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Polymorphism of Myostatin Gene (MSTN) Coding Region in Batur Sheep

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Abstract. The present study was to identify the Myostatin gene polymorphism in Batur sheep, Batur lambs were reared under intensive feed system. Body weight measured monthly after weaning until six months of age. DNA Extraction used 200 ul of whole blood samples. To amplify the exon 3 region of MSTN gene a specific primer designed using the Primer3 software. Volume 25 µl contained 25 ng genomic DNA, with a 2x Reaction mix 12.5 µl for each primer. The PCR cycling protocol was 35 cycles with denaturation at 94 °C, 73.9 annealing for 45 sec, extended at 72°C, with a final extension at 72°C. Eleven polymorphic sites were observed in the third exon region transversions at c.*121 G instead of A, del-T at c.*129, one individual at c.*139 and one individual at c.*158 positions, however, one individual sequence disrupted reading frame in whole *MSTN* sequenced, MSTN gene was polymorphic in Batur sheep, however, frequency of allele A compare to allele B is low in diversity, this gene may not be a marker-assisted in the future selection program.

Keywords: Identification, MSTN gene, Polymorphism and Batur sheep

Abstrak. Tujuan dari penelitia ini adalah untuk mengidentifikas Polimorfisme gen myostatin pada domba Batur, 30 ekor Batur semua domba diberi pakan konsentrasi. Ekstraksi DNA digunakan 200 µL sampel darah. Untuk memperkuat ekson 3 daerah MSTN gen dirancang primer tertentu menggunakan Primer3. 25 µL mengandung 25 ng DNA genom, 12,5 µL 2x campuran reaksi dari masing-masing primer. Protokol *cycling* adalah 5 menit pada 95 ° c sebagai denaturasi awal, 35 siklus mendenaturasikan pada 94°C selama 45 detik, Anealing di 73,9 selsma 45 detik, ekstensi pada 72°C, dan diekstensi akhir pada 72°C. Sebelas polimorfik situs yang diamati di dalam 3 ekson wilayah transversi di c.*121 G bukan A, del-T di c.*129, satu individu di c *139 dan satu individu di c.*158 posisi Namun, satu urutan individu terganggu membaca frame di seluruh dearah sekuen gen *MSTN*. Gen MSTN bersifat polimorfik pada domba Batur, namun, frekuensi alel A dibandingkan dengan alel B rendah dalam keragaman. Gen ini mungkin tidak menjadi penanda bantuan dalam program seleksi di masa depan.

Kata kunci: Identifikasi, Gen MSTN, Polimorfisme, Domba Batur

Introduction

Myostatin MSTN gene is known as growth and differentiation factor 8 (GDF8), which negatively regulates skeletal muscle growth (McPherron et al., 1997). It is may also contribute to the adipogenesis regulation (Lee and McPherron, 2001) and the regulation and function of tendon structure during both postnatal and prenatal growth (Mendias et al., 2008). The variations in the myostatin gene (MSTN) have been associated with muscling in difference mammalian species including humans (Schuelke et al., 2004), mice (McPherron et al., 1997), pigs (Stinckens et al., 2008), cattle (Dunner et al., 2003; Grobet et al., 1997), dogs (Mosher et al., 2007), and sheep (Kijas et al., 2007; Boman and Våge, 2009; Johnson et al., 2009; Hickford et al., 2010).

Myostatin plays a critical role in muscular hypertrophy, hyperplasia, and myogenic differentiation seen in animals that lack functional myostatin is due to deregulated differentiation and proliferation of myoblasts (Rehfeldt et al., 2000; Parker and Rudnicki 2003). Genetic diversity often happens within the coding regions, there are only three variations described in the coding region of ovine MSTN. These include; c.960delG which

disrupt the reading frame from aa320 to a premature stop codon at aa359 (Boman et al., 2010), c.101G>A result in a missense substitution of glutamic acid to glycine at amino acid 34 (aa34/codon 34/c34) (Zhou et al., 2008); and c.120insA results in a premature stop codon at aa49 and leads to a nonfunctional MSTN completely due to the bioactive carboxy-terminal end of the protein not being produced (Boman & Våge, 2009).

The c.120insA and c.960delG are associated with reducing carcass fat and increases of the muscle mass and in the Norwegian White breeds (Boman et al., 2010) and Norwegian Spælsau (Boman & Våge, 2009) respectively. There are intensive studies on genetic variations of the MSTN gene in Texel sheep (Walling et al., 2001; Marcq et al., 2002; Johnson et al., 2005; Clop et al., 2006; Kijas et al., 2007). The bases of genetic evolution and diversity in the coding regions may alter negatively or positively the function of protein products therefore, production characteristics (Smith et al. 2000). Consequently, coding region variants or variations with negative unwanted influences are systematically eliminated from breeding flocks while those with positive impacts on traits are held, however, the majority of DNA variants with deep influence on production traits are located in the exons (Smith et al. 2000). The present study aimed to identify the polymorphism of the myostatin gene in Batur sheep.

Materials and Methods

Experimental Design

Batur sheep are the predominant sheep in the upland areas of Banjarnegara – Batur village - where they are well adapted to the cold humid environment. 30 heads of Batur sheep used for this experiment, blood samples were collected from the jugular vein about 3 ml of each head of experimental lambs into Vacutainer tubes containing anticoagulant EDTA. For the DNA Extraction used manufacturer protocol (Lab P.T Genetika science); 200 ul of whole blood samples. To amplify an exonic region of MSTN gene a specific primer designed using the Primer3 software from the NCBI website, primer, forward 5´-TGCGGTAGGAGAGTGTTTGG-3´ and primer, reverse 5´-AAAATTGTTGAGGGGAAGACC-3´ with product size 487 bp and molting temperature 61,2 oC and 59,3 oC respectively. The concentration and purity of isolated DNA were measured by Nano-drop 8000 Spectrophotometer via Absorbance method, thereby, the DNA concentration was calculated (ng/µl) and DNA purity (A260/280) to remove any contaminants such as a protein, agarose, phenol, or other nucleic acids).

PCR Conditions

The total volume of 25 ul contained 25 ng of DNA genomic, 0.1 units of Taq DNA polymerase, and 12.5 ul 2x reaction mix for each primer. The protocol cycling was denaturated 5 minutes at 95°C as initial, 35 cycles of denaturation at 94°C for 45 sec, annealing at 73.9 for 45 sec, extended at 72°C for 40 seconds, with a final extension at 72°C for 10 min.

All samples sent to Malaysia for sequencing and used BioEdit program to identify mutation or nucleotide substitution, then sequenced results compared with MSTN gene reference coding region. Used the chromosome no. 2 of the MSTN region to identify candidacy gene by Ensembl database (www.ensembl.org) which chosen based on its known function or potential involvement with growth and muscularity.

Calculations of alleles, genotypes frequencies, heterozygosity, and chi-square tests were performed.

$$\text{Genotypic frequency} = \frac{total\ number\ of\ individuals\ of\ a\ particular\ genotype}{total\ number\ of\ individuals\ of\ all\ genotype}$$

Allele frequency = P+1/2 H

Where, P= frequency of homozygote, H= frequency of heterozygote

Results and Discussion

This study investigated the variation in the third exon of the Myostatin gene in Batur sheep breed at Banjarnegara – Batur village. PCR products were chosen to identify the single nucleotide polymorphism (SNP), a 487 bp fragment of the 3rd exon of the MSTN locus in Batur sheep was performed by manual PCR technique in this study. The sequences were aligned and screened for SNP. The analysis revealed a total of seventeen polymorphic sites in the MSTN coding region (figure 1). There are only two alleles observed (A and B) resulting in three genotypes, the animals with both alleles were considered as AB genotype, whereas those having only A or B alleles assigned as AA or BB genotypes. However, it is shown in chromatogram "GG, GC, and CC" as in (figure 1). Eleven polymorphic sites were observed in the in 3rd exon region transversions at locus c.*121 G>C, one individual G>A which disrupted the reading frame in whole MSTN sequenced, and one individual polymorphic site seen as a del-T at c.*129, c.*139 and c.*158 positions as in the figure.

In an investigated population, this locus is in Hardy-Weinberg equilibrium with X2 test of 0.0034 and probability p=0.95. Table 1 indicated that genotypic and allele frequencies in the 3rd exon of the Myostatin genotype were 0.552 AA, 0.379 AB, and 0.069 BB, where the allele frequency was 0.74 A and 0.26 B. this is a pre-result for ongoing study of association of this gene with production traits.

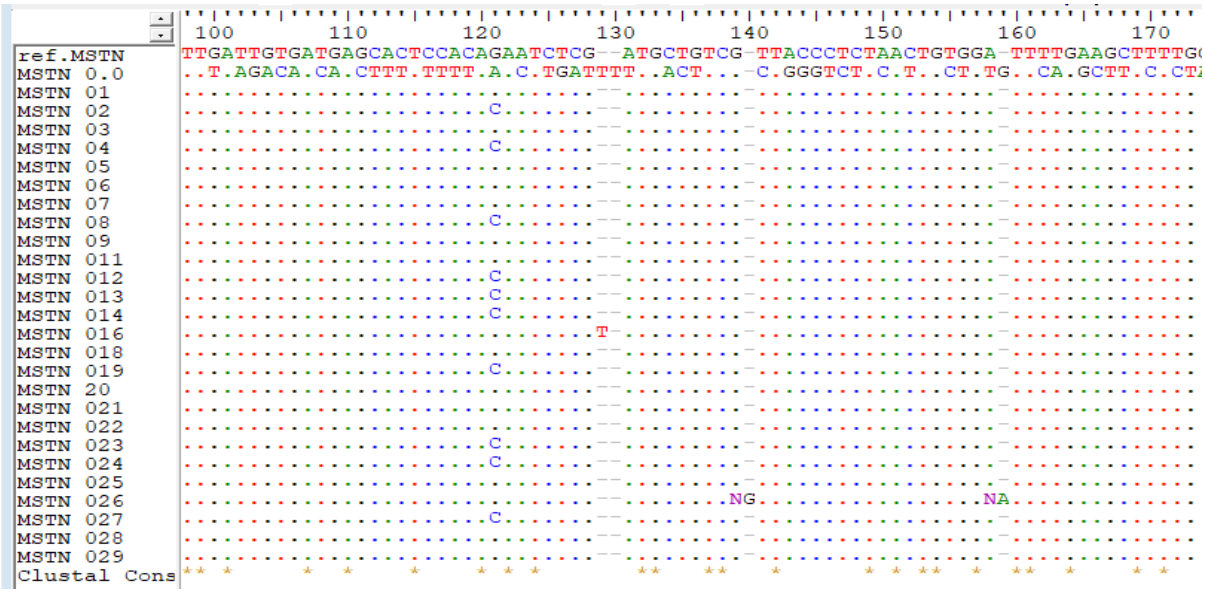

Figure 1. the BioEdit program used to alignment the 3rd exon of MSTN gene, all sequence plotted to a standard as dot, compared to the reference sequence (NCBI) there are 11 different variants on the matched sequence positions appeared at c.*121, del-T at c.*129, one individual at c.*139 and one individual at c.*158 positions, however, one individual sequence disrupted reading frame in MSTN.

Table 1. Genotypic, allelic frequencies and heterozygosity

Genotype	(16) AA	(11) AB	(2) BB	A	B	p-value
Genotypic frequency	0.552	0.379	0.069	0.74	0.26	0.95
Expected frequency	0.549	0.384	0.067			

The disability to find an association of genotypic and allelic in the 3rd exon of the MSTN gene in Batur sheep in present study might be due to the breed-specific influence of the locus or loci under study. Sumantri et al. (2008) reported that genetic variation of the MSTN c.del960G locus based on a molecular marker in Indonesian local sheep is very low, this is subjected to the value of a genotype frequency and an allele which has a value of 1, which make the fixation process. The deletion or absence in one base pair at the MSTN c.del960G locus could be caused by a tropical adaptation process which assumed that the animals live in this environment are having small performance. In this situation, the presence of the c.del960G variant at the coding region in both alleles means that no functional myostatin protein is expected to be produced (Boman et al., 2010). If myostatin not be expressed, so the negative growth regulation will fail and the number of muscle fibers are increasing (hyperplasia).

The high similarity of the MAST gene in Batur sheep because of their coding region is similar moreover, this breed has a close breeding system which is a result of the inbreeding effect, as it is assumed by Hardy-Weinberg Batur sheep experiencing a random mating system at a small scale then, the fixation process is expected, if it is on a large scale hence, the alleles deviation will occur and the population would be disequilibria. A genetic diversity found at the exonic region may not affect mRNA splicing and consequently influence the amino acid sequences produced from a process of the transcription.

Conclusion

In conclusion, this study is a preliminary state to identify the candidacy gene for future selection plans and meat characteristics. The significance of present research lies in realizing the genotypic and allelic effects of the MSTN gene coding region in Baur sheep. MSTN gene was polymorphic, but the frequency of allele A compared to allele B is low in diversity. This gene may not be a marker for future selection program.

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