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THE SEASONAL EFFECTS ON IMF CONTENT OF *FAS* mRNA EXPRESION IN MUSCLES OF MONGOLIAN SHEEP

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ABSTRACT

This survey's aim was to investigate the seasonal effect of Fatty acid synthesis (FAS) expression on intramuscular fat (IMF) content in grazing Mongolian, Urumqi and grain-fed Ujumqin sheep. Different skeletal muscles of sheep (biceps femoris, longissimus dorsi and triceps brachii) were sampled to measure IMF content and total RNA was extracted to determine FAS mRNA expression levels by real-time PCR. The result revealed that: (1) the difference in IMF content in the muscles of Mongolian sheep grazed in summer was observed to be much higher than sheep grazed in winter ($p<0.01$). Also there was significant difference in IMF content in the muscles of Ujumqin sheep grazed in summer than winter ($p<0.05$). The mRNA expression level of FAS in muscles of winter grazing Mongolian sheep was significantly higher than summer ($p<0.05$). The seasonal effects on IMF content in the muscles and its FAS mRNA *gene expression was all negative correlated between the sheep breeds. Accordingly, grazing Mongolian sheep's FAS gene expression level was very high negatively correlated ($r=-0.964$). This study suggested that grazing Mongolian and Ujumqin sheep able to store large amount of IMF under depends on seasonal effects.*

KEY WORDS: Mongolian sheep, Urumqi sheep, IMF content, *FAS*, correlation

INTRODUCTION

Mongolia has a long tradition of raising livestock. Its pastoral production system dates back to at least thousand years. Connecting to the specific nomadic lifestyle and pasture-raising livestock of Mongolia, meat is a staple for the country people. Mongolian consumer high amounts (100kg per capita) of red and fatty meat from five species of pasture-fed livestock animals, especially of sheep meat [6]. There are increasing concerns of society towards the consumption of animal products which have been produced and transformed in a sustainable manner. This trend influences consumer and their purchasing decision, particularly in developed countries [12]. Sheep and beef meats are also a rich source of protein, providing 20 g/100 g of consumed meat, and necessary micronutrients (iron, zinc, selenium

and vitamins) to human life, which are not present in vegetables or they have a low bioavailability [1]. The mutton production in the world is increasing rapidly and in developed countries the key point of sheep husbandry has shifted from wool to mutton products [16]. So, more attention is paid on improving the mutton quality and to provide more technical support for breeding. One of the main factors affecting meat quality is intramuscular fat (IMF) content that is positively correlated with lipogenesis in mammals. It is noteworthy that nearly every tissue in the human body has some level *FAS* expression, but it is highly expressed in tissues like liver adipose and lactating mammary glands [3, 4]. There have been some studies on the relationship between IMF content and FAS mRNA expression level in the mammals such as pigs, rats, sheep and chicken [4, 13]. There are many articles on *FAS* gene of humans, rodents, pigs, chicken, mice, but rarely on ovine. In this article, we reared two breeds and 3 kind's Mongolian sheep (grazing Mongolian, Ujumqin and grain fed Ujumqin) to be investigated the effects of seasonal changes on the IMF content and to study the expression of *FAS* mRNA in different skeletal muscles.

MATERIALS AND METHODS

Animals: Two breeds and 3 kinds of sheep were selected for the study. Fifty four 2 year old male sheep were taken for the experimental procedure at different seasons (winter and summer); 18 Mongolian sheep (grazing grassland in Galshir sum, Khentii aimag, Mongolia), 18 Ujumqin sheep (grazing grassland in Shiliin gol, Inner Mongolia, China) and 18 grain-fed Ujumqin sheep (MAO's ranch, Shilinhot, Inner Mongolia, China; Feedstuffs-Zhendgda feed) in Inner Mongolia). The animals were slaughtered for three different body parts sampling like *biceps femoris, longissimus dorsi and triceps brachii* muscles and were stored at −20^{0}C for detecting IMF contents using Soxhlet petroleum-ether extraction, and the residuals were snap-frozen in liquid nitrogen and then stored at −80^{0}C for total RNA analysis at a later date. ***Primer design:*** According to the published sequences of ovine *FAS* and β-actin gene mRNA at GenBank, the oligonucleotide primer set for the three genes were designed using Primer premier 5.0 software. The following specific primers were used: for *FAS* (Genbank accession No: AF479289; product size: 188bp) forward: 5'-CCCAGCTCAACGAAACCA-3′, reverse: 5'-GACGAGGTCAACACCCTTCC-3′, for *β-actin* (Genbank accession No: NM001009784; product size: 103bp) forward: 5'-AGAGCAAGAGAGGCATCC-3', reverse: 5'--TCGTTGTAGAAGGTGTGGT-3'. ***Total RNA extraction and reverse transcription polymerase chain reaction (RT-PCR):*** Total RNA of different skeletal muscles (*biceps femoris, longissimus dorsi and triceps brachii*) was extracted using the acid-guanidineium-thioc-yanatephenol-chloroform extraction. The extracted RNA was dissolved in DEPC-treated water and the concentration, purity, and integrity were assessed using a spectrophotometer at 260/280 nm (*OD*260/*OD*280 =1.8–2.0), and by electrophoresis with ethidium bromide staining. 1 μg of total RNA was used for reverse transcription (BioRT Two Step) in a final volume of 10 μL containing 5U AMV reverse transcriptase, 40U RNase inhibitor (Bioer, China), 2 μL of 5 × RT buffer (250 mmol/LTris-HCl pH 8.3, 50 mmol/L MgCl2, 250 mmol/L KCl, 50 mmol/L DTT, 2.5 mmol/L Spermidine), 1 μL dNTP mixture(10mM), 0.5 μL of oligo-dT, 0.5 μL RNase inhibitor (40U/μL), 0.5 μL AMV Reverse Transcriptase, and 4.5 μL each of RNase free H_2O. The RNA sample, random primer, dNTP, and sterile H_2O (final volume was 4.5μL) were mixed in a 0.5 mL micro centrifugal tube. The centrifugal of the reagents were then added into the reaction tube with a final volume of 10μL and incubated at 45^{0}C for 45min, and the reaction was terminated by heating at 95^{0}C for 5 min and quickly cooled on ice. RT production was stored at −20^{0}C. 0.8 μL of RT reaction mix was used for PCR in a final volume of 10 μL containing 5 μL 2xRealtime PCR Master Mix, RNase free water 3.8 μL and 0.2 μL each of gene-specific primers. The following amplification conditions were used: one cycle of 1 min at 95^{0}C followed by 40 PCR cycles of 20 s at 95^{0}C, 30 s at the annealing temperature (57^{0}C) of the primers, 30 s at 72^{0}C, and a final extension for 5 min at 72^{0}C. Correct length of the products was confirmed on

10% polyacrylamide gel, which was subsequently analyzed with a computer flatbed scanner after silver staining. ***Statistical analyses:*** Data were described as $x \pm Sd$ and statistically analyzed using SPSS16 For Windows Software. Differences of the IMF content and gene expression level between season (winter and summer) in the different skeletal muscles same kind sheep and those at the different kinds between in different skeletal muscles at the same season were analyzed by one-way ANOVA and independent-sample *t*-test, respectively. Significant and extreme differences were set at $P<0.05$ and $P<0.01$, respectively. Bivariate correlations were used to evaluate the relativity between IMF content and gene expression level.

RESULT AND DISCUSSION

Fat content in skeletal muscle The effect of season changes in the muscles of grazing and grain-fed diet on IMF content in the winter and summer seasons in three kinds of Mongolian sheep are presented in table 1. Result show that there was significant difference in IMF content ($p<0.01$) between season in different skeletal muscle of grazing Mongolian sheep, but no such extreme differences ($p>0.01$) was observed in different muscles of grain-fed Ujumqin sheep. The IMF content in different skeletal muscle of grazing Mongolian and Ujumqin sheep were greater than grain-fed Ujumqin sheep in the summer season. But the IMF contents were similar in the winter season in muscle of three kind sheep. Based on this result, the differences of IMF depends on season effect natural grazing Mongolian and Ujmuqin sheep may be due to the characteristics of adipose accumulation in different seasons. There are many factors effecting the intramuscular fat accumulation, such as breeds, ages, nutrition, environmental factors, seasonal effect, and so on proposed that the property of IMF in Erualian boars increased during early growing period while Large White boars kept steadily, suggesting that the IMF accumulation in boars was obviously breeds-dependent. Yong *et al.* suggested the IMF content in creased continuously with growth and showed significant difference ($p>0.05$) between different age groups in male in Kazak sheep [7]. Hao *et al.* also proved that the IMF contents of the three different parts of muscle increased during early development term in Hu lamb [9].

Table 1

IMF content in different skeletal muscles of Mongolian sheep, %

Sheep kinds	seasons	m.biceps femoris	m.longissimus dorsi	m.triceps brachii
MGL	winter	2.89±0.36^{ab}	2.93±0.10^{a}	2.94±0.20^{a}
	summer	4.58±0.21^{c}	4.62±0.24^{d}	4.89±0.17^{c}
UJQ1	winter	2.62±0.20^{ab}	2.73±0.20^{a}	2.92±0.15^{a}
	summer	4.11±0.24^{c}	4.18±0.32^{c}	4.33±0.17^{b}
UJQ2	winter	2.41±0.19^{a}	3.43±0.26^{b}	2.95±0.23^{a}
	summer	2.98±0.25^{b}	3.68±0.15^{b}	3.02±0.15^{a}

Means with different common letters are significantly different ($p<0.05$); MGL, grazing Mongolian sheep; UJQ1, grazing Ujumqin sheep; UJQ2, grain-fed Ujumqin sheep;

Gene expression in skeletal muscle The total RNA of Mongolian sheep muscles was used as the initial sample to amplify *FAS* and *β-actin* genes by RT-PCR, and cDNA fragments, respectively with a size of 188 and 103 bp were produced (Fig. 1).

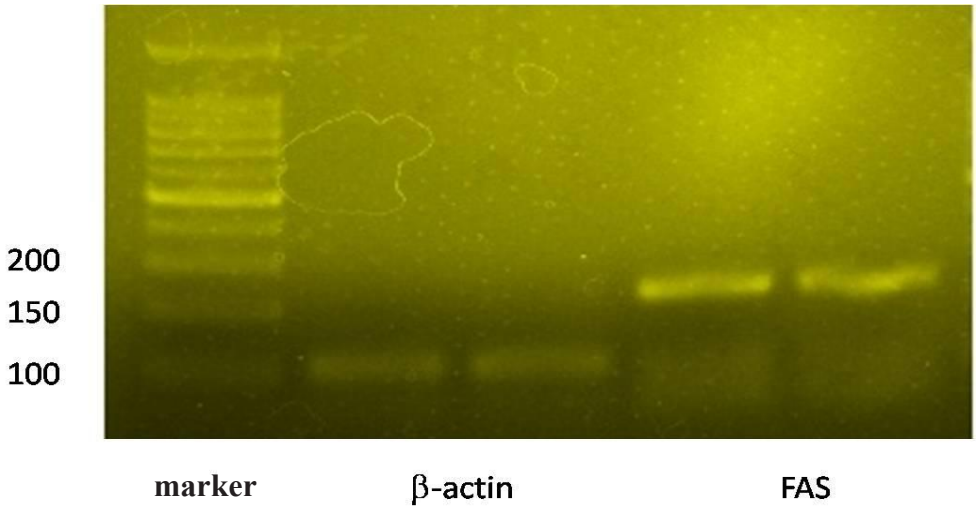

Figure1. RT-PCR of FAS and and β-actin of sheep muscle

Fig 2 shows season changes mRNA levels of *FAS* in different skeletal muscles of grazing Mongolian and Ujumqin sheep compared to grain-fed Ujumqin sheep. In the winter, the mRNA expression level of *FAS* in muscle of grazing Ujumqin sheep was highest another kinds sheep and sheep different skeletal muscles have significant differences ($p<0.05$). In the summer, the mRNA expression level of *FAS* in muscles of grain-fed Ujumqin sheep was greatest another kinds sheep and observed significant differences ($p<0.05$).

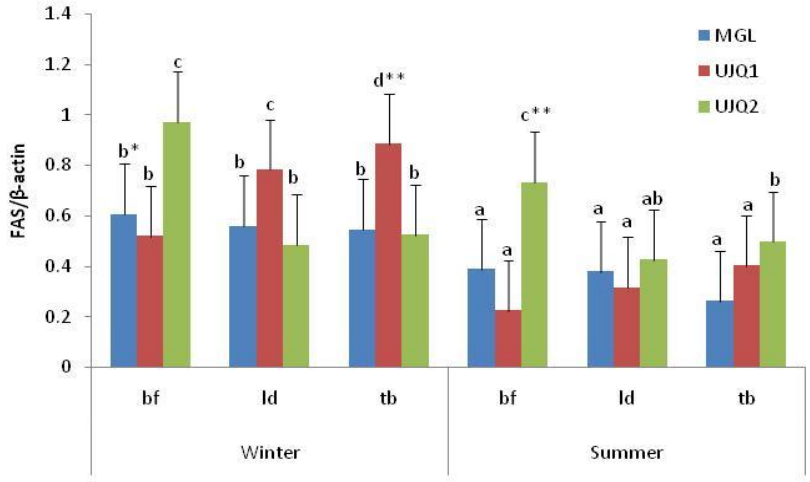

Figure 2. mRNA expression of *FAS* in different skelatal muscles of Mongolian sheep
*indicates significant differences ($p<0.05$) between season at the same kind; **indicates significant differences ($p<0.05$) between muscles of same kinds at the same season; bf,m.biceps femoris; ld, m.longissimus dorsi; tb, m.triceps brachii;

The important enzyme in adipocytes metabolism, *FAS* plays the rate-limiting role in the homeostasis of accumulation and metabolism of intramuscular adipose, which include both the fatty acid's synthesization and hydrolyzation. The increased *FAS* expression may lead to obesity through the accumulation of triglyceride in human [7]. Nadeau KJ *et al*. pointed out, the exercise training increased intramuscular triglyceride in the skeletal muscle with *FAS* protein expression increased in rats and calorie restriction in monkeys. Kim TS and Ding ST J reported *FAS* is highly expressed in pig adipose tissue and to a much lesser extent in liver [11]. This is different from other species (humans, poultry, chicken and rats) in which *FAS* is highly expressed in the liver and in rabbit is expressed mainly in both adipose tissues and liver. Cui HX *et al*. suggested *FAS* mRNA was dominantly expressed in liver of chicken during all developmental stages detected in both BJY chicken and AA broiler [4]. And *FAS* mRNA expressed in breast and thigh tissues as well, but the expression level was fairly low and relatively

stable compared to those in the liver. The expression of *FAS* may be controlled by many factors, such as insulin and *PPARγ* [8]**.** Yong Q *al*. found that *PPARγ* had similar expression model as *FAS* in Kazak sheep muscle [16].***Correlation between FAS mRNA expression levels and intramuscular fat content*** The mRNA expression level of *FAS* in muscles of grazing Mongolian sheep was higher between seasons also much higher negative correlated (r=-0.964 (P=0.021) with IMF content (Table 2). This suggests that natural grazing Mongolian sheep is able to store large amount of IMF in the between seasons. Chen J *et al*. (2004) reported, there was no obvious relationship between *FAS* expression and intramuscular fat contents in swine's longissimus dorsal muscle. A novel negative correlation between *FAS* mRNA level and IMF were detected in this study (P = 0.02). It suggested that *FAS* may function as an enzyme of intramuscular fat storage and has diverse influence in different species.

Table 2

Correlation between *FAS* mRNA expression levels and fat content in muscles of Mongolian sheep

	Sheep kinds	***FAS* mRNA level**
IMF content	MGL	r=-0.964 (P=0.021)
	UJQ1	r=-0.787 (P=0.063)
	UJQ2	r=-0.632 (P=0.178)

CONCLUSIONS

The changes on seasonal effects in IMF accumulation ability between grazing and grain-fed diets sheep was accompanied with differences in fat related *FAS* gene expression and correlation in different skeletal muscles. IMF content is an important determinant of meat eating quality. In present study, we revealed that season effects, could lead to difference the IMF content of sheep feed on different diets. The result indicates that IMF content in the natural grazing diet could be due to the characteristics of adipose accumulation by seasonal depends effect.The important enzyme in adipocytes metabolism, FAS play the rate-limiting role in the homeostasis of accumulation and metabolism of intramuscular adipose. It was detected that the *FAS* mRNA level was negatively correlated with IMF contents in this study (P=0.021, P=0.063, P=0.178). It suggested that FAS may function as an enzyme of intramuscular fat storage. This study identified the diversity of IMF accumulating between in muscles of grazing and grain-fed sheep as that described in previous articles, which suggested the seasonal effects of animal adipocyte cells, especially intramuscular adipocytes.

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