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The Expression of Myogenin and Myostatin Genes in Baluchi Sheep

Research Article

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ABSTRACT

Myogenin gene (MYoG) affects the synthesis of muscle myofibrillar growth and increase of meat production. The myostatin (MSTN) gene is identified as a specific negative regulator of skeletal muscle growth. Reduction of the expression level of MSTN through mutation in the sequence of this gene leads to an increase of myogenesis and regeneration of muscle cells during the postnatal growing period of sheep. The Baluchi sheep are among the most popular breeds of sheep for breeding in Iran and have an important portion in meat production industry of the country. In present work the relative expression level of the two candidate genes have been studied at two age intervals (9 and 12 months) in male and female Baluchi sheep. In order to analyze the relative expression level of MYoG and MSTN gene in Baluchi sheep's longissimus muscles, quantitative real-time polymerase chain reaction (qRT-PCR) reaction has been applied. Results of RT-PCR for sex effect showed MSTN and MYoG expression were not highly expressed in ram' longissimus dorsi compared to ewe at the same age stages (P>0.05) and there were no significant differences between male 12 months comparing to female 12 months (P>0.05) and male 9 months with female 9 months (P>0.05). For the effect of age, relative expression of MYoG and MSTN genes on Baluchi sheep longissimus muscles did not show significant differences between males or females (P>0.05).

KEY WORDS Baluchi, gene expression, myogenin (MYoG), myostatin (MSTN), RT-PCR, sheep.

INTRODUCTION

The Baluchi sheep is one of the popular breeds in eastern Iran and southwest Pakistan and has an important portion in meat production industry of Iran with a body size varies between 35-38 kg in adult ewes and 45-48 kg in rams (Yazdi *et al.* 1997). The mayogenin gene (MYoG) is a positive regulator and a member of myogenic differentiation (MYoD) genes (MYoG, MYF-5, MYF-6 and MYoD1). The MYoG is known as myogenic factor 4 and has direct effect on myogenesis in skeletal muscle myofibril. MYoG is effective on synthesis and myogenesis of skeletal muscles's myofibrils (Zhihong *et al.* 2009; Femanda *et al.* 2013). MYoG is closely associated with the number of muscle fibers at birth time, which is most important in determination of maximal lean meat growth capacity in pigs (Handel and Stickland, 1988). The myogenesis synthesizes myofibrillar proteins in the skeletal muscles and regulates the number of their myofibers. It has positive effect on meat percentage and number of myofibrils (Sun *et al.* 2012). The myostatin (MSTN) gene has negative direct effect on development and regeneration of the skeletal muscles. The gene is known as a negative regulator. The MSTN protein is one of the transforming growth factor-β (TGF-β) superfamily that includes a group of development, differentiation and growth factors. The gene is an important inhibitor on the embryonic development and meat percentage (Lin *et al.* 2002).

MSTN involved in early birth, maturation age for improvement and development of skeletal muscles. MSTN gene also known as growth / differentiation factor 8. Myostatin in early birth, growth and maturation age for improvement many of muscles (Florent *et al.* 2007). The gene production (i.e. MSTN protein) inhibits growth of the animal and mediates the formation of muscle fiber. It worth mentioning that feeding and situation of rearing have direct effect on the gene expression at growing stages (Yingying *et al.* 2015). During the life of the animal the gene can be active or deactive (Kobolok *et al.* 2002). Therefore, this study was aimed to investigate the relative expression level of MSTN and MYoG genes in male and female Baluchi sheep's longissimus muscles at two age intervals (9 and 12 months) .

MATERIALS AND METHODS

Animals and tissue collection

A number of 18 (9 male and 9 female) nine months old Baluchi lambs with initial body in rams (29±0.21 kg) and female (21±0.35 kg) reared for 3 months with the same conditions. They were fed adequately and their diet were assigned with vital nutrients and important supplements for growing period (Sun *et al.* 2012). Samples were taken from the dorsi muscles tissues of the lambs at the start and end of experimental period. The animals were locally anesthetized by the injection of 0.06 mg/kg of Xylazine and then 2 cm incision has been made at the point of injection to separate skin from meat tissues. All tissue samples were immediately frozen in liquid nitrogen tank and stored until RNA isolation (Sun *et al.* 2012).

RNA isolation and quantitative real-time PCR

Total RNA was extracted from muscle tissue using Trizol reagent according to Trizol Regent Kit instructions (Qiagen, TRI Reagent, RNeasy Plus Mini Kit). The concentration of total RNA isolated was determined by absorbance at 260 nm and purity (A260/A280) of > 1.8. The first strand complementary DNA (cDNA) was synthetized from 5 µg of total RNA using Cinnagen reverse transcript kit (Cinnagen, Tehran, Iran). After reverse transcription analysis of gene, expression of myostatin and myogenin were performed through qRT-PCR reaction by making use of Eppendrof apparatus (Applied Biosystem Inc). Total volume of PCR reaction system was 20 µL including 10 PCR-master mix (2X) of Cinnagen, 1 µL cDNA, 1 µL primer (10 pmol) and 8 µL dH_2O. PCR condition for myogenin and myostatin genes were as following: The real-time PCR reaction was carried out for 1 cycle 15 min at 95 ˚C, 40 cycle 25 sec at 95 ˚C and 40 cycle 60 sec at 62 ˚C. A housekeeping gene RPL19 was used as normalizing control. The primers were design by primer express software (Applied Biosystems) (Table 1). After amplification, 6 µL of each PCR product was analyzed on agarose gel electrophoresis 2% and vitalized with red gel (Sigma) under UV light (Figure 1).

Statistical analysis

Difference in relative gene expression levels were analyzed using the Ct value and standard error between samples (Joshua *et al.* 2006). Myogenin and myostatin genes were analyzed based on ΔCt as Ct (MYoG/MSTN gene)-Ct (RPL19 gene). Gene expression data were expressed as means ± SE.

Two-factor analysis of variance ANOVA ($P<0.05$) was used to measure the interaction between gender (ΔCt 12 month female–ΔCt 9 month female), (ΔCt 12 month male–ΔCt 9 month male) (Figure 2) and various developmental stages after 6 months (ΔCt male–ΔCt female), (ΔCt 12 mo male–Ct 12 mo female), (ΔCt 9 mo male–Δ Ct 9 mo female) and no significant interactions were observed in MYoG and MSTN (Figure 3).

Myostatin expression

The expression of the MSTN gene in Baluchi sheep showed no significant difference between males and females at total number of animals ($P>0.05$), 9 months of age and 12 months of age (Figure 3) and showed no significant difference between male animals and between female ones at 12 months age ($P>0.05$), (Table 2, Figure 2).

This shows that MSTN gene expression in male ram's longissimus muscles was not higher than the females at two ages (Figure 3). In addition, 12 month old males showed no significant differences when compared with 12 months old females ($P>0.05$).

Myogenin expression

The expression of the MYOG gene in Baluchi sheep showed no significant difference between males and females at the total animals ($P>0.05$), 9 mo of age and 12 mo of age (Figure 3) and no difference between male and female at 12 mo of age ($P>0.05$), (Tables 3 and 4, Figure 2).

RESULTS AND DISCUSSION

The myostatin gene is a regulator factor in the muscle that causes to achieve highest amount of muscle mass. The gene is expressed in many tissues. If the MSTN gene undergoes mutation, the negative regulating function of the gene does not work (Kobolok *et al.* 2002). Myogenin (MYoG) is a transcription factor that has direct effect on skeletal muscle which means that with expressed MYoG gene the muscle mass increases. The variations of MYoG gene may be relative to myogenesis process and cause variations in muscle quality (meat percentage).

Table 1 Sequence of real-time PCR primers used in this study

Gene	Gen bank accession No.	Forward primer	Reverse primer	Product size (bp)
Myogenin	443185	GGAGAAGCGCAGACTCAAGAAG	CTATGGGAGCTGCATTCACTGG	231
Myostatin	443449	ATCCGATCTCTGAAACTTGACAT	AGTCCTTCTTCTCCTGGTTCTG	182
RPL19	100270789	AGCCTGTGACTGTCCATTCC	ACGTTACCTTCTCGGGCATT	126

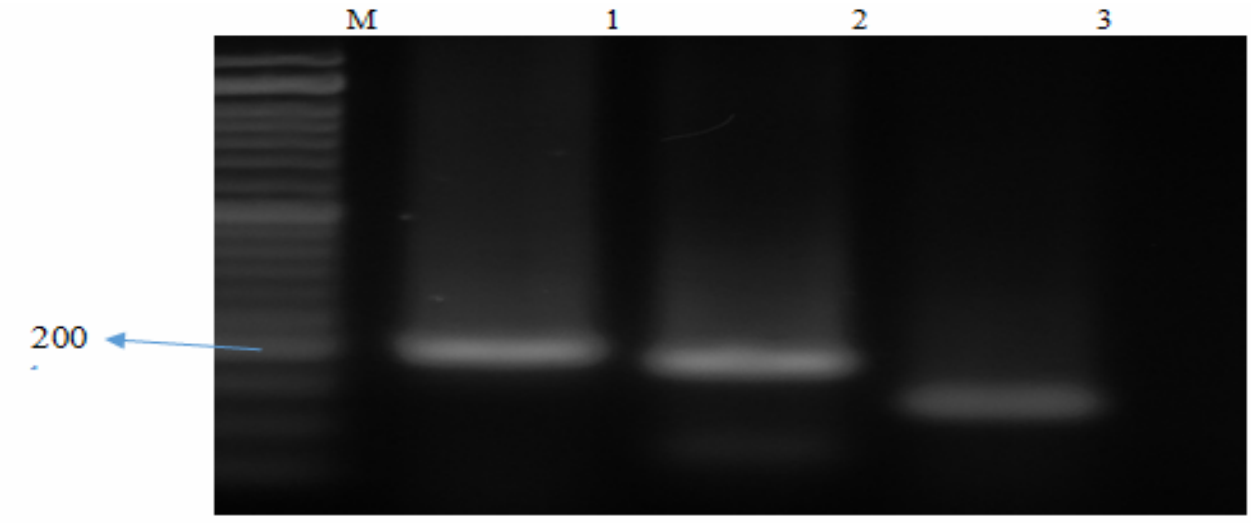

Figure 1 Agarose gel electrophoresis of 1) MSTN, 2) MYoG and 3) RPL19 gene
M: molecular ladder

Table 2 Variance analysis of MSTN expression among different developmental stages in the same sex ($2^{-\Delta\Delta}$Ct)

Gene	Gender	Total	9 mounts old	12 months old
MSTN	Female	1	1	1
	Male	0.41±0.17	0.42±0.08	0.63±0.41

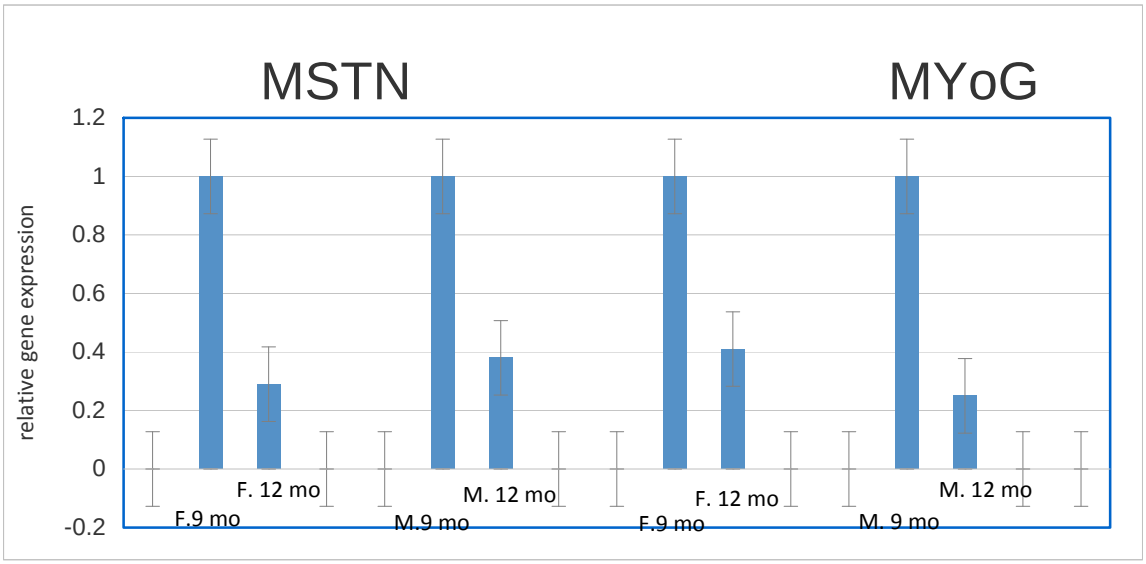

Figure 2 MYoG and MSTN genes expression in 12 month of age of Baluchi sheep in male and female sex ($2^{-\Delta\Delta}$Ct) method, 9 months for the control group)

There was no significant difference (P>0.05) between males and females at two growth stages (9 and 12 months old) in expression of the MYoG gene.

Yang *et al.* (2006) determined the developmental changes of MSTN and MYoG genes expression in longissimus dorsi muscle of Erhualian and Large white pigs.

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

Gene	Gender	Total	9 mounts old	12 months old
MYoG	Female	1^{m}	1^{m}	1^{m}
	Male	0.86±0.16	0. 51±0.587	0. 11±0.07

Table 4 Variance analysis of MSTN and MyoG expression among different developmental stages in the same sex ($2^{-\Delta\Delta}$Ct method)

Gene	Gender	9 months old	12 months old	Gene	Gender	9 months old	12 months old
MSTN	Male	1	0.38±0.09	MYoG	Male	1	0. 25±0.01
	Female	1	0. 29±0.04		Female	1	0. 41±0.02

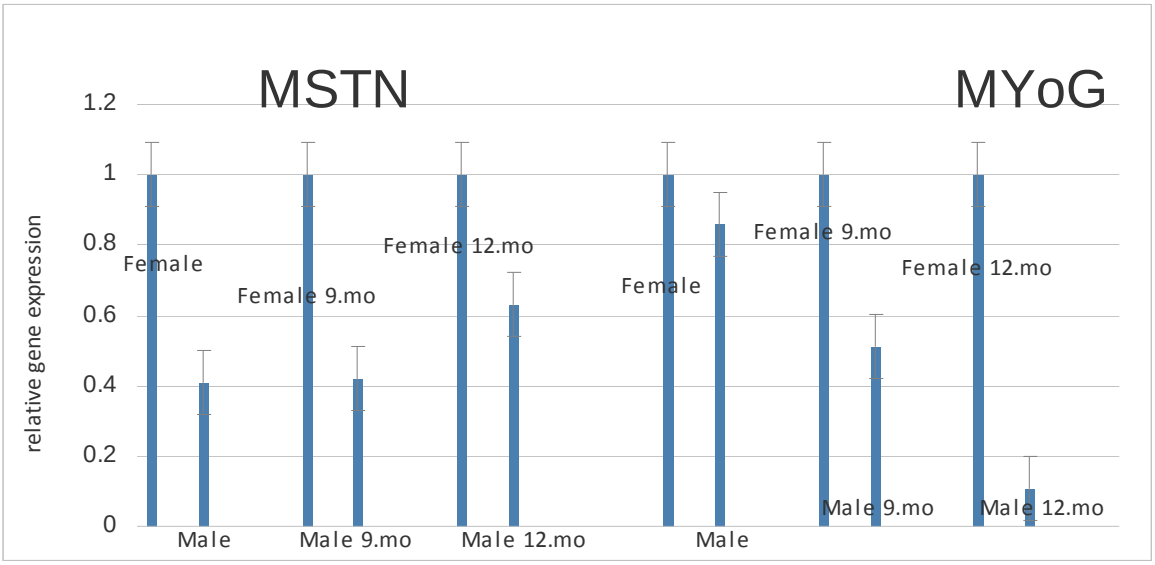

Figure 3 MYoG and MSTN genes expression in males of Baluchi sheep in the same growth stage (12 and 9mo) and total population ($2^{-\Delta\Delta}$Ct method, ewe for the control group)

They indicated that risen level of myogenin and myostatin genes expression might have important regulation's effect on maturation of myofibrils during postnatal stage and no observed meat percentage with changing age. In the present study, the results for MSTN and MYoG genes expression is similar to Yang *et al*. (2006). Hasty *et al*. (1993) reported that the expression of the MYoG gene has negative relation with lean meat percentage and age in Landrace pigs and breed had no effect on the result and the result of (Sun *et al*. 2012) showed that the expression of MYoG in male sheep' longissimus dorsi muscle was non-significantly (P>0.05) higher than female after birth. Both of these results are similar to our results about comparison of MYoG expression for between 12 and 9 mo of age at the same sex (P>0.05). The variation in MSTN and MYoG mRNA levels were related neither to age nor to age of the animals within the ranges covered in the study. In the present study, with the comparison of the male and female, myostatin and myogenin expression were not highly expressed in ram' longissimus dorsi compared to ewe at different growth stages after 3 months, it seems sex and age has no direct effect on MSTN and MYoG expression in this breed.

Shibata *et al*. (2006) showed that myostatin gene could increase adipogenesis in muscle marbling, and that the myostatin and MRF genes might have an effect at an early stage of skeletal muscle regeneration. 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*. 2012). (Shan *et al*. 2009) reported the MYoG expression in longissimus dorsi muscle (LDM) of Jinhua pig and Landrace pig (35, 80, 125 days of age) and determined that with an increase in the age of pigs the expression of the myogenin gene increased and caused to increase lean meat percentage, the expression of myogenin and meat percentage had positive correlation, in contrast the expression of MYoG gene with an increase in age decreased and lean meat percentage in Landrace pigs and observed negative correlation in the expression of MYoG and muscle percentage in Landrace breed. Results of MYoG expression in longissimus dorsi muscle of Baluchi sheep breed were different from MYoG mRNA expression LDM Jinhua pig. (Su *et al*. 2014) determined association between IGF-I and DLK1 gene expression and meat quality in Hu sheep.

Results showed that just growth stage have significantly affected IGF-I and DLK1 expression (P<0.01) while sex had no effect on meat quality. (Su *et al*. 2014) suggested that in Hu sheep different growth ages had significant effect on DLK1 and IGF-I relative gene expression in Hu sheep's muscle.

In another research (Sun *et al*. 2014) analyzed developmental changes in myogenin and IGF-I gene expression and their association with meat traits in Hu sheep. Compression expression of the myostatin gene in Hu sheep were significantly different between rams and ewes at the 2 day (P<0.05), 1 month (P<0.05) and 3 month (P<0.01) ages.

Myostatin gene expression in rams longissimus muscles was higher than that of ewes at all ages (P<0.05), except for the 3 month age (P>0.05) and there were no significant difference (P>0.05) between rams and ewes at any age in expression of the myogenin gene. The result of (Su *et al*. 2014) and (Sun *et al*. 2014) are different from our results about MSTN gene expression in Baluchi sheep and about myogenin gene expression, the result of (Sun *et al*. 2014) were similar .

CONCLUSION

The expression of the myogenin gene was generally equal in male Baluchi sheep at 12 and 9 months of age. Similar expression of the myostatin and myogenin in male and female Baluchi sheep at 12 mo of age, showed a new expression pattern of these genes between different sexes which may indicate the regulatory role of contribution between MSTN and MYoG gene expression on skeletal muscle formation. While in other researches MSTN and MYoG genes have positive correlation, and the expression of these two genes in myofibrils have direct effect on meat percentage, in our results about direct effect of expression of these genes on growth stages and sex of sheep, the differences might be related to the experimental situation and selection of ages and measurement methods may be the reason of these difference. We guess that the change in algorithms of body in higher age in adult sheep (9-12 mo) inclines to save fat in adipocyte instead of increasing myofibrils (Yingying *et al*. 2015). Many important genes have been discovered to be involved in controlling meat traits in livestock like MSTN and MYoG genes which can subsequently affect other gene expression and change their expression in comparison. These results about MSTN and MYoG gene expression of Baluchi sheep can provide a theoretical basis for further research and provide genetic data for Baluchi sheep. The results of present study can also be helpful to provide information for other genes that affect the breeding strategies for meat percentage and carcass traits.

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