

Full Length Research Paper

Meat tenderness and water holding capacity are associated with a 959 A → G mutation in the MyoG gene of Chinese indigenous cattle

Ujan, J. A.^{1*}, Zan, L. S.^{1,2*}, Shengjuan Wei¹, Adoligbe, C.¹ and Wang, H. B.¹

¹College of Animal Science and Technology, Northwest A and F University, 712100, Yangling, Shaanxi, P. R. China.

²National Beef cattle improvement centre, 712100, Yangling, Shaanxi, P. R. China.

Accepted 26 April, 2011

Myogenin (MyoG) gene has mapped at 25 to 73 cm interval on BTA 16 where several quantitative trait loci for carcass weight and marbling are located. In this study, we determined the associations between gene-specific single nucleotide polymorphisms (SNP) in MyoG gene, to investigate whether this polymorphism affected meat quality characteristics and to evaluate the allelic and genotypic frequencies of six native Chinese cattle breeds. The breeds were Jiaxian red (JXR), Luxi (LX), Nan-yang (NY), Qinchuan (QC), Xia-Nan (XN) and Xue long (XL). Our results suggested a transition of A → G at position 959 in exon 1 of the MyoG gene in cattle that caused the substitution (⁹⁵⁹Serine/⁹⁵⁹Cysteine). The A959G SNP was significantly associated with water holding capacity and meat tenderness ($P < 0.05$), while no effect of genotype on back fat thickness, rib area, loin eye height, eye muscle width and marbling was disclosed ($P > 0.05$). The χ^2 -test revealed that the genotype distributions among the five cattle breeds (JXR, LX, NY, QC and XL) agreed with Hardy-Weinberg equilibrium ($P > 0.05$), although, one breed (XN) was not in Hardy-Weinberg equilibrium ($P < 0.01$). We concluded that, A959G SNP can be used as an efficacious genetic marker for meat quality traits in native Chinese cattle breeds but a much large number of animals are required for Marker assisted selection.

Key words: Cattle, genotypic frequencies, myogenin (MyoG) gene, meat quality, single nucleotide polymorphism.

INTRODUCTION

The candidate gene approach permits designation of SNPs in genes which may cause variations in a phenotypic attributes (Shin and Chung, 2007). Moreover, the candidate genes located within a QTL region are positional candidate genes and relationship of these genes with production properties provides an excellent means for marker assisted selection (Khatib et al., 2007; Dario et al., 2009).

Meat quality is affected by genetic and environmental factors which estimate the metabolic process in muscle tissue and in postmortem transition of muscle in to meat. Postnatal tissue growth and myogenesis are determined

by the myogenic determination gene family (MyoD), comprising of four genes MyoD1 (MyF-3), Myf-4 or MyoG (Myogenin), Myf-5 and MyF-6.

MyoG gene is mapped on BTA16 and diverse QTLs for carcass weight and marbling are situated at 25 to 73 cm interval (Casas et al., 2004). Myogenin is considered as a candidate gene for meat production and quality traits because of its probable role in muscle fiber development (Wyszynska-Koko et al., 2006; Verner et al., 2007). Therefore, Myogenin is a possible positional candidate gene for meat quality traits in cattle.

Chinese indigenous cattle are chiefly used for beef production. It has high nutritional value, high protein content, high ratio of meat to bone in the carcass and large loin eye area. However, it tends to have lower carcass fat content and intramuscular adipose tissue deposition. This influences eat quality and consumer acceptability and is

*Corresponding author. E-mail: zanls@yahoo.com.cn. Tel: +86-29-87091923. Fax: +86-29-87091148.

Table 1. Oligonucleotide primers, amplicon sizes and corresponding annealing temperature for bovine MyoG gene.

Gene (accession number)	Oligonucleotide sequence (5' to 3')	Amplicon size (bp)	Annealing temperature (°C)
MyoG (NW_001501985.1)	F: TGTAAGAGGAAGTCGGTGTC R: CGATGTACTGGATGGCACT	171	52
	F: AACCAGGAGGAGCGTGAC R: TGGCACCTGCACCAAC	159	54
	F: GGGGTCCAGAAGCAAGTC R: GGTGAAGGAGGCAGAGTGT	246	52.5
	F: CACAGGTACTTCTGCCCACT R: TTGGAGCCAAGGTTACCAG	204	55
	F: GCCTGACTGGTAACCTT R: TAAGAGGGGAGAACCTG	183	47
	F: CTCACGGCTGACCCTACA R: CCCAGAGCTGGCTTCTCA	231	56

a key factor constraining the entry of Chinese indigenous cattle into the market for top-grade beef. Thus, uninterrupted efforts have been paid to improve its growth performance and meat quality in order to meet the increasing inquires of consumers for meat of high quality. In previous studies, influences of Myogenin genotypes on birth weight, growth rate, carcass weight, back fat thickness and lean weight of pigs were studied in the Netherlands Te Pas (1999). Furthermore, identification of SNPs in MyoD gene family and their associations with carcass traits in cattle have been reported in Korean cattle (Bhuiyan et al., 2009). Therefore, the objective of this study was to investigate the relationship between meat quality traits and polymorphism of Myf-4 gene in crossbred Chinese indigenous cattle breeds.

MATERIALS AND METHODS

Animals and sample collection

A total of 520 cattle belong to six genotypically different (Heterogeneous) Chinese indigenous cattle breeds were used to analyze the allelic frequencies of MyoG gene. The breeds were Jia-xian red (JXR n= 96), Luxi (LX n= 99), Nan-yang (NY n= 60), Qinchuan (QC n= 74), Xia-Nan (XN n= 148) and Xue long (XL n= 43). The animals were selected at the age of two and half year. The JXR cattle breed were from the breeding farm of Jiaxian cattle (Jiaxian county, Henan Province, P. R. China); LX cattle breed were obtained from the reserved center of LX (Heze city, Shandong province, P. R. China); NY animals were obtained from the breeding centre of Nanyang cattle (Nanyang city, Henan Province, P. R. China); QX animals were obtained from the reserved farm of QC (Weinan city, Shaanxi Province, P. R. China); XN animals were from the breeding farm of Xianan cattle (Biyang city, Henan province,

China) and finally, the XL animals were obtained from Dalian city, Liaoning province. All of the animals were cared according to the standards set out by the Canadian council on Animal care McNeil and Newman (1994). Moreover, the polymorphism and its association with meat quality traits were analyzed in 428 individuals. Following meat quality traits were accounted for statistical analysis as previously described by Wheeler et al. (1994), taking on back fat thickness (BFT), loin eye height (LEH), loin eye area (LEA), marbling (Mb) and meat tenderness (MT), rib area (RA) and water holding capacity (WHC). In this study, Mb for meat quality grade was counted on a cross section of the loin muscle between the 12th and 13th rib, that is scored on a scale from 1 to 5.

DNA extraction and PCR amplification

Five hundred and twenty (520) blood samples were collected from Six Chinese indigenous cattle breeds. DNA samples were extracted from leucocytes and assorted from acid citrate dextrose (ACD) blood samples (ACD: blood is 1:6) then treated with 2% heparin, and finally stored at -80°C, complying the standard method prescribed by (Sambrook and Russell, 2002). A total of six primer pairs were planned based on NCBI database, as per the sequence of bovine MyoG gene (Gene bank accession number NW_001501985.1) to amplify both exon and intron regions in MyoG gene employing the program primer 3 (<http://frodo.wi.mit.edu/>). More elaborated information for oligonucleotide primers, amplicon size and corresponding annealing temperature is described in Table 1.

The PCR amplifications was executed in a 20 µl reaction mixture containing 50 ng templates DNA, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM MgCl₂, 16 µl 10×PCR buffer and 0.5 U taq DNA polymerase (TaKaRa, Dalian, China). PCR reaction were performed under the following conditions: initial denaturation was performed at 95°C for 5 min, 31 cycles of denaturation at 94°C for 30 s, 52°C annealing temperature for 30 s, extension at 40°C for 30 s and a final extension at 72°C for 10 min. The PCR product was operated on 1.5% agarose gels (containing 200 ng/ml ethidium

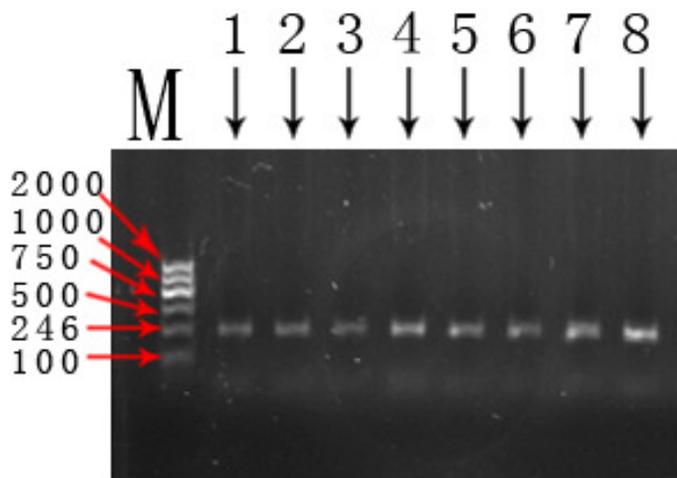


Figure 1. PCR product of MyoG gene exon1 and its flanking region. M, Marker; lanes 1-2, PCR products of the MyoG gene intron1 and its flanking region.

bromide) using 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na₂EDTA).

The purification of the PCR product were done with Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology, P. R. China) and sequenced by ABI PRIZM 3730 DNA sequencer (Perkin-Elmer Shanghai Sangon Biological Engineering Technology, Ltd.). Analysis of the DNA polymorphisms were exerted by comparing the obtained sequence data with the published sequence in the NCBI database (<http://www.ncbi.nlm.nih.gov>) by applying DNAMAN software (version 6.0). The sequencing map for this novel SNP of bovine MyoG intron 1 region revealed an A>G mutation at 959 bp using Chromas 2.33 version (<http://www.technelysium.com.au/>) software (Figure 3).

PCR-SSCP analyses

For single-strand conformation polymorphisms (SSCP), aliquots of 4 µl of PCR products were mixed with 8 µl of denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), denatured at 98°C for 10 min, chilled on ice simultaneously. The denatured DNA were loaded onto 10% PAGE gel in 1×TBE buffer at a constant voltage of 120 V for 12 h and the gel was stained with 0.1% silver nitrate and visualized and developed with 2% NaOH solution (containing 0.1% formaldehyde), according to Zhang et al. (2007).

Statistical analyses

Genotype distribution was determined for Hardy-Weinberg equilibrium as proposed by Falconer and Mackay (1996). Population genetic indices likewise, *H_e* (gene heterozygosity), *H_o* (gene homozygosity), *N_e* (effective allele numbers) and PIC (polymorphism information content), were evaluated according to Nei and Roychoudhury (1974) and Nei and Li (1979). Differences in genotypic frequencies of SNP were prescribed for deviations from Hardy-Weinberg equilibrium by mean values ± S.E.M, which was performed by using SPSS version 17.0. Statistical analysis for associations was also made between MyoG genotypes and meat quality traits of Chinese indigenous cattle using SPSS software.

The following model was applied for statistical analysis, which included fixed effects of sex, age and genotype.

$$Y_{ijk} = \mu + A_i + G_j + S_k + E_{ijk}$$

Where, *Y_{ijk}* is the observed value of meat quality traits; μ is the overall population mean; *A_i* is the fixed effect of the *i*th age; *G_j* is the fixed effect of the *j*th genotype (AA and AB genotype); *S_k* is the fixed effect of the sex and *E_{ijk}* is the random error.

RESULTS AND DISCUSSION

SNP, genotype frequencies of different breeds and χ^2 test

Primer pair MyoG-P2 amplified a 246 bp fragment of the MyoG gene from nt 744 to 989 bp, which includes a part of exon1 as shown (Figure 1). Afterwards the amplification and sequencing of 246 bp fragment, a novel SNP at A959G in intron 1 of MyoG gene (Gen bank accession number NW_001501985.1) in 520 animals was discovered. The results of PCR-SSCP are shown in Figure 2. This A→G point mutation induced a change of serine to cysteine and this SNP exhibited two different patterns one with single band and another with two bands as shown in Figures 2 and 3. In order to identify them, we have assigned AA to denote the single band and AB to denote the bands. The sequencing map of this novel SNP also supports the synonymous mutation A>G (Figure 3).

Subsequently, allelic frequencies of this SNP have been determined by χ^2 test in all cattle breeds (JXR, LX, NY, QC and XN), and results varied from 0.804 to 0.904 as shown in Table 2. The χ^2 -test suggested that the genotype distributions among the 5 cattle breeds, JXR, LX, NY, QC and XL were in Hardy-Weinberg equilibrium and did not showed any significant differences in allelic frequencies (*P* > 0.05) whereas, the contrary results were shown by the XN breed, it does not agreed with Hardy-Weinberg equilibrium and revealed significant differences in allelic frequencies (*P* > 0.05). Genotypic frequencies in five cattle breeds (JXR, LX, NY, QC and XL) showed small diversity, whereas, only the XN breed showed a moderate diversity (0.25 < PIC < 0.5). The AA genotype was the most dominant genotype in all populations examined ranging from 0.608 to 0.808; Contrarily AB genotype frequency was lower in all the breeds studied ranging from 0.218 to 0.391. The BB homozygous genotype was not found in all the populations, possibly because: (1) the very low frequency of the BB genotype in homozygous condition; (2) homozygous BB null is lethal for Myogenin in Chinese cattle breeds.

Furthermore, gene heterozygosity (*H_e*), effective allele numbers (*N_e*) and PIC (polymorphism information content) of MyoG gene locus in six cattle breeds deviated from 0.173 to 0.315, 1.209 to 1.460 and 0.158 to 0.265, respectively. Generally, PIC is classified into three cases: low polymorphism (PIC < 0.25), median polymorphism

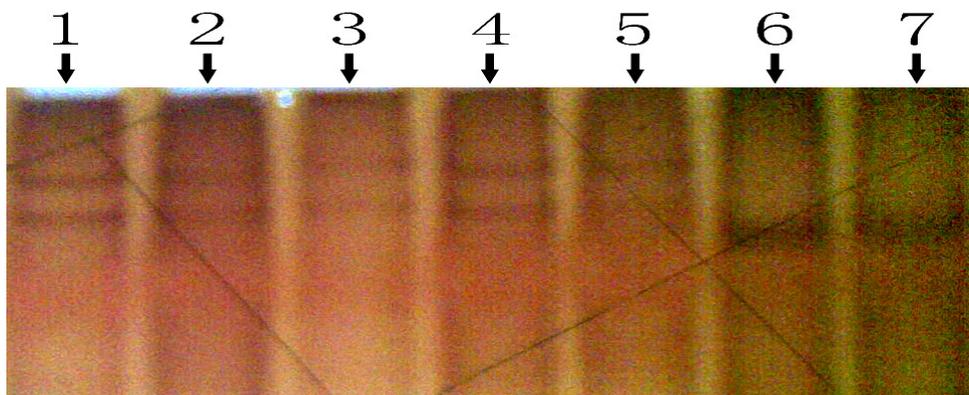


Figure 2. The PAGE electrophoresis patterns of PCR-SSCP intron1 of bovine MyoG gene. Lane 1, 2, 3, 4 and 5: Genotype AA; while 6 and 7; genotype AB.

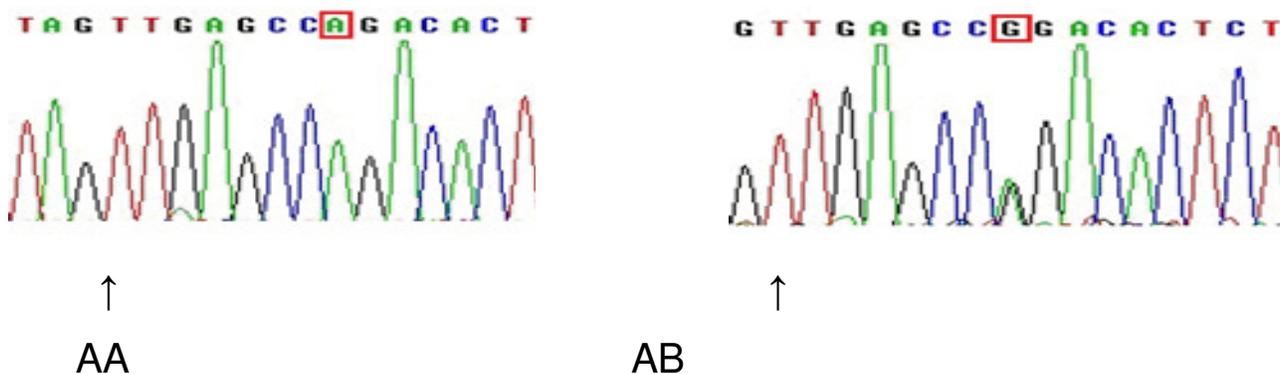


Figure 3. The sequencing map of the novel SNP of bovine MyoG intron1 region. Note. The map reveals A→G mutation at 959 bp.

Table 2. Mendelian factor determination (Polymorphic indexes) allelic, genotypic frequencies and polymorphic indices of MyoG gene in all six Chinese indigenous cattle breeds.

Breed	Genotypic frequency (N)		Total	Allelic frequency		Effective allele number Ne	PIC	He	X ² (HWE)
	AA	AB		A	B				
JXR	0.781(75)	0.218(21)	96	0.890	0.109	1.242	0.175	0.194	0.544
LX	0.808(80)	0.191(19)	99	0.904	0.096	1.209	0.158	0.173	0.289
NY	0.700(42)	0.300(18)	60	0.850	0.150	1.342	0.222	0.255	0.868
QC	0.648(48)	0.351(26)	74	0.824	0.175	1.407	0.247	0.289	2.228
XN	0.608(90)	0.391(58)	148	0.804	0.195	1.460	0.265	0.315	8.789
XL	0.651(28)	0.348(15)	43	0.825	0.174	1.404	0.246	0.288	0.883

JXR, Jia-xian red cattle; LX, Luxi cattle; NY, Nan yang cattle; QC, Qinchuan cattle; XN, Xia-nan cattle; XL, Xue long cattle. N, number of observations; X² (HWE), Hardy–Weinberg equilibrium, X² value. Its P value for JXR, LX, NY, QC and XL were greater than 0.05 and only for XN was below than 0.01.

(0.25 < PIC value < 0.5) and high polymorphism (PIC > 0.5). On the basis of the aforementioned, PIC classification JXR, LX, NY, QC and XL demonstrated low polymorphism level and the only XN breed proved median polymorphism level as shown in Table 2. Thus, our findings indicated that the high frequency of MyoG-A

allele could be used to characterize the Bos tarus breeds.

SNP marker associations

Genotypic data of 428 Chinese indigenous cattle breeds

Table 3. Association analyses, least square means and standard errors for meat quality of MyoG genotypes for Chinese indigenous cattle.

Genotype	Meat quality trait (cm, Mean \pm SE)						
	BT	LEH	RA	MB	LEA	WHC	MT
AA	0.971 \pm 0.085	6.19 \pm 0.290	17.35 \pm 0.460	2.49 \pm 0.174	80.62 \pm 4.48	0.219 \pm 0.008	2.17 \pm 0.115 ^a
AB	1.12 \pm 0.12	5.73 \pm 0.406	16.81 \pm 0.643	2.26 \pm 0.244	71.12 \pm 6.26	0.195 \pm 0.011	1.80 \pm 0.161 ^b
P value	0.209	0.237	0.620	0.235	6.03	0.02	0.019

MT, Scored from 1 (extremely tender) to more than 11 (extremely tough); MB, score was measured from 1 (abundant) to 5 (poor); BFT, back fat thickness; LEH, loin eye height; LEA, loin eye area; MB, marbling; MT, meat tenderness; RA, rib area; WHC, water holding capacity. P value criteria: Significant association with any trait ($P < 0.05$); highly significant association with any trait ($P < 0.01$); a, b means with different superscripts were significantly different ($P < 0.05$).

were compared with their phenotypic data for the following meat quality traits; back fat thickness (BFT), loin eye height (LEH), loin eye area (LEA), marbling (Mb), meat tenderness (MT), rib area (RA) and water holding capacity (WHC). Least squares analysis showed that the A \rightarrow G mutation at 959 bp is significantly associated with water holding capacity and meat tenderness ($P < 0.05$), but did not show any significant association with back fat thickness, eye muscle width, loin eye height, loin eye area and marbling. Moreover, results also showed that the AA genotype had higher WHC and MT ($P < 0.05$) than individuals with genotype AB.

Currently, breeding goals are shifting from high yield to more meat quality concerned attributes (van Wijk et al., 2005). According to McIlveen and Buchanan (2001), flavor, tenderness and juiciness are considered to be the three most crucial determinatives of sensory enjoyment for the United Kingdom consumers'. Among these three qualities, beef tenderness has been ascribed as the primary determinant of satisfaction among beef consumers (Ouali, 1990; Warkup et al., 1995; Szczesniak, 1998; Koohmaraie, 1998). In Norway, a latest research showed that the beef consumers were willing to pay 50% more for very tender meat and 25% more for tender meat compared with less tender beef (Alfnes et al., 2005). Consequently, providing systematically tender beef should be the main priority for the meat industry. Although, many productive efforts at improving the tenderness of meat research has revealed that an unacceptable level of variability still exists in beef tenderness (Maher et al., 2004). Research of associating candidate genes with meat quality is a step towards better apprehension of the genetic basis of productive traits (O'vilo et al., 2006). Candidate gene mutation and its association with economic attributes have been executed to ascertain the genetic basis of quality traits and also used to develop marker assisted selection. Postnatal tissue growth and myogenesis are determined by the myogenic determination gene family (MyoD), comprising of four genes MyoD1 (MyF-3), Myf-4 or MyoG (Myogenin), Myf-5 and Myf-6. Mutation in these genes have been identified in several pig breeds and pig lines which affect the meat dethronement in carcass (Cieoelak et al., 2002; KuryŁ et al., 2002) and the

micro-structural features of muscle tissue (KŁOsowska and Fiedler, 2003; KŁOsowska et al., 2001, 2004).

Meat quality traits in cattle are determined by the number of embryonically developed myocytes, as demonstrated in double-muscled cattle which have a much greater number of prenatally developed myofibers than other cattle (Swatland and Kiefer, 1974; Hanset et al., 1982). The Myogenin gene has been suggested as a candidate gene for meat characteristics since it is involved in the control of the exit of myoblasts from the cell cycle and their differentiation state in the myogenesis (Buckingham, 1992). Any deviation in the expression of this gene or its structure, caused by mutations, could influence the process of differentiation and in the end, meat quality.

MyoG gene was the subject of several earlier studies in porcine research (Ernst et al., 1993; Soumillion et al., 1997; te Pas et al., 1999; Cieoelak et al., 2000), as the gene having a crucial role in the genesis of muscle tissue, unreplaceable by other genes (Hasty et al., 1993, Nabeshima et al., 1993). Soumillion et al., (1997) noticed a number of mutations in the MyoG locus, but only one, situated about 3 kb downstream of the STOP codon. It has been found to be highly polymorphic in European meat pig breeds. Moreover, there have been few studies for the MyoG gene in cattle and particularly in Chinese indigenous cattle breeds.

In this study, the results suggested an association between single nucleotide polymorphisms in the bovine Myogenin (MyoG) gene and meat quality traits in Chinese indigenous cattle (Table 3). This study furnished a new selective information in this regard that the intron1-959 bp A \rightarrow G synonymous mutation was highly significantly associated with water holding capacity and meat tenderness but at the same time dissimilar results for back fat thickness, loin eye height, Loin eye area and Marbling, rib area have been observed. In accordance with this study, several other studies also reported significant associations with carcass and meat quality traits (Te Pas et al., 1999; Kapelanski et al., 2005; Wyszynska-Koko et al., 2006; Verner et al., 2007). MyoG has been previously mapped on BTA16 and this chromosome harbors several QTLs for carcass weight, marbling and

percentage of kidney, pelvic and heart fat (KPH) relative to carcass weight at 25 to 73 cm interval (Casas et al., 2004). Another study (Kapelański et al., 2005) indicated a highly significant association between MyoG gene and meat quality traits. Furthermore, dissimilar results have shown that polymorphisms in MyoG gene did not have significant effects on any of the traits investigated (Bhuiyan et al., 2009). Some attempts were also made to discover a relation between meat quality traits and effects of Myogenin and MyF-3 polymorphisms (Kapelański et al., 2001, 2004), however, the results showed a lack of significant associations. On the basis of our findings, it is inferred that mutations in non-coding regions of MyoG gene can significantly bring about change in meat characteristics and quality.

Conclusion

In this study, the association between single nucleotide polymorphisms of MyoG gene and meat quality traits was studied in Chinese indigenous cattle. It was found that the genotypes were highly significantly associated with the water holding capacity (WHC) and meat tenderness (MT), but at the same time dissimilar results for back fat thickness (BFT), loin eye height (LEH), loin eye area (LEA) and Marbling (MB) rib area (RA) have been observed. Our results confirm legion of the previously reported significant associations, but diverge with respect to others demonstrating that further investigations are required on the effect in meat quality traits of genetic variation in the bovine MyoG gene. It is also suggested that this SNP could be used for marker-assisted selection, but a huge number of samples would be required for this job.

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