Identification of myostatin gene on Kacang and Peranakan Etawah goats in Deli Serdang District, North Sumatera, Indonesia

Hasyim, A.R.[1*], Tafsin, M.[2] and Batubara, A.[3]
[1]Graduate Magister of Animal Science Program, Faculty of Agriculture, University of North Sumatera, Medan-Indonesia, [2]Animal Science Program, Faculty of Agriculture, Universty of North Sumatera, Medan-Indonesia, [3]Loka Research Center Kambing Potong Sei Putih, North Sumatera, Indonesian
*Corresponding author: aulianursyahhasyim@gmail.com

Received: 27 February 2018. Accepted: 30 May 2018.

Abstract

One of the important genes that affects the growth of goats is the myostatin(MSTN) gene. The purpose of this study was to detect the polymorphism of the MSTN gene and identify SNP in the fragment target MSTN gene exon 1 in Kacang and Peranakan Etawah goats in Deli Serdang District, Sumatra Indonesia. The number of samples used was 60 goats consisting of 30 Kacang goats and 30 Peranakan Etawah (PE) goats from Galang, Hamparan Perak and Namorambe, Deli Serdang district. The MSTN gene forward primer was 5'-AGA ACA GCG AGC AGA AGG AA-3' and reverse primer 5'-CGT CGT AAC GTG GTA GTC AT-3', the enzyme for restriction *HinIII*. To determine Hardy-Weinberg equilibrium status, both allele and genotype frequencies were analyzed. To align sequencing results BioEdit and MEGA6 softwares were used. The results showed that there were MSTN gene exon 1 found and SNP in 53 points in Kacang and PE goat populations in the district sampled and was monomorphic. Only one genotype from MSTN exon 1 was discovered, that was AA. Heterozygosity and HW equilibrium was 0, because only one genotype was found in MSTN gene in this study. Myostatingene at Kacang and PE goat populations in Deli Serdang District is therefore found to be monomorphic.

Keywords : Myostatingene, local goats, genetic polymorphism.

Introduction

Goats have a good adaptability to various environmental circumstances. Goat development has a good prospect because in addition to meet the demand for meat in the country, also has an opportunity as an export commodity. According to Batubara *et al*. (2004) there are two most popular goat breeds in Indonesia, there are the Peranakan Etawah (PE) and Kacang goats. Kacang goats have a smaller body size compared to Peranakan Etawah goats. One of the regions with the largest goat population in North Sumatra is Deli Serdang district. But the potential of the goats has not been explored optimally. Improvement of genetic quality of goats can be done through molecular approach based on Marker Assisted Selection (MAS) (Azrai, 2005). Selection by utilizing MAS initiative had been done in cattle (Rezende *et al*., 2012; Alwiyah *et al*., 2016) chicken (Lahav *et al*., 2006) and buffalo (Sarika *et al*., 2013). One of the potential measurements of goats can be observed through the nature of growth. Growth is a trait controlled by many genes. One of the important genes that affects the growth of

goats is the myostatingene. Lee and McPherron (2001) mentioned myostatingene (MSTN) or commonly known as growth and differentiation factor 8 (GDF8) which is a member of the superfamily transforming growth factor β (TGF-β.) It is involved in mediating cell growth and development through signal transduction. The MSTN gene works as an inhibitor (negative regulator) of myogenesis and inhibits myoblast poliferation during cell cycle and myogenic differentiation (Miyake *et al*., 2010). Research on the MSTN gene had been done in cattle (Sarti *et al.,* 2014, Coles *et al*., 2015). However, the study of MSTN gene in goats in Indonesia is still rare, therefore this study aims to detect the polymorphism of the MSTN gene and identify Single Nucleotide Polymorphism (SNP) in the fragment target MSTN gene exon 1 in Kacang and Peranakan Etawah goats in Deli Serdang District, Sumatra Indonesia.

Materials and Methods

The study was divided into two stages, namely stage 1 field test to obtain phenotypic characteristics and blood sampling, and continued with step 2 to determine the results of MSTN gene diversity in the studied sample.

Phase I Field Research

Phase 1 research was conducted at community farms in three sub-districts in Deli Serdang District. Maintenance of the goats was done by intensive system in which farmers collected feedstuffs in the field which were then fed to the animals. The local goat blood sample size was 60 heads: 20 heads from Galang, 20 heads from Perak and 20 heads from Namorambe districts comprising of 30 Kacang and 30 PE goats. Blood samples were taken through jugular vein using venoject needles and collected in EDTA vacuttainer tubes.

Phase II Laboratory Testing

Total DNA Extraction

DNA extraction was based on the method of Sambrook *et al*. (1989). Blood was taken as much as 200 µl, then was added 1000 µl DW in 1.5 ml eppendorf tube, then vortexed and allowed to stand for 5 min. Then the sample was centrifuged at 8000 rpm for 5 min and the supernatant formed was discarded. The precipitate was added 40 µl SDS 10%, 10 µl proteinase-K (5 mg/ml) and 1 × STE to 400 µl, then incubated at 55°C for 2 h while gently rocking using a tilter. The degradation of organic matter was done by adding 400 µl phenol solution, 400 µl of CIAA, and 40 µl NaCl 5M, then rocked for one hour at room temperature. The DNA particle was separated from phenol by means of centrifugation at a rate of 12000 rpm for 5 min to form a DNA phase (clear). The DNA phase was transferred to a new tube before adding 800 µl EtOH absolute 70% and 40 µl NaCl 5M, then chilled (overnight). The DNA was pelleted at a rate of 12000 rpm for 5 min to separate the absolute EtOH. The deposited supernatant was removed. The remainder was allowed to dry to be suspended in 100 µl TE 80%. DNA samples were stored in a freezer.

DNA Amplification

The primer used on MSTN exon 1 gene was based on Zhang *et al*. (2012), forward 5'-AGAACAGCGAGCAGAAGGAA-3' and reverse 5'-CGTCGTAACGTGGTAGTCAT-3', which was cut off with HinIII restriction enzyme. A 2 µl extracted DNA sample was inserted into the PCR tube, and then added 13 µl of the premix solution. Premix solution contained 0.3 µl primer, 5.2 µl DW, 7.5µl

Green Master Mix. This mixture was incubated in a thermocycler for the amplification process. The initial denaturation was carried out at 95°C for 5 min, subsequently adding for 10 sec at the same temperature. Primary attachment for the MSTN gene at 60°C lasted for 2 sec. Extension temperature was 72°C for 30 sec, and final extension for 5 min. Amplification process was 35 cycles.

Reaction Fragment Lengh Polymorphism (RFLP)

Genotyping was done by RFLP technique. A total of 5 µl amplycans were added 1 µl DW, 0.7 µl buffer, and 0.3 µl restriction enzyme and then incubated. HinIII enzyme on MSTN exon 1 gene cutting was incubated at 37°C overnight. Each sample was re-electrophoresed as much as 5 µl at 100 V for 35-45 min on agarose gel 2%. The resulting products were visualized under UV light. The size was estimated by comparing to the marker 100 bp.

Sequencing

The purified PCR products of PE and Kacang goats were sent to First Base, Malaysia DNA sequencing provider service (ABI PRISM Genetic Analyzer) and was conducted both in foward and reverse.

Data Analysis

Data analysis in this study was done by comparing the diversity of MSTN exon 1 gene in the form of genotype and allele frequencies and also conducted descriptive statistical analysis on the diversity of heterozygosity degree and population balance.

Frequency of Alleles and Genotypes

Allele frequency was calculated using the formula of Nei and Kumar (2000) as follows:

$$x_i = \frac{\left(2n_{ii} + \sum_{i \neq j} n_{ij}\right)}{2N}$$

The genotype frequency was calculated according to the following formula (Nei and Kumar, 2000):

$$x_{ii} = \frac{n_{ii}}{N}$$

where

x_i = ith allele frequency
x_{ii} = frequency of ith genotype
n_{ii} = total number of ii genotypes
n_{ij} = total number of ij genotypes
N = total number of samples

Sequencing Analysis of MyostatinGene

The analysis was performed on sequence results with BioEdit programme and analyzed using BLAST method (www.ncbi.nhl.nih.gov./BLAST) to determine the similarity with Myostatingene in GenBank. The presence of mutations or SNP in the Myostatingene fragment sequence was analyzed using the Molecular Evolutionary Genetic Analysis 5 (MEGA5) programme (Tamura *et al*., 2011).

Results and Discussion

In this study goats used for research were Kacang and Peranakan Etawa derived from the sub-districts of Galang, Hamparan Perak, and Nomorambe. In general, Kacang goats in these three areas have relatively small body size with a mature weight of about 20-25 kg (Faozi et. al., 2013). PE is a cross between Kacang and Etawah goats. In addition, the

management applied was intensive maintenance. In general, people in the area raise goats for savings.

DNA Amplification

The myostatingene was successfully amplified with annealing temperature 60°C for 20 sec and with 35 cycles (Figure 1). The Figure shows the marker used as a standard for calculating the base length of the target genes. The marker used was 100 bp in length, so the marker displayed reached 1000 bp. Samples 1 to 8 were samples of Kacang goats and samples 9 to 16 were samples of PE goats. The PCR product of the myostatingene in PE goats and Kacang goats was about 272 bp. Zhang *et al.* (2012) reported the myostatingene in the studied goats was amplified at 57°C. Annealing temperature is very influential on the success of the amplification process. During the PCR process the optimum temperature for the primer can be attached to the targeted DNA sequence that is called annealing temperature. The primary or annealing attachment temperature ranges from 36-72°C, but the commonly used temperature is 50-60°C (Muladno, 2002).

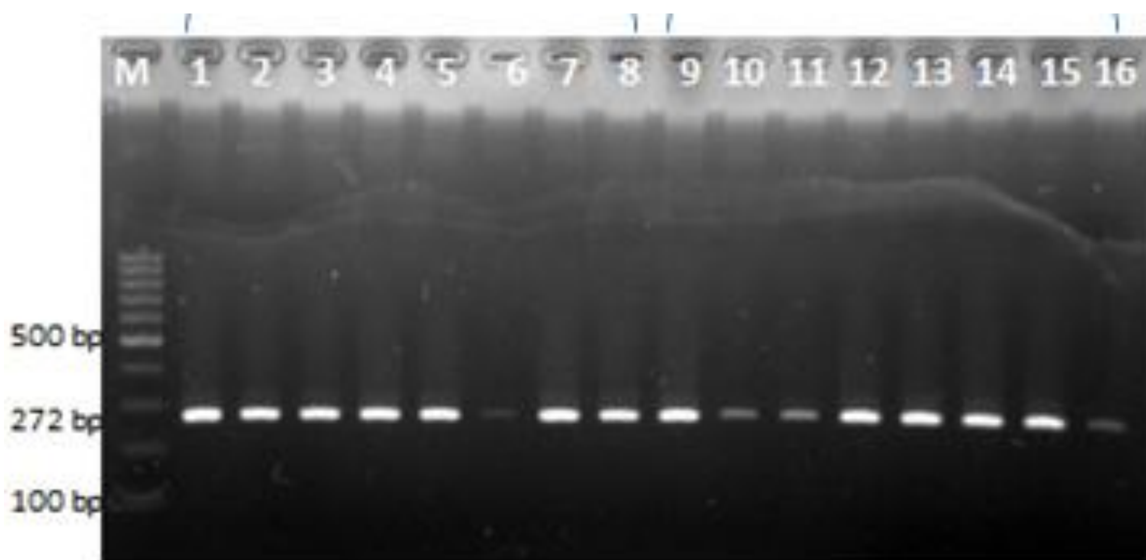


Figure 1. Amplification product of MSTN gene on goats in Deli Serdang at 272 bp (M=marker, sample 1-8 PE goat, sample 9-16 Kacang goat.)

One SNP was found in exon 1 MSTN goat gene. SNP in exon 1 gene MSTN was identified using restriction enzyme HinIII with cutting site AAGCT|T. Based on the genotyping result, there was a base mutation of Tymine (T) to Adenine (A) on the 53rd base. The mutation found in this study was transversion mutation, i.e., the change of base from purine to pyrimidine or vice versa. The genotype found for MSTN gene in both PE and Kacang goats was only AA genotype. The genotyping of the exon 1 MSTN gene is shown in Figure 2.

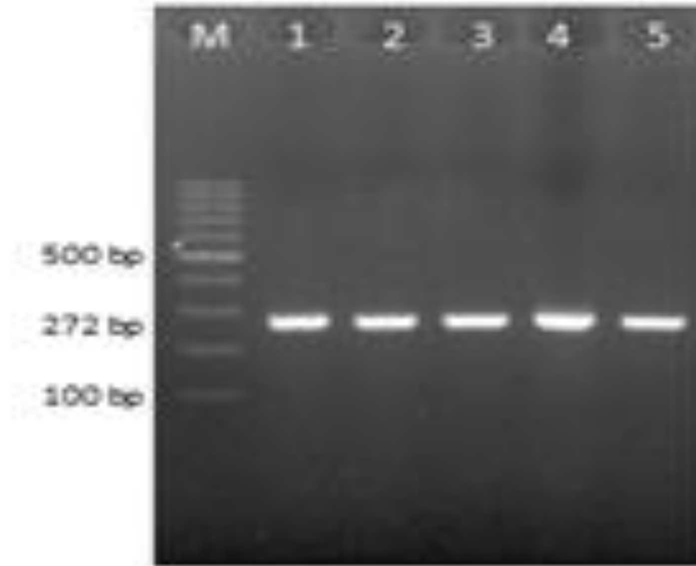


Figure 2. Visualitation PCR-RFLP MSTN gene exon 1 cut by *HinIII* enzyme in PE and Kacang goats in Deli Serdang district at agarose gel 2%; M = marker.

All samples of Kacang and PE goats were not successfully cut by the *HinIII* enzyme. This happenned because the enzyme was not able to find the point of cut that was A | ATGCTT. Meanwhile, if the MSTN gene was cut with a band length of 219 pb and 53 pb then it showed the T allele. PCR-RFLP results were verified to explore with sequencing and alignment of MSTN gene sequencing results in goats with sequences from GenBank with access number EF591039. Based on the results of sequencing it was evident that the analyzed fragment was a fragment of the MSTN gene and found the position of the T -->A mutation on the base to 53. The result of sequencing the MSTN gene is shown in Figure 3.

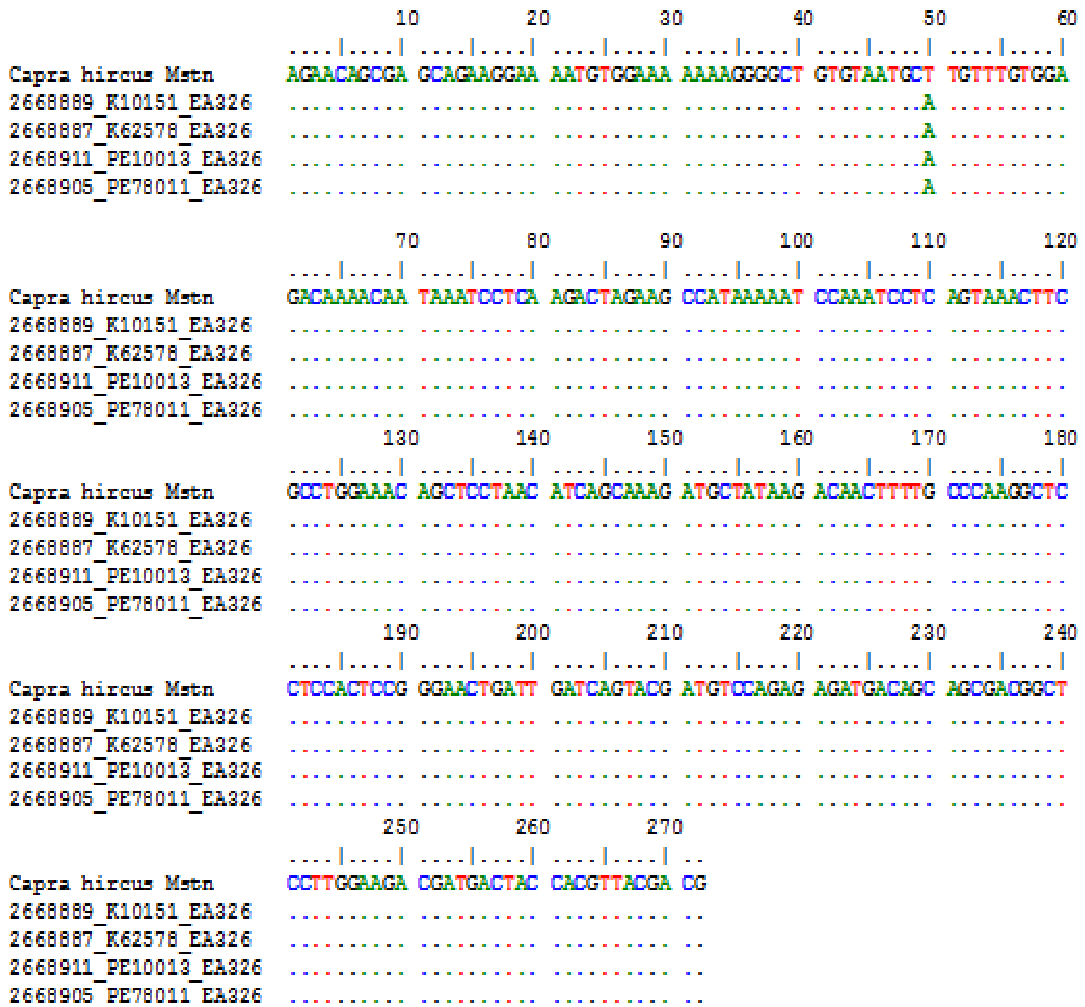
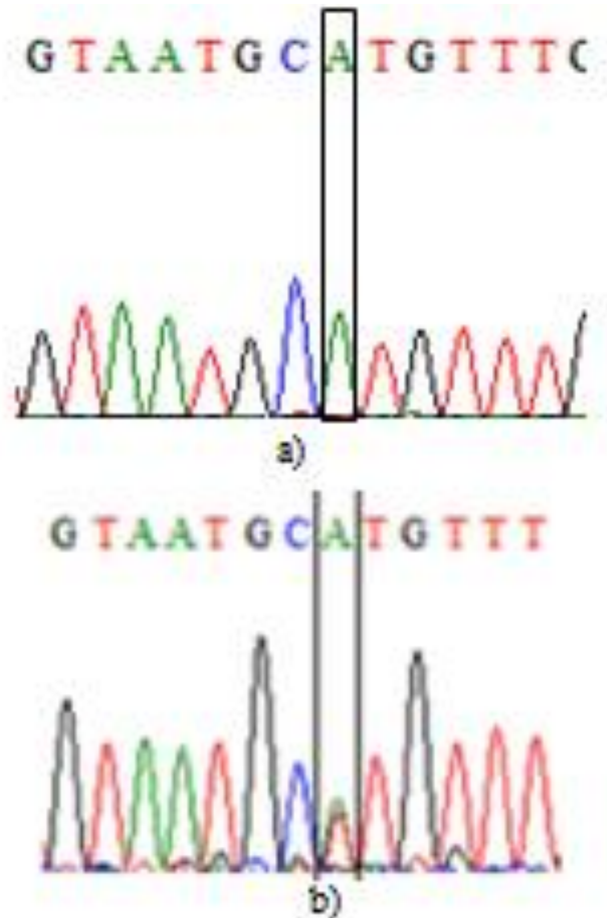


Figure 3. Sequence of MSTN gen with acsess number *Genbank* EF591039 on Kacang and PE goats in Deli Serdang District

From the results of the tracing known, both Kacang and PE goats from various regions in Deli Serdang had the same results as that of allele A. The results of this study are contrary to the results of Zhang *et al.* (2012) that in this study all Kacang and PE goat populations which originated from different regions had allele A while in the study of Zhang *et al.* (2012) allele A frequency was very low in Boer goat population, whereas in Matou goats, Haimen and Nubi, allele A was not found or in other words was monomorphic at allele T. It becomes unique for Kacang and PE goats in Indonesia, especially in Deli Serdang because in general the allele frequency was very low and didn't even exist, but in these goat populations had a high allele frequency. The peak form of the mutation point was found as presented in Figure 4. In this case the mutations contained in the results of this study were transversal mutations.



d) Genotype AA b) Genotype AT (Zhang *et al.* 2012)

Figure 4. Comparison of peaks from sequence between AA and AT genotypes in MSTN gene at mutation point 53

Polymorphism of MyostatinGene

An analysis of the diversity of the exon 1 MSTN gene in goats was performed using genotype and allele frequencies as presented in Table 1. From Table 1 it can be observed that from a total of 30 Kacang goats from various populations there was only one genotype emerging, i.e., the genotype AA (100%), so the frequency of the A allele was 1.00. These results indicated that the absence of genetic diversity of MSTN exon 1 gene sequence used and not found in the T allele in the entire Kacang goat population under study and the population was monomorphic for the exon 1 MSTN gene.

Table 1. Polymorphisme of exon 1 MSTN gene in goat populations and results from other research at same mutation point

Breed/district	Genotype			Alelle		Source
	TT	TA	AA	T	A	
Kacang/Galang (10)	0	0	1.00	0	1.00	Present study
Kacang/Hamparan Perak (10)	0	0	1.00	0	1.00	
Kacang/ Namorambe (10)	0	0	1.00	0	1.00	
PE/ Galang (10)	0	0	1.00	0	1.00	
PE/ Hamparan Perak (10)	0	0	1.00	0	1.00	
PE/ Namorambe (10)	0	0	1.00	0	1.00	
Boer	0.66	0.31	0.03	0.82	0.18	Zhang *et al.*(2012)
Matou	1.00	0	0	1.00	0	Zhang *et al.*(2012)
Haimen	1.00	0	0	1,00	0	Zhang *et al.*(2012)
Nubi	1.00	0	0	1.00	0	Zhang *et al.*(2012)

The same finding was found for PE goats in Galang Subdistrict, Hamparan Perak and Namorambe, Deli Serdang District, where only one genotype, AA, was discovered. The frequency of genotype AA was 1.00. The results of the Table 1 show that PE goats in Deli Serdang District was monomorphic for exon 1 MSTN gene. Noor (2010) mentioned that gene frequencies can undergo changes if there is selection, mutation, mixing of populations, inner and outer crossings, and genetic drift.

In contrast to Zhang *et al*., (2012) Boer goats were found to have three genotypes, namely, TT, TA and AA. However, the TT genotype was higher when compared with TA and AA genotypes. The T allele frequency was 0.82 and the A allele frequency was only 0.18. Unlike the goats Matou, Haimen and Nubi, the three goat breeds had only one genotype TT (100%). The diversity of MSTN exon 1 gene in goats is still rare, researchers generally do research on the MSTN gene in exon 2 or 3, even in Indonesia there is no publication about the exploration of exon 1 MSTN gene. In this case there is still a need to explore the MSTN exon 1 gene so that it will be able to provide important information related to this MSTN gene. MSTN exon 1 gene has a significant effect on birth weight, birth height, and body weight at 90 d. In general, the MSTN gene studied at only exons 2 and 3 only.

Heterozygosity

Genetic diversity of a population can be measured using heterozygosity. MSTN gene in the 3 different sub-districts had a low diversity based on its heterozygosity value. Heterozygosity values indicated that the MSTN exon 1 gene in the goats in the 3 different sub-districts was not diverse (0.00) or uniform, since the gene had only one genotype AA. Heterozygosity value was 0.000 due to the absence of a heterozygous individual on the SNP. It is known that the SNP is a monomorphic. According to Allendorf and Luikart (2007) if the low heterozygosity value is below 0.5 then it indicates a low frequency gene in the population.

McPherron *et al*. (1997) have found that the absence of myostatincould result in skeletal muscle mass twice as large in mice as compared to wild-type. In some cattle, double muscle characteristic is caused by mutations that result in loss of function of myostatingene in Belgian Blue and Peidmontese cattle (Dierks *et al*., 2014). Mutations in the exon region 1 myostatingene in goats are known to cause increased muscle mass to form different phenotypes. Mutations in the myostatingene can produce non-functional proteins that lead to double-muscling cases (Dierks *et al*., 2014).

Conclusion

Myostatingene exon 1 at the point of the 53rd mutation in Kacang and PE goats in Deli Serdang, North Sumatra was found to be monomorphic. Only one genotype AA of the exon 1 MSTN gene was discovered in both Kacang and PE goats.

References

Allendorf, FW and Luikart, G. 2007. Conservation and the genetics of populations. Oxford (UK): Blackwell Publishing.

Alwiyah, Naraini, H, Agung PP, and Jakaria. 2016. Polymorphism stearoyl-coa desaturase (SCD) gene and associaton with characteristics meat in Bali cattle. J. Indonesian Trop. Anim. Agric. 41(4):188-195.

Azrai, M. 2005. Utilization of molecular markers in the process of plant breeding selection. Agro Biogen. 1(1):26-37.

Batubara, A, Doloksaribu and M, Bess, T. 2014. Potential diversity of genetic resources of local Indonesian goats. JIVET. 19:206-214.

Coles, CA, Wadeson, J, Leyton, CP, Siddle, JP, Greenwood, PL, White, JD, and McDonagh, MB. 2015. Proliferation rates of bovine primary muscle cells relate to live weight and carcase weight in cattle. Plosone. 10(4):1-14.

Dierks, C, Eder, J, Glatzer, S, Lehner, S and Distl. 2014. A novel myostatinmutation in double-muscled German Gelbvieh. Stichting International Foundation for Animal Genetics. 46: 91–99.

Faozi, A.N., Priyono, A. and Yuwono, P. 2013. Ukuran Vital Tubuh Cempe Pra Sapih dan Hubungannya dengan Bobot Tubuh Berdasarkan Tipe Kelahiran pada kambing Peranakan Etawah. Fakultas Peternakan Universitas Jenderal Soedirman, Purwokerto. Jurnal Ilmiah Peternakan 1 (1) :184-194.

Lahav, T, Atzmon, G, Blum, S, Ben-ari, G, Weigend, S, Cahaner, A, Lavi, U, and Hillel, J. 2006. Marker-assisted selection based on a multi-trait economic index in chicken: Experimental results and simulation. Animal Genetics. 37: 482-488.

McPherron, AC and Lee, SJ. 1997. Double muscling in cattle due to mutations in the myostatingene. PNAS. 94: 12457–12461.

Miyake, M, Hayashi, S, Taketa, Y, Iwasaki, S, Watanabe, K, Ohwada, S, Aso, H, and Yamaguchi, T. 2010. Myostatindown-regulates the igf-2 expression via alksmad signaling during myogenesis in cattle. J. Anim. Sci. 81:223 –229.

Muladno. 2010. Genetic Engineering Technology. Second Edition. Bogor (ID): IPB Pr.

Nei, M, and Kumar, S. 2000. *Molecular Evolution and Phylogenetics*. New York (US): Oxford University Pr.

Noor, RR. 2010. Animal Genetics. Jakarta (ID): Penebar Swadaya.

Pelt-Verkuil, EV, Belkum, AV, and Hays, JP. 2007. Principles and technical aspects of pcr amplification. Netherlands (GB): Springer.

Rezende, FM, Ferraz JBS, Eler JP, Silva RCG, Mattos EC, and N Iba´n˜ez-Escriche. 2012. Study of using marker assisted selection on a beef cattle breeding program by model comparison. J. Livestock Sci. 147:40–48.

Sambrook, J, Fritsch, EF, and Medrano, JF. 1989. Molecular Cloning: a Laboratory Manual. 2nd ed. New York (US): Cold Spring Harbor Laboratory Pr.

Sarika, Arora V, Iquebal M, Anil R, and Dinesh K. 2013: In silico mining of putative microsatellite markers from whole genome sequence of water buffalo (*Bubalus bubalis*) and development of first BuffSatDB. BMC Genomics. 14:43.

Sarti, FM, Lasagna, E, Ceccobelli, S, Lorenzo ,P, Filippini, F, Sbarra, F, and Giontella, A. 2014. Influence of single nucleotide polymorphism in myostatinand myogenic factor 5 muscle growth-related genes on the performance traits of Marchigiana beef cattle. J. Anim. Sci. 92: 3804-3810.

Tamura, K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. J. Mol. Biol. 28(10): 2731–2739.

Zhang, C, Liu, Y, Xu, D, Wen, Q, Li, X, Zhang, W, and Yan, L. 2012. olymorphisms of myostatingen (MSTN) in four goat breeds and their effects on Boer goat growth performance. Mol. Biol. Rep. 39: 3081–308.