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Molecular Characterization of MSTN Gene in Holstein Friesians and Brown Swiss Cattle Breeds

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ABSTRACT

This research was carried out to investigate the polymorphisms of Myostatin gene in Holstein Friesian and Brown Swiss cattle breeds. PCR-RFLP methods were used to investigate of MSTN gene. *DraI* restriction enzyme was used to detect of MSTN gene polymorphism. The result showed that there were two genotypes (AA and AB) of MSTN gene in two cattle breeds. The allele frequencies in Holstein Friesians were 0.97 and 0.03 while the allele frequencies in Brown Swiss were 0.88 and 0.12.Genotype frequencies in Holstein Friesian were 0.94 and 0.06 while the genotype frequencies in Brown Swiss were 0.77 and 0.23. The genotype distributions for these alleles in two cattle breeds were in agreement with Hardy-Weinberg equilibrium (P>0.05). If there is statistical significance in association analysis with this gene for meat characteristics and growth characteristics, this result can be used in the future to improvement of meat properties and growth characteristics in the cattle. Also, this study is also important in determining the status of these two breeds raised in Turkey and to shed light on those who will work on this issue.

1. Introduction

Improvement of livestock has focused on the selective breeding of individuals with superior phenotypes. With the development of increasingly advanced statistical methods that maximize selection for genetic gain, this simple approach has been extremely successful in increasing the quantity of agricultural output (Williams, 2005). Marker assisted selection (MAS) is a novel technique that can complement traditional breeding methods for rapid genetic gains. Genetic gain through selective breeding is the objective of a breeder to achieve long term improvement in animal and plant genomes; however the pace of improvement is inversely proportional to the generation interval. Genetic improvement in livestock, particularly those with long generation intervals, requires decades for tangible results. Successful MAS breeding programmes require gene mapping, marker genotyping, quantitative trait loci (QTL) detection, genetic evaluation and finally MAS (Moniruzzaman et al., 2014). Use of these can aid on the selection of animals with highest breeding values. To determine the best genotypes carrying alleles by taking into account the phenotypic values of animals in quantitative characters are difficult (Aytekin and Boztepe, 2013).

Myostatin or GDF-8 concerning with economic characteristics of farm animals have been studied for marker assist selection (MAS). This gene is the part of the transforming growth factor beta (TGF-β) superfamily. This superfamily is cytokines, whose function is predominantly to control cell growth, apoptosis, cell differentiation and proliferation from embryonic to mature cells. Myostatin has the specific function of negatively regulating muscle growth and muscle homeostasis through its interactions with myoblasts during their proliferation to myotubes (Rasmussen, 2016). The amino acid sequence of myostatin is conserved across many species (human, cow, rat, mouse, monkey, dog, chicken and turkey). Typically, it consists of three exons and two introns, the length and sequence of which can vary slightly. Bovine MSTN is located near the centromere on chromosome 2 (2q14-q15) and has a total length of 6673 base pairs (bp), of which 2767 bp make up the coding-region of the myostatin protein.

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Exon 1 is 5063 bp, intron 1 is 1840 bp, exon 2 is 374 bp and intron 2 is 2033 bp. Exon 3 has a variable length of 1701 bp, 1812 bp or 1887 bp, depending on where the polyadenylation site is located (Jeanplong et al., 2001). The objective of this study was to analyze the molecular characterization of MSTN gene in Holstein Friesians and Brown Swiss cattle breeds.

2. Material and method

In this study, 103 Friesian and 125 Brown Swiss were used. Disodium EDTA containing tubes were used to prevent coagulation of blood during collection of samples. Then, blood samples storage was carried out at -20^{c} until DNA extraction procedures Blood samples were taken from the Tail Vein cattle. Genomic DNA was extracted from whole blood using the Quick Gene DNA whole blood kit S (DB-S) (KURABO, Japan). Amplification of fragments of MSTN gene was carried out with expected amplicon sizes of 1346 bp. Myostatin gene, forward:

5′-CCCTACAGAGGCCACTTCAA-3′ and reverse:

5′-CTCGCTGTTCTCATTCAGATC-3′, were designed by Zhang et al., (2007).

The PCR was done in a reaction volume of 10 µL according with some modifications. The reaction consists of 5µL of 2X Dream Taq Green PCR Master Mix (Thermo Scientific, USA), 0.30µL primer each primer forward and reverse (10 pmol) (Macrogen, Turkey) and 3.4µL ddH2O which finally added to 1 µL genomic DNA. thermal cycling program denaturation at 94°C for 3 min, followed by 39 cycles at 94°C for 30 sec, annealing temperature 63°C for 40 sec and extension at 72°C for 1 min, final step is the extension at 72°C for 10 min. The PCR product of each sample (5 µL) and 100 bp DNA ladder (Vivantis, Malaysia) were loaded in 2% (w/v) agarose gels in 0.5X Tris-Borate-EDTA (TBE) buffer staining using ethidium bromide. The electrophoresis was carried out for 45 min at 100 V. The electrophoresis gel was examined on an UV transilluminator and bands were visualized and photographed. The PCR products of MSTN gene were cleaved by fast digest; amplified fragments were digested with *DraI* (Thermo Scientific, #FD0224) at 37°C. The reaction volume was 15 µL consisted of 5 µL PCR product, 8.5 µL ddH2O, 1 µL 10X buffer and 0.5 µL restriction enzyme. The polymorphism of the cleaved fragments recognition was carried out by %2 agarose gel electrophoresis then their polymorphic pattern was obviously envisioned under U.V by gel documentation system.

3. Result and Discussion

Two polymorphisms, MSTN-*DraI* were observed after products were digested with enzymes. The polymorphism of MSTN-*DraI* is caused by T/A transversion at position -371 (relative to ATG start codon) that introduces a site for *DraI* restriction enzyme (Crisa et al., 2003). Digestion of the PCR fragment of MSTN promoter with *DraI* resulted in fragment lengths of 505, 427, 321,93 and 62 bp for phenotype AA, and 505, 427, 365, 321, 93 and 62 bp for phenotype AB (Figure 1). The frequency of allele A and B is given in (Table 1).

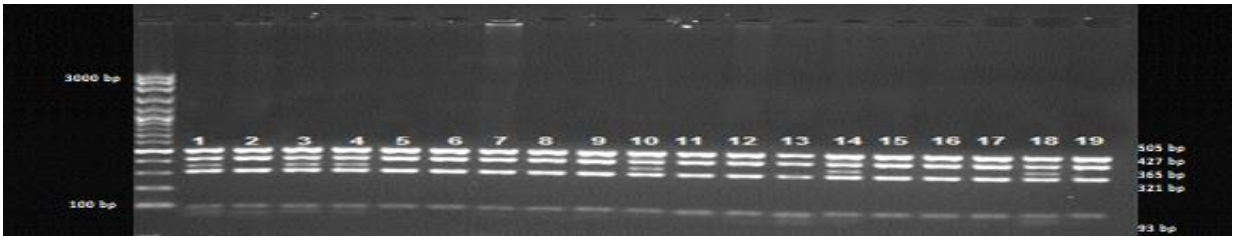

Figure 1 Agarose gel electrophoresis (2%) of PCR fragment of MSTN gene digested with *DraI*. Genotype AA is in lanes 2, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 19. Genotype AB is in lanes 1, 3, 4, 10, 14, 18. M is the 100bp marker.

Table 1. Allele and genotype frequencies at MSTN gene in two cattle breeds

Breed	Individuals (N)	Genotype frequencies			Allele frequencies		χ^2
		AA	BB	AB	A	B	
HF	103	0.94 (97)	0	0.6 (6)	0.97	0.3	0.76
BS	125	0.77 (96)	0	0.23 (29)	0.88	0.12	0.14

HF: Holstein Friesians; BS: Brown Swiss; NS; not significant (P>0.05); χ^2: test of Hardy-Weingberg equlibrium,

Myostatin has been identified as the factor causing a phenotype known as double muscling, in which a series of mutations render the gene inactive, and therefore, unable to regulate muscle fibre deposition (Bellinge, 2005). Myostatin is involved in double muscling as it functions like a negative regulator of the muscle cell growth, thus inhibiting myoblast proliferation and differentiation (Crisa, 2003). Crisa et al.,

(2003) did not find statistically significant differences with the mutation between the genotypes of three breeds of cattle. But there is a significant difference between individuals who carry the mh/+ combination in third exon and AA in the *DraI* site, with + / + in exon 3 and AB or BB in the *DraI* site. Zhang et al., (2007) found three genotypes in three Chinese cattle breeds (AA, AB and BB) but no statistically significant differences in growth traits were observed between the genotypes of the Jiaxian breed at MSTN loci. However, there were statistically significant differences between the genotypes at MSTN locus of the Nanyang breed for withers height, heart girth, heart girth index and ratio of heart girth and body length ($P<0.05$), and the traits affected significantly were different at different growth stages in Nanyang cattle breeds. Genotypic frequencies of AA, AB and BB for the Nanyang, Qinchuan and Jiaxian breeds were 0.91, 0.94 and 0.93 for AA; 0.09, 0.05 and 0.05 for AB and 0.000, 0.000 and 0.009 for BB, respectively. These reported values are similar to those obtained values in the current study. Nasr *et al*., (2016) reported that allele and gene frequencies were homozygot for MSTN gene in Holstein bull (AA). Han et al. (2012) investigated that the relationship between MSTN g.-371T>A gene and carcass characteristics in Korean cattle. For this purpose, three different genotypes using *DraI* restriction enzymes have identified in Holstein, Juju Black Cattle and Hanwoo breeds. Genotype frequencies were 0.001, 0.063 and 0.016 (AA); 0.053, 0.312 and 0.212 (AT) and 0.946, 0.625 and 0.772 (TT) for all three breeds, respectively. Allele frequencies were 0.028, 0.219 and 0.122 (A); 0.972, 0.781 and 0.878 (T) in the same order, respectively. In this study, two genotypes (AA and AB) were identified. It was observed that there was a difference between two cattle breeds. This population showed high frequencies of alleles which can be used in genetic improvement programs. This study thus provides base line data for future genetic assessments of this population. This result can be used in the future to improvement of meat properties and growth characteristics in the cattle. This study is also important in determining the status of these two breeds raised in Turkey and to shed light on those who will work on this issue. As a result, it can be said that more work needs to be done in this issue.

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6. References

Aytekin İ, Boztepe S (2013). Associations of Pit-1 Gene Polymorphism with Milk Yield and Composition Traits in Brown Swiss Cattle. *J. Anim. Plant Sci*. 23 (5): 1281-1289.

Bellinge RH,Liberles S,Iaschi DA, O'brien SPA, PA, Tay GK (2005). Myostatin and its implications on animal breeding: a review. *Animal genetics*36(1): 1-6.

Crisa A, Marchitelli C, Savarese MC, Valentini A (2003). Sequence analysis of myostatin promotor in cattle. *Cytogenet, Genome Res*102:48-52.

Han SH, Cho IC, Ko MS, Kim EY, Park SP, Lee SS, Oh HS (2012). A promoter polymorphism of MSTN g.− 371T> A and its associations with carcass traits in Korean cattle. *Molecular biology reports* 39(4): 3767-3772.

Jeanplong F, Sharma M, Somers WG, Bass JJ, Kambadur R (2001). Genomic organization and neonatal expression of the bovine myostatin gene. *Molecular and Cellular Biochemistry*220(1): 31-37.

Moniruzzaman M, Khatun R, Mintoo AA (2015). Application of marker assisted selection for livestock improvement in Bangladesh. *Bangladesh Veterinarian* 31(1): 1-11.

Nasr SM, AteyaAI, Sadek KM, Radwan HA (2016). Effect of genetic polymorphisms in GH/HpaII and MSTN/*DraI* loci on body weight in Friesian bull calves. *Pak. J. Biol. Sci*19: 338-344.

Rasmussen, KL (2016). Investigation of variation in the promoter region of the myostatin gene (GDF-8) in New Zealand cattle. PhD thesis, Lincoln University.

Williams JL (2005). The use of marker-assisted selection in animal breeding and biotechnology. *Revue Scientifique et Technique-Office International des Epizooties* 24(1): 379.

Zhang RF, Chen H, Lei CZ, Zhang CL, Lan XY, Zhang YD, Wang XZ (2007). Association between polymorphisms of MSTN and MYF5 genes and growth traits in three Chinese cattle breeds. *Asian Australasian Journal Of Animal Sciences* 20(12): 1798.