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The Correlation Between Polymorphisms of the *MSTN* Gene and Slaughter Traits in Sansui Ducks

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Authors' Contribution

ZHZ collected samples, performed the experiments, analyzed the data and wrote the article. HL is the project leader and corresponding author. BXP and HJY collected samples, performed the experiment and analyzed the data.

Key words

Sansui duck, *MSTN*, SNPs, slaughter traits, myostatin.

ABSTRACT

Direct sequencing of PCR amplicons was used to identify single nucleotide polymorphisms (SNPs) in the Sansui duck (Sansui Sheldrake duck) myostatin (*MSTN*) gene exons. In addition, the genetic effects of the *MSTN* gene on slaughter traits were analyzed. The results showed that in the studied sample groups, six SNPs (g.106G>A, g.120A>G and g.159G>A of exon1; g.5368G>A, g.5389A>C and g.5410G>A of exon3) were found in the *MSTN* gene. The G/A mutation in the g.106G>A SNP resulted in the change of codon from GGC to AGC, and the coding amino acid from Gly to Ser. Additionally, the association tests between SNPs and slaughter traits showed that g.120A>G affected the breast muscle percentage and lean meat percentage; and g.5368G>A/g.5389A>C affected the semi-eviscerated weight and eviscerated weight. We conclude that the *MSTN* gene could be regarded as a useful candidate gene for carcass traits in Sansui ducks.

INTRODUCTION

The myostatin (*MSTN*) gene encodes a growth factor, sometimes referred to as growth differentiation factor 8 (GDF-8). The *MSTN* gene was discovered by McPherron and Lee (1997a) in a study of the transforming growth factor-β (TGF-β) superfamily. They showed that MSTN is a negative regulator of muscle growth. The *MSTN* gene is associated with the growth and differentiation of animal skeletal muscle, and the loss of gene function would cause skeletal muscle growth abnormalities (Lee and McPherron, 1999; McPherron and Lee, 1997b; Kambadur *et al.*, 1997). The *MSTN* gene is highly conserved in different species, *e.g.* the human, pig, cow and chicken genes all contain three exons and two introns. Studies in cattle (Di-Stasio and Rolando, 2005), pig (Jiang *et al.*, 2002) and chicken (Gu *et al.*, 2003) showed that the *MSTN* gene is an important candidate gene for carcass and meat traits. Research into *MSTN* gene polymorphisms in duck is comparatively rare. In GenBank, there are only two *MSTN* transcript variants of Peking duck, which were obtained as using a whole genome shotgun strategy. For other breeds, only partial coding sequences have been deposited. The correlation between polymorphisms in the *MSTN* gene and slaughter traits has not been reported.

Sansui ducks are a local breed of Guizhou province. Currently, studies of Sansui ducks have been limited to the impact of breed characteristics, body measurements, slaughter traits, and muscle and egg nutritional components. Studies at the molecular level are also limited. Therefore, in this study we used 17-week-old Sansui ducks to study the association of single nucleotide polymorphisms (SNPs) in MSTN exon and slaughter traits. The aims of this study were to find a marker related to slaughter traits of Sansui ducks, to provide basic data for marker-assisted selection of ducks, and to provide a reference for further study of the *MSTN* gene in other breeds.

MATERIALS AND METHODS

Test materials

The population used for the association analysis comprised 60 pure bred Sansui ducks (50% male and 50% female), but individuals within gathering colonies repelled one another. The treatment conditions were the same for all animals, and the ducks were slaughtered at 17 weeks old. The measured carcass traits were: weight before slaughter, carcass weight, half carcass weight, eviscerated weight, breast muscle weight and leg muscle weight, dressing percentage, semi-eviscerated percentage, eviscerated weight percentage, leg muscle percentage, breast muscle percentage, lean meat percentage. The experimental procedures were performed according to the protocols approved by the Biological Studies Animal Care and Use Committee of Guizhou, People's Republic of China.

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Table I.- PCR primer information.

Primer name	Primer sequences (5′–3′)	Product size	Annealing temperature (°C)	Amplified region
P1	F: GAACTGAAAGAAAAGGGGAAAG R: GACGAAAGCAGCAGGGTT	472bp	50.6°C	g.54–g.525
P2	F: TTTGTTCCCTGTTCAGTAAT R: CAAGTTAAATGCTTTCCAAT	480bp	56.5°C	g.2575–g.3054
P3	F:GAGACTTGTAGGAGGATAAAG R:ACAGTTTCAAAGATGGGTG	638bp	57.0°C	g.5051–g.5688

Table II.- PCR reaction conditions.

Primer name	Pre denaturation at 95°C	Denaturation at 94°C	Annealing (°C /s)	Extend at 72°C	Cycle number	Again extend at 72°C
P1	4min	40s	50.6°C /40s	45s	35	5min
P2		35s	56.5°C /40s	40s	30	
P3		45s	57.0°C /50s	50s	30	

Genomic DNA extraction

A genomic DNA extraction kit (Tiangen Biotech, Beijing, China) was used to extract DNA from blood samples, and the products were detected using 1.0% agarose gel electrophoresis. We measured their optical density (OD) using a spectrophotometer, and determined that their concentrations were 100–200μg/μL.

Primer design

The primers used in the PCR amplification are listed in Table I. The primers for the three exons of *MSTN* were designed using Primer Premier5.0 and Primer3.0 and the chicken *MSTN* gene compete coding region (GenBank Accession No.: AF346599.2) and partial duck sequences (GenBank Accession Nos.: AY329600.1, DQ355160.1, DQ419906.1, AF440861.1 and NW_0046766457). Invitrogen (Shanghai, China) synthesized the primers. PCR primer sequences, the product size, annealing temperature and product-specific information are shown in Table I.

PCR amplification

PCR was performed in a volume of 30μl, containing 2×Taq PCR MasterMix 15μl (Tiangen Biotech), 0.3μM primers and 20ng of genomic DNA. Water was added to 30μl. The PCR amplification conditions are listed in Table II; the PCR products were analyzed by 1.0% agarose gel electrophoresis.

Sequencing of the PCR products

The PCR products were purified and sequenced (Invitrogen). The sequences were analyzed to identify the SNPs sites in the coding region of the *MSTN* gene using the DNAStar software.

Statistical analysis

The association analysis between single marker and traits were performed by using the GLM procedure in the SAS software package (SAS Inst. Inc., Cary, NC, USA). The linear model was as follows:

$$Y=\mu+G+S+G\times S+e$$

where Y is the dependent variable (analyzed traits); μ is the population mean; G is the SNP genotype; S is the sex of the sample; G×S is the interaction effect between genotype and sex, and e is the random residual.

RESULTS

The results of PCR amplification

Figure 1 shows the results of PCR amplification using the three pairs of primers. The amplicons were of the expected sizes. Three pairs of primers were used in PCR, and the PCR products were detected using 1% agarose gel electrophoresis (Fig.1). The results were consistent with purpose fragment.

Sequencing of the PCR products

Three polymorphisms were identified in exon 1: g.106G>A, g.120A>G, and g.159G>A (compared with GenBank: NW_004676457.1, Fig. 2A-C), and the G/A mutation in the g.106G>A SNP resulted in a change of codon from GGC to AGC, and the coding amino acid from Gly to Ser. There was no SNP in the second exon.

The third exon contained three SNPs: g.5368G>A, g.5389A>C and g.5410G>A (compared with GenBank: NW_004676457.1, Fig. 2D-F). GenBank entry NW_004676457.1, has the genotype GG, AA, GG, GG, AA and GG for the six identified SNPs.

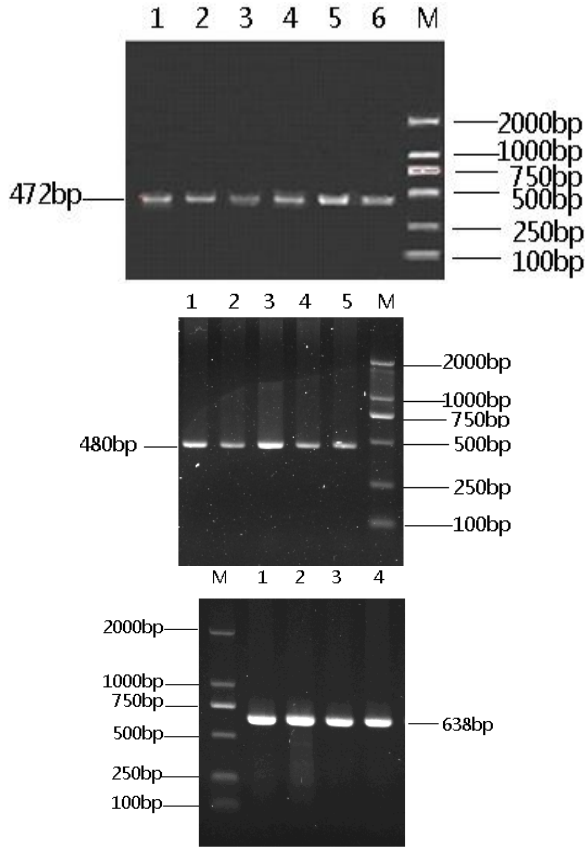

Fig. 1. The electrophoresis results of PCR amplicons for duck *MSTN* gene exons. (M: DNA molecular weight marker DL2000.).

The linkage disequilibrium analysis of the SNPs

We performed the linkage disequilibrium analysis using *Haploview4.0* to understand the interrelationships among the six SNPs detected in the coding regions of the *MSTN* gene. The results are shown in Figure 8.There was a complete linkage disequilibrium (r^2=1) between g.5368G>A and g.5389A>C.

The allele frequencies and genotype frequencies of the MSTN gene

The number of the different genotypes was counted and the genotype frequencies and allelic frequencies of the six SNPs were calculated (Table III). We observed that allele G of locus g.5368G>A and allele A of locus g.5389A>C had the same allelic frequency, and allele A of locus g.5368G>A and allele C of locus g.5389A>C had the same allelic frequency. This proved that the validity of the linkage disequilibrium analysis (Fig. 3). Chi squared tests showed that the other five allele frequencies, except g.5410G>A, were in accordance with the assumption of the Hardy–Weinberg equilibrium (P > 0.05) in the experimental population.

The homozygosity, heterozygosity, effective number of alleles and polymorphism information content of the *MSTN* gene polymorphic loci are shown in Table IV. The heterozygosities of the g.106G>A, g.159G>A, g.5368G>A and g.5389A>C loci (*He*) were high relativity, which showed the large variance in Sansui duck populations. For each locus, the *PIC* was different (two loci had low polymorphism information contents (*PIC*<0.25) and other four loci had intermediate polymorphism (0.25<*PIC*<0.5)) It could offered a certain amount of genetic information.

Association analysis of SNPs with slaughter traits

The association analysis between the polymorphisms of the *MSTN* gene and the slaughter traits of Sansui ducks are shown in Tables V and Table VI. The results indicated that *MSTN* g.106G>A had a significant association with slaughter traits in ducks ($p<0.05$), and the association of individuals with the GG genotype was significantly higher than GA and AA genotype ($p<0.05$). The *MSTN* g.120A>G had a significant association with slaughter weight, breast muscle weight, dressing percentage, breast muscle percentage and lean meat percentage ($p<0.05$). The ducks had significant differences in dressed percentage and slaughtering weight, and the female ducks' breast muscle percentages were significant difference in different genotypes: individuals with the AG genotype were always significantly higher than those with the AA genotype ($p<0.05$), which might be caused by a dominant effect. The *MSTN* gene g.5368G>A and g.5389A>C SNPs were in complete linkage, and they had significant associations with weight, slaughter weight, semi-eviscerated weight, eviscerated weight and breast muscle percentage ($p<0.05$). Individuals with the AA/CC genotype showed significantly higher associations compared with those with GG/AA and GA/AC genotype ($p<0.05$) for drakes' weight, slaughter weight, semi-eviscerated weight, eviscerated weight, and female ducks' breast muscle percentage. The results showed that sex had an effect on weight, slaughter weight, semi-eviscerated weight and eviscerated weight. Female breast muscle percentage was significantly higher than that of the male. There were no significant differences for the other traits. The g.5410G>A mutation site had no significant effect on slaughter traits.

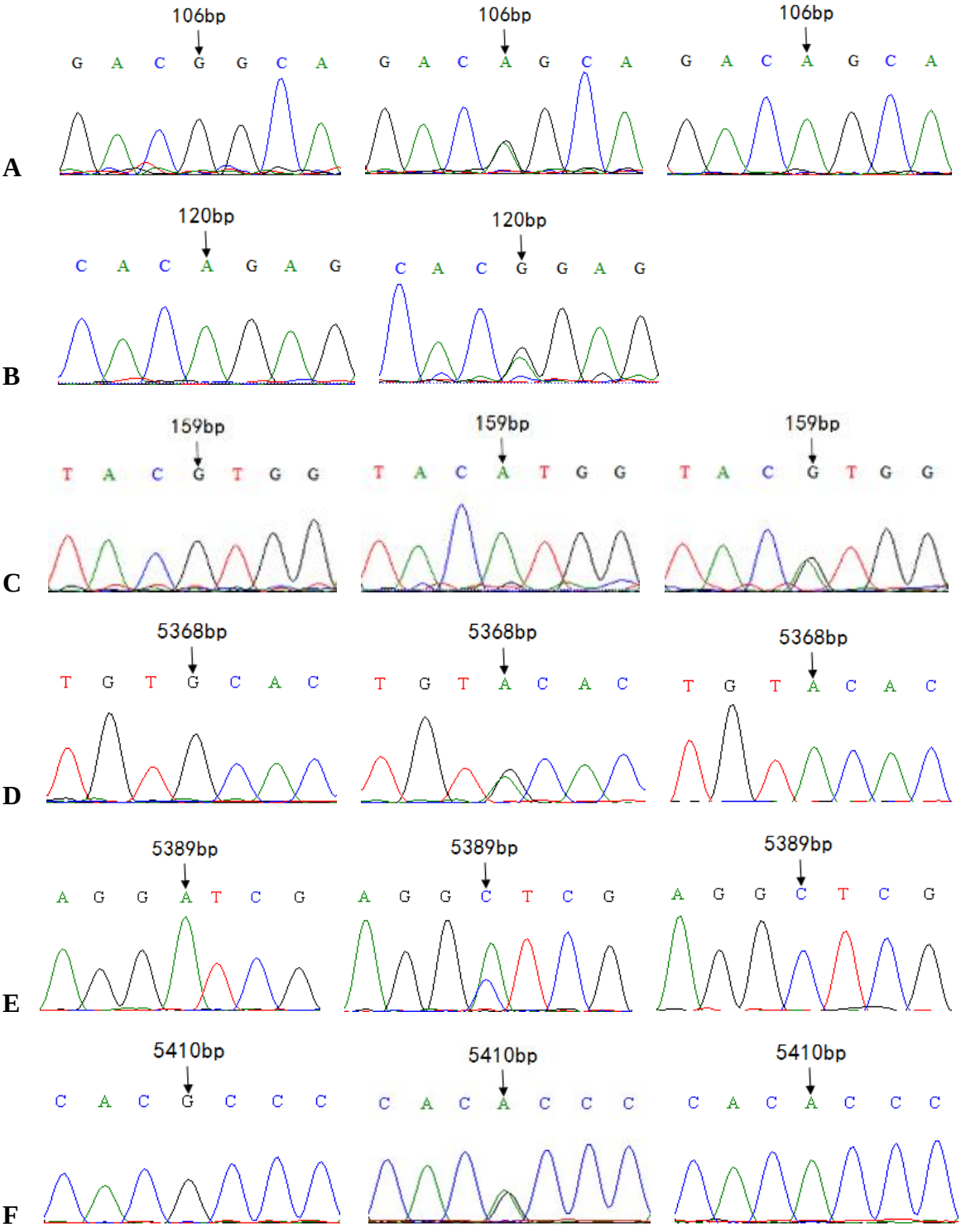

Fig 2. The sequencing map of the *MSTN* gene at position 106 (A), position 120 (B), position 159 (C), position 5368 (D), position 5389 (E), and position 5410 (F).

Table III.- The genotype frequencies and allelic frequencies of each SNP .

SNP	Genotype frequency			allele frequency		χ^2 P value
g.106G>A	GG (11):0.1834	GA (35):0.5833	AA(14):0.2333	G:0.4750	A:0.5250	1.7257(0.4220)
g.120A>G	AA (50):0.8333	AG (10):0.1667	GG (0):0	A:0.9167	G:0.0833	0.4964 (0.7802)
g.159G>A	GG (14):0.2333	GA (34):0.5667	AA(12):0.2000	G: 0.5167	A: 0.4833	1.0869 (0.5807)
g.5368G>A	GG (27):0.4500	GA (25):0.4167	AA (8):0.1333	G: 0.6583	A: 0.3417	0.3267 (0.8493)
g.5389A>C	AA (27):0.4500	AC (25):0.4167	CC (8):0.1333	A:0.6583	C:0.3417	0.3267 (0.8493)
g.5410G>A	GG (47):0.7833	GA (9):0.1500	AA (4):0.0667	G: 0.8583	A: 0.1417	8.8075 (0.0122)

Note: df=2, $\chi^2_{0.01(2)}$=9.21, $\chi^2_{0.05(2)}$=5.99, χ^2 of the different genotype distribution of Hardy–Weinberg equilibrium test.

Table IV.- Population genetic parameters

SNP marker	Homozygosity (*Ho*)	Heterozygosity (*He*)	Effective number of allele (*Ne*)	polymorphism information content (*PIC*)
g.106G>A	0.5012	0.4988	1.9950	0.3744
g.120A>G	0.8403	0.1597	1.1901	0.1597
g.159G>A	0.5006	0.4994	1.9978	0.3747
g.5368G>A	0.5501	0.4499	1.8177	0.3487
g.5389A>C	0.5501	0.4499	1.8177	0.3487
g.5410G>A	0.7568	0.2432	1.3213	0.2136

Note: PIC<0.5 means high diversity,0.25<PIC<0.5 means moderate diversity, PIC<0.25 means low diversity.

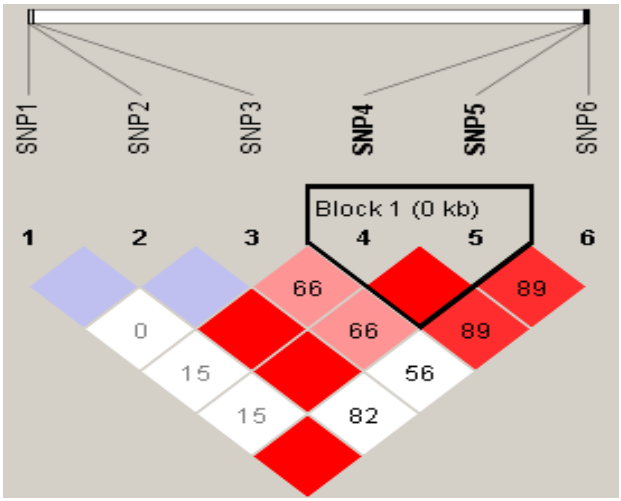

Fig. 3. The result of linkage disequilibrium analysis of six identified SNPs

DISCUSSION

Myostatin is a cytokine that plays an important role in growth and development of skeletal muscle. The *MSTN* gene of cattle was associated with the double muscular phenotype (Grobet *et al.*, 1997). At the same time, PCR amplification of the MSTN gene of cattle indicated that its overexpression in skeletal muscle of the Belgian Blue cattle might reflect a lack of inhibition of *MSTN* in skeletal muscle cells (McPherron and Lee, 1997). Sellick *et al.* (2007) reported that silencing of the bovine *MSTN* gene could lead to double muscular cattle, which was significant in the production of beef cattle. *MSTN* gene polymorphisms have been used for marker-assisted selection in Boer, Nubi, Matou and Haimen goats (Zhang *et al.*, 2012). Gu (2002) proposed that the chicken *MSTN* gene was an important candidate gene that affected meat traits, and the effect was different according to the different genetic backgrounds. Guernec *et al.* (2004) studied the expression of the *MSTN* gene in chickens of different ages using RT-PCR. The results showed that there were reduced *MSTN* mRNA levels in adult chickens in the fasting state. The chicken *MSTN* exon 1 was subjected to PCR-restriction fragment length polymorphism analysis, which showed that two polymorphic loci had significant or extremely significant relationships with breast muscle weight, leg muscle weight, abdominal fat weight, breast muscle rate and slaughter percentage (Zhu *et al.*, 2007). Hu *et al.* (2003) demonstrated high expression levels of the chicken *MSTN* gene in skeletal muscle. In a study of the SNPs of the *Myostatin* gene coding region of the Yangzhou goose, Wanxi white goose and Wulong goose, the results showed that the coding regions were high conserved among geese:

Table V.- The association between the polymorphisms of *MSTN* gene and Sansui duck slaughter traits.

Traits	g.106G>A						g.120A>G						g.5368G>A / g.5389A>C					
	Gene		Sex		Gene × Sex		Gene		Sex		Gene × Sex		Gene		Sex		Gene × Sex	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Weight	0.180	0.836	2.537	0.117	2.103	0.132	0.111	0.741	1.733	0.193	1.107	0.297	2.294	0.111	6.173	0.016	3.276	0.045
Slaughter (g)	0.535	0.589	4.029	0.050	2.703	0.076	0.287	0.594	4.521	0.038	2.804	0.100	2.525	0.090	9.630	0.003	4.293	0.019
Semi-eviscerated (g)	0.171	0.843	1.048	0.311	0.629	0.537	0.126	0.724	0.486	0.489	0.064	0.801	3.373	0.042	5.958	0.018	2.946	0.061
Eviscerated (g)	0.173	0.842	1.292	0.261	0.603	0.551	0.077	0.783	0.504	0.481	0.011	0.916	3.388	0.041	6.202	0.016	2.627	0.082
Leg muscle (g)	0.103	0.902	0.092	0.762	1.341	0.270	0.633	0.430	0.138	0.712	0.026	0.872	1.161	0.321	2.242	0.140	0.876	0.422
Breast muscle (g)	2.015	0.143	0.014	0.905	1.047	0.358	1.616	0.209	3.499	0.067	4.137	0.047	0.729	0.487	0.148	0.702	0.399	0.673
Dressing (%)	0.712	0.495	1.708	0.197	2.054	0.138	0.143	0.706	5.915	0.018	3.249	0.077	0.622	0.541	2.958	0.091	0.413	0.664
Semi-eviscerated (%)	2.659	0.079	0.598	0.443	1.121	0.334	1.977	0.165	0.998	0.322	1.980	0.165	0.885	0.418	0.100	0.753	0.446	0.642
Eviscerated (%)	2.317	0.108	0.246	0.622	1.210	0.306	1.404	0.241	0.848	0.361	2.675	0.108	0.869	0.425	0.186	0.668	0.257	0.775
Leg muscle (%)	0.001	0.999	0.139	0.711	1.443	0.245	1.676	0.201	0.000	0.995	0.037	0.848	0.007	0.993	0.002	0.967	0.045	0.956
Breast muscle (%)	2.620	0.082	0.978	0.327	0.659	0.522	4.309	0.043	9.251	0.004	7.803	0.007	0.374	0.690	4.721	0.034	2.029	0.141
Lean meat (%)	0.955	0.391	0.685	0.411	1.270	0.289	4.314	0.042	3.088	0.084	3.053	0.086	0.094	0.910	1.529	0.222	0.916	0.406

Notes: F means F test (analysis of variance) and Sig. means the *P* value

Table VI.- Association results between the genotype of the *MSTN* gene and slaughter traits.

		g.106G>A			g.120A>G		g.5368G>A / g.5389A>C		
		GG(11)	GA(35)	AA(14)	AA(50)	AG(10)	GG/AA(27)	GA/AC(25)	AA/CC(8)
Weight (g)	♀	/	/	/	/	/	1390±83(15)	1332±92(9)	1320±125(6)
	♂	/	/	/	/	/	$1388±135^{A}$(12)	$1357±124^{A}$(16)	$1595±339^{B}$(2)
	T	/	/	/	/	/	1389±107	1348±112	1389±209
Slaughter weight (g)	♀	1213±66	1271±84	1259±114	1264±89	1216±88	1283±67	1237±102	1220±113
	♂	$1410±262^{B}$	$1280±114^{A}$	$1267±110^{A}$	$1283±118^{A}$	$1376±260^{B}$	$1290±109^{A}$	$1276±127^{A}$	$1533±390^{B}$
	T	1303±201	1276±100	1263±108	1274±104	1296±201	1286±86	1262±118	1298±228
Semi-eviscerated weigh (g)	♀	/	/	/	/	/	1153±79	1106±101	1113±104
	♂	/	/	/	/	/	$1170±105^{A}$	$1114±130^{A}$	$1367±339^{B}$
	T	/	/	/	/	/	$1160±90^{B}$	$1111±118^{A}$	$1177±195^{B}$
Eviscerated weight (g)	♀	/	/	/	/	/	1021±71	982±96	999±91
	♂	/	/	/	/	/	$1040±98^{A}$	$997±114^{A}$	$1219±287^{B}$
	T	/	/	/	/	/	$1030±83^{B}$	$992±106^{A}$	$1054±168^{B}$
Dressing percentage (%)	♀	/	/	/	92.75±2.64	91.31±0.90	/	/	/
	♂	/	/	/	$93.39±3.24^{A}$	$95.59±3.59^{B}$	/	/	/
	T	/	/	/	93.07±2.95	93.45±3.34	/	/	/
Breast muscle percentage(%)	♀	/	/	/	$10.87±1.59^{A}$	$13.26±0.88^{B}$	$10.70±1.51^{A}$	$11.50±2.05^{B}$	$12.32±1.45^{C}$
	♂	/	/	/	10.75±1.27	10.39±1.55	10.80±1.57	10.72±1.15	9.74±0.14
	T	/	/	/	$10.81±1.43^{A}$	$11.83±1.92^{B}$	10.74±1.51	11.00±1.54	11.68±1.71
Lean meat percentage (%)	♀	/	/	/	22.24±2.70	25.49±1.39	/	/	/
	♂	/	/	/	22.23±2.26	22.52±2.78	/	/	/
	T	/	/	/	$22.24±2.46^{A}$	$24.00±2.60^{B}$	/	/	/

Note: Lin data with different uppercase symbols without the same superscripts differ significantly (P<0.05), within a line, data followed by the same letters indicate no significant difference at 0.05 level (P>0.05). “T” means male and female.

one mutation was detected at 263bp of the third exon, and individuals with the AB genotype had higher meat production performance in Wulong Geese (Yang *et al.*, 2007). In addition, recent studies have shown that the SNPs in the *MSTN* gene affect animals' production performance. PCR-single strand conformation polymorphism (PCR-SSCP) analysis of SNPs in the *MSTN* gene confirmed that several haplotypes of Romney sheep had obvious effects on slaughter traits (Han *et al*., 2015). At roughly the same time, other researchers showed that a polymorphism of the *MSTN* gene affects Romney sheep's growth and carcass muscle traits (Wang *et al*., 2015). Silencing of *MSTN* changed the development of duck embryonic myoblasts by regulating the expression level of the *MYOD* and *MYF5* genes, and affected skeletal muscle growth (Tao *et al*., 2015). All of these results revealed that SNPs in the *MSTN* gene probably have value as genetic markers for improving animals.

There have been few studies concerning the duck *MSTN* gene. Dai (2006) analyzed a partial sequence of *MSTN* exon 3 and intron 2 by PCR-SSCP in Peking ducks and Cherry Valley ducks. The results showed that polymorphisms in the *MSTN* gene had a significant effect on the breast muscle weight, breast muscle percentage, leg muscle weight and leg muscle percentage. Analysis of the 5′regulatory region of the *MSTN* gene showed that polymorphisms were associated with the breast muscle percentage and abdominal fat rate (Lu *et al*., 2008). A G→A mutation at 2701bp in exon 3 of the *MSTN* correlated with the abdominal fat rate in Gaoyou ducks (Liu *et al.*, 2012). Zhang *et al.* (2013) studied polymorphisms of the 5′-regulatory region of the *MSTN* gene in Putian black duck, Liancheng white duck, Shaoxing duck, Youxian duck, Jianchang duck, Gao You and Pekin duck, which identified seven variations of the C1024G loci representing three genotypes. Research into the correlation between all the exons of the *MSTN* gene and slaughter traits in duck has not been reported. Thus, in this study all the exons of the *MSTN* gene were amplified by PCR and sequenced. We identified six SNPs in the first and the third exons, the g.5368G>A and g.5389A>C were in complete linkage disequilibrium, and in addition, the g.5410G>A locus deviated from the Hardy–Weinberg equilibrium. Four loci showed a significant effect ($P<0.05$) on leg muscle weight, leg muscle percentage and dressing percentage. However, there were some limitations to this study, such as the small sample size and the lower abdominal fat rate. Combined with previous results, we hypothesized that the *MSTN* gene probably has an association with muscle growth traits in ducks, but the result may be not exact because of the small number of samples; therefore, we intend to perform further studies with larger sample sizes under different conditions. In conclusion, *MSTN* gene polymorphisms have a close association with slaughter traits in Sansui ducks.

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Conflict of interest declaration

The authors have no conflicts of interest. The manuscript has been submitted solely to the Pakistan Journal of Zoology and is not being published, considered for publication, or submitted elsewhere.

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