



## Developmental changes in IGF-I and MyoG gene expression and their association with meat traits in sheep

W. Sun<sup>1,2</sup>, R. Su<sup>1</sup>, D. Li<sup>1</sup>, H.H. Musa<sup>3</sup>, Y. Kong<sup>1</sup>, J.T. Ding<sup>1</sup>, Y.H. Ma<sup>2</sup>, L. Chen<sup>4</sup>, Y.F. Zhang<sup>4</sup> and W.Z. Wu<sup>4</sup>

<sup>1</sup>Animal Science and Technology College, Yangzhou University, Yangzhou, China

<sup>2</sup>Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China

<sup>3</sup>Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan

<sup>4</sup>Animal Science & Veterinary Medicine Bureau of Suzhou City, Suzhou, China

Corresponding author: W. Sun  
E-mail: dkxmsunwei@163.com

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**ABSTRACT.** In the present study, real time-polymerase chain reaction was applied to analyze the expression of IGF-I and MyoG genes in Hu sheep longissimus dorsi at different growth stages and their association with meat traits. Expression of the IGF-I gene in Hu sheep differed significantly between males and females at the two day-old ( $0.01 < P < 0.05$ ), one-month old ( $0.01 < P < 0.05$ ), and three month-old ( $P < 0.01$ ) stages. IGF-I gene expression in male longissimus muscles was higher than that of females at all growth stages, except for the three month-old stage. There was no significant difference ( $P > 0.05$ ) between males and females at any growth stage in expression of the MyoG gene. MyoG gene expression in male longissimus muscles tended to be higher than that of females at all growth stages, except for the six month-

old stage. IGF-I gene expression was significantly and positively correlated with live weight ( $P < 0.01$ ) and carcass weight ( $0.01 < P < 0.05$ ), and was non-significantly positively correlated with net meat weight ( $P > 0.05$ ). In contrast, MyoG gene expression was non-significantly and positively correlated with live weight, carcass, and net meat weight ( $P > 0.05$ ). Carcass traits showed highly significant positive correlations ( $P < 0.01$ ). Furthermore, expressions of IGF-I and MyoG genes showed highly significant positive correlations ( $P < 0.01$ ). We conclude that the expressions of IGF-I and MyoG genes are significantly and positively correlated with early muscle traits of Hu sheep.

**Key words:** Hu sheep; IGF-I; MyoG; Gene expression; Carcass trait

## INTRODUCTION

Because the profitability of sheep production for meat depends largely on lamb weight, selection objectives should concentrate on these traits (Tosh and Kemp, 1994). Quantitative traits are often controlled by multiple genes. Localization of quantitative trait loci can be accomplished by linkage disequilibrium analysis or by adopting a candidate gene approach. Candidate genes are those with known biological functions related to the development or physiology of an important trait (Rothschild et al., 1997). The gene encoding insulin-like growth factor 1 (IGF-1) is seen as a promising candidate gene for marker-assisted selection of growth traits (Zhang et al., 2008). IGF-1 is a mediator of many biological effects; for example, it increases the absorption of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes, increases the synthesis of lipids, stimulates the production of progesterone in granular cells, and intervenes in the synthesis of DNA, protein, RNA, and in cell proliferation (Reyna et al., 2010). In humans, pigs, goats, rats, and chickens, the IGF1 nucleotide sequence is approximately 70-90 kb in length (Shimatsu and Rotwein, 1987; Kajimoto and Rotwein, 1991; Rose, 2002). The exon number of this gene differs among species; goats, pigs, and sheep have 1-6 exons (Mikawa et al., 1995), and humans and rats have 1-55 exons (Rotwein et al., 1986).

The skeletal muscle-specific gene, *myogenin* (*Myog*), is a key developmental regulator of skeletal muscle formation, and is one of the best-studied tissue-specific genes. *Myog* encodes a transcription factor of the basic-helix loop-helix (bHLH) protein family (Meadows et al., 2011). Expression of the *Myog* gene is restricted to skeletal muscle cells, where the transcriptional activator turns on a gene expression program that permits the transition from proliferating myoblasts to differentiating myotubes (Cao et al., 2010). The strict temporal and spatial regulation on *Myog* expression in the embryo makes it an ideal gene with which to study the developmental regulation of tissue-specific expression (Faralli and Dilworth, 2012).

Hu sheep from China is one of the few high-fecundity sheep breeds in the world. It is characterized by early sexual maturity, high fecundity, low lean meat percentage, and low growth rate. Therefore, it is considered as one of the protected sheep breeds in China (Zhang, 2003). Several studies related to tissue expression profiles in domestic sheep

have been conducted recently, with most focusing on the expression of intramuscular fat content. In the present study, we investigated the effect of sex and different growth stages (two day-old, one month-old, two month-old, three month-old, four month-old, and six month-old) on the expression of IGF-I and MyoG genes in longissimus dorsi tissues of Hu sheep.

## MATERIAL AND METHODS

### Experimental animals

The 36 Hu sheep used in this study were reared under the same conditions, and their diets contained adequate minerals and vitamins. Animals were slaughtered at six different growth stages (2 days, 1, 2, 3, 4, and 6 months), including six animals per stage with equal numbers of males and females. Samples were collected from longissimus dorsi muscle tissues, and all tissue samples were immediately frozen in liquid nitrogen after collection and stored at  $-80^{\circ}\text{C}$  until RNA isolation. Live weight, carcass weight, and net meat weight were measured from all animals.

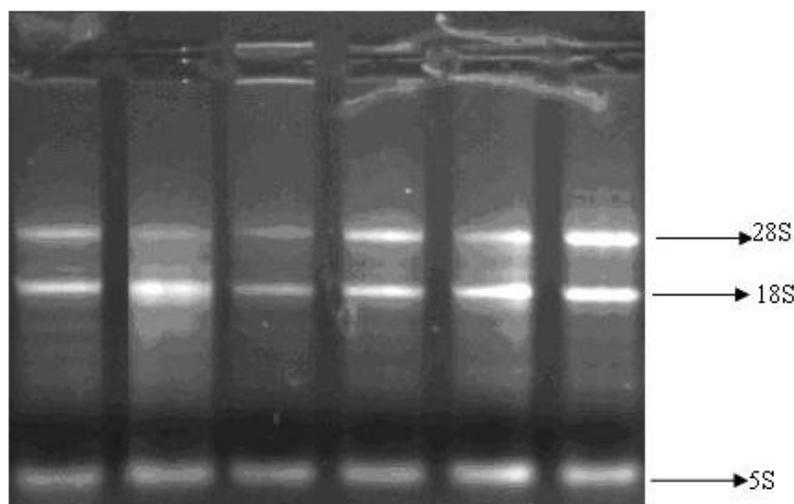
### Real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from longissimus dorsi muscle tissues according to Trizol Regent Kit instructions (Takara Biotechnology Dalian, Co. Ltd., China). The concentration of RNA was measured using a Nano Drop ND-1000 Spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA) with a purity ( $A_{260}/A_{280}$ ) of  $>1.8$ , and total RNA was detected by 1.2% agarose gel electrophoresis (Figure 1). Two hundred fifty nanograms total RNA from each sample were transcribed into cDNA using the reverse transcription kit according to manufacturer instructions. Real-time PCR was performed on the FQ-PCR ABI 7900 system for IGF-I, MyoG, and 18S genes, according to standard protocols with the primers indicated in Table 1. The 18S ribosomal RNA gene (eukaryon) was used as a reference gene to analyze the expression of IGF-I and MyoG genes. Annealing temperatures ranged from  $53^{\circ}\text{C}$ - $63^{\circ}\text{C}$ , and primer concentrations were optimized according to the SYBR Green I kit. The optimal reaction used was 10  $\mu\text{L}$ , containing 0.2  $\mu\text{L}$  each primer, 0.2  $\mu\text{L}$  ROX Reference Dye, 3.4  $\mu\text{L}$   $\text{H}_2\text{O}$ , 5  $\mu\text{L}$  SYBR Green Real Time PCR Master Mix, and 1  $\mu\text{L}$  cDNA template. The PCR conditions were 40 cycles of 15 s at  $95^{\circ}\text{C}$ , 5 s at  $95^{\circ}\text{C}$ , and 30 s at  $60^{\circ}\text{C}$ . For the negative control, 1  $\mu\text{L}$  sterile water (instead of template) was used, and three parallel experiments were run for every sample. The fluorescent signal was analyzed with computer software, and signals were transformed into cycle threshold (Ct) values for IGF-I and MyoG genes.

### Statistical analysis

SPSS 16.0 was used to calculate Ct values and standard errors among replicated samples. Difference in relative gene expression levels were analyzed using the  $2^{-\Delta\Delta\text{Ct}}$  method (Liu, 2007):  $\text{Ct}_{\text{target gene}} - \text{Ct}_{\text{reference gene}}$ . For comparisons of Hu sheep of the same age but different gender, the  $\Delta\Delta\text{Ct}$  was calculated as:  $\Delta\text{Ct}_{\text{male}} - \Delta\text{Ct}_{\text{female}}$ . For comparisons of Hu sheep of the same

sex but different ages, the  $\Delta\Delta Ct$  was calculated as:  $\Delta Ct_{\text{all other stages}} - \Delta Ct_{\text{2 day-old stage}}$ . The  $2^{-\Delta\Delta Ct}$  value represented differential expression of the target gene between the experimental group and control group. Two-factor analysis of variance (ANOVA) was used to measure the interaction between gender and various developmental stages after birth, and no significant interactions were observed either in MyoG ( $F = 1.58$ ,  $P = 0.102$ ) or IGF-I ( $F = 1.22$ ,  $P = 0.306$ ). We next focused on the comparison between genders at the same growth stage and different growth stages within the same gender, respectively. The goal of these analyses was to explore the effect of gender and growth stage on expressions of the MyoG and IGF-I genes. Data of sheep of the same age but different genders were compared with the Student's *t*-test, whereas data of individuals within the same sex but of different ages were compared by one-way ANOVA. The mRNA transcriptional quantity is represented as means  $\pm$  standard error. Furthermore, the  $\Delta Ct$  histogram was used to verify whether  $\Delta Ct$  was negatively related with transcriptional quantity.



**Figure 1.** Agarose gel electrophoresis of total RNA.

**Table 1.** Primer sequence of IGF-I, MyoG, and 18S genes for RT-PCR.

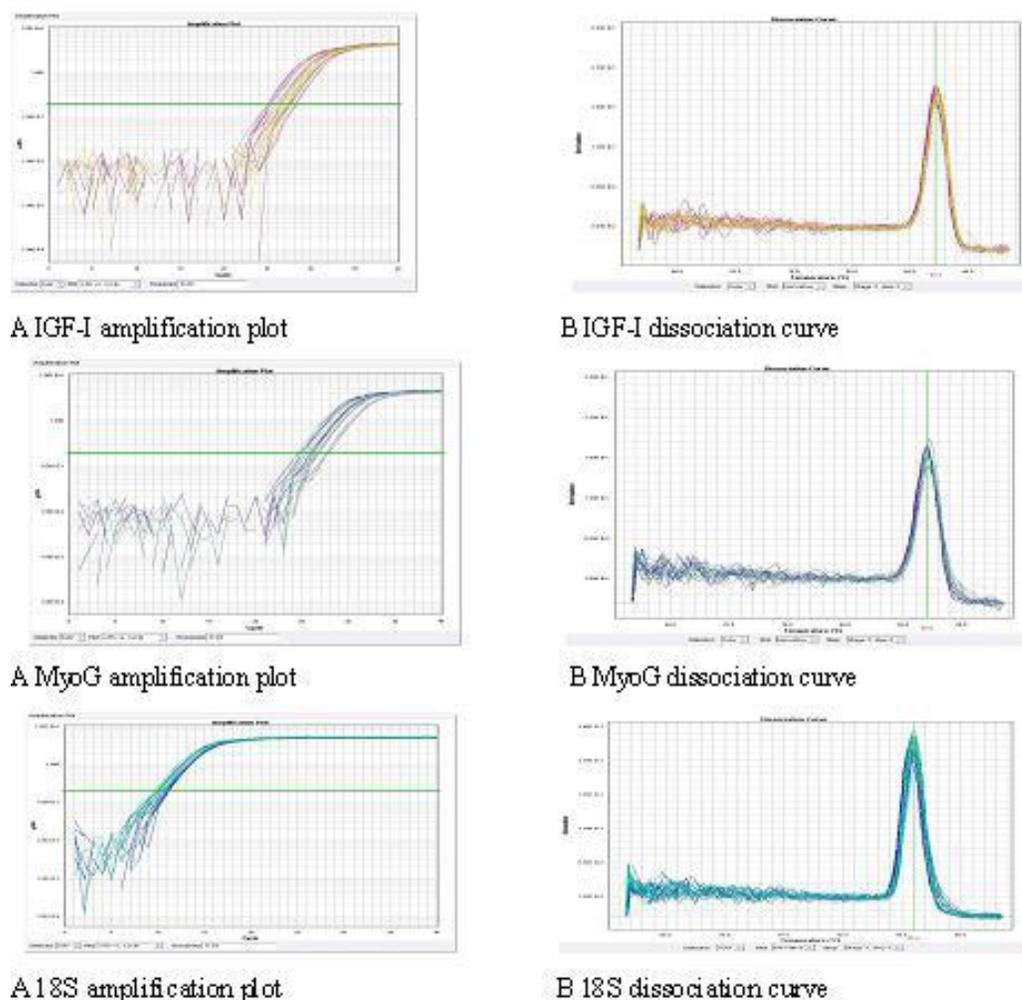
Gene	Reference sequence	Primers (forward primer SF, downstream primer SR)	Products (bp)
IGF-I	M30653	SF: TCCAGTTCGTGTGCGGAGA SR: TCCTCAGATCACAGCTCCGG	126
MyoG	AF433651	SF: AATGAAGCCTTCGAGGCC SR: CGCTCTATGTACTGGATGGCG	101
18S (eukaryon)	AY753190	SF: CGGCTACCACATCCAAGGAA SR: GCTGGAATTACCGCGCT	187

## RESULTS

### Specificity of the amplified fragment

The dissociation curve of IGF-I, MyoG, and 18s PCR products showed a sharp single

peak (Figure 2). The potential for producing primer dimers and non-special products was avoided, since primers were highly specific and the PCR reaction was optimized. The solution temperatures of IGF-I, MyoG, and 18S genes were 86.7°, 87.2°, and 85.0°C, respectively, and no amplification product was generated in the negative control. The efficiencies of IGF-I, MyoG, and 18S gene amplification were 99.7, 99.8, and 99.8%, respectively (Figure 2).



**Figure 2.** Amplification plot and dissociation curve of IGF-I, MyoG, and 18S.

### IGF-I gene expression

The expression of the IGF-I gene in Hu sheep showed a significant or extremely significant difference between males and females at the two day-old ( $0.01 < P < 0.05$ ), one month-

old ( $0.01 < P < 0.05$ ), and three month-old ( $P < 0.01$ ) stages (Table 2). The results of multiple comparisons of different age groups within a sex are shown in Table 3. In females, IGF-I expression differed significantly between the six month-old stage and the four, three, two, one month-old, and two day-old stages ( $P < 0.01$ ). Similarly, expression in four months old females differed significantly with those of three months old and two days old females ( $0.01 < P < 0.05$ ). In males, IGF-I expression differed significantly between the six month-old stage and the four, three, two month-old, and two day-old stages ( $P < 0.01$ ). In addition, one month old males showed significant differences when compared with three months old and two days old males ( $0.01 < P < 0.05$ ). These results suggested that age and sex had significant effects on the expression of the IGF-I gene in sheep muscle tissue.

**Table 2.** Variance analysis of IGF-I expression between males and females in the same developmental stage ( $2^{-\Delta\Delta Ct}$  method).

Gene	Gender	Two days old	One month old	Two months old	Three months old	Four months old	Six months old
IGF-1	Female	1 <sup>m</sup>					
	Male	1.264 ± 0.112 <sup>n</sup>	2.095 ± 0.501 <sup>n</sup>	1.204 ± 0.123 <sup>m</sup>	0.730 ± 0.048 <sup>N</sup>	1.180 ± 0.242 <sup>m</sup>	1.350 ± 0.310 <sup>m</sup>

Serial of M (m) and N(n) shows the results of multiple comparison of the same stage of different sex. Means with the different lower-case within the same column shows that there are significant differences between different rows. Means with the different capital superscripts within the same column shows there are extremely significant differences between different rows.

**Table 3.** Variance analysis of IGF-I expression among different developmental stages in the same sex ( $2^{-\Delta\Delta Ct}$  method).

Gene	Gender	Two days old	One month old	Two months old	Three months old	Four months old	Six months old
IGF-1	Female	1 <sup>Bc</sup>	1.383 ± 0.168 <sup>Bbc</sup>	1.669 ± 0.143 <sup>Bbc</sup>	1.983 ± 0.368 <sup>Bb</sup>	2.060 ± 0.295 <sup>Bb</sup>	3.445 ± 0.598 <sup>Aa</sup>
	Male	1 <sup>Bc</sup>	2.264 ± 0.541 <sup>ABab</sup>	1.599 ± 0.163 <sup>Bbc</sup>	1.033 ± 0.069 <sup>Bc</sup>	1.787 ± 0.366 <sup>Bbc</sup>	3.403 ± 0.780 <sup>Aa</sup>

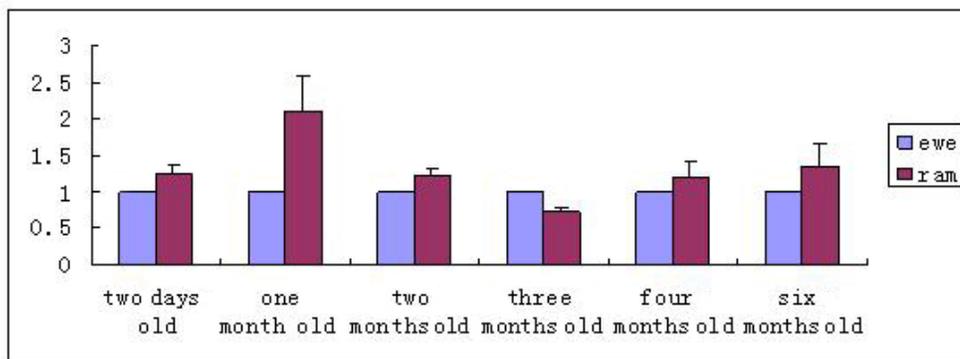
Serial of A(a), B(b) and C(c) shows the results of multiple comparison of same sex at different stages; Means with the different lower-case within the same row shows there are significant difference between different columns. Means with the different capital superscripts within the same row shows there are extremely significant differences between different columns.

IGF-I gene expression in male (rams) longissimus muscles tended to be higher than that in females (ewes) at all growth stages except for the three month-old stage (Figure 3). IGF-I gene expression in female longissimus dorsi gradually increased with the development of their growth, whereas changes in IGF-I gene expression in male longissimus dorsi were unstable (Figure 4).

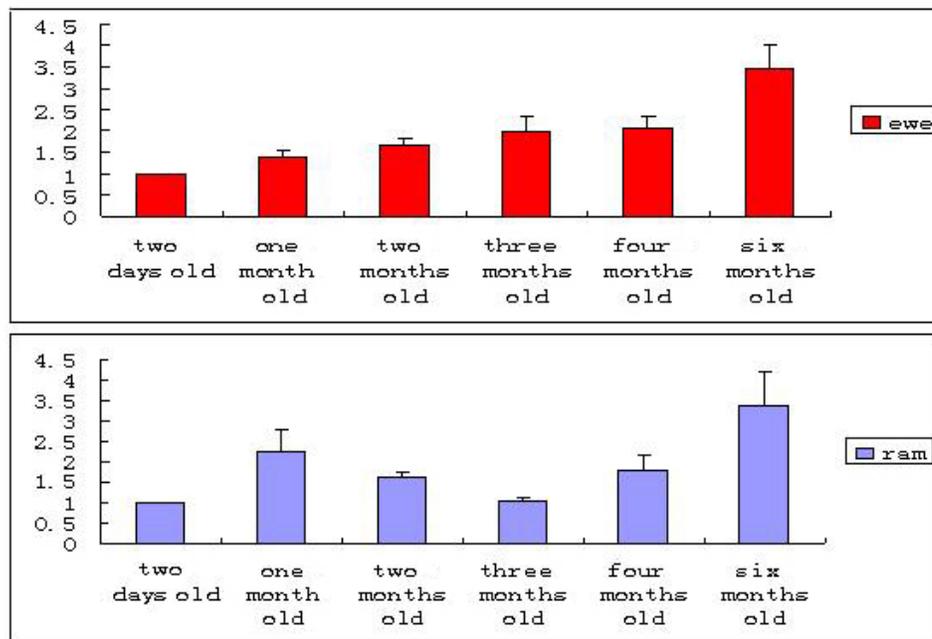
### MyoG gene expression

There was no significant difference ( $P > 0.05$ ) in the expression of the MyoG gene between males and females at any growth stage, although males showed higher MyoG gene expression levels compared to females (Table 4). Multiple comparison analysis of the same sex at different growth stages showed significant differences in the expression of MyoG between females at the six month-old stage with the four, two month-old, and two day-old stages ( $0.01 < P < 0.05$ ), the three month-old stage with the one month-old stage ( $0.01 < P < 0.05$ ),

the four month-old stage with the three month-old and one month-old stages ( $0.01 < P < 0.05$ ), and the two month-old stage with the one month-old and three month-old stages ( $0.01 < P < 0.05$ ). Similarly, extremely significant differences were found when six months old females were compared to two and four months old females, when four months old females were compared to three months old females, and when three months old females were compared to two days old females ( $P < 0.01$ ). The expression of MyoG in males showed extremely significant differences when comparing six months old individuals to two days old individuals ( $P < 0.01$ ) (Table 5).



**Figure 3.** IGF-I gene expression in males and females of Hu sheep at the same age ( $2^{-\Delta\Delta Ct}$  method, ewe for the control group).



**Figure 4.** Changes of IGF-I expression among Hu sheep different developmental stages in the same gender ( $2^{-\Delta\Delta Ct}$  method, 2-day for the control group, A = ewe, B = ram).

**Table 4.** Variance analysis of MyoG expression between males and females in the same developmental stage ( $2^{-\Delta\Delta C_t}$  method).

Gene	Gender	Two days old	One month old	Two months old	Three months old	Four months old	Six months old
MyoG	Female	1 <sup>m</sup>					
	Male	1.437 ± 0.338 <sup>m</sup>	1.096 ± 0.277 <sup>m</sup>	1.235 ± 0.238 <sup>m</sup>	1.075 ± 0.201 <sup>m</sup>	1.536 ± 0.257 <sup>m</sup>	1.013 ± 0.138 <sup>m</sup>

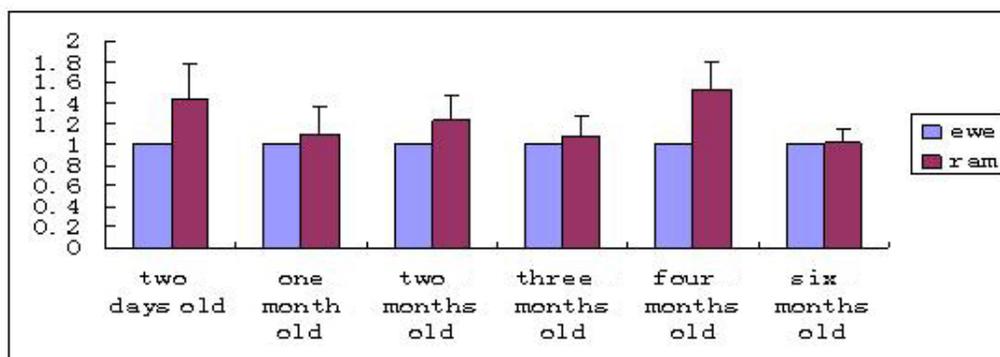
Serial of M(m), N(n) shows the results of multiple comparison of same stage of different sex. Means with the different lower-case within the same column shows that there are significant differences between different rows. Means with the different capital superscripts within the same column shows that there are extreme significant differences between different rows.

**Table 5.** Variance analysis of MyoG expression among different developmental stages in the same sex ( $2^{-\Delta\Delta C_t}$  method).

Gene	Gender	Two days old	One month old	Two months old	Three months old	Four months old	Six months old
MyoG	Female	1 <sup>Bc</sup>	1.731 ± 0.124 <sup>ABab</sup>	1.381 ± 0.084 <sup>ABbc</sup>	2.053 ± 0.505 <sup>Ab</sup>	0.984 ± 0.129 <sup>Bc</sup>	2.276 ± 0.226 <sup>Aa</sup>
	Male	1 <sup>Bb</sup>	1.809 ± 0.457 <sup>ABab</sup>	1.634 ± 0.315 <sup>ABab</sup>	1.380 ± 0.258 <sup>ABab</sup>	1.379 ± 0.231 <sup>ABab</sup>	2.161 ± 0.295 <sup>Aa</sup>

Serial of A(a), B(b) and C(c) shows the results of multiple comparison of same sex at different stages; Means with the different lower-case within the same row shows that there are significant difference between different columns. Means with the different capital superscripts within the same row shows that there are extremely significant differences between different columns.

MyoG gene expression levels in male longissimus muscles tended to be higher than those of ewes at all growth stages, except for the six month-old stage (Figure 5). However, the changes in MyoG gene expression in female and male longissimus dorsi were unstable (Figure 6).

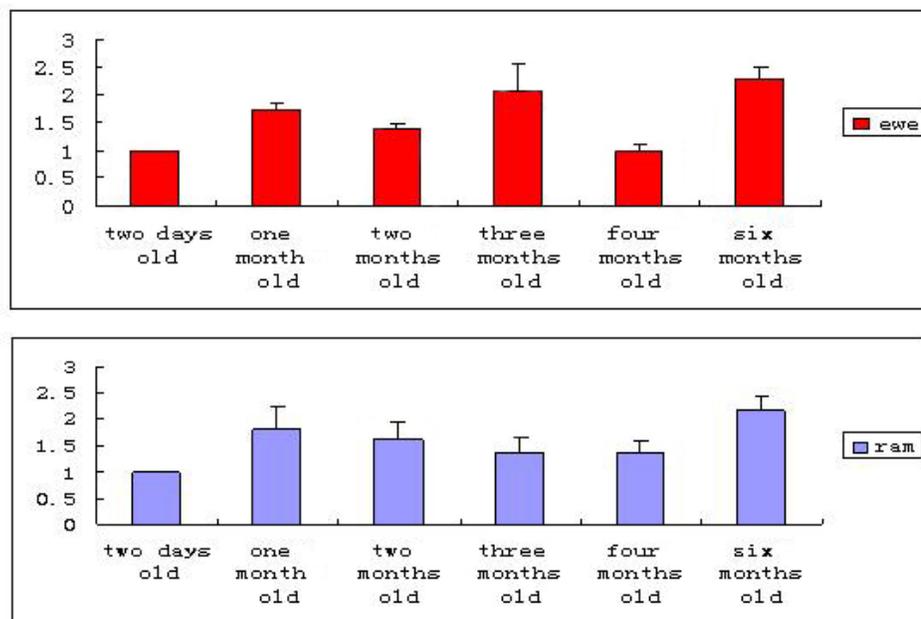


**Figure 5.** MyoG gene expression in males and females of Hu sheep in the same growth stage ( $2^{-\Delta\Delta C_t}$  method, ewe for the control group).

### Association of IGF-I and MyoG gene expressions with carcass traits

IGF-I gene expression was significantly and positively correlated with live weight ( $P < 0.01$ ) and carcass weight ( $0.01 < P < 0.05$ ), and was non-significantly but positively correlated with net meat weight ( $P > 0.05$ ). In contrast, MyoG gene expression was non-

significantly and positively correlated with live weight, carcass weight, and net meat weight ( $P > 0.05$ ). An extremely significant positive correlation was found among the carcass traits ( $P < 0.01$ ). Furthermore, there was an extremely significant positive correlation between the expressions of IGF-I and MyoG genes ( $P < 0.01$ ) (Table 6).



**Figure 6.** Changes of MyoG expression among Hu sheep different developmental stages in the same gender ( $2^{-\Delta\Delta Ct}$  method, 2 days for the control group, A = ewe, B = ram).

**Table 6.** Association of IGF-I and MyoG genes expression with carcass traits.

Index	IGF-I	MyoG	Live weight	Carcass weight	Net meat weight
IGF-I	1	0.464**	0.450**	0.409*	0.247
MyoG	0.464**	1	0.249	0.246	0.083
Live weight	0.450**	0.249	1	0.993**	0.824**
Carcass weight	0.409*	0.246	0.993**	1	0.848**
Net meat weight	0.247	0.083	0.824**	0.848**	1

\*There is significant relationship between two different index ( $0.01 < P < 0.05$ ). \*\*There is extreme significant relationship between two different index ( $P < 0.01$ ).

## DISCUSSION

IGF-I is an important regulatory factor for body growth, development, and metabolism. IGF-I mRNA expression in longissimus dorsi of pigs showed no significant difference ( $P > 0.05$ ) between males and females (Xu, 2002). In the present study, with the exception of the three month-old stage, IGF-I was more highly expressed in male longissimus dorsi compared to females at different growth stages after birth. This may indicate that IGF-I has a greater effect on early sheep muscle growth in males than it does in females. Gerrard et al. (1998) demon-

strated that IGF-I mRNA expression increased gradually from the 44th day of pregnancy to the late pregnancy stage in pig fetal semitendinosus muscles, further increased after birth, reaching a peak at 21 days old, and then declined in adulthood. Gotz et al. (2001) showed that the expression levels of IGF-I were similar in pig skeletal muscle and in blood from 11 to 22 weeks old. Xu (2002) showed that the expression of IGF-I did not change significantly from birth to one month old in the longissimus dorsi of both Erhualian and Yorkshire pigs, and then significantly increased from the one month old to the three month-old stage, and remained at a high level. Gu et al. (2009) found that the expression of IGF-I increased gradually after birth in both Landrace and Meishan pigs. Huang and Xie (2009) found that the expression of IGF-I showed large fluctuations in Xinjiang fine-wool sheep; it slightly declined from the two day-old to the one month-old stage, reached a peak at two months-old, and then dropped to the lowest level before increasing again over the subsequent growth stages. In the present study, the expression of the IGF-I gene in ewes' longissimus dorsi gradually increased with growth development and reached its peak at six months old, confirming results obtained by Gu et al. (2009). In agreement with Huang and Xie (2009), the expression of IGF-I in rams increased during the first two stages, dropped to the level of the two days-old stage at three months old, and then gradually increased thereafter in the following stages, reaching its peak at the six months-old stage. IGF-I functions to increase amino acid utilization during protein synthesis and inhibits protein degradation, and thus, accordingly, promotes the proliferation of bone and muscle cells. Much research has been conducted recently regarding the correlation between polymorphisms of the IGF-I gene with carcass traits of poultry and pigs. Although several studies have investigated the expression of IGF-I in sheep muscle, few have evaluated the correlation between this expression with different developmental stages and carcass traits. In the present study, the relative expression of the IGF-I gene showed an extremely significant positive correlation with live weight ( $P < 0.01$ ) and with carcass weight ( $0.01 < P < 0.05$ ).

Myogenin (MyoG) is an important transcription factor that regulates skeletal muscle development, and is expressed in all skeletal muscle cells in the MyoD family. Genetic variation of MyoG might be related with myogenesis, and it could ultimately lead to variations in meat quality. Yablonka-Reuveni and Paterson (2001) noted that in chicken embryo-type myoblast cultures, MyoG appeared to be terminally differentiated, while in adult-type myoblast cultures, MyoG was expressed in early myogenic cell lineages. In the present study, the expression of MyoG in rams' longissimus dorsi was non-significantly ( $P > 0.05$ ) higher than that in ewes after birth. In contrast, extremely significant ( $P < 0.01$ ) or significant ( $0.01 < P < 0.05$ ) differences were observed between males and females at the same growth stage in Erhualian pigs and Hu sheep (Yang et al., 2006; Sun et al., 2010). The reason for these different results might be related to the choice of experimental methods. Yang et al. (2006) and Sun et al. (2010) both used the semi-quantitative gray scale analysis method, while we used the  $2^{-\Delta\Delta Ct}$  method to analyze relative expression differences. Shan et al. (2009) investigated MyoG mRNA expression in longissimus dorsi muscles of Jinhua and Landrace pigs, and found that the expression of the MyoG gene increased with age and lean meat percentage of the carcass. In contrast, Hasty et al. (1993) found that the expression of the MyoG gene decreased with age and lean meat percentage of the carcass in Landrace pigs, which were negatively correlated, but did not display breed differences. In the present study, the expression of the MyoG gene was non-significantly ( $P > 0.05$ ) and positively correlated with live weight, carcass, and net meat weight.

During the skeletal muscle growth process, IGF-I regulates the growth of skeletal muscle and stimulates myoblast terminal differentiation by inducing the expression of MyoG (Xue, 2004). In the present study, we found that the expression of IGF-I and MyoG genes were positively correlated, and the expression of these two genes in muscle affected carcass traits. This study could provide a theoretical basis for further research and provides genetic data for Hu sheep carcass trait breeding.

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