Developmental changes in fatty acid-binding protein (H-FABP) mRNA expression and intramuscular fat (IMF) content in Oula sheep

X. Lang,* C. Wang,* P. J. Wu,*[1] and D. P. Casper†

*Institute of Animal & Pasture Science and Green Agriculture, Gansu Academy of
Agricultural Science, No. 1 Agricultural Academy Village of Anning, Lanzhou, Gansu, People's
Republic of China 730070; and †Furst-McNess Company, 120 East Clark Street, Freeport, IL 61032

ABSTRACT: Increasing meat consumption by Chinese people has created a focus for improving meat quality for increasing quality of life. Twenty-five Male Oula sheep were selected at 2, 21, 56, 84, and 112 d to investigate the developmental changes associated with age on the intramuscular fat (IMF) content of heart fatty acid-binding protein (H-FABP) mRNA expression in muscle. Longissimus dorsal muscle and biceps femoris muscle were sampled to measure IMF concentrations and total mRNA was extracted to measure H-FABP mRNA expression using real-time polymerase chain reaction (PCR). Growing male Oula sheep demonstrated that the IMF concentrations continuously increased with age and significant differences ($P < 0.05$) were detected among the age groups; 2. The IMF concentrations among tissues were different; 3. The development changes in H-FABP mRNA expression in longissimus dorsal muscle and biceps femoris muscle were similar with a decrease from 2 to 21 d, followed by continuously increasing concentrations being significant different ($P < 0.05$) among age groups; 4. The H-FABP mRNA expression in the longissimus dorsal muscle tissue was significantly ($P < 0.05$) higher compared to the biceps femoris muscle; 5. The muscle H-FABP mRNA expression concentration was positively correlated with IMF concentrations from d 21 to 112; 6. The correlation coefficients were significantly ($P < 0.01$) different between H-FABP gene mRNA expression in the longissimus dorsal muscle and IMF concentration of 0.815 compared to the biceps femoris muscle and IMF concentration of 0.787,which indicated that the H-FABP gene may be affecting the IMF concentrations in the early developmental stages of Oula sheep. These results support the hypothesis that H-FABP gene and its expression in muscle tissue is related to the IMF concentration of meat.

Key words: Fatty acid-binding protein, Intramuscular fat, Oula sheep, real-time PCR.

INTRODUCTION

China is almost self-sufficient in meat production with people becoming more interested in improving meat quality for quality of life. Breeding programs are rapidly developing to improve meat quality. One of the key factors affecting meat quality is the intramuscular fat (IMF) concentrations that are positively correlated with tenderness, juiciness, and taste (Wood et al., 2008). This focuses attention on understanding and manipulating the genes responsible for IMF deposition in sheep.

Fatty acid-binding proteins (FABP) are small intracellular proteins having a molecular size of 14 to 16 kDa with 126 to 134 amino acids. The FABP are responsible for fatty acid transport from the cell membrane to the intracellular sites of fatty acid utilization. Currently, 11 identified FABP are expressed in different tissues, but the adipocyte-FABP (A-FABP) and heart-FABP (H-FABP) are the 2 FABP influencing IMF concentrations (Huang and Zhuang, 2004). The H-FABP gene is expressed pre-dominantly in muscle cells, whereas the A-FABP gene is almost exclusively adipocytes (Huang and Zhuang, 2004).

Peroxisome proliferator-activated receptors (PPAR) are members of the nuclear receptor super-family. Three PPAR subtypes have been characterized

as PPARα, PPARβ (also called PPARδ or NUC-1), and PPARγ (also called PPARγ). The PPAR, have important roles in lipid metabolism, are encoded by separate genes with distinct tissue distributions, unique functions, and ligand specificities. Adipose tissue contains mainly PPARγ, which regulates gene expression encoding several proteins involved in adipocyte differentiation, which could affect IMF concentrations (Huang and Zhuang, 2004).

The literature contains little information on the relationship of H-FABP and PPARγ genes in the ovine. Oula sheep are a special native breed to the Tibetan plateau in China's Gansu Province. These sheep are reared for meat production and were selected to study H-FABP mRNA gene expression on muscle IMF concentrations for focusing future breeding programs to improve the breed.

MATERIALS AND METHODS

Animal Description

Oula sheep (Fig. 1) are a special native breed of Tibetan sheep found across Maqu County in the eastern Qinghai-Tibet plateau in China's Gansu Province that are raised for their excellent growth rates and meat quality. The natural breeding selection over time on the Tibetan plateau has resulted in a very strong adaptability to the alpine habitat. The average body weight of an adult male Oula sheep is 80 kg, while the ewe will average 65 kg. These Oula sheep play an important and active role in improving the local economy and maintaining social stability for Tibetan livestock

Figure 1. Adult male Oula sheep.

producers (Lang, 2009). The environmental characteristics for the Tibetan plateau are given in Table 1.

Sampling Periods

The lamb experiment was conducted according to the Chinese Standards for the Use and Care of Research Animals (He et al., 2016). Twenty-five male Oula sheep in groups of 5 were selected on 2, 21, 56, 84, and 112 d of age from the Oula pasture in Maqu County of China's Gansu Province. The animals were transported to the research laboratory, final body weights recorded and harvested with subsamples collected from the longissimus dorsal muscle, biceps femoris muscle, and heart. Subsamples of the sampled tissues were stored at –20°C for measurement of IMF concentrations via Soxhlet petroleum-ether extraction (Lang, 2009) with the residual

Table 1. Environmental information of area for Oula sheep

Location: east longitude	100°45′45"—102°29′00"	Location: north latitude	33°06′30"—34°30′15"
Altitude	3,400m~4,800m	Mean temperature of the warmest month (July)	10.7°C
Climate type	Cold climate on the Qinghai-Tibet plateau	Extreme maximum temperature	23.6°C
Mean annual temperature	0.5~1.1°C	Period below 0°C in a year for month	5 mo
Mean temperature of the coldest month (January)	–9.6°C	Annual accumulated temperature above 0°C	1361.1°C
Absolute lowest temperature	–29.6°C	Rainfall	615.5mm
Relativehumidity(RH)	40~80%	Agrotype	Alpine meadow soil, sub-alpine meadow soil, sub-alpine scrub meadow soil, meadow soil, boggy soil, peat soil, dark brown earth
Frostlessseason	No absolute frotless season	Pasturage type	Gramineae, Cyperaceae, Polygonaceae, Ranunculaceae, Compositae, Rosaceae
Grassland type	Alpine meadow, sub-alpine meadow, mountain grassland	Cover degree of pasturage	> 85%
Growing period of pasturage	Germinating at the last 10 d of April, reviving at the last 10 d of May, growing period is 5 mo,	Average height of turf	About 30cm
Crop	Oat	Fresh yield	> 4 ton/ha

being snap-frozen in liquid nitrogen and subsequently stored at –80°C for later analysis of total RNA.

Primer Design

According to the published sequences of ovine H-FABP and GAPDH (glyceraldehyde-3-phos-phatedehydrogenase) mRNA at GenBank (Kijas et al., 2006), the oligonucleotide primer sets for the 2 genes were designed using Primer premier 5.0 software (Premier Biosoft International, Palo Alto, CA) and described in detail in Table 2. The mRNAs of GAPDH were used as an internal standard for determination of targeted mRNA levels.

Total RNA Extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA Extraction: The total RNA of longissimus dorsal muscle and biceps femoris muscle was extracted by using the acid-guanidineium-thiocyanate-phenol-chloroform extraction (Chengli et al., 2008). The extracted RNA was dissolved in DEPC-treated water and the concentration, purity, and integrity were assessed using a spectrophotometer (WD-9403D, Liuyi Instruments, Beijing, China) set at a wavelength of 260/280 nm (OD260/OD280 = 1.8 to 2.0) and by electrophoresis with ethidium bromide staining (Gerbens et al., 2001).

Reverse Transcription (RT): A 2-μg aliquot of total RNA was used for reverse transcription in a final volume of 25 μL containing 200 UMMLV reverse transcriptase (Promega Corp., Madison, WI), 20 URNase inhibitor (Promega Corp.), 1 μg of random primer, 5 μL of 5 × RT buffer (250 mmol/L Tris-HCl pH 8.3, 50 mmol/L $MgCl_2$, 250 mmol/L KCl, 50 mmol/L DTT, 2.5 mmol/L Spermidine), and 0.4 mmol/L each of dNTP. The first step was to mix the RNA sample, random primer, dNTP, and sterile H_2O (final volume was 10 μL) in a 0.5 mL micro-centrifuge tube (brand) and incubated at 70°C for 5 min, removed and cooled on ice for 2 min. Then, the remaining reagents were added to the reaction tube to achieve a final volume of 25 μL and incubated at 37°C for 1 h. The reaction was terminated by heating at 95°C for 5 min, removed and quickly cooled on ice and the remaining RT products were stored at –20°C.

Polymerase Chain Reaction (PCR): Upon thawing, 0.5 μL of RT reaction products were mixed with 1 U Taq DNA polymerase (TaKaRa Bio Inc., Kusatsu, Japan), 1 μL of 10 × PCR buffer (100 mmol/L Tris-HCl pH 8.3, 500 mmol/L KCl), 0.25 mmol/L each of dNTP, 1.25 mmol/L $MgCl_2$, and0.5 μmol/L to achieve a final volume of 10 μL containing each of gene-specific primers for PCR.

The following amplification conditions were used: one PCR cycle of 1 min at 94°C followed by 40 PCR cycles of 30 s at 94°C, 30 s at the annealing temperature of the primers, 30 s at 72°C, and a final extension for 5 min at 72°C. Correct length of the products was confirmed on an 8% polyacrylamide gel, which was subsequently analyzed with a computer flatbed scanner after silver staining (Sambrook and Russell, 2001).

Cloning and Sequence Analysis of the Amplified PCR Fragments

Products were determined after being confirmed by electrophoresis on 1% agarose gel and purified by V-gene DNA Purification Kit (V-gene Bio-Technology Ltd., Hangzhou, China) following the supplier's procedures, then cloned into a pMD18-T simple vector. Subsequently, the ligation products were transformed into JM109 cells. Positive clones on the basis of blue-white selection were picked out for plasmid extraction by the V-gene Kit (V-gene Bio-technology Ltd., Hangzhou, China) following the manufacturer's procedures and then identified by PCR, using gene-specific primers. Plasmids containing inserts of the right size were sequenced by Invitrogen Bio-technology Co. Ltd (Shanghai, China) following the procedures of Huang (2005), to verify that the assay was measuring the specific genes.

Real-Time Polymerase Chain Reaction (Real-Time PCR)

The abundance of H-FABP mRNAs was assessed by real-time RT-PCR using a fluorescence temperature cycler (DNA Engine Opticon Real-time PCR Systems, MJ Research Inc., Waltham MA). The final reaction volume was20 μL containing 1 μL of RT reaction mix, 1 U EXTaq HS DNA polymerase (TaKaRa, Bio Inc., Kusatsu, Japan), 4 μL of5 × PCR buffer, 0.3 mmol/L each of dNTP, 3.75 mmol/L $MgCl_2$, 0.5 μmol/L each

Table 2. Parameters of primer pairs for the target genes.

Target genes	mRNA accession number	Primer sequence	Product size	Annealing temperature
H-FABP	GenBank AY 157617	Forward: 5'-TGACCAAGCCTACCACAA-3' Reverse: 5'-CACTATTTCCCGCACAAG-3'	224 bp	54°C
GAPDH	GenBank AF030943	Forward: 5'-ACTTTGGCATCGTGGAGG-3' Reverse: 5'-GAAGAGTGAGTGTCGCTGTTG-3'	379 bp	54°C

of primers and 1 μL of 20 × SYBR green. The PCR reaction conditions were as follows: one PCR cycle of 1 min at 95°C; 45 PCR cycles of 10 s at 95°C,10 s at the annealing temperature of the primers, 15 s at 72°C, then plates were read; followed by an extension of 10 min at 72°C; followed by plate reading every other 0.2°C from 65°C to 94°C for drawing melting curves; the reaction was terminated with an extension of 5 min at 72°C. The reactions were repeated twice for every sample. Plasmid DNA with targeted DNA fragments was diluted to gradient concentrations, which were used to draw quantitative standard curves. Prior to start of sample analyses, the house keeping gene was tested and no effects was found ($P > 0.10$).

Analyzing Intramuscular Fat (IMF) Concentrations

The Soxhlet petroleum-ether extraction method was used to measure the IMF concentrations (Sañudo et al., 2000). Sample preparation was accomplished by grinding through a meat grinder and the subsamples of longissimus dorsal muscle and biceps femoris muscle stored at –20°C. Then 10 g of sample was weighed and dried to a constant weight in a drying oven (Dongguan Zhongzhi Testing Instruments Co., Guangdong, China) at 65°C. Another subsample, weighing 2 to 3 g was weighed into a fat bag (bag doesn't absorb fat) and dried to a constant weight at 65°C, and then samples were (muscle sample and bag) were placed in ether for 24 h. Samples were then ether extracted at 80°C for 10 h, after which the bags were placed in a well ventilated room to volatilize the ether for 30 min. Subsequently, samples were dried to a constant weight in a drying oven (Dongguan Zhongzhi Testing Instruments Co.) at 105°C for 3 h, and finally weighed.

Statistical Analyses

All data were subjected to one way ANOVA using the One Way Procedure of SPSS version 19.0 (IBM SPSS Statistics for Windows, Version 19.0, Armonk, NY). Differences among means were separated using an independent-sample t test (Steel and Torrie, 1980). Bivariate Correlation was used to evaluate the relativity between IMF content and gene expression level.

Means are presented with significant differences being declared at $P < 0.05$ and $P < 0.01$.

RESULTS

Muscle IMF Concentrations in Oula Sheep

The IMF concentration in longissimus dorsal muscle and biceps femoris muscle of male Oula sheep continuously increased with age due to growth resulting in significant ($P < 0.05$) differences (Table 3). The IMF concentration of longissimus dorsal muscle and biceps femoris muscle at the same age were similar ($P > 0.05$), however, the IMF concentration in longissimus dorsal muscle was always numerically greater compared to the IMF concentration in biceps femoris muscle. The muscle IMF concentration continued to increase through the end of the 112 d study, thus the peak IMF concentrations may not have been reached.

RT-PCR of H-FABP and GAPDH Genes

The total RNA of male Oula sheep muscle was used as the initial sample to amplify H-FABP and GAPDH genes by RT-PCR, and cDNA fragments, respectively, with a size of 224, and 379 bp were produced (Fig. 2).

Sequence Analysis of the Amplified Fragments

The amplified DNA fragments of the H-FABP and GAPDH genes were cloned into a PGM-T simple vector. After the ligation products were identified by PCR using gene-specific primers, plasmids containing inserts of the right size were sequenced. Then the sequences of amplified fragments were aligned using DNASTAR software (DNASTAR Inc., Madison, WI) with the corresponding reported sequences (sequences of ovine H-FABP and GAPDH) according to which gene-specific primers were designed. The results demonstrated that: 1) there was 98.75% sequence identity between the amplified H-FABP gene cDNA fragment and the published ovine H-FABP gene sequence; and 2) there was 100% sequence identity for GAPDH. The above results indicated that the amplified cDNA fragments of the 3 genes were gene-specific products.

Table 3. Percentage of intermuscular fat concentration of harvested Oula lambs

Age(d)	2	21	56	84	112
Longissimus thoracis	0.61 ± 0.27[e]	0.86 ± 0.29[d]	1.19 ± 0.35[c]	1.38 ± 0.47[b]	1.62 ± 0.65[a]
Biceps femoris	0.58 ± 0.22[e]	0.79 ± 0.27[d]	1.15 ± 0.31[c]	1.32 ± 0.43[b]	1.51 ± 0.58[a]

[a–e]Values in the same row differ, different ($P < 0.05$).

a)

b)
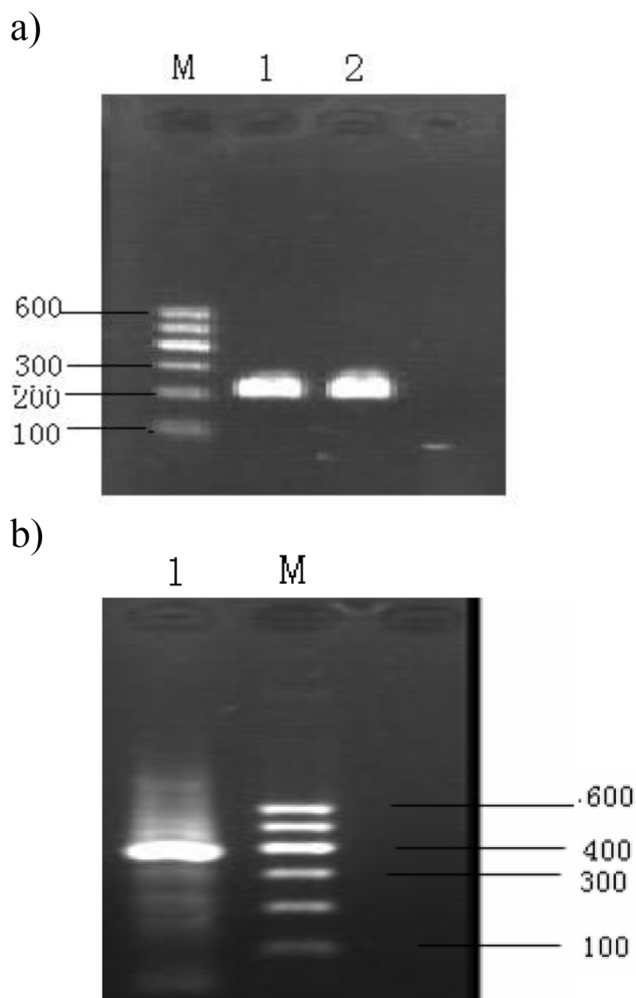
Figure 2. a) Electrophoresis result of H-FABP PCR products M.DNA Marker, 1, 2. Products of H-FABP PCR. b) Electrophoresis result of GAPDH PCR products, M.DNA Marker; 1, 2. Products of GAPDH PCR.

Developmental Changes of H-FABP mRNA Expression

The developmental changes associated with the H-FABP mRNA expression levels were measured using real-time PCR (Fig. 3). The R value for the standard curve of the 2 genes was > 0.99, which can be used as a standard. There was only one large peak on the melting curve of H-FABP and GAPDH gene, so without using specific fluorescence, the quantification was deemed accurate. For growing Oula sheep the developmental changes in muscle H-FABP mRNA expression was greater ($P < 0.05$) for longissimus dorsal muscle and biceps femoris muscle compared to the heart muscle (Table 4). The changes in H-FABP mRNA expression with age for longissimus dorsal, biceps femoris, and heart muscle were similar in that the expression decreased from d 2 to 21 and then increase through d 112 with significant ($P < 0.05$) differences occurring between each d of measurement.

The Relationship between H-FABP mRNA Expression and IMF Concentration

Bivarate correlation was used to determine the relationship between H-FABP mRNA expression and IMF concentrations (IBM SPSS Statistics for Windows, Version 19.0, Armonk, NY). The mRNA expression level of the H-FABP gene in longissimus dorsal muscle and biceps femoris muscle was positively correlated ($r = 0.815$ and $r = 0.787$, respectively; $P < 0.01$) with the IMF concentrations from d 21 to 112.

DISCUSSION

The IMF concentrations of male Oula lambs continuously increased with increasing age from d 2 to 112 due to growth. At the same time, the IMF concentration varied between the different muscle tissues, in that fat was deposited in the longissimus dorsal muscle tissue before the biceps femoris muscle. Thus, male Oula sheep initiated IMF accumulation in the early stage of life, which is consistent with the animal's evolution to adapt to the natural environment. Thus, to prepare for adaptation to possible nutritional shortages in the cold winter, it would be necessary to graze heavily for energy intake to be deposited as fat in the summer and autumn. Oula sheep are native to the Qinghai-Tibet plateau and a valuable genetic resource based on natural selection. In the winter, feed supply may be short and the climate was cold, so Oula sheep adapted by depositing energy as fat in the body depots at an early stage of life for meeting the nutrient requirements later when the environmental Qinghai-Tibet plateau were less favorable for energy intake.

The IMF concentrations are an energy source for that animal, but also play an important role when the animal is harvested for meat. The IMF concentrations in meat are positively correlated with tenderness, juiciness, and taste, such that, the greater the meat IMF concentrations, the more the favorable the taste (Patrici, 1985). The IMF concentrations can be affected by many factors, such as breed, age, sex, tissue depot, nutritional intake, etc. For example, for the Danmark pig, improving meat taste was directly proportional to increasing IMF concentrations, while at the same time, meat tenderness was improving (Patrici, 1985). Huang and Li (2006)reported that the IMF concentrations varied with different tissue depots, which is consistent with our research results. The IMF concentrations in longissimus dorsal muscle of Kazak sheep increased with the age when growing from d 2 to 90, whereas, Xinjiang fine wool sheep demonstrated little difference (Huang, 2005). The IMF concentration of longissimus dorsal muscle of Manchego lamb was greater than the IMF concentrations in the quad-
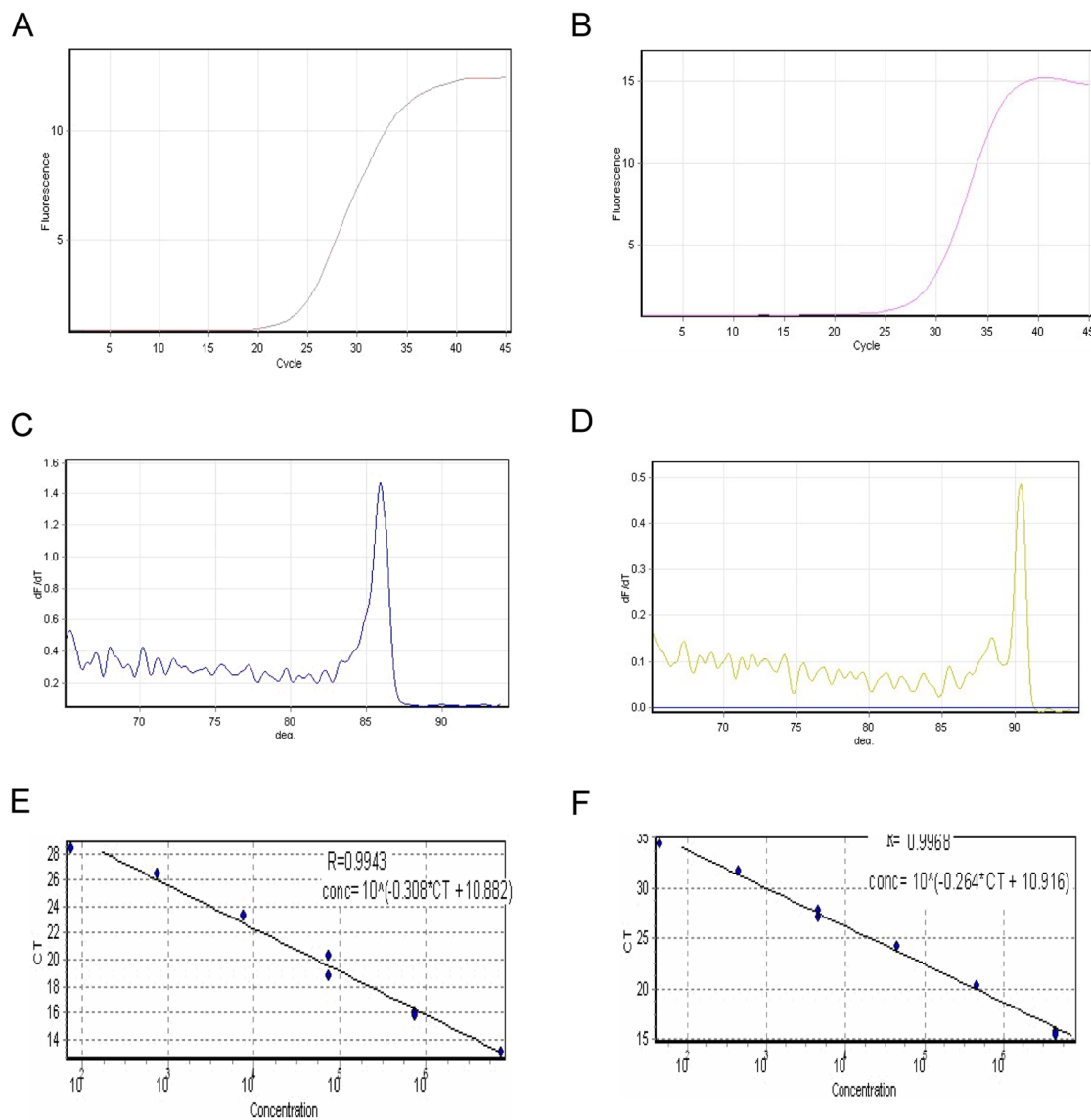
Figure 3. Graphs A, C, and E, respectively are the amplification, standard and melting curve of H-FABP. Graphs B, D, and F, respectively are the amplification, standard and melting curve of GAPDH.

riceps (Cañeque et al., 2005). In agreement, the IMF concentration in the longissimus dorsal muscle of Hu sheep was greater than the IMF concentration in the biceps femoris muscle (Chengli et al., 2008). These data taken together indicate that age and breed affect IMF deposition, which is in agreement with the literature (Patrici, 1985; Wood et al., 2008).

All the members of the FABP family are closely related to IMF deposition, of which, H-FABP played an important role in IMF concentrations (Gerbens et al., 1999; Wood et al., 2008). Previous research in this subject area has focus mainly on humans, rodents, and pigs, but rarely ovines (Wood et al., 2008). The H-FABP and A-FABP genes were significantly associated with the

Table 4. Development changes of H-FABP mRNA expression in longissimus thoracis, biceps femoris muscle and heart of different days lambs

Age(d)	2	21	56	84	112
Longissimus thoracis, copies/µl[1]	1.95 ± 0.37^e	1.15 ± 0.21^d	1.81 ± 0.19^c	2.17 ± 0.32^b	2.89 ± 0.27^a
Biceps femoris muscle, copies/µl[2]	1.83 ± 0.25^c	1.07 ± 0.17^e	$1.72 \pm 0.0.08^d$	1.95 ± 0.29^b	2.57 ± 0.32^a
Heart	0.92 ± 0.21^b	0.11 ± 0.07^e	0.42 ± 0.35^d	0.87 ± 0.55^c	1.38 ± 0.45^a

[a–e]Values in the same row differ, different ($P < 0.05$).

[1]mean $\times 10^4$.

[2]mean $\times 10^5$.

genetic variation of IMF concentrations in a Duroc pig population and the authors speculated that the H-FABP gene was a candidate to account for the variation in IMF accretion (Gerbens et al., 1999). In a later study, Gerbens et al. (2000) using F2 crossbreeds of Meishan and Western pigs reported that the H-FABP gene resided within the large chromosomal region containing a putative QTL affecting IMF content. Further work demonstrated significant differences in mRNA, but not in protein expression levels between H-FABP HaeIII PCR-RFLP genotype classes. Thus, it was mRNA expression, but not protein expression that was significantly related to IMF concentrations (Huang and Zhuang, 2004). The H-FABP gene exerts important effects on the IMF concentration and loin eye muscle area (Ovilo et al., 2005). The difference in H-FABP tissue expression between different pig breeds was studied using RT-PCR and the results demonstrated that the H-FABP expression in the heart was higher than in muscle ($P < 0.01$), while the H-FABP expression in heart and muscle of Yanan pigs was greater than in DLY muscle (Luo et al., 2006). The H-FABP expression in muscle was highest on d 2 and demonstrated variation between ages in male Kazak sheep, as well as, Xinjiang fine wool sheep (Chengli et al., 2008). In that study, the expression was the lowest on d 30 and then continuously increased. But, in the Xinjiang breed the expression declined continuously from d 2 to 90 and then increased. It was reported that the muscle H-FABP mRNA expression was highly and positively correlated with the IMF concentration for d 30 to 90 for male Kazak sheep, but not in the other breeds. These data may indicate that the gene may start influencing the IMF concentrations in the early life stages of the Kazak breed, but not the other breeds. Kang (2008) discovered that the correlation coefficient between muscle H-FABP expression of Mongolia sheep from d 90 to 160 with IMF concentrations was 0.988.

The present study using growing Oula sheep demonstrated that the developmental changes in H-FABP mRNA expression in the longissimus dorsal muscle and biceps femoris muscle was similar, in that the trend was a decrease from d 2 to 21, thereafter, continuously increasing. In addition, the H-FABP mRNA expression was greater for the longissimus dorsal muscle depot compared to the biceps femoris muscle depot. The heart muscle H-FABP mRNA expression was higher than both the longissimus dorsal and biceps femoris muscle. We also found in male Oula sheep that the muscle H-FABP mRNA expression was highly and positively correlated with IMF concentrations from d 21 to 112. The conclusion would be that the gene may be affecting the IMF concentrations in the early life stages of Oula sheep. These results also support the hypothesis that the H-FABP gene is a candidate for regulating the IMF depots within the muscle.

Further work should explore these gene effects on the later growth stages of Oula sheep.

Conclusions

China is almost self-sufficient in meat production with people becoming more interested in improving meat quality for quality of life. Oula sheep, which are native to the Tibetan plateau in China's Gansu Province, are reared for meat production. These results demonstrate that there is a linkage between the H-FABP gene and IMF, which indicates that it may be a candidate for regulating the IMF deposition within the muscle, which will affect meat tenderness, juiciness, and taste. The gene appears to be affecting the muscle IMF concentrations in the early stages of life. Thus, the future development of Olua sheep breeding programs it appears to be possible to modify the H-FABP mRNA gene expression to influence IMF concentrations that would improve meat's attributes for meeting people's quality of life requirements.

LITERATURE CITED

Cañeque, V., M. T. Diaz, I. Alvarez, and S. Lauzurica. 2005. The influences of carcass weight and depot on the fatty acid composition of fats of suckling Manchego lambs. J. Meat Sci. 70:373–379. doi:10.1016/j.meatsci.2005.02.003

Chengli, H., L. Qifa, and Q. Yong. 2008. Association o fthe H-FABP and PPARy gene expression with intramucalar fat content in Hu sheep muscles. Scientia Agric. Sinica. 41:3776-3783. doi:10.3864/J.issn.0578-1752.2008.11.045

Gerbens, F., A. J. van Erp, F. L. Harders, F. J. Verburg, T. H. E. Meuwissen, J. H. Veerkamp, and M. F. W. te Pas. 1999. Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. J. Anim. Sci. 77:846–852. doi:10.2527/1999.774846x

Gerbens, F., D. J. Koning, F. L. Harders, T. H. E. Meuwissen, L. L. G. Janss, M. A. M. Groenen, J. H. Veerkamp, J. A. M. van Arendonk, and M. F. W. te Pas. 2000. The effect of adipocyte and heart fatty acid-binding protein genes on intramuscular fat and backfat content in Meishan crossbred pigs. J. Anim. Sci. 78:552–559. doi:10.2527/2000.783552x

Gerbens, F., F. J. Verburg, H. T. Van Moerkerk, B. Engel, W. Buist, J. H. Veerkamp, and M. F. Te Pas. 2001. Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat content in pigs. J. Anim. Sci. 79:347–354. doi:10.2527/2001.792347x

He, Z. M., G. P. Li, and D. S. Zhu. 2016. Laboratory animals management and use guidelines. Science Press, Beijing, China.

Huang, Z., and X. Zhuang. 2004. The research development of livestock and poultry adipose related gene. J. Anim. Husb. Vet. Med. 36:41–43.

Huang, Z., and X. Li. 2006. The developmental changes and effect on IMF content of H-FABP and PPAR mRNA expression in sheep muscle. J. Acta. Genetica. Sinica. 33:507–514.

Huang, Z. 2005. Research on the developmental changes of MRNAS expression level of the genes related to growing or intramuscular fat (IMF) in sheep. D. Nanjing Agricultural University. Nanjing, Jiangsu, China.

Kang, K. 2008. The Development Rule and Relativity on IMF Content of H-FABP and PPAR-γ mRNA Expression Level in Tan Sheep and MengGu Sheep Muscle. D. Yangling: Northwest Agriculture and Forestry University. Xianyang, Shaanix, China.

Kijas, J. W., M. Menzies, and A. Ingham. 2006. Sequence diversity and rates of molecular evolution between sheep and cattle genes. Anim. Genet. 37:171–174. doi:10.1111/j.1365-2052.2005.01399.x

Lang, X. 2009. Study on genetic resources of sheep in Gansu Province. Beijing, China Agric. Sci. Techn. Press, p. 185-186.

Luo, X., D. Chen, and K. Zhang. 2006. Porcine H-FABP gene expression in different genotypes and muscular tissues. Acta. Vet. Zootech. Sinica.37:727–730.

Ovilo, C., A. Fernandez, J. L. Noguera, C. Barragan, R. Leton, C. Rodriguez, A Mercade, E. Alves, J. M. Folch, L. Varona, and M. Toro. 2005. Fine mapping of porcine chromosome 6 QTL and LEPR effects on body composition in multiple generations oan Iberian by Landrace intercross. Genetics Reseach 85:57-67. doi:10.1017/S0016672305007330.

Patrici, A. 1985. Eating quality of pork in Denmark. Pig Farming.10:56–57.

Sambrook, J., and D. W. Russell. 2001. Molecular Cloning: A Laboratory Manual (third edition). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sañudo, C., M. E. Enser, M. M. Campo, G. R. Nute, G. Maria, I. Sierra, and J. D. Wood. 2000. Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. Meat Sci. 54:339–346. doi:10.1016/S0309-1740(99)00108-4

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill Book Co., New York.

Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hughes, and F. M. Whittington. 2008. Far deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343–358. doi:10.1016/j.meatsci.2007.07.019