

Genes Expression Related to Intramuscular Fat Deposition in Muscles of Small Tail Han Sheep

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Abstract: Intramuscular Fat (IMF) is an important factor affecting meat quality. The objective of this study was to investigate the expression changes of genes related to IMF formation in muscles of Small Tail Han sheep. These genes include Acetyl CoA Carboxylase (ACC), Fatty Acid Synthase (FAS), Diacylglycerol Acyltransferase 1 (DGAT1), Heart Fatty Acid Binding Protein (H-FABP), CCAAT-Enhancer-Binding Protein α (C/EBP α), Peroxisome Proliferator-Activated Receptor γ (PPAR γ), Malic Enzyme (ME) and Lipoprotein Lipase (LPL). In the longissimus dorsi, IMF content continuously increased with growth and was significantly different ($p < 0.05$) at all 4 months; however, IMF content reached a maximum at 5 months in the gluteus maximus. The gene expression patterns of the 8 genes involved in IMF synthesis mainly followed one of 2 trends: gene expression tended to be lowest at 4 or 5 months and then subsequently increase in the longissimus dorsi whereas gene expression tended to peak at 4 or 5 months and then subsequently decrease in the gluteus maximus. Intramuscular fat content correlated with the expression levels of all genes in the longissimus dorsi and all genes except DGAT1, H-FABP in the gluteus maximus. Remarkably, all of the above correlations between IMF and gene expression levels were positive. In conclusion, the correlation between gene expression levels and IMF content indicate that these genes play an important role in the deposition of IMF in Small Tail Han sheep.

Key words: Gene expression, gluteus maximus, intramuscular fat, longissimus dorsi, muscle, sheep

INTRODUCTION

Intramuscular Fat (IMF) content is an important factor affecting meat quality and is associated with tenderness, juiciness and palatability (Wang *et al.*, 2009). Earlier studies have shown that IMF contains large amounts of flavor-forming precursor material and positively correlates with meat quality and taste (Westerling and Hedrick, 1979). Intramuscular fat also defined as marbling is located within the perimysium which surrounds muscle bundles (Warner *et al.*, 2010). Electron microscopy studies have clearly indicated that development of the IMF disorganizes the structure of intramuscular connective tissue and separates the perimysium into thinner collagen fibrils; therefore, increasing meat tenderness (Nishimura *et al.*, 1999).

Research on IMF has gradually increased in recent years. A large number of candidate genes and pathways involved in IMF formation have been identified (Arnyasi *et al.*, 2006; Lowe *et al.*, 2011; Urban *et al.*, 2002) and it is known that IMF deposition is regulated by a wide range of factors via a mechanism which varies from the processes regulating fat deposition in other tissues (Kokta *et al.*, 2004). The generation and deposition of IMF is a complex process involving many genes.

Local Chinese species exhibit a number of advantages compared to livestock breeds which have been introduced from overseas. Small Tail Han is a famous indigenous Chinese sheep breed used for meat which has a high prolificacy and excellent meat quality (Chu *et al.*, 2002). Small Tail Han sheep grow and develop rapidly, fatten easily, mature early and have an excellent meat quality attributes. The meat of Small Tail Han sheep is very tender and contains high levels of IMF. In this study, researchers characterized and discussed the gene expression changes involved in IMF deposition in the longissimus dorsi and gluteus maximus of Small Tail Han sheep.

MATERIALS AND METHODS

The Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China) were followed when caring for the animals.

Animals and sampling: Sixteen 2 months of age male Small Tail Han sheep of a similar weight (13.0 kg) were selected from a farm in Beijing. All sheep were housed indoors and feeding twice every day, free of drinking. Diets and nutrient composition in Table 1.

At 3-6 months of age, 4 sheep were randomly selected, slaughtered and the longissimus dorsi and gluteus maximus were sampled. Tissue samples were immediately frozen in liquid nitrogen and stored at -80°C for total RNA extraction, or stored at -20°C for the determination of IMF content.

Measurement of slaughter weight and intramuscular fat content: About 3.0 g of each sample, eliminating obvious fat was minced thoroughly after being thawed. All samples were dried in two 10-12 h stages (65 then 105°C) and cooled in a desiccator for at least 30 min. The IMF contents of longissimus dorsi and gluteus maximus were measured by the Soxhlet Method (AOAC, 1990) using anhydrous ether as the solvent and were calculated using the following equation:

$$\text{IMF content} = \frac{\text{Total weight before extraction} - \text{Total weight after extraction}}{\text{Total weight of dry tissue prior to extraction}}$$

Table 1: Composition and nutrient levels of diets (DM %)

Ingredients	Content (%)
Hay	67.50
Corn	18.50
Soybean meal	3.20
Cottonseed cake	2.20
Wheat bran	7.50
Premix ²	0.50
NaCl	0.25
Limestone	0.35
Total	100.00
Nutrition level (%)	
ME (MJ kg ⁻¹) ¹	10.50
Crude protein	15.30
Neutral detergent fiber	37.20
Acid detergent fiber	18.10
Calcium	0.44
Phosphorus	0.34
Non-fiber carbohydrates ¹	38.30

¹ME and NFC are calculated values, other nutrient levels are measured values. ²Provided per kg of premix: VA 1000 KIU, VD 3250 KIU, VE 2400 mg, Fe 2000 mg, Cu 3000 mg, Zn 14000 mg, Mn 3000 mg, I 180 mg, Se 100 mg, Co 40 mg

RNA isolation and cDNA synthesis: Total RNA was extracted from muscles using the Rneasy Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol, dissolved in RNase-free water and examined using a UV spectrophotometer at 260/280 nm to determine the total RNA purity. The amount of total RNA was quantified by spectrophotometric OD260 measurement. RNA integrity was checked by electrophoresis of 500 ng RNA on 0.8% agarose ethidium bromide-stained gels.

The QuantiTech Reverse Transcription kit (QIAGEN, Hilden, Germany) was used to synthesize cDNA. Briefly, the genomic DNA elimination reaction was performed by incubating 6 µL (0.8 µg) template RNA, 2 µL g DNA wipeout buffer and 6 µL RNase-free water in a total volume of 14 µL for 2 min at 42°C and then placed immediately on ice. For reverse-transcription, the 14 µL template RNA was mixed with 1 µL reverse-transcription master mix containing RNase inhibitor and dNTPs, 4 µL Quantiscript RT Buffer, 1 µL RT primer mix and incubated for 15 min at 42°C followed by incubation at 95°C for 3 min to inactivate the reverse transcriptase. The reverse transcription products were stored at -20°C. Reverse transcription was confirmed by PCR amplification of β-actin using 1 µL of the RT reaction mix in a final reaction volume of 20 µL containing 1 U Taq DNA polymerase (TIANGEN, Beijing, China).

Real-time quantitative PCR: Oligonucleotide primer sets (Table 2) were designed using Primer Express 3.0 (Applied

Table 2: Real-time quantitative PCR primer sequences

Gene ¹	Accession No.	Primer sequence	Product size (bp)	Ta ³ (°C)
<i>ACC</i>	X80045.1	F:5'-CGGAAGGGACAGTAGAAATCAA-3' R:5'-CCCAGTCGCTCAGCCAAGT-3'	97	60
<i>FAS</i>	AF479289.1	F:5'-CAACTCCCTGGCGGAAGAG-3' R:5'-GGTGGTTGTTGAAAGGTCAA-3'	101	60
<i>DGATI</i>	NM_001110164.1	F:5'-AGTTTGAGACCGCGAGTTCT-3' R:5'-AGTGTCTGAGGCACCACTTGTG-3'	105	60
<i>H-FABP</i>	AY157617.1	F:5'-AGACCACAGCAGATGACAGGAA-3' R:5'-CCCGCACAAGTGATGTCTCTT-3'	105	60
<i>C/EBPα</i>	NM_176784.2	F:5'-GAGAGCGCCATCGACTTCAG-3' R:5'-CGCTTTGTGGTTGCTGTTAAAG-3'	111	62
<i>PPARγ</i>	AY137204.1	F:5'-GGTTGACACAGATGCCGTTT-3' R:5'-CGGTGGTGAAGGGCTTGATA-3'	110	60
<i>ME</i>	EU646206.1	F:5'-CAGCGGCACTCTTTTGATC-3' R:5'-ACACGCCACGACCAAGA-3'	112	60
<i>LPL</i>	NM_001009394.1	F:5'-CCCTAACGGAGGCACTTCC-3' R:5'-GCTCGTGGGAGCACTTCACT-3'	110	60
<i>β-Actin²</i>	NM_001009784.1	F:5'-GCCCTGAGGCTCTCTCCA-3' R:5'-GGATGTCGACGTCACTTCA-3'	99	60

¹ACC: Acetyl CoA Carboxylase; FAS: Fatty Acid Synthase; DGATI: Diacylglycerol Acyltransferase 1; HFABP: Heart Fatty Acid Binding Protein; C/EBPα: CCAAT-enhancer-binding protein α; PPARγ: Peroxisome Proliferator-Activated Receptor γ; ME: Malic Enzyme; LPL: Lipoprotein Lipase. ²β-Actin was used as housekeeping gene. ³Ta: Annealing Temperature

Biosystems, Foster city) according to the published sheep mRNA sequences in GenBank and synthesized by Invitrogen (Beijing, China). These primers were validated through product sequencing and the slope of each validation curve was close to -3.3.

The efficiency of them for the target and internal control genes is closed to 100%. The housekeeping gene *β-actin* (Sturzenbaum and Kille, 2001; Suzuki *et al.*, 2000) was used as internal control. Target gene expression levels relative to *β-actin* gene were quantified using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster city) following the manufacturer's standard protocol in 15 μL reaction volumes containing 7.5 μL 2X Power SYBR Green Real-Time PCR Master Mix (Applied Biosystems, Foster city), 0.5 pmol gene-specific primers and 1 μL template cDNA with an initial denaturation for 4 min at 95°C, 40 cycles of 30 sec at 95°C, 1 min at 60°C followed by a final extension at 72°C for 10 min.

Statistical analysis: The IMF content and the expression levels of all genes are described as means±SD and were analyzed using SAS 8.2 for Windows (SAS Institute Inc., Cary, NC). The IMF content and gene expression levels in different tissues of the same age and the same tissues at different ages were analyzed using the General Linear Model (GLM) according to the following Statistical Model:

$$y = u + s + t + s \times t + e$$

Where:

- y = The observed IMF content or gene expression level
- u = Mean of the population
- s = The growth stage
- t = The tissue tested
- s×t = The interaction of stage and tissue
- e = Random residual error

The relationship between gene expression levels and IMF content were determined by linear regression analysis (CORR, SAS, V8.2) and pearson correlation coefficients (r) were calculated. Statistically significant was considered as p<0.05

RESULTS

Changes in saughter weight with increasing age: The average Small Tail Han slaughter weights at 3-6 months old were 15.56±1.43, 20.87±1.70, 26.37±2.06 and 31.37±1.70 kg, respectively. The mean weight gains of 3-4, 4-5 and 5-6 months were 5.31, 5.50 and 5.00 kg, respectively. Small Tail Han sheep slaughter weight showed a linear increase from 3-6 months of age (Fig. 1).

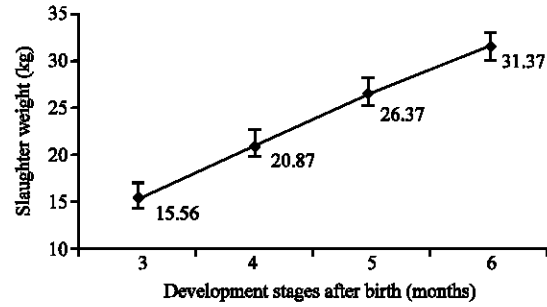


Fig. 1: Changes in Small Tail Han sheep slaughter weight with age. Four Small Tail Han sheep were used at each stage

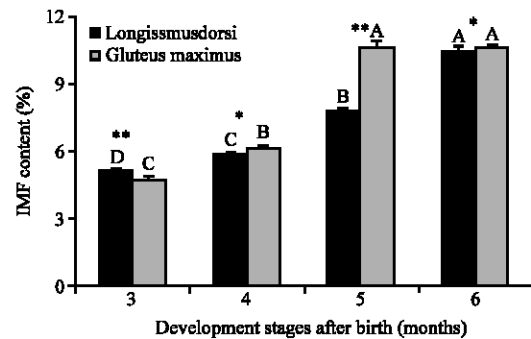


Fig. 2: IMF content during growth and development of the longissimus dorsi and gluteus maximus. Four Small Tail Han sheep were used at each stage; Significant differences between the values obtained in a single muscle over time are denoted with different letters (p<0.05). Significant differences between the different muscles at each time point are indicated as *p<0.05 and **p<0.01. IMF: Intramuscular Fat

Changes in IMF content with increasing age: Intramuscular fat content was determined in two different muscles (the longissimus dorsi and gluteus maximus) at 4 growth stages (3-6 months) in Small Tail Han sheep (Fig. 2). The IMF content varied significantly at all growth stages in the longissimus dorsi (p<0.01). The largest increase in the IMF content of the longissimus dorsi occurred between 5 and 6 months. In the gluteus maximus IMF content significantly increased between 3-5 months (p<0.05) and did not further significantly increase by 6 months (p>0.05).

Intramuscular fat content varied in the longissimus dorsi and gluteus maximus at different developmental stages (p<0.05). At 3 months IMF content was higher in the longissimus dorsi than the gluteus maximus; however, at 4-6 months the IMF content was higher in the gluteus maximus than the longissimus dorsi.

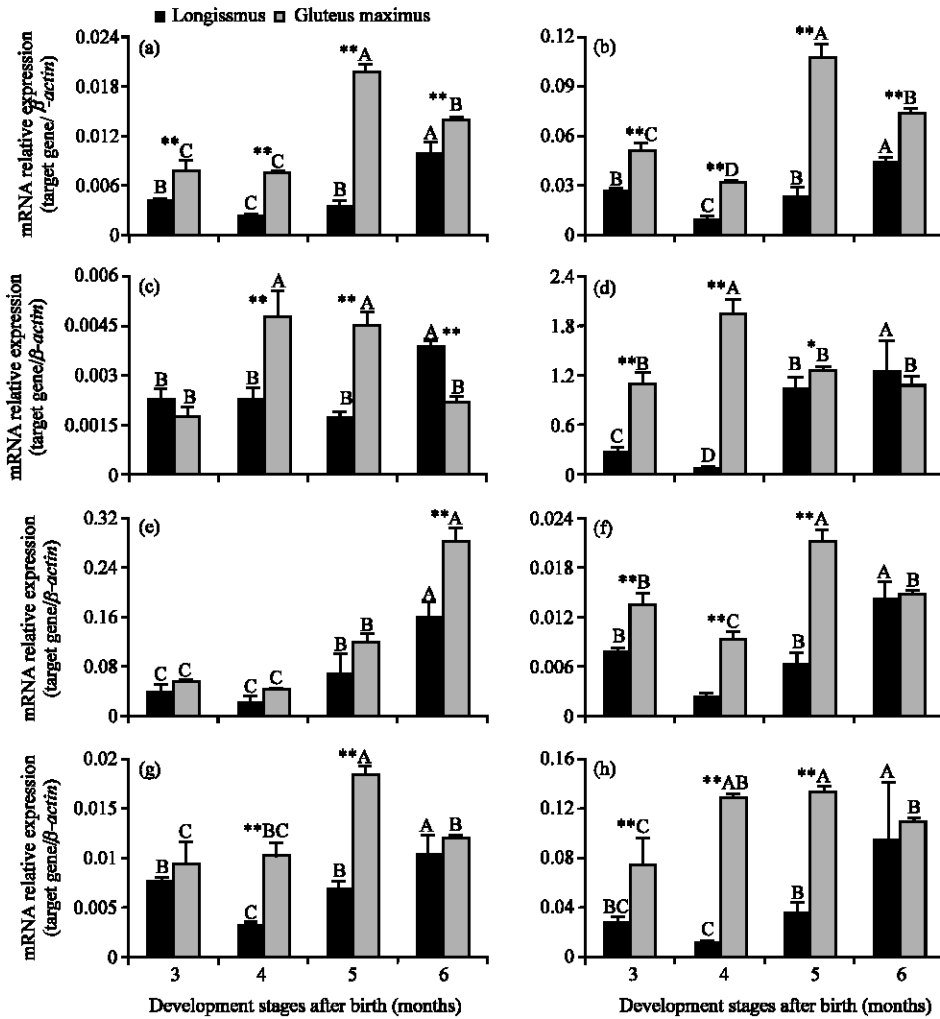


Fig. 3: The expression patterns of genes involved in IMF synthesis. Four Small Tail Han sheep were used at each stage; Expression levels are normalized to housekeeping β -actin. Significant differences between the values obtained in a single muscle over time are denoted with different letters ($p < 0.05$). Significant differences between the different muscles at each time point are indicated as * $p < 0.05$ and ** $p < 0.01$; a) ACC: Acetyl CoA Carboxylase; b) FAS: Fatty Acid Synthase; c) DGAT1: Diacylglycerol Acyltransferase 1; d) HFABP: Heart Fatty Acid Binding Protein; e) C/EBP α : CCAAT-Enhancer-Binding Protein α ; f) PPAR γ : Peroxisome Proliferator-Activated Receptor γ ; g) ME: Malic Enzyme; h) LPL: Lipoprotein Lipase

Developmental gene expression pattern of ACC and FAS:

The expression levels of Acetyl CoA Carboxylase (ACC) and Fatty Acid Synthase (FAS) followed a similar trend during muscle growth. In the longissimus dorsi, ACC and FAS expression levels were moderate at 3 months, reduced at 4 months and then steadily increased at 5 and 6 months (Fig. 3). In the gluteus maximus, ACC and FAS expression were moderate at 3 and 4 months, peaked at 5 months and then reduced at 6 months. At each growth stage, the expression levels of ACC and FAS in the longissimus dorsi and gluteus maximus were significantly different ($p < 0.01$).

Developmental gene expression pattern of DGAT1 and H-FABP:

In the longissimus dorsi, Diacylglycerol Acyltransferase 1 (DGAT1) expression decreased between 3 and 5 months and then markedly increased at 6 months (Fig. 3). Heart Fatty Acid Binding Protein (H-FABP) showed a similar trend in the longissimus dorsi; however, the minimum H-FABP expression level was observed at 4 months after which time H-FABP expression increased. In the gluteus maximus, DGAT1 and H-FABP expression increased and peaked at 4 months and then decreased. There was no significant difference in DGAT1 expression in the longissimus dorsi and gluteus maximus

at 3 months; however, DGAT1 expression in the two muscles varied significantly at 4, 5 and 6 months ($p < 0.05$). The expression of H-FABP varied significantly in the longissimus dorsi and gluteus maximus at 3-5 months ($p < 0.05$); however, there was no significant difference at 6 months.

Developmental gene expression pattern of C/EBP α and PPAR γ : CCAAT-Enhancer-Binding Protein α (C/EBP α) displayed a similar expression pattern in the longissimus dorsi and gluteus maximus (Fig. 3). C/EBP α expression reached a minimum at 4 months and then significantly increased at 5 and 6 months. The expression profile of Peroxisome Proliferator-Activated Receptor γ (PPAR γ) also varied in the longissimus dorsi and gluteus maximus. In the longissimus dorsi, PPAR γ expression was lowest at 4 months and increased at 5 and 6 months. In the gluteus maximus, PPAR γ expression showed an undulating pattern; decreased between 3 and 4 months, peaked at 5 months and then decreased again at 6 months.

The expression of C/EBP α in the longissimus dorsi and gluteus maximus was significantly different at 5 and 6 months ($p < 0.05$) but was not significantly different at 3 and 4 months. The expression of PPAR γ was significantly different in the longissimus dorsi and gluteus maximus at 3-5 months ($p < 0.01$) but was not significantly different at 6 months.

Developmental gene expression pattern of ME and LPL: Malic Enzyme (ME) and Lipoprotein Lipase (LPL) displayed similar expression patterns in the longissimus dorsi and gluteus maximus (Fig. 3). In the longissimus dorsi, the minimal ME and LPL expression values occurred at 4 months then gradually increased and peaked at 6 months. In the gluteus maximus, ME and LPL expression gradually increased during growth, peaking at 5 months and then decreasing at 6 months. The expression of LPL in the longissimus dorsi and gluteus maximus was significantly different at 3 months ($p < 0.01$) whereas ME expression in the longissimus dorsi and gluteus maximus was significantly different 4 and 5 months ($p < 0.01$).

Correlation between gene expression and IMF content: The correlation between IMF content and gene expression levels were analyzed and varied relationships were observed in the different muscles: IMF content in longissimus dorsi exhibited a correlation with the expression levels of a larger number of genes and displayed higher correlation coefficients than the gluteus maximus (Table 2). In the longissimus dorsi, all of the genes exhibited a significant correlation with IMF content.

In the gluteus maximus, significant correlations were observed between the IMF content and ACC, FAS, C/EBP α , PPAR γ , ME, LPL expression levels but not DGAT1, H-FABP. Remarkably, all of the above correlations between IMF and gene expression levels were positive.

DISCUSSION

This study demonstrates that both slaughter weight and IMF content substantially increase in Small Tail Han sheep from 3-6 months of age. The transcription levels of genes involved in IMF formation researchersre quantified and different gene expression patterns were observed at each stage of growth in the longissimus dorsi and gluteus maximus. Additionally, researchers observed significant correlations between the IMF content and expression level of most of the genes tested in both muscles.

There is a close relationship between the IMF content and slaughter weight. The present study demonstrates that the IMF content of Small Tail Han sheep continues to rise with age until at least 6 months of age. A study of the IMF content in 72 pigs from 3 different breeds indicated that the IMF content increases in parallel to weight gain in the Brazilian indigenous breed Piau but not in commercial and crossbred breeds (Serao *et al.*, 2011). Bruns *et al.* (2004) evaluated the relationship between IMF content and the body weight of Angus steers and observed that greater increases in marbling relative to total slaughter fatness occurred at Hot Slaughter Weights (HCW) < 300 kg. Taken together, this data indicates that the relationship between body weight and IMF content varies in different species, breeds and at different stages of growth.

The IMF content of Small Tail Han sheep is higher than other breeds of sheep at the same age. The IMF content of the longissimus dorsi on 4 months in male Kazak sheep and Xinjiang fine wool sheep are 1.40 and 0.60%, respectively (Huang *et al.*, 2006). Hao (2008) reported that the IMF content of the longissimus dorsi of Hu sheep was 4.60% at 6 months of age. In this research, the IMF content of the longissimus dorsi muscle of Small Tail Han sheep reached 5.86% at 4 months and 10.4% at 6 months of age. This variation in IMF content may be due to breed-specific differences as breed is an important factor which affects the IMF content. Small Tail Han sheep are bred for meat while Kazak and Xinjiang fine wool sheep are bred for wool and have to synthesize and transport fat for the growth of wool. In addition, it is worth noting that Small Tail Han sheep are a Chinese local breed of sheep and many Chinese local breeds have a

characteristically high IMF content including Rongchang swine (5.77%), Dahuabai swine (4.16%) (Lu *et al.*, 2008) and individuals of the Laiwu swine breed which can reach >10.0% IMF (Guo *et al.*, 2007).

This study demonstrates that IMF content continuously increases in both the longissimus dorsi and gluteus maximus; however, a different pattern of IMF deposition was observed in each muscle. The IMF content reached a high steady state at 5 months of age in the gluteus maximus but significantly increased between 5 and 6 months in the longissimus dorsi of Small Tail Han sheep. The IMF content in the gluteus maximus was higher than the longissimus dorsi after 4 months with large differences observed at 5 months of age. Research has indicated that IMF content of different muscles varies. In Manchego sheep the IMF content of the longissimus dorsi is higher than the quadriceps femoris (Caneque *et al.*, 2005) and similarly in cattle, Pannier *et al.* (2010) reported that the IMF content of the longissimus thoracis muscle is higher than the semimembranosus muscle.

Changes in IMF content are closely related to triglyceride and fatty acid synthesis. Increased IMF content is almost entirely reflected by a higher muscle triglycerides content and free fatty acids (Fernandez *et al.*, 1999). Body fat deposition occurs due to fat cell differentiation, increased numbers of fat cells and fat cell hypertrophy which are regulated by altered gene expression of a series of fat synthesis-related genes. Acetyl CoA carboxylase and fatty acid synthase constitute the main enzymes in fatty acid synthesis. Acetyl CoA carboxylase carboxylates acetyl-CoA and catalyzes the production of malonyl-CoA, an essential substrate for fatty acid synthesis (Brun *et al.*, 1993, 1997). Fatty acid synthase is the rate limiting enzyme in fatty acid synthesis and catalyzes the conversion of malonyl-CoA into palmitate (Joseph *et al.*, 2002; Ortega *et al.*, 2010). Acetyl CoA carboxylase and fatty acid synthase are involved in the preparation of Free Fatty Acid (FFA) for the synthesis of triglycerides. In this study, researchers observed that the expression levels of ACC and FAS were both closely related to IMF content in the longissimus dorsi and gluteus maximus. Similarly, FAS expression exhibits a strong positive correlation with IMF in Kazak sheep but not Xinjiang fine wool sheep (Qiao *et al.*, 2007). Canovas *et al.* (2009) reported that ACC expression did not correlate with the semimembranosus muscle IMF content in the purebred Duroc pig. The expression of FAS significantly correlates with hepatic fat content but not with the IMF content in breast and thigh tissues of chicken (Cui *et al.*, 2011a). Therefore, the correlation between ACC or FAS expression and IMF may vary in different tissues, breeds and species.

Diacylglycerol acyltransferase is a key enzyme required to catalyze the final step of triglyceride synthesis (Cases *et al.*, 1998) and diacylglycerol acyltransferase catalyzes the formation of triglycerides from diacylglycerol in the final rate-limiting step of the triglyceride synthesis pathway in skeletal muscle (Ikeda *et al.*, 2002). Research on the relationship between DGAT1 mRNA expression and the IMF content in 3 breeds of pig (Laiwu pig, Lulai Black and Large White) indicated that DGAT1 expression was maximal in the Laiwu pig but did not correlate with IMF content (Cui *et al.*, 2011b). Similarly, researchers observed no correlation between the expression level of DGAT1 and IMF content in gluteus maximus of Small Tail Han sheep. Heart-fatty acid binding protein is a type of Fatty Acid Binding Protein (FABP) which is expressed in the heart, skeletal muscle and the lactating mammary gland. Heart fatty acid binding protein transports fatty acids outside the cell membrane to sites of triglyceride and phospholipid synthesis and combines with fatty acids in the cell in order to maintain a concentration gradient and promote the uptake of fatty acids. Gerbens *et al.* (2001) reported that H-FABP mRNA expression levels were positively correlated with IMF content in the pig (Table 3).

The formation of adipocytes from preadipocytes involves a complex and highly orchestrated program of gene expression (Lowe *et al.*, 2011). Two principal adipogenic factors, C/EBP α and PPAR γ regulate the terminal adipocyte differentiation process (Farmer, 2006). Peroxisome proliferator-activated receptor γ is a member of the nuclear-receptor superfamily and the master regulator of adipogenesis (Rosen and MacDougald, 2006) and is an essential regulator of adipogenesis (Rosen *et al.*, 2000). CCAAT-enhancer-binding protein α

Table 3: Correlation between gene expression and intramuscular fat content
Correlation coefficient and p-value

Gene ²	Longissimus dorsi		Gluteus maximus	
	Correlation	Values	Correlation	Values
ACC	0.801**	p = 0.0002	0.878**	p < 0.0001
FAS	0.751**	p = 0.0008	0.799**	p = 0.0002
DGAT1	0.673**	p = 0.0043	0.168	p = 0.5329
H-FABP	0.860**	p < 0.0001	-0.318	p = 0.2292
C/EBP α	0.893**	p < 0.0001	0.748**	p = 0.0013
PPAR γ	0.770**	p = 0.0005	0.679**	p = 0.0038
ME	0.634**	p = 0.0083	0.725**	p = 0.0015
LPL	0.772**	p = 0.0005	0.540*	p = 0.0308

¹The Correlation between gene expression and intramuscular fat content are marked with (*), one (*) indicate significant correlation (p < 0.05); double (**) indicate extreme significant correlation (p < 0.01); ²ACC, Acetyl CoA Carboxylase; FAS: Fatty Acid Synthase; DGAT1: Diacylglycerol Acyltransferase 1; HFABP: Heart Fatty Acid Binding Protein; C/EBP α : CCAAT-Enhancer-Binding Protein α ; PPAR γ : Peroxisome Proliferator-Activated Receptor γ ; ME: Malic Enzyme; LPL: Lipoprotein Lipase

and PPAR γ cooperate to regulate the differentiation and maturation of adipocytes. Peroxisome proliferator-activated receptor γ leads to increased expression of C/EBP α in fibroblasts and both C/EBP α and PPAR γ induce the expression of a range of genes in developing and mature adipocytes (Hamm *et al.*, 1999). CCAAT-enhancer-binding protein α induces PPAR γ -dependent adipogenesis and can not promote adipogenesis in the absence of PPAR γ ; however, PPAR γ can promote adipogenesis in C/EBP α -deficient cells (Rosen *et al.*, 2002). Therefore, C/EBP α and PPAR γ act synergistically to regulate a wide range of genes involved in development of mature adipocytes (Lefterova *et al.*, 2008; Rosen *et al.*, 2000). The study showed that there was a positive correlation between the expression level of C/EBP α and IMF content and still, PPAR γ expression level showed positive correlation with IMF content in Small Tail Han sheep. CCAAT-enhancer-binding protein α and PPAR γ cooperate and interact to regulate maturation and differentiation of fat cells and the experiments indicated a similar expression pattern of these genes during IMF deposition. The expression of C/EBP α is regulated in a tissue-specific but not breed-specific manner in the longissimus muscle and adipose tissues of 18 months old Holstein and Charolais bulls (Xu *et al.*, 2009). A large number of pathways, metabolites, cofactors and modifications including acetylation, phosphorylation and methylation regulate PPAR γ and C/EBPs making adipogenesis a complex process (Lowe *et al.*, 2011) and further research is required to understand the mechanism regulating the varied pattern of C/EBP α and PPAR γ expression during IMF deposition in Small Tail Han sheep.

CONCLUSION

In this study, researchers quantified the expression patterns of genes involved in IMF deposition and discussed the relationships between gene expression and IMF content during the growth of Small Tail Han sheep. Characteristic correlations between IMF content and gene expression were observed in the longissimus dorsi and gluteus maximus. Most gene expression patterns followed a similar trend and showed the highest expression levels at 4 and 5 months of age during the period of the fastest increase in IMF content. This research can contribute to knowledge of the expression pattern of genes involved IMF formation and facilitate further research on the molecular mechanisms of IMF deposition in sheep. Further studies are required to investigate how the varied expression patterns of these genes in the longissimus dorsi and gluteus maximus correlate with IMF deposition.

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