatty acids with a single double bond (C14:1~C20:1); ΣPUFA, polyunsaturated fatty acid, all fatty acids with 2 or more double bonds (including C18:2n6c, C20:3n3, C20:4n6 and C20:5n3); EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. ω6 (C18:2n6c + C20:4n6); ω3 (C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3).

RNA extraction and cDNA preparation

Total RNA from muscle tissuses was extracted using the AxyPrep Total RNA Isolation Kit. The total RNA concentration and purity was measured using Maestro Nanomicro spectrophotometer (MaestroGEN, Las Vegas, NV, USA). Total RNA for inverse transcription was carried out by TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd., Beijing). The product was stored at -80°C for further analysis.

Primer design

Real-time PCR primers were designed on the basis of the mRNA sequences of the candidate genes by Primer Premier 5.0 software. The sequences of *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* were published in NCBI (GenBank), while the *β-actin* was used for an internal reference. Primers synthesized by Sangong Biotech (Shanghai, China). The sequences, annealing temperatures and PCR product lengths of primers are shown in Table 2.

Table 2. Primer sequences used for RT-PCR of Intramuscular fat and fatty acid candidate genes for sheep.

Gene name	Primer sequence (5'→ 3')	Accession	Product size (bp)
PPARγ	F: ACGGGAAAGACGACAGACAAA R: AAACTGACACCCCTGGAAGATG	NM_001100921	150
C/EBPα	F: CAAGAACAGCAACGAATAC R: AGGCGGTCATTGTCACTGGT	NM_001308574	135
FAS	F: CCCAGCAGCATTATCCAGTGT R: ATTCATCCGCCATCCAGTTC	NM_001012669.1	87
LPL	F: CCCAGCAGCATTATCCAGTGT R: ATTCATCCGCCATCCAGTTC	NM_001009394	87
FABP4	F: GGGATGGGAAATCAACCACCA R: TGGTAGCAGTGACACCGTTC	NM_001114667	87
ACC	F: GGCTCGTGCGTGGAAGTTGAC R: GTGGTGTAACTGCTGCCGTCATAG	NM_001009256	80
β-actin	F: TGAACCCCAAAGCCAACC R: AGAGGCGTACAGGGACAGCA	NM_001009784.1	107

Real‑time fluorescence quantitative PCR (qPCR) system

The reaction system for real-time PCR was 25 mL by SYBR Premix Ex Taq™ II. The PCR cycle programs were 95°C for 30 s, 50 cycles of 95°C for 5 s, 60°C for 34 s, and 72°C for 30 s. A dissociation curve analysis was carried out at 95°C for 10 s and at 60°C for 1 min, and then raise the temperature from 60°C to 95°C with a rate 0.5°C/10 s.

Statistical analysis

Data were analyzed using one-way ANOVA and Duncan test, and different muscle tissues of Tan sheep were compared with SPSS 16.0 software (SPSS Inc., Michigan Avenue, Chicago, IL, USA). The relative mRNA levels of the candidate genes were calculated by the method of $2^{-\Delta\Delta CT}$. Correlations was executed by the Pearson test. Statistical significance was noted $P < 0.05$ and $P < 0.01$. All images were produced by GraphPad Prism 5.0a (GraphPad Software, Inc.).

RESULTS

IMF content

IMF amounts in longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus are shown in Figure 1. The highest IMF content was found in longissimus dorsi

muscle (10.4%) but the lowest in biceps femoris (5.37%). The IMF amounts in longissimus dorsi muscle was 45.4% higher than that in triceps brachii ($P < 0.05$), 48.4% higher than that in biceps femoris, and 20.1% higher than that in supraspinatus.

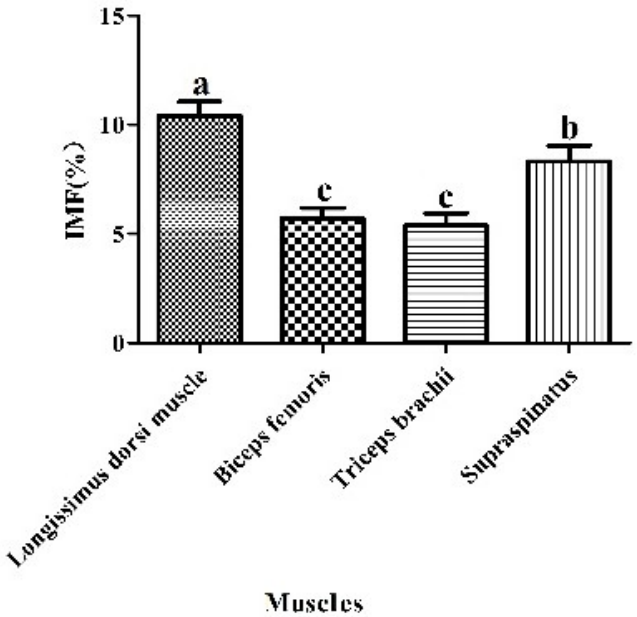

Figure 1. Intramuscular fat (IMF) in % in longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus of Tan sheep. Each bar represents means ± SEM. Different lowercase letters indicate significant differences.

FA composition

The weight concentrations (mg/100g) of the FA composition in longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus of Tan sheep are demonstrated in Table 1. Laurate acid (C12:0) was 9.45 and 11.42 times ($P < 0.05$) higher in longissimus dorsi muscle and supraspinatus, respectively. Myristic acid (C14:0) was higher ($P < 0.05$) in longissimus dorsi muscle than in biceps femoris and supraspinatus. Pentadecanoic acid (C15:0) and palmitic acid (C16:0) were higher ($P < 0.05$) than in longissimus dorsi muscle, triceps brachii and supraspinatus. Palmitoleic acid (C16:1) was higher ($P < 0.05$) in supraspinatus than in longissimus dorsi muscle, triceps brachii and biceps femoris. Conversely, Stearic acid (C18:0) was lower ($P < 0.05$) in supraspinatus than in the other muscles. Arachidic acid (C20:0) was found in triceps brachii and it was 1.5 times lower ($P < 0.05$) in supraspinatus. Eicosenoic acid (C20:1) was 3.3 times ($P < 0.05$) higher in biceps femoris than in longissimus dorsi muscle.

EPA content was 2.7, 3.6 and 5.9 times higher ($P < 0.05$) in biceps femoris than in triceps brachii, supraspinatus and longissimus dorsi muscle, respectively. Total SFA content differed significantly between triceps brachii and supraspinatus and between biceps femoris and supraspinatus ($P < 0.05$). By contrast, total MUFA content differed significantly between supraspinatus and longissimus dorsi muscle and between biceps femoris and longissimus dorsi muscle ($P < 0.05$). Total PUFA content was 2.1 and 2.8 times higher ($P < 0.05$) than in longissimus dorsi muscle and supraspinatus, respectively. Total ω-3 content was 1.5, 2.8 and 3.5

times ($P < 0.05$) higher in biceps femoris than in triceps brachii, supraspinatus, and longissimus dorsi muscle, respectively. Total ω6 content differed significantly ($P < 0.05$), being 1.2, 2.1 and 3.1 times higher in triceps brachii than in biceps femoris, longissimus dorsi muscle, and supraspinatus, respectively.

PUFA/SFA ratio in our trial was significant in triceps brachii and longissimus dorsi muscle, triceps brachii and supraspinatus, biceps femoris and longissimus dorsi muscle, and biceps femoris and supraspinatus ($P < 0.05$). In our study, the highest ω6/ω3 ratio (5.06; $P < 0.05$) was found in longissimus dorsi muscle compared with 4.47 in triceps brachii, 2.44 in biceps femoris, and 2.82 in supraspinatus.

mRNA abundance in longissimus dorsi muscle, biceps femoris, triceps brachii and supraspinatus

Gene (i.e., mRNA) expressions in longissimus dorsi muscle, biceps femoris, triceps brachii and supraspinatus are given in Figure 2.

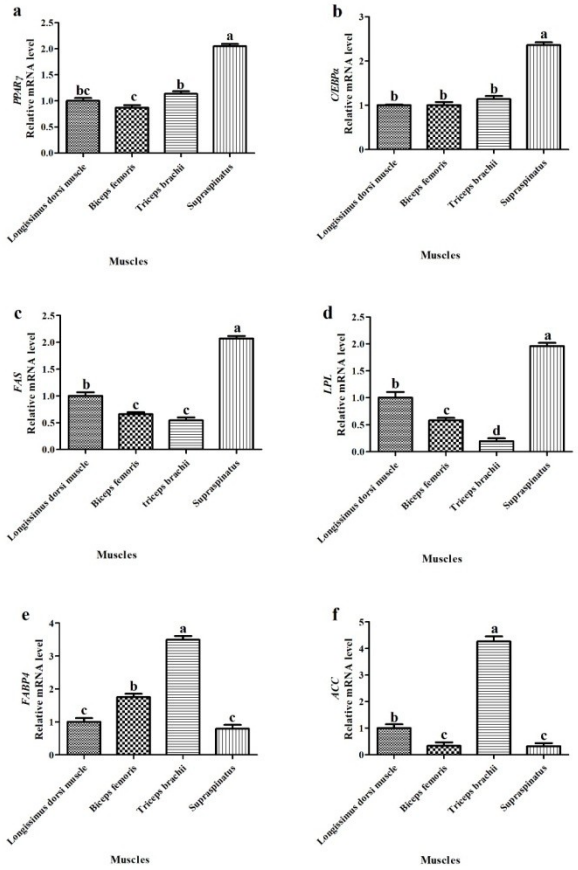

Figure 2. *PPARγ* (a), *C/EBPα* (b), *FAS* (c), *LPL* (d), *FABP4* (e) and *ACC* (f) relative mRNA expression level in longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus of Tan sheep. Data are ratios of *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4* and *ACC* genes' relative mRNA levels normalized to *β-actin* (housekeeping gene) mRNA levels. Each bar represents means ± SEM. Lowercases $P < 0.05$.

Expression of *PPARγ*, *C/EBPα*, *FAS* and *LPL* in supraspinatus differed ($P < 0.05$) from that in longissimus dorsi muscle, biceps femoris, and triceps brachii. The expression of *PPARγ* mRNA differed significantly ($P < 0.05$) in biceps femoris and triceps brachii. Expression of *FAS* mRNA in longissimus dorsi muscle was significantly different ($P < 0.05$) from that in biceps femoris and triceps brachii. Expression of *LPL* mRNA differed significantly among the four muscles ($P < 0.05$). Expression of *FABP4* and *ACC* in triceps brachii was significantly different from that in longissimus dorsi muscle, biceps femoris, and supraspinatus ($P < 0.05$), and expression of *FABP4* in biceps femoris differed from that in longissimus dorsi muscle and supraspinatus ($P < 0.05$). Finally, expression of *ACC* was significantly different in longissimus dorsi muscle from that in biceps femoris and supraspinatus ($P < 0.05$).

Correlations of gene mRNA level with IMF content in different muscle types

Correlations of *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* mRNA and IMF contents in longissimus dorsi muscle, biceps femoris, triceps brachii, and supraspinatus are given in Tables 3 IMF was positively correlated with expression levels of *PPARγ* ($P < 0.01$), *C/EBPα* ($P < 0.05$), *FAS* ($P < 0.05$), *LPL* ($P < 0.01$), *FABP4* ($P < 0.01$), and *ACC* ($P < 0.01$) in longissimus dorsi muscle. IMF was positively correlated with the mRNA level of *FAS* ($P < 0.05$), *LPL* ($P < 0.05$) in biceps femoris. IMF was positively correlated with mRNA level of *C/EBPα* ($P < 0.01$) and *FAS* ($P < 0.01$) in triceps brachii. IMF was positively correlated with the mRNA level of *PPARγ* ($P < 0.01$) and *FABP4* ($P < 0.01$) in supraspinatus. IMF was negatively correlated with the expression of *C/EBPα* ($P < 0.01$) and ACC ($P < 0.01$) in supraspinatus.

Correlations of genes (mRNA) expression with FA composition in different muscles

The correlation between *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* mRNA expression and FA composition in longissimus dorsi muscle are shown in Table 3. C12:0 and C14:1 contents were positively correlated with mRNA level of *FAS* ($P < 0.05$). Negative correlations were found for C17:0 content and expression of *LPL* ($P < 0.05$), *FABP4* ($P < 0.05$), and *ACC* ($P < 0.05$); for C18:0 content and expression of *PPARγ* ($P < 0.01$), *LPL* ($P < 0.05$), *FABP4* ($P < 0.05$), and *ACC* ($P < 0.05$); for C18:3n3 content and expression of *PPARγ* ($P < 0.01$), *C/EBPα* ($P < 0.05$), *FAS* ($P < 0.05$), *LPL* ($P < 0.01$), *FABP4* ($P < 0.01$), and *ACC* ($P < 0.01$); and for C20:0 content and expression of *PPARγ* ($P < 0.01$), *FAS* ($P < 0.05$), *LPL* ($P < 0.05$), *FABP4* ($P < 0.05$), and *ACC* ($P < 0.05$). C20:4n6 content was positively correlated with the mRNA level of *FAS* ($P < 0.05$) and *LPL* ($P < 0.05$). C20:5n3 content was negatively correlated with the m RNA level of *PPARγ* ($P < 0.05$), *FAS* ($P < 0.01$), *LPL* ($P < 0.01$), *FABP4* ($P < 0.05$), and *ACC* ($P < 0.05$), and the total SFA content was negatively correlated with the mRNA level of *PPARγ* ($P < 0.01$), *FAS* ($P < 0.05$), *LPL* ($P < 0.05$), and *FABP4* ($P < 0.05$). Positive correlations were found for ω6 content with expression of *FAS* ($P < 0.05$) and for the rate of ω6/ω3 with expression of *LPL* ($P < 0.05$) and *ACC* ($P < 0.05$).

Table 3. The association of gene expression with intramuscular fat content and fatty acid composition in longissimus dorsi muscle in Tan sheep.

Item	*PPARγ*	*C/EBPα*	*FAS*	*LPL*	*FABP4*	*ACC*
IMF (%)	0.886**	0.612*	0.672*	0.864**	0.887**	0.879**
Fatty acid (mg/100g)						
C12:0 Laurate acid	-0.006	-0.387	0.592*	0.380	0.272	0.308
C14:0 Myristic acid	0.211	0.020	0.407	0.395	0.357	0.374
C14:1 Myristoleic acid	0.187	-0.172	0.514*	0.367	0.303	0.316
C15:0 Pentadecanoic acid	-0.309	0.106	-0.612*	-0.475	-0.413	-0.424
C16:0 Palmitic acid	-0.059	-0.252	0.418	0.319	0.237	0.274
C16:1 Palmitoleic acid	0.141	-0.219	0.493	0.324	0.256	0.270
C17:0 Margaric acid	-0.376	0.053	-0.689	-0.579*	-0.515*	-0.529*
C17:1 Heptadecanoic acid	0.082	-0.129	0.376	0.297	0.243	0.263
C18:0 Stearic acid	-0.676**	-0.421	-0.476	-0.572*	-0.595*	-0.578*
C18:1n9t Elaidic acid	-0.276	0.068	-0.256	-0.092	-0.083	-0.058
C18:1n9c Oleic acid	-0.602*	-0.249	-0.496	-0.483	-0.487	-0.469
C18:2n6c Linolenic acid	-0.503*	-0.221	-0.422	-0.425	-0.428	-0.415
C18:3n3 Linolenic acid	-0.763**	-0.544*	-0.512*	-0.676**	-0.708**	-0.694**
C20:0 Arachidic acid	-0.706**	-0.358	-0.563*	-0.604*	-0.614*	-0.597*
C20:1 Eicosenoic acid	0.050	-0.087	0.284	0.241	0.199	0.217
C20:3n3 Eicosatrienoic acid	0.169	0.326	0.084	0.300	0.318	0.334
C20:4n6 Arachidonic acid	0.345	0.105	0.527*	0.542*	0.504	0.521
C20:5n3 EPA	-0.568*	-0.027	-0.776**	-0.646**	-0.595*	-0.594*
C22:6n3 DHA	0.080	-0.186	0.364	0.232	0.178	0.190
∑SFA	-0.645**	-0.291	-0.508*	-0.512*	-0.521*	-0.500
∑MUFA	0.240	0.068	0.467	0.494	0.452	0.476
∑PUFA	0.163	0.029	0.353	0.365	0.330	0.349
∑ω-3	-0.260	-0.005	-0.085	0.043	0.024	0.060
∑ω-6	0.300	-0.220	0.568*	0.502	0.448	0.464
PUFA/SFA ratio	0.132	-0.042	0.362	0.329	0.287	0.305
ω6/ω3 ratio	0.312	0.114	0.509	0.546*	0.508	0.529*

Note: Pearson correlation coefficients are across all treatments. Number of observations = 24. ns shows that there are no significant relationship between two different index ($P > 0.05$); * shows that there are significant relationship between two different indexes ($0.01 < P < 0.05$) ; ** shows that there are extreme significant relationship between two different indexes ($P < 0.01$).

The correlation between *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* mRNA expression and FA composition in biceps femoris are shown in Table 4. Positive correlation was found for C17:0 content with expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.01$), *FABP4* ($P < 0.01$), and *ACC* ($P < 0.05$); for C18:0 and C18:1n9c contents and expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.01$), and *FABP4* ($P < 0.01$); for C18:1n9t content and expression of *FAS* ($P < 0.01$) and *LPL* ($P < 0.05$); for C18:2n6c content and mRNA level of *FAS* ($P < 0.01$); for C18:3n3 and C20:0 contents and expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.01$), *FABP4* ($P < 0.01$), and *ACC* ($P < 0.01$); for C20:5n3 and total SFA contents and expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.01$), *FABP4* ($P < 0.01$), and *ACC* ($P < 0.05$). The total ω-6 contents were negatively correlated with expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.01$), *LPL* ($P < 0.05$), and *FABP4* ($P < 0.05$).

The correlation between *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* mRNA expression and FA composition in triceps brachii are shown in Table 5. The positive correlation was found between C14:0 content and mRNA level of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.01$), and *LPL* ($P < 0.05$); for C17:1 content and expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.05$), and *ACC* ($P < 0.05$); for C18:1n9t and mRNA level of *C/EBPα*

($P < 0.01$) and *ACC* ($P < 0.01$); for C20:3n3 content and expression of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.05$), and *ACC* ($P < 0.05$); for total PUFA content and mRNA level of *C/EBPα* ($P < 0.01$) and *ACC* ($P < 0.05$); for total ω3 content and expression of *C/EBPα* ($P < 0.01$) and *FAS* ($P < 0.05$); for total ω6 content and mRNA level of *C/EBPα* ($P < 0.01$) and *ACC* ($P < 0.01$); for PUFA/SFA ratio and mRNA level of *C/EBPα* ($P < 0.01$), *FAS* ($P < 0.05$), and *ACC* ($P < 0.05$). The negative correlation was found between the content of C16:1 and mRNA level of *C/EBPα* ($P < 0.01$) and *FAS* ($P < 0.01$); for C18:2n6c content and mRNA level of *C/EBPα* ($P < 0.01$) and *ACC* ($P < 0.01$); for C20:0 content and mRNA level of *C/EBPα* ($P < 0.01$) and *FAS* ($P < 0.05$); for total SFA content and mRNA level of *C/EBPα* ($P < 0.05$) and *FAS* ($P < 0.05$); for total MUFA content and mRNA level of *C/EBPα* ($P < 0.05$) and *FAS* ($P < 0.01$); for ω6/ω3 ratio and expression of *C/EBPα* ($P < 0.05$).

Table 4. The association of gene expression with intramuscular fat content and fatty acid composition in triceps brachii in Tan sheep.

Item	*PPARγ*	*C/EBPα*	*FAS*	*LPL*	*FABP4*	*ACC*
IMF (%)	0.434	0.313	0.585*	0.552*	0.251	0.377
Fatty acid (mg/100g)						
C12:0 Laurate acid	0.022	-0.346	-0.280	-0.046	-0.523	-0.357
C14:0 Myristic acid	-0.195	-0.173	-0.192	-0.251	-0.327	-0.206
C14:1 Myristoleic acid	-0.185	-0.243	-0.283	-0.256	-0.378	-0.284
C15:0 Pentadecanoic acid	0.303	0.538*	-0.767**	0.450	0.464	0.544*
C16:0 Palmitic acid	0.083	0.122	-0.007	0.066	0.055	-0.302
C16:1 Palmitoleic acid	-0.224	-0.217	-0.298	-0.298	-0.330	-0.290
C17:0 Margaric acid	0.203	0.670**	0.786**	0.354	0.661**	0.573*
C17:1 Heptadecanoic acid	-0.249	-0.106	-0.118	-0.292	-0.270	-0.112
C18:0 Stearic acid	0.225	0.685**	0.764**	0.372	0.689**	0.511
C18:1n9t Elaidic acid	0.470	0.489	0.635**	0.596*	0.478	0.296
C18:1n9c Oleic acid	0.214	0.685**	0.750**	0.359	0.699**	0.502
C18:2n6c Linolenic acid	0.291	0.443	0.668**	0.423	0.401	0.512
C18:3n3 Linolenic acid	0.065	0.645**	0.787**	0.216	0.626**	0.696**
C20:0 Arachidic acid	0.161	0.756**	0.879**	0.325	0.707**	0.639**
C20:1 Eicosenoic acid	-0.316	-0.035	-0.083	-0.353	-0.186	-0.064
C20:3n3 Eicosatrienoic acid	-0.249	0.017	0.041	-0.264	-0.174	0.020
C20:4n6 Arachidonic acid	-0.038	-0.280	-0.252	-0.103	-0.448	-0.326
C20:5n3 EPA	0.165	0.817**	0.897**	0.327	0.751**	0.574*
C22:6n3 DHA	-0.351	-0.115	-0.269	-0.420	-0.191	-0.218
∑SFA	0.179	0.726**	0.781**	0.327	0.733**	0.533*
∑MUFA	-0.091	-0.260	-0.323	-0.168	-0.379	-0.392
∑PUFA	-0.303	-0.184	-0.328	-0.382	-0.259	-0.285
∑ω-3	0.338	0.500	0.459	0.419	0.476	0.018
∑ω-6	-0.413	-0.625**	-0.809**	-0.563*	-0.534*	-0.422
PUFA/SFA ratio	-0.178	-0.044	-0.076	-0.214	-0.206	-0.154
ω6/ω3 ratio	0.325	0.384	0.374	0.382	0.278	-0.097

Note: Pearson correlation coefficients are across all treatments. Number of observations = 24. ns shows that there are no significant relationship between two different indexes ($P > 0.05$); * shows that there are significant relationship between two different indexes ($0.01 < P < 0.05$) ; ** shows that there are extreme significant relationship between two different indexes ($P < 0.01$).

The correlation between *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4*, and *ACC* gene mRNA expression and FA composition in supraspinatus are shown in Table 6. The positive correlation was found between C14:0, C15:0 and C18:0 contents and expression of *PPARγ* ($P < 0.01$); for C20:4n6 content and expression of *C/EBPα* ($P <$

0.05), *FAS* ($P < 0.05$), and *LPL* ($P < 0.05$); for total SFA content and mRNA level of *PPARγ* ($P < 0.01$). C18:1n9t content was positively correlated with expression of *PPARγ* ($P < 0.01$), but negatively correlated with mRNA level of *FAS* ($P < 0.05$). The total MUFA content was positively correlated with mRNA level of *PPARγ* ($P < 0.01$), but negatively correlated with mRNA level of *FAS* ($P < 0.05$). The total PUFA content was positively correlated with mRNA level of *PPARγ* ($P < 0.05$), but negatively correlated with mRNA level of *FAS* ($P < 0.05$). The total ω-3 content was positively correlated with mRNA level of *PPARγ* ($P < 0.01$), but negatively correlated with mRNA level of *FAS* ($P < 0.05$). The total ω-6 content was positively correlated with mRNA level of *C/EBPα* ($P < 0.05$), *FAS* ($P < 0.05$), and *LPL* ($P < 0.01$), but negatively correlated with mRNA level of *PPARγ* ($P < 0.05$). PUFA/SFA ratio was positively correlated with expression of *C/EBPα* ($P < 0.01$) and *ACC* ($P < 0.01$), but negatively correlated with mRNA level of *FAS* ($P < 0.05$) and *FABP4* ($P < 0.01$). ω6/ω3 ratio was positively correlated with mRNA level of *C/EBPα* ($P < 0.05$), *FAS* ($P < 0.05$), and *LPL* ($P < 0.01$), but negatively correlated with mRNA level of *FAS* ($P < 0.05$) and *PPARγ* ($P < 0.05$).

Table 5. The association of gene expression with intramuscular fat content and fatty acid composition in biceps femoris in Tan sheep.

Item	*PPARγ*	*C/EBPα*	*FAS*	*LPL*	*FABP4*	*ACC*
IMF (%)	-0.368	0.768**	0.735**	0.046	0.251	0.367
Fatty acid (mg/100g)						
C12:0 Laurate acid	0.015	-0.777	-0.413	0.181	-0.438	-0.620*
C14:0 Myristic acid	-0.388	0.887**	0.691**	0.538*	0.018	0.336
C14:1 Myristoleic acid	-0.122	0.916**	0.480	0.279	0.222	0.564*
C15:0 Pentadecanoic acid	0.364	-0.525*	-0.649**	0.128	-0.130	-0.231
C16:0 Palmitic acid	0.125	-0.788**	-0.495	0.025	-0.294	-0.535*
C16:1 Palmitoleic acid	0.374	-0.645**	-0.655**	-0.108	-0.023	-0.266
C17:0 Margaric acid	-0.094	0.974**	0.479	0.348	0.386	0.596*
C17:1 Heptadecanoic acid	-0.202	0.974**	0.585*	0.352	0.286	0.541*
C18:0 Stearic acid	0.431	-0.562*	-0.726**	0.062	-0.120	-0.206
C18:1n9t Elaidic acid	-0.085	0.988**	0.497	0.227	0.392	0.643**
C18:1n9c Oleic acid	0.339	-0.585*	-0.659**	0.145	-0.216	-0.291
C18:2n6c Linolenic acid	-0.022	-0.754**	-0.384	0.262	-0.499	-0.639**
C18:3n3 Linolenic acid	-0.380	0.883**	0.753**	0.279	0.246	0.390
C20:0 Arachidic acid	0.291	-0.642**	-0.620*	0.093	-0.204	-0.350
C20:1 Eicosenoic acid	0.270	-0.571*	-0.583*	0.174	-0.210	-0.326
C20:3n3 Eicosatrienoic acid	-0.175	0.965**	0.527*	0.459	0.230	0.524*
C20:4n6 Arachidonic acid	-0.183	0.969**	0.550*	0.393	0.240	0.539*
C20:5n3 EPA	0.425	-0.507	-0.671**	0.000	0.012	-0.159
C22:6n3 DHA	-0.283	0.922**	0.611*	0.475	0.104	0.434
∑SFA	0.241	-0.619*	-0.558*	0.114	-0.194	-0.367
∑MUFA	0.487	-0.526*	-0.744**	-0.021	0.000	-0.132
∑PUFA	-0.075	0.904**	0.415	0.315	0.202	0.572*
∑ω-3	-0.246	0.947**	0.586*	0.483	0.160	0.469
∑ω-6	0.067	0.866**	0.320	0.006	0.392	0.690**
PUFA/SFA ratio	-0.166	0.980**	0.547*	0.363	0.290	0.562*
ω6/ω3 ratio	0.187	-0.546*	-0.509	0.279	-0.292	-0.376

Note: Pearson correlation coefficients are across all treatments. Number of observations = 24. ns shows that there are no significant relationship between two different indexes ($P > 0.05$); * shows that there are significant relationship between two different indexes ($0.01 < P < 0.05$) ; ** shows that there are extreme significant relationship between two different indexes ($P < 0.01$).

Table 6. The association of gene expression with intramuscular fat content and fatty acid composition in supraspinatus in Tan sheep.

Item	*PPARγ*	*C/EBPα*	*FAS*	*LPL*	*FABP4*	*ACC*
IMF (%)	0.849**	-0.727**	0.373	0.319	0.920**	-0.863**
Fatty acid (mg/100g)						
C12:0 Laurate acid	0.436	0.112	-0.793**	-0.616*	-0.119	0.067
C14:0 Myristic acid	0.783**	-0.119	-0.440	-0.335	0.189	-0.163
C14:1 Myristoleic acid	-0.808**	0.522	0.383	0.573*	-0.096	0.368
C15:0 Pentadecanoic acid	0.654**	0.102	-0.480	-0.283	0.095	0.020
C16:0 Palmitic acid	0.549*	-0.344	-0.681**	-0.832**	-0.184	-0.169
C16:1 Palmitoleic acid	0.680**	0.007	-0.561*	-0.463	-0.022	0.014
C17:0 Margaric acid	-0.651**	0.714**	0.406	0.663**	-0.147	0.558*
C17:1 Heptadecanoic acid	-0.582*	0.578*	0.542*	0.775**	0.021	0.398
C18:0 Stearic acid	0.829**	-0.284	-0.295	-0.170	0.470	-0.412
C18:1n9t Elaidic acid	0.702**	-0.052	-0.520*	-0.362	0.172	-0.133
C18:1n9c Oleic acid	0.585*	-0.025	-0.610*	-0.406	0.175	-0.149
C18:2n6c Linolenic acid	0.691**	0.005	-0.526*	-0.378	0.090	-0.046
C18:3n3 Linolenic acid	-0.646**	0.699**	0.452	0.632*	-0.251	0.619*
C20:0 Arachidic acid	0.500	0.165	-0.716**	-0.575*	-0.198	0.175
C20:1 Eicosenoic acid	0.624*	-0.206	-0.680**	-0.601*	0.106	-0.239
C20:3n3 Eicosatrienoic acid	-0.785**	0.655**	0.386	0.582*	-0.221	0.539*
C20:4n6 Arachidonic acid	-0.484	0.574*	0.608*	0.806**	0.001	0.433
C20:5n3 EPA	0.773**	-0.186	-0.515*	-0.456	0.139	-0.194
C22:6n3 DHA	0.739**	-0.140	-0.507*	-0.377	0.220	-0.214
∑SFA	0.778**	-0.016	0.110	0.145	0.209	-0.004
∑MUFA	0.644**	0.037	-0.571*	-0.397	0.078	-0.033
∑PUFA	0.598*	0.136	-0.535*	-0.311	0.079	0.034
∑ω-3	0.662**	0.046	-0.523*	-0.340	0.110	-0.034
∑ω-6	-0.585*	0.567*	0.561*	0.777**	0.010	0.400
PUFA/SFA ratio	-0.321	0.783**	-0.458*	-0.353	-0.871**	0.933**
ω6/ω3 ratio	-0.628*	0.540*	0.590*	0.740**	-0.075	0.432

Note: Pearson correlation coefficients are across all treatments. Number of observations = 24. ns shows that there are no significant relationship between two different indexes ($P > 0.05$); * shows that there are significant relationship between two different indexes ($0.01 < P < 0.05$) ; ** shows that there are extreme significant relationship between two different indexes ($P < 0.01$).

DISCUSSION

The Chinese Tan sheep is recognized for its meat quality, which is characterized by featured flavors of mutton. However, under stall-feeding conditions, the muscle of Tan sheep contains less IMF, which has became a significant problem (Xu et al., 2018; Gao et al., 2014). Previous studies suggested that the flavor of meat is significantly related to IMF content and FA composition (Qian, 2007; Wood et al., 2008; Wang et al., 2017; Yang et al., 2017;). We evaluated the IMF content and FA profile of longissimus dorsi muscle, biceps femoris, triceps brachii, and supraspinatus in Tan sheep and the role of genes in lipid metabolism. It is well known that IMF is an important factor in meat quality (Fortin, 2005), and deposit of IMF directly influences meat quality and its economic value. Genetic improvement of sheep in the past few decades has focused on growth rate, feed conversion, and lean ratio, but has resulted in substantial negative effects on sensory characteristics, nutritional value, and eating quality of mutton. In recent years, researches on enhancing the quality of sheep meat have focused on fat deposition and its regulation (Hopkins, 2006; Karamichou et al., 2006).

We found that IMF content was higher in longissimus dorsi muscle than in other muscles, indicating that the speed of IMF deposit in different muscles differed, and that IMF deposits in Tan sheep exhibits a tissue-specific manner. This result is consistent with the role of IMF in Hu sheep, in which IMF content increased during developmental progress and was higher in longissimus dorsi muscle and psoas major muscle than in biceps brachii muscle at the same age (Hao et al., 2008). IMF is one of the most important flavor precursors, and the role of IMF in flavor is related not only to the IMF, but also to the composition and content of FA in the IMF.

The generation of aroma during cooking and storage of meat primarily depends on the amount of fatty acid oxidation products and the balance of products from the Maillard reaction. During this process, phospholipids participate in oxidation and Maillard reactions (Elmore, 2002; Gorraiz, 2002). The volatile products of phospholipids originates from the oxidation of unsaturated fatty acids. When these compounds accumulate to a certain level, they adversely produce undesirable flavors or odors. These compounds derive from the oxidation of n-6 or n-3 PUFA, and the type of oxidation product is related to the type of FA (Scollan et al., 2006). Volatile substances produced by lipids during heating can generate characteristic flavors of meat for different animal species (Gounaris, 2010). The substances produced are saturated and unsaturated aldehydes, ketones, esters, alkanes, and volatile fatty acids of C6 to C10 (Hu et al., 2011). They are all important components of meat volatile components, which substantially impacts meat flavor. Fatty acids in goat meat include palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). Stearic acids and palmitic acids are SFA, oleic acids and palmitic acids are MUFA, and linolenic acids (C18:3) and linoleic acids are PUFA (Banskalieva, 2000; Coltro et al., 2005; Peña et al., 2009; Ebrahimi, 2012). The study found that ruminant fat is often perceived as having a negative impact on human health; however, the composition of the fat is under complex biochemical control and can be improved through strategic manipulation of the animal's diet. There were two major objectives of this study, namely (i) to develop and validate a primary bovine intramuscular adipocyte cell line and (ii) to examine the effect of eicosapentaenoic acid (EPA) on the transcriptional regulation of Δ-9 desaturase in vitro using the novel cell line (Waters et al., 2009). In our study, the highest content of palmitic acid was found in biceps femoris. Oleic acid (C18:1n9c) was higher in supraspinatus than in longissimus dorsi muscle, triceps brachii, and biceps femoris. One of the effects of plasma cholesterol is the FA composition of fat. Palmitic acid is associated with plasma cholesterol levels, which can be reduced by oleic acid (French, 2002; Allman-Farinelli, 2005). Among the different muscle tissues evaluated in our study, the highest oleic acid content was detected in supraspinatus, indicating that the meat quality of the supraspinatus can reduce cholesterol level in human blood. All SFA have an influence on cholesterol levels, expressed by the ratio of PUFA/SFA (Zhao et al., 2013).

The type and composition of FA in meat and the its fat content have a direct influence on meat quality. To improve the flavor and quality of meat, it's important to study its FA composition, especially the effect of fatty acids on the formation of meat flavor. *PPARγ* and *C/EBPα* are important transcription factors for adipose differentiation, and they can directly or indirectly cooperate with other transcription factors that regulate fat differentiation (Rosen et al., 2002). The expression of genes encoding proteins involved in triacyglyceride and fatty acid synthesis and storage in cattle muscle are correlated with intramuscular fat (IMF)%. Are the same genes also correlated with IMF% in sheep muscle,

and can the same set of genes be used to estimate IMF% in both cattle and sheep (Guo et al., 2014). Expression of *PPARγ* was positively associated with fat deposits. The correlation of *PPARγ* expression in sheep muscle and its IMF content indicate that *PPARγ* expression may have some effect on IMF deposits. Moreover, *C/EBPα* plays a critical role in adipocyte proliferation and maturation. It can directly promote the expression of adipogenesis gene (Katsumata, 2011). Therefore, *PPARγ* and *C/EBPα* are major genes affecting IMF content. FABPs can specifically bind to FA and are considered as important proteins that transport FA into cells; they play an important role in fat metabolism, and *H-FABP* and *A-FABP* are deemed as candidate genes to infer IMF content (Chen, 2013).

Lipid metabolism in adipose tissue involves several key factors. ACC and FAS are lipogenic enzymes whose activity changes can change the synthesis rate of FA (Clarke, 1993; Liu, 1994; Smith, 2003). *LPL* is considered to limit the rate at which FA transfer into tissues (Mersmann, 1998). Our research evaluated the effects differential expression of *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4* and *ACC* on IMF and FA composition in different muscles in Tan sheep. We detected strong and positive correlations between *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4* and *ACC* gene mRNA level and IMF content in longissimus dorsi muscle. Significant positive correlations were found between *FAS* mRNA level and C12:0, C14:1, C20:4n6, and ω-6 concentrations. Significant positive correlations were also found between *LPL* mRNA level and C20:4n6, and the ratio of ω6/ω3 concentrations.

In biceps femoris, IMF was positively correlated with *FAS* and *LPL* mRNA level. Significant positive correlations were found between *C/EBPα* mRNA level and C15:0, C17:0, C18:0, C18:1n9c, C18:3n3, C20:0, C20:5n3, and total SFA contents. Significant positive correlations were found between *FAS* mRNA level and C17:0, C18:0, C18:1n9t, C18:1n9c, C18:2n6c, C18:3n3, C20:0, C20:5n3, and total SFA contents. Significant positive correlations were found between *FABP4* mRNA level and C17:0, C18:0, C18:1n9c, C18:3n3, C20:0, C20:5n3, and total SFA contents. Finally, we found significant positive correlations for *ACC* mRNA level with C15:0, C17:0, C18:3n3, C20:0, C20:5n3, and total SFA contents.

Expression of C/EBPα and FAS mRNA level was correlated with IMF content in triceps brachii. Significant positive correlations were found for *C/EBPα* mRNA level and of C14:0, C14:1, C17:0, C17:1, C18:1n9t, C18:3n3, C20:3n3, C20:4n6, C22:6n3, total PUFA, total ω3 and ω6 contents, and PUFA/SFA ratio. This is closely related to the tenderness, texture and potential oxidation of the meat (Wood et al., 1999). Thus, the *C/EBPα* gene may affect meat tenderness, texture, and potential oxidation. Significant positive correlations were found between *FAS* mRNA level and C14:0, C17:1, C18:3n3, C20:3n3, C20:4n6, C22:6n3, total ω3 contents, and PUFA/SFA ratio. The significant positive correlations found between *ACC* mRNA level and of C14:1, C17:0, C17:1, C18:1n9t, C20:3n3, C20:4n6, total PUFA, total ω-6 contents, and PUFA/SFA ratio. This may suggest that *ACC* regulates lipolysis and the deposition of MUFA rather than SFA. SFA content is a health concern in meat because it is positively correlated with blood cholesterol levels, which contributes to cardiovascular disease. MUFA plays an important role in decreasing blood low density lipoprotein (LDL) cholesterol by increasing liver LDL receptor activity (Zhang et al., 2009).

In supraspinatus, we detected positive correlation for *PPARγ* and *FABP4* mRNA level with IMF content. Significant positive correlations were found between *PPARγ* mRNA level and C14:0, C15:0, C16:0, C16:1, C18:0, C18:1n9t, C18:1n9c, C18:2n6c,

C20:1, C20:5n3, C22:6n3, total SFA, total MUFA, total PUFA and total ω-3 contents. This is consistent with the results of Xue et al. (2015), in which *LIPE* was found to be significantly associated with the C12:0 and C14:0 content and tended to be of associated with C18:0 content in pigs (Xue et al., 2015). It indicated that *PPARγ* gene polymorphism affects some SFA contents in sheep. Significant positive correlations were found between *C/EBPα* mRNA level and of C17:0, C17:1, C18:3n3, C20:3n3, C20:4n6, total ω-6 contents, and the PUFA/SFA and ω6:ω3 ratios. Significant positive correlations were found between *FAS* mRNA level and of C17:1, C20:4n6, total ω-6 contents, and the ratio of ω6/ω3. Significant positive correlations were found between *LPL* mRNA level and of C14:1, C17:0, C17:1, C18:3n3, C20:3n3, C20:4n6, total ω-6 contents and the ratio of ω6/ω3. Significant positive correlations were found between *ACC* mRNA level and of C17:0, C18:3n3, C20:3n3 contents and PUFA/SFA ratio in our study. The PUFA/SFA ratio was highest in triceps brachii and biceps femoris, and the highest ω6/ω3 ratio was found in longissimus dorsi muscle, indicating that triceps brachii and biceps femoris could significantly affect cholesterol levels. *ACC* gene polymorphism was highly correlated with total MUFA and total PUFA content, but had no correlation with SFA, revealing that *ACC* gene affects intramuscular lipid composition. This suggests that the polymorphism of the *ACC* gene may affect the deposition of MUFA and PUFA in adipose tissue, rather than the deposition of SFA. Taken together, our results indicate that *PPARγ*, *C/EBPα*, *FAS*, *LPL*, *FABP4* and *ACC* play important roles in different muscle types of Tan sheep in healthy development of meat.

CONCLUSIONS

In short, this study demonstrates that tissue type affects IMF content and FA composition. We investigated the relationship between lipid metabolism, fat deposition, fatty acid distribution and gene mRNA level in different muscle types of Tan sheep. *FAS*, *LPL* and *FABP4* were associated with intramuscular fat deposition, and *PPARγ*, *C/EBPα*, *LPL*, *FABP4* and *ACC* with fatty acid content of longissimus dorsi muscle, triceps brachii, biceps femoris and supraspinatus. We conclude that the above candidate genes are effective markers for selecting good flavored sheep meat and for improving the quality of sheep meat. These data may be used to control or offer useful information to increase IMF deposition and FA accumulation in muscle.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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