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# mRNA transcription and protein expression of PPARγ, FAS, and HSL in different parts of the carcass between fat-tailed and thin-tailed sheep

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ABSTRACT

*Background:* The objective of this study was to compare the level differences of mRNA transcription and protein expression of PPARγ, FAS and HSL in different parts of the carcass in different tail-type sheep. Six Tan sheep and six Shaanbei fine-wool sheep aged 9 months were slaughtered and samples were collected from the tail adipose, subcutaneous adipose, and longissimus dorsi muscle. The levels of mRNA transcription and protein expression of the target genes in these tissues were determined by real-time quantitative PCR and western blot analyses.
*Results:* The results showed that PPARγ, FAS, and HSL were expressed with spatial differences in tail adipose, subcutaneous adipose and longissimus dorsi muscle of Tan sheep and Shaanbei fine-wool sheep. Differences were also observed between the two breeds. The mRNA transcription levels of these genes were somewhat consistent with their protein expression levels.
*Conclusion:* The present results indicated that PPARγ, FAS and HSL are correlated with fat deposition, especially for the regulating of adipose deposition in intramuscular fat, and that the mRNA expression patterns are similar to the protein expression patterns. The mechanism requires clarification in further studies.

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## 1. Introduction

Fatty deposits have been attracting increasing attention in recent years [1,2,3,4]. Fat deposition is closely correlated with the relative protein expression levels of peroxisome proliferator-activated receptor (PPARγ), fatty acid synthase (FAS), and hormone-sensitive lipase (HSL), which are the most important transcription factors and key enzymes during adipose deposition [5,6,7]. PPARγ, a member of nuclear receptor family, is considered to be the main regulator of adipogenesis and is expressed in adipose tissue at a high level [8]. FAS is a key enzyme in fatty acid synthesis [9] and catalyzes acetyl coenzyme A, malonyl coenzyme A and nicotinamide adenine dinucleotide 2′-phosphate to synthesize fatty acids [10,11]. HSL is the rate-limiting enzyme in initiating triglyceride polymerization to form fat and influences the adipose deposition rate in mammalian tissue [12]. The fact that a knockout of HSL can significantly decrease the rates of fat hydrolysis, lipid synthesis, and adipose metabolism, suggests that HSL plays an important role in these processes [13].

The deposition efficiency of tail fat in fat-tailed sheep such as Tan sheep is higher than that in other parts of the carcass, such as subcutaneous adipose and longissimus dorsi muscle. Meanwhile, in thin-tailed sheep such as Shaanbei fine-wool sheep, the deposition efficiency of tail fat is far lower than that in other parts of the carcass. Therefore, there are likely to be remarkable differences in fat metabolism among different parts within the same breed. In addition, adipose tissue is likely to have biological effects on the "part deposition" in metabolic processes and lead to biodiversity in the animal body. Thus, to compare the distribution differences in the carcass between Tan sheep and Shaanbei fine-wool sheep is significant for theoretical research and practical applications. Many previous studies have paid attention to subcutaneous adipose [14], intramuscular adipose [15], and visceral adipose [16], while studies about tail adipose especially comparative studies of tail adipose, subcutaneous adipose and longissimus dorsi muscle, between fat-tailed and thin-tailed sheep are rare.

In this study, the levels of mRNA transcription and protein expression of PPARγ, FAS and HSL in different parts of carcass between fat-tailed Tan sheep and thin-tailed Shaanbei fine-wool sheep were determined by real-time quantitative PCR and Western blot analyses. In addition, the differences among these levels were

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compared to provide experimental data for the "part deposition" in sheep for further theoretical research.

## 2. Materials and methods

### 2.1. Reagents

All reagents were of analytical grade and of the highest purity commercially available. A PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) and SYBR Premix Ex TaqTM II were purchased from Takara Biotechnology (Dalian, China). ProteoJETTM Mammalian Cell Lysis Reagent was purchased from Fermentas Scientific Molecular Biology Corporation (Fermentas, EU). A Western Lightning ECL Kit was purchased from Perkin Elmer Corporation (Foster City, CA).

### 2.2. Animal treatment

Six Tan sheep from Ningxia Tianyuan Agriculture Science and Technology Development Limited Company and six Shaanbei fine-wool sheep from Shaanxi Dingbian breeding farm aged 9 months were used in this study. The animals were slaughtered according to the National Standard of China (GB 13078-2001 and GB/T 17237-1998) and Agriculture Standards of China (NY 5148-2002-NY 5151-2002). As soon as possible after slaughter, approximately 300 mg samples from tail adipose, subcutaneous adipose and longissimus dorsi muscle were extracted, packed with foil paper, placed in liquid nitrogen, and stored at -80°C until further use.

### 2.3. Design and synthesis of primers

Using Primer 5.0 software, primers for real-time PCR were designed based on the mRNA sequences of the target genes, PPARγ, FAS and HSL, published in NCBI (GenBank), and the β-actin gene as an internal reference. The primers were synthesized at Sangong Biotech (Shanghai, China). The sequences of the primers, annealing temperatures and the lengths of the PCR products are shown in Table 1.

### 2.4. Extraction and reverse transcription of RNA

Total RNA was extracted from the tail adipose, subcutaneous adipose, and longissimus dorsi muscle samples, using Trizol (TaKaRa, Tokyo, Japan), and the concentration and purity of the extracted total RNA were determined with a Maestro Nanomicro-spectrophotometer (MaestroGEN, Las Vegas, NV). Reverse transcription of the total RNA was carried out using the PrimeScript RT Reagent Kit and the products were stored at -20°C until further analysis.

### 2.5. Quantitative PCR

Real-time PCR was performed in a 25-ml reaction system by using SYBR Premix Ex TaqTM II. The PCR cycling conditions were 95°C for 30 s followed by 50 cycles of 95°C for 5 s, 60°C for 34 s and 72°C for 30 s. A melting curve analysis was performed at 95°C for 10 s and 60°C for 1 min, followed by a decrease in the temperature from 60°C to 95°C at a rate of 0.5°C/10 s.

### 2.6. Protein extraction

Total protein was isolated from the frozen tissues using ProteoJET™ Mammalian Cell Lysis Reagent which was added phenylmethanesulfonyl fluoride (PMSF) at 1:100 before use. The protein quantity was determined with the Maestro Nanomicro-spectrophotometer. The protein products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

### 2.7. Western blot analysis

Protein samples (80 μg protein) were separated by SDS-PAGE in a 12% gel using a voltage of 80 V, and then transferred to a polyvinylidene fluoride (PVDF) membrane by electroblotting. Western blotting was performed as follows: The transferred membrane was blocked with 5% bovine albumin blocking agent (BSA) for 2 h, followed by incubation with primary antibodies against PPARγ (MB0080; Bioworld Technology, Beijing, China), FAS (ab22759; Abcam, Hong Kong, China), HSL (sc-25843; Santa Cruz Biotechnology, Beijing, China) and GAPDH (bsm-0978M; Biosynthesis Biotechnology, Beijing, China) for 2 h at room temperature. The membrane was then incubated with appropriate secondary antibodies for PPARγ (CW0102; CWBIOTECH, Beijing, China), FAS (CW0105; CWBIOTECH, Beijing, China), HSL (CW0103; CWBIOTECH, Beijing, China), and GAPDH (CW0102; CWBIOTECH, Beijing, China) for 2 h at room temperature. After washing with phosphate-buffered saline (PBS), the membranes were processed for chemical luminescence with enhanced chemiluminiscent (ECL; Amersham, USA) for 3 min followed by a 1-min exposure to X-ray film. The film was developed and fixed.

### 2.8. Statistical analyses

The experiments were repeated three times, and the mean ± standard deviation was evaluated. Data were analyzed using SPSS software (version 10.1.0, SPSS Science, USA), and values of $P < 0.05$ were considered to indicate statistical significance. The relative expression amounts of the target genes were determined by the $2^{-\Delta\Delta CT}$ method [17,18]. Immunoblotting was analyzed by the optical density values determined by Image J software (Toronto Western Research Institute University Health Network).

**Table 1**
Primer sequences used for RT-PCR.

| Gene | GenBank accession number | Oligos sequences | Product size (bp) | Tm (°C) |
|---|---|---|---|---|
| PPARγ | NM001100921.1 | F: 5′-ACGGGAAAGACGACAGACAAA-3′<br>R: 5′-AAACTGACACCCCTGGAAGATG-3′ | 150 | 62 |
| FAS | NM001012669.1 | F: 5′-CCCAGCAGCATTATCCAGTGT-3′<br>R: 5′-ATTCATCCGCCATCCAGTTC-3′ | 87 | 62 |
| HSL | NM001128154.1 | F: 5′-CTTTCGCACCAGCCACAAC-3′<br>R: 5′-CTCGTCGCCCTCAAAGAAGA-3′ | 136 | 62 |
| β-Actin | NM001009784.1 | F: 5′-TGAACCCCAAAGCCAACC-3′<br>R: 5′-AGAGGCGTACAGGGACAGCA-3′ | 107 | 61 |

F. Forward primers; R. Reverse primers.

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