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Polymorphism of MyoG Gene in Sheep and Its Association with Meat Quality Traits

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ABSTRACT

Background: At present, our mutton production cannot keep up with the needs of consumers. This study aims to analyze the polymorphism of intron II in the MyoG gene of sheep and investigate its relationship with production traits. It hopes to provide some theoretical research on the supply of mutton in China to serve the production of mutton.

Methods: Five breeds of sheep, including Small-tailed Han sheep, Large-tailed Han sheep, Yuxi fat-tailed sheep, Mongolian sheep and Lanzhou Large-tailed sheep, were selected as the subjects. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used to genotype the intron II of the MyoG gene. Meat samples were collected from the back of the sheep to determine drip loss, shear force, moisture content, cooking loss and colour. The genetic diversity of the MyoG intron II in the sheep population was calculated using Popgene software and the association between different genotypes and sheep growth traits was analyzed using SPSS 17.0 software.

Result: The results showed that both Small-tailed Han sheep and Lanzhou Large-tailed sheep had three genotypes in the MyoG intron II: AA (368, 540 bp), AB (908, 368, 540 bp) and BB (908 bp). Large-tailed Han sheep, Yuxi fat-tailed sheep and Mongolian sheep had two genotypes in the MyoG intron II: AB (908, 368, 540 bp) and BB (908 bp). The association analysis showed that individuals with the AA and BB genotypes had significantly higher moisture content in their meat than individuals with the AB genotype ($p<0.05$). The cooking loss in the meat of individuals with the AB and BB genotypes was significantly higher than that of individuals with the AA genotype ($p<0.05$). The colour of the meat in individuals with the AA genotype and AB genotype was significantly higher than that in individuals with the BB genotype ($p<0.05$). In conclusion, the polymorphism studied provides valuable information for improving the production performance of sheep.

Key words: Association analysis, MyoG Gene, Polymorphism, Production trait, Sheep.

INTRODUCTION

Muscle mass is determined by the quantity and dimensions of muscle fibers, as well as the number of cells within the muscle tissue. The MyoD gene plays a critical role in muscle growth; its suppression or withdrawal leads to the immediate cessation of myoblast division and differentiation. Myogenin (MyoG) is an essential gene regulating muscle growth and development, with profound theoretical implications for practical applications in production systems.

Myogenic regulatory factors (MRFs) are nuclear phosphoproteins characterized by conserved structural features, including a basic amino acid-enriched domain and a helix-loop-helix (HLH) motif. The basic region is responsible for specific DNA recognition and binding, while the HLH motif facilitates heterodimer formation. These heterodimers bind to promoter or enhancer sequences upstream of muscle-specific genes, thereby conferring the capacity to effectively promote skeletal muscle development (Jia *et al*., 1992; Piette *et al*., 1990). As a key member of the MRF family, MyoG serves as a critical regulator of myoblast differentiation by modulating the expression of muscle-specific proteins such as myosin light chain, functioning as a positive regulator in skeletal muscle development (Francetic and Li, 2011). Its role cannot be supplanted by other MRFs, as it is indispensable from muscle fiber formation to the functional and structural maturation of the organism (Hughes, 1999). Furthermore, MyoG not only activates the transcription of muscle-specific genes but also enhances cellular differentiation (Sabourin *et al*., 1999). Studies employing distinct shRNA constructs revealed variable silencing efficiencies for myostatin in goat fetal fibroblasts, ranging from 55.1% to 91.5% ($p<0.01$), accompanied by differential upregulation of myogenic genes. Notably, MyoD expression increased by 7.97% to 111.67%, MyoG by 77.0% to 319.47% and Myf5 by 16.67% to 138.0%. Pearson correlation analysis revealed a negative association between myostatin and the investigated genes, highlighting their regulatory interactions (Borah *et al*., 2018).

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The MyoD gene family encodes four myogenic transcription factors: MyoD, MRf4, Myf5 and MyoG. Among these, MyoG holds particular significance as it begins exerting its effects during fetal development. The MyoG gene plays a pivotal role in muscle differentiation by orchestrating the fusion of myoblasts and the formation of muscle fibers. Numerous studies have explored the role of MyoG employed polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis to investigate polymorphisms at the Msp I site in the 3′ end of the MyoG gene in some pig breeds (Zhu and Li, 2005). Their results demonstrated that the N allele significantly increased the cross-sectional area of the longissimus dorsi muscle and the proportion of lean meat, which contributed to increased meat yield, reduced subcutaneous fat deposition and enhanced carcass quality. Deng analyzed the influence of the MyoG gene on mutton production traits by leveraging sequence information from other species available in GenBank (Deng, 2006). Through PCR-SSCP analysis and sequence comparison, they amplified, cloned, sequenced and examined the polymorphism of previously unreported portions of the MyoG gene in sheep. Their findings revealed that the known MyoG gene sequence in sheep exhibited a GC content of 58.6%, with exons possessing a significantly higher GC content (65.4%) compared to introns. Comparative analysis of exon sequences across species showed homologies of 99.22%, 91.31%, 89.41% and 87.51%, indicating a high degree of conservation. Chu designed two primer sets and employed PCR-SSCP technology to examine the polymorphisms of MyoG gene exons in four sheep breeds (Chu *et al.,* 2005). Polymorphisms were detected with the second primer set, revealing three genotypes: AA, AB and BB. Among these, the AA genotype exhibited the highest frequency and the frequency of the A allele was markedly higher than that of the B allele. The MyoG gene has been extensively studied and mapped to chromosome 9 in pigs (Ernst *et al.,* 1998). It has been confirmed to influence carcass traits, growth traits (Xue and Xu, 2007) and reproductive traits (Zhao *et al.,* 2005). Furthermore, preliminary analyses of polymorphisms in partial sequences of the MyoG gene have been conducted in goats, sheep and quails (Bai *et al*., 2020a, 2020b; Gao *et al.,* 2009; Liu *et al.,* 2009). Currently, domestic mutton production cannot meet the growing consumption demands. Research on the MyoG intron II gene in sheep aims to provide theoretical insights to support mutton supply and facilitate the production needs of the sheep industry in China.

Materials and Methods

The experimental animals included 32 Small-Tailed Han Sheep, 38 Large-Tailed Han Sheep, 38 Yuxi Fat-Tailed Sheep, 36 Mongolian Sheep and 32 Lanzhou Large-Tailed Sheep. Blood samples were collected *via* jugular venipuncture using ACD as an anticoagulant. Samples were transported at low temperature in an ice container to the laboratory and stored at -20°C for genomic DNA extraction. This experiment was completed in the Laboratory of Animal Genetics in 2023.

The primers for amplifying the MyoG gene intron II were derived from Liu *et al*. (2011), with a product size of 908 bp. The sequences of the primers were as follows: forward primer, 5′-TCTTCCCTCTTCCCTTATTC-3′; reverse primer, 5′-GTCCCTTGCTTTATCTCC-3′. The primers were synthesized by Shanghai Sangon Biotech Co., Ltd.

The PCR amplification was performed in a 15 µL reaction mixture, which consisted of 1.0 µL of DNA, 0.6 µL of forward primer, 0.6 µL of reverse primer, 5.3 µL of deionized water and 7.5 µL of 2×Taq PCR Green Mix. The PCR conditions were as follows: pre-denaturation at 94°C for 4 min; denaturation at 94°C for 40 s; annealing at 58°C for 1 min; extension at 72°C for 1 min 20 s, for 35 cycles; and a final extension at 72°C for 5 min.

The PCR products of MyoG intron II were digested in a 20 µL reaction system containing 2 µL of 10× Buffer, 10 µL of PCR product, 0.2 µL of Eco72 I restriction enzyme and 7.8 µL of sterilized double-distilled water. The reaction mixture was incubated at 37°C for 3 h in a water bath. The digested products were analyzed by electrophoresis on a 3% agarose gel.

Meat samples were collected from Small-Tailed Han Sheep immediately after slaughter at a sheep slaughter house. The samples were excised from the dorsal region and were subjected to measurements of meat quality traits, including drip loss, shear force, moisture content, cooking loss and color. The measurement methods were adapted from the performance measurement protocol described by Liu *et al*. (2007).

The association analysis between MyoG intron II genotypes and sheep morphometric traits was performed using the two-factor variance analysis module in SPSS software. Duncan's multiple range test was used for one-way analysis. The factors considered included gender and MyoG intron II genotype.

Results and Discussion

The restriction digestion results for MyoG intron II in Small-Tailed Han Sheep are shown in Fig 1. As observed in the figure, three genotypes were identified for MyoG intron II in Small-Tailed Han Sheep, designated as AA (368 bp, 540 bp), AB (908 bp, 368 bp, 540 bp) and BB (908 bp). Samples 1, 3, 5 and 11 were classified as genotype AA, while samples 2, 4, 6, 7, 8, 9, 10 and 12 were classified as genotype AB.

The frequencies of the three genotypes (AA, AB and BB) in the five sheep breeds-Small-Tailed Han Sheep, Large-Tailed Han Sheep, Yuxi Fat-Tailed Sheep, Mongolian Sheep and Lanzhou Large-Tailed Sheep-are presented in Table 1. As illustrated in Table 1, only two genotypes (AB and BB) were detected in Large-Tailed Han Sheep, Yuxi Fat-Tailed Sheep and Mongolian Sheep, whereas three genotypes (AA, AB and BB) were identified in Small-Tailed

Han Sheep and Lanzhou Large-Tailed Sheep. Among the five breeds, the BB genotype was predominant in Lanzhou Large-Tailed Sheep, with a frequency of 0.591, while the AB genotype was dominant in the other four breeds. The frequencies of the AB genotype in Small-Tailed Han Sheep, Large-Tailed Han Sheep, Yuxi Fat-Tailed Sheep and Mongolian Sheep were 0.767, 0.850, 0.917 and 0.583, respectively.

In terms of allele frequencies, Small-Tailed Han Sheep exhibited equal frequencies for A and B alleles (0.500 and 0.500, respectively). However, in Large-Tailed Han Sheep, Yuxi Fat-Tailed Sheep, Mongolian Sheep and Lanzhou Large-Tailed Sheep, the B allele was dominant, with frequencies of 0.571, 0.542, 0.708 and 0.682, respectively.

Analysis of the data in Table 1 revealed that the genetic heterozygosity values for the five sheep breeds were approximately 0.5, indicating moderate genetic variation in the MyoG intron II region. The effective allele values for Small-Tailed Han Sheep, Large-Tailed Han Sheep and Yuxi Fat-Tailed Sheep were close to the observed value of 2, while Mongolian Sheep and Lanzhou Large-Tailed Sheep showed some deviation but remained relatively close to the observed value. The polymorphism information content (PIC) values ranged from 0.329 to 0.375, indicating that MyoG intron II exhibits moderate polymorphism.

Chi-square analysis was employed to test the significance of differences in genotype distribution for MyoG intron II among the five sheep breeds. As shown in Table 2, Lanzhou Large-Tailed Sheep exhibited highly significant differences in genotype distribution compared to Large-Tailed Han Sheep, Small-Tailed Han Sheep and Yuxi Fat-Tailed Sheep ($P<0.01$) and significant differences compared to Mongolian Sheep ($P<0.05$). Furthermore, highly significant differences ($P<0.01$) were observed between Mongolian Sheep and Yuxi Fat-Tailed Sheep, while significant differences ($P<0.05$) were found between Mongolian Sheep and both Large-Tailed Han Sheep and Small-Tailed Han Sheep. No significant differences ($P>0.05$) were detected between the remaining breed pairs.

As shown in Table 3, the association analysis between MyoG intron II genotypes and meat quality traits revealed that MyoG intron II significantly influenced water content, cooking loss and color in sheep meat but did not have a significant effect on other meat quality traits ($P>0.05$). The AA and BB genotypes exhibited significantly higher water content in meat compared to the AB genotype ($P<0.05$). Meanwhile, the AB and BB genotypes displayed significantly higher cooking loss than the AA genotype ($P<0.05$). Additionally, the AA and AB genotypes produced meat with significantly better color compared to the BB genotype ($P<0.05$).

Research indicates that muscle fibers in vertebrates begin to form during the embryonic stage and the number of muscle fibers remains constant after birth, with muscle growth relying on the hypertrophy of existing fibers rather than an increase in fiber number (Han *et al*., 2016). The MyoG gene is the only member of the MRFs family that is expressed universally in all skeletal muscle cells. MyoG

Table 1: Polymorphism information content of sheep.

Populations	Genotype frequency			Gene frequency		Heterozygosity	Number of effective alleles	Polymorphism information content
	AA	AB	BB	A	B			
Small-tailed han sheep	0.117	0.767	0.117	0.500	0.500	0.500	2.000	0.375
Large-tailed han sheep	0.000	0.850	0.150	0.425	0.575	0.489	1.956	0.369
Yuxi fat-tailed sheep	0.000	0.917	0.083	0.458	0.542	0.496	1.986	0.373
Mongolian sheep	0.000	0.583	0.417	0.292	0.708	0.413	1.705	0.329
Lanzhou big-tailed sheep	0.227	0.182	0.591	0.318	0.682	0.434	1.766	0.340

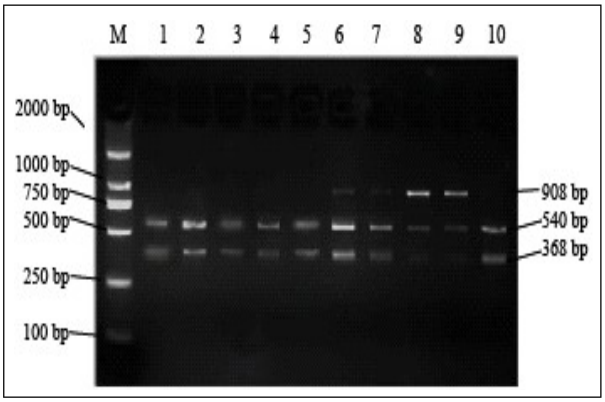


Fig 1: Digestion results of MyoG intron II in Small-tailed han sheep.

regulates myoblast differentiation by promoting the fusion of mononuclear myoblasts into multinucleated myotubes and halting the proliferation of myoblasts (Han, 2016). Liu identified three genotypes (AA, BB and AB) of the MyoG exon 1 region in four sheep populations, with the BB genotype detected only in Mongolian Sheep, Tosa Sheep and White Suffolk Sheep (Liu *et al*., 2007). Bai reported the presence of three genotypes (AA, BB, AB) and two alleles (A and B) in six sheep populations, including Large-Tailed Han Sheep, Small-Tailed Han Sheep, Yuxi Fat-Tailed Sheep, Lanzhou Large-Tailed Sheep, Mongolian Sheep and Tong Sheep (Bai *et al*., 2017). Li found three genotypes (AA [368/540 bp], AB [908/368/540 bp], BB [908 bp]) of MyoG intron II in five sheep populations, with the AB genotype being predominant across populations (Li *et al*., 2022).

In this study, agarose gel electrophoresis of restriction enzyme-digested PCR products revealed three banding patterns in Small-Tailed Han Sheep and Lanzhou Large-Tailed Sheep, while only two patterns were observed in the other breeds. This disparity may be attributed to interspecies genetic differences. Among the five sheep breeds, the B allele was dominant in all populations except Small-Tailed Han Sheep, where A and B allele frequencies were equal. The prevalence of the B allele aligns with the findings of Liu in goats, where the B allele was identified as dominant (Liu *et al*., 2011). The genetic heterozygosity of the five sheep breeds was approximately 0.5, suggesting moderate genetic variation in the MyoG intron II region. This finding is consistent with the results of Liu regarding Boer goats, which showed a genetic heterozygosity value close to 0.5, are consistent with the results observed in this study (Liu *et al*., 2011). The effective allele values for Small-Tailed Han Sheep, Large-Tailed Han Sheep and Yuxi Fat-Tailed Sheep were approximately equal to the observed value of 2, while Mongolian Sheep and Lanzhou Large-Tailed Sheep displayed slight deviations but remained relatively close to the observed values. This observation aligns with the study by Liu, which reported effective allele values near 2 in Boer goats (Liu *et al*., 2011). However, a substantial discrepancy was noted when compared to the effective allele values of hybrid goat offspring, which were closer to 1. The polymorphism information content (PIC) for the five sheep breeds was generally low, ranging from 0.329 to 0.375, indicating that the MyoG intron II region exhibits moderate polymorphism. This conclusion is consistent with Liu, who confirmed that this locus is moderately polymorphic in goats (Liu *et al*., 2011).

Wang investigated the expression of MyoG in the longissimus dorsi muscle of Hu Sheep at different growth stages using RT-PCR (Wang *et al*., 2017). Their study revealed that MyoG expression is influenced by sex and age and is involved in muscle growth and development. Li found that Saltwater Black-bone Chicken exhibited superior body weight, body size and slaughter traits compared to Daweishan Miniature Chicken (Li *et al*., 2020). They also observed higher MyoG expression in the breast, leg muscles and liver of Saltwater Black-bone Chicken, with significant positive correlations between MyoG expression and growth traits ($P<0.05$). Moreover, relative MyoG expression was significantly correlated with all traits except chest depth ($P<0.01$). In studies by Chai, single nucleotide polymorphisms (SNPs) in the MyoG gene were detected and analyzed for their association with body size traits in yaks (Chai *et al*., 2018). Four mutation sites (g.757T>C, g.662G>A, g.539A>G, g.2216A>G) were identified, each containing three genotypes and conforming

Table 2: Significance test of the genotype distribution of MyoG intron II in different varieties.

Populations	Small-tailed han sheep	Large-tailed han sheep	Yuxi fat-tailed sheep	Lanzhou big-tailed sheep
Small-tailed han sheep				
Large-tailed han sheep	5.080			
Yuxi fat-tailed sheep	5.055	0.807		
Lanzhou big-tailed sheep	25.220**	28.434**	27.046**	
Mongolian sheep	7.257*	3.936*	7.200**	7.042*

Note:** Indicating a highly significant correlation ($P<0.01$);* Indicate significant correlation ($0.01<P<0.05$); Other insignificant correlations ($P>0.05$).

Table 3: Association between genotype of MyoG intron II and meat traits of sheep.

Traits	AA genotype	AB genotype	BB genotype
Drip loss	45.806±0.628[a]	45.527±0.395[a]	48.319±2.205[a]
Shear force	2.744±0.179[a]	2.890±0.135[a]	3.591±0.861[a]
Moisture	75.550±0.243[a]	74.598±0.226[b]	75.742±0.885[a]
Cooking loss	32.884±0.697[b]	31.810±0.471[a]	33.273±2.111[a]
Color	3.800±0.187[a]	3.670±0.128[a]	2.170±0.601[b]
Crude protein	21.143±0.235[a]	21.261±0.198[a]	-
Crude fat	1.523±0.038[a]	1.513±0.039[a]	-

Note: Different lowercase letters indicate significant differences ($0.01<P<0.05$), while the same lowercase letters indicate insignificant differences ($P>0.05$).

to Hardy-Weinberg equilibrium. The polymorphism at g.757T>C, g.662G>A and g.539A>G loci was higher in Shenza and Pali yaks compared to other populations. Statistical tests revealed significant associations between these loci and height traits (P<0.05), suggesting that the MyoG gene may be a major gene influencing height in yaks or is linked to such loci.

CONCLUSION

The MyoG intron II region was found to contain two alleles (A and B) and three genotypes (AA, BB and AB) across the five sheep populations studied. This study analyzed the association between the MyoG intron II region and sheep meat quality traits, revealing that the MyoG intron II region significantly affects water content, cooking loss and color but has no significant impact on other traits. These findings suggest that the MyoG intron II region plays a substantial role in influencing sheep meat quality. Future studies should expand the sheep populations examined to deepen the understanding of this gene's effects.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal careand handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish,or preparation of the manuscript.

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