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Short Communication: Polymorphism at third exon of the Myostatin gene and its association with growth and carcass traits in Batur sheep

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Abstract. *Haren HIH, Purwantini D, Sumaryadi MY, Prayitno. 2020. Short Communication: Polymorphism at third exon of the Myostatin gene and its association with growth and carcass traits in Batur sheep. Biodiversitas 21: 2074-2078.* The present study aimed to investigate the association between myostatin (MSTN) genotype and two traits, growth and carcass, in Batur sheep. Blood samples representing thirty head were collected and genomic DNA was extracted. A specific primer designed to amplify the MSTN gene, samples sequenced then, used the BioEdit program to identify any mutation. Calculation of genotypes, gene and allele frequencies, heterozygosities, and Chi-square test was performed. Only two alleles observed (G and C) resulted in three genotypes. 11 polymorphic sites were observed, transversion at c.*121G>C, and one individual G>A which disrupted reading frame of whole MSTN sequenced, genotypic and allelic frequencies were 0.552 GG, 0.379 GC, and 0.069 CC, where the allele frequency was 0.741 G and 0.259 C. Lambs that carrying genotype GC had slightly more pre-slaughter weight, hot carcass weight, cold carcass weight, carcass length, and total yield (lion, shoulder, leg yield, and thigh) compared to those carrying genotype GG. There was no significant effect of the MSTN genotype on carcass traits (p=0.05). Polymorphic site c.*121 G>C is present in Batur sheep for the first time about the association with the MSTN gene however, it has not effects weaning weight, 6-month weight, and studied carcass traits.

Keywords: Batur sheep, carcass traits, growth traits, MSTN gene, third exon

INTRODUCTION

Myostatin which is also known as a growth and differentiation factor 8 (GDF8), acts as a negative regulator of skeletal muscle growth. Variation in the Myostatin gene (MSTN) has been associated with muscling in mammalian including mice (McPherron et al. 1997), cattle (Grobet et al. 1997; Dunner et al. 2003), humans (Schuelke et al. 2004), dogs (Mosher et al. 2007), and sheep (Kijas et al. 2007; Boman and Våge 2009; Johnson et al. 2009; Hickford et al. 2010; Han et al. 2013). The polymorphism of this gene has been determined in various breeds of sheep (Zhou et al. 2008) and goat (Han et al. 2015). The ovine MSTN gene is 4991bp in size and is present on chromosome no. 2 (Boman et al. 2009). Single Nucleotide Polymorphisms (SNPs) within the coding region of the Myostatin gene are associated with double muscling (Hadjipavlou et al. 2008). Moreover; the Myostatin sequence analysis of double-muscled European breed revealed 7 DNA sequence polymorphisms and concluded that five of them were responsible for modulating the functions of protein (Joulia-Ekaza and Cabello 2007). Different methods for determining carcass and body composition of domestic animals have been extensively studied because of their nutritional and economic importance (De Paula et al. 2013). However, some methods are limited to use in laboratory conditions, and others due to their expensive cost (Scholz et al. 2015). Carcass quality traits are important for predicting the final amount of saleable meat per animal. These traits are correlated with live weight, affecting sheep farmers' income. Live measures (weights at different stages; ultrasound images of muscle transversal area and fat thickness) and post-mortem traits (carcass weight, length, and conformation; Knight et al. 2014; Ciappesoni et al. 2014) help to assess muscularity, fattening, and other carcass properties, and are usually included in genetic evaluation systems. Only three polymorphisms have been observed in the coding region of the ovine MSTN gene so far. One nonsynonymous single-nucleotide polymorphism (SNP) in the 34th codon was identified in the New Zealand Romney sheep (Zhou et al. 2008). Moreover, a deletion of one base pair in the position of the 960th nucleotide (c.960delG) was found in Norwegian White sheep (Boman et al 2009), whereas insertion of one base pair in the position of the 120th nucleotide was identified in the Norwegian Spælsau (Boman and Våge 2009). These two nucleotide variations, located in the coding region of the MSTN gene, resulted in nonfunctional protein formation (Boman et al. 2010). The present study was aimed to investigate the association

between MSTN genotype, and growth traits, and carcass traits in Batur lambs.

MATERIALS AND METHODS

Animals and Experimental Design

Batur sheep are the predominant breed in the upland areas of Banjarnegara - Indonesia - where they are well adapted to the local cold and humid environment. This breed developed by crossing between local breeds (Fat and Thin Tailed Sheep) and imported breed (Merino) (Prayitno 2010). Thirty heads of Batur sheep used for this experiment, the number of lambs born for every birth of each ewe was recorded and the suckling program of the lambs lasted for three months (90th day). Batur lambs were reared at the Batur area under an intensive feeding system until six months of age. All lambs were fed by concentrated feed consist of mix 3% feedstuff gave 3% of their body weight. Bodyweight measured monthly after weaning to six months of age. Male lambs were slaughtered by the Islamic method at weights (ranged from 25 to 45 kg). Then, the carcasses were split off into two identical longitudinal halves and sectioned into five regions (neck, shoulder, ribs, loin, and leg). Hot carcass weights were measured directly at slaughter, HCW is the weight in kilograms of the carcass components minus the pelt, head, and gut. Then, carcasses chilled under -4^{o}C for 24 hours in the big refrigerator room by hanging them up. Cold carcass weight measured 24 hours after slaughtering, other carcass data including loin yield, leg yield, total yield, and shoulder yield. The total yield is the sum of the leg, loin and shoulder yield for any given carcass.

DNA Extraction

Blood samples (3 ml) were collected from the jugular vein of each head of experimental lambs and put it into the vacutainer tubes contained EDTA (10 mL EDTA spray dried). For DNA extraction 200 μl of whole blood samples were used and performed according to the manufacturer protocol (Genetika science). To amplify the exon 3 region of MSTN gene a specific primer designed using the Primer3 software from the NCBI website in (Table 1).

The concentration and purity of isolated DNA were measured using Nano-Drop 8000 Spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA) by Absorbance method. About 1.5 µl isolated DNA for measuring the concentration and purity in each spectrophotometer well. The absorbance reading recorded at A260/A280 nm. Then, the DNA concentration (ng/µl) and DNA purity A260/280 were calculated.

Polymerase Chain Reaction Conditions

Each 25 µl PCR reaction contained 25 ng of genomic DNA, 12.5 µl 2x Reaction mix of each primer, and 1.0 its of Taq DNA polymerase as shown in table 2, The cycling protocol was 5 minutes at 95°C as an initial denaturation, 35 cycles of denaturing at 94°C for 45 seconds, annealing at 73.9 for 45 seconds, extending at 72°C for 40 s, with final extension for 10 minutes.

Coding region of MSTN sequencing

All samples were sent to Malaysia for sequencing and the BioEdit program was used to identify a nucleotide substitution or mutation, and sequenced results compared with MSTN gene reference (accession number DQ530260) coding regions. The chromosome ovr2 of the MSTN region used to identify a candidate gene by the Ensembl database (www.ensembl.org). A candidate gene was chosen based on their known function or potential involvement with growth and muscularity.

Data analysis

Genotypes, and allele frequencies, heterozygosity rates were counted and Chi-square test was performed. The mathematical model for gene and allele frequency (Nei and Kumar 2000) as:

$$\text{Genotype frequency} = Xi = \frac{Gi}{N} \times 100\%$$

$$\text{Allele frequency} = Xi = \frac{2nii + 2nij}{2N}$$

Where:
Xi = Genotype or allele frequency,
i^{th} = homozygous alleles,
j^{th} = heterozygous alleles,
Gi = number samples of i genotype
N = total samples.

Statistical analysis using ANOVA procedure (software SPSS program version 17.0) was utilized to determine the correlation between MSTN genotype and body weight and weaning weight.

Table 1. Primer forward and reverse for MSTN Amplification

Amplified fragment	Size (bp)	Primer name	Primer sequence	Tm (°C)
MSTN	487	Forward :	5´-TGCGGTAGGAGAGTGTTTGG-3´	61.2
		Reverse:	5´-AAAATTGTTGAGGGGAAGACC-3´	59.3

Table 2. PCR reaction mixture for amplification of MSTN gene of sheep

Reaction components	Quantity (μL)
Kapa mix	12.5
Forward primer	1.0
Reverse primer	1.0
DNA template	1.0
dH2O	9.5
Total	25

RESULTS AND DISCUSSION

MSTN Genotyping

A 487 bp fragment for the 3rd exon of MSTN locus in Batur sheep was proliferated by manual PCR technique. The analysis revealed a total of seventeen polymorphic sites in the MSTN coding region (Figure 1). There were only two observed alleles (G and C) resulting in three genotypes, the animals with both alleles were considered as GC genotype, whereas those possessed only G or C alleles assigned as GG or CC genotypes. Eleven polymorphic sites were observed in the 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 sites seen a del-T at c.*129, c.*139 and c.*158 positions as in the figure. In an investigated population, this locus was in Hardy-Weinberg equilibrium with X^2 test of 0.0034 and probability of (p=0.95). This confirmed that factors leading to disequilibrium, especially selection and migration, may affect the genetic structure of the population. The studied population showed a low degree of genotypic variability for the MSTN gene. This may be explained by the conservation and no breeding plans have been applied, because there are many rams that have been used randomly as sires in a breeding system which is a result of the inbreeding effect. As previously reported by the authors (Haren et al. 2019) the high similarity of the MSTN gene in Batur sheep observed because of their coding region are similar, a genetic variation found at the 3rd exon region may not influence mRNA splicing and therefore affect the amino acid sequences produced from a process of the transcription. Moreover, a breed-specific influence of the locus under study.

Effect of MSTN genotype on growth traits

Table 3 indicates that genotypic and allele frequencies in the 3rd exon of Myostatin genotype were 0.552 (GG), 0.379 (GC), and 0.069 (CC), where the allele frequency was 0.74 G and 0.26 C, however; weaning weight of Batur lambs were 19.99 GG, 21.3 GC, and 19.7 CC and 6-month weight 30.49 (GG), 31.87 (GC), and 29.9 (CC), respectively. Lambs with heterozygous (GC) genotype were heavier than homozygous (GG) and recessive (CC) genotype by 1.6 and 2 kg at weaning and 6-months weight, respectively. However, there was no significant difference at weaning weight (p=0.99), 6-month body weights (p=0.98) at a locus (c.*121G>C) of the MSTN gene in Batur sheep. Similar findings for the non-significant effect of genetic variants in exon 3 of the MSTN gene with growth traits were reported in Zel sheep (Dehnavi et al. 2012).

Effect of MSTN genotype on carcass traits

The carcass traits and their proportion yield are present in Table 4, lambs that carry the GC genotype have slightly more pre-slaughter weight, hot carcass weight, cold carcass weight, carcass length and total yield (loin, shoulder, leg yield, and rump) comparing to those carry's the GG genotype. The results showed there was no significant effect of the MSTN genotype on carcass traits (P > 0.05). It might be due to the increase in muscle mass.

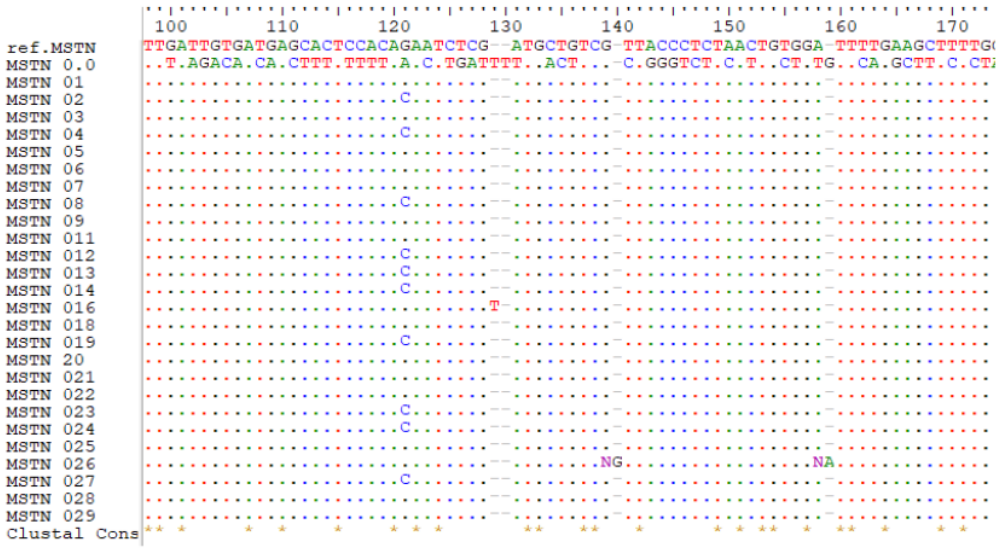

Figure 1. Chromatogram of BioEdit program used to identify if any nucleotide substitution in the 3rd exon of MSTN gene, all sequence plotted to a standard as a dot, compared to the reference sequence (NCBI) there are 17 different variants on the matched sequence

positions, 11 variants 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 3. Genotypic, allelic frequencies and heterozygosity of exon 3 of locus c.*121G>C and its association with weaning and body weight in Batur sheep

Genotype	(16) GG	(11) GC	(2) CC	G	C	X^2	p-value
Genotypic frequency	0.552	0.379	0.069	0.74	0.26		-
Expected frequency	0.549	0.384	0.067			0.0034	0.95
Weaning	19.99±1.3	21.3±1.5	19.7±4.5				0.99
6-month weight	30.49±1.6	31.9±1.9	29.9±1.8				0.98

Table 4. Means, standard deviation (S.D), minimum and maximum weights (kg) for carcass traits

	Genotype GG				Genotype GC				
	Mean	S. D	Min	Max	Mean	S. D	Min	Max	p-value
PSW (kg)	32.7	2.8	30.7	34.7	34.3	2.85	31	36.3	0.59
HCW (kg)	13.4	0.0	13.4	13.4	14.6	1.55	13.1	16.2	0.38
CCW (kg)	13.3	0.07	13.2	13.3	14.4	1.50	13	16	0.37
Carcass length (cm)	75	0.0	75.0	75.0	78.0	3.46	74	80	0.33
Total yield (kg)	8.05	0.07	8.00	8.10	8.10	1.49	6.4	9.2	0.97
Lean yield (kg)	7.4	0.85	6.80	8.00	8.87	0.95	7.8	9.6	0.18
Bone yield (kg)	3.6	0.28	3.40	3.80	3.87	1.03	3.0	5.0	0.58
Fat yield (kg)	2.1	0.14	2.00	2.20	2.07	0.61	1.4	2.6	0.95
Dressing %	41	0.04	39	44	43	0.03	40	46	0.64
Boneless %	56	0.07	51	61	61	0.025	60	64	0.26

Note: HCW = hot carcass weights, CCW = cold carcass weight, PSW = Pre-slaughter weight

Discussion

The inability to find any association of weaning and body weight with the polymorphic status of the MSTN gene in the present study might be due to the breed-specific effect of the locus under study. This study in line with (Sahu et al. 2017) which mentioned that, a non-significant effect of this mutation that observed on birth, weaning (three months) and six months weight in Indian Mecheri and Madras Red sheep. (Sumantri et al. 2008) reported that genetic diversity based on molecular marker MSTN c.del960G locus in Indonesia local sheep are very low. This is indicated by the value of one genotype frequency and allele which has a value of 1, which marks the fixation process. The absence of deletion in 1-bp deletion at MSTN c.del960G can be caused by a tropical adaptation process which suggested that the animal which can survive in this environment is having small performance. In this case, presence of the c.960delG mutation in both alleles means that no functional Myostatin protein is expected to be produced (Boman et al. 2009).

If Myostatin did not express, so the negative growth regulation will fail and the number of muscle fibers increases (hyperplasia). (Boman et al. 2010) reported the homozygous c.960delG (AA) animals had lower daily gain and weaning weight, but higher carcass weight. While the genotypes (del-G)-AG and (del-G)-GG resulted in significant ($p < 0.001$) effects, towards more meat and less fatty animals. However, mutations in the third exon affect conformation and fat class in NWS lambs, yielding a carcass with less fat and increased muscle mass these findings are not far to our study concerning fat, muscles, and carcass yield.

In conclusion, the present study investigated the allelic and genotypic effects of the MSTN gene on growth and carcass traits, polymorphism is present in Batur sheep for the first-time regarding assessment of the association with the 3rd exon of the MSTN gene. Moreover, non-significant genotypic effects of the MSTN gene have detected concerning weaning weight, 6-month weight, and studied carcass traits. Moreover, these results may not be useful for developing future selection programs unless further investigation of the MSTN gene and it is interaction performed with other genes that involve in muscle growth and carcass traits through analyzing association studies at large scale.

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