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Genetic Polymorphisms at Myostatin Locus in Fat Tailed Sheep, Dorper and Texel Sheep

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Abstract: ***The objectives of the research was to analyze genetic diversity and genetic polymorphisms at Myostatin gene locus in Fat Tailed Sheep, Dorperand Texel Sheep. Research was conducted in the village area of Pandesari, Pujon District, Malang Regency, East Java Indonesia. Research was carried out in two stages, (1) field research, to observe the linear measurements of Fat Tailed Sheep, Dorper Sheep and Texel Sheep. Blood samples were taken from 91 sheep consisting of the three breeds35 (Texel, 19 Dorper and 37 Fat Tailed Sheep). (2) Laboratory research was conducted to analyze DNA polymorphism with target gene was the myostatin gene on exon 3, using PCR-RFLP analysis with Msp1 restriction enzyme was conducted to analyze polymorphism at the myostatin gene in these sheeps. The Msp1 was used to cut the mutated fragments. Data analysis used (1). Analysis of % PIC to analyze degree of polymorphism, (2) For linear measurements data such as chest girth, body length, body height, tail circumference was analysed using Minitab software version 17. Results showed that from the aspect of the phenotype, Texel, Dorper and DEG sheep have a high level of diversity, which is quite high (above 9%). The results of PCR-RFLP obtained monomorphicsite in all breeds of sheep so that the correlation between MSTN gene polymorphisms and sheep growth could not be analyzed. Because the Myostatin gene of the three breeds was monomorphic. It was concluded that the exon 3 area in all breeds was a conserved region that rarely occurs mutations.***

Keywords: Polymormorphism, Myostatin gene, PCR-RFLP, Msp1, restriction enzyme

1. Introduction

Indonesia is known as a country with high biodiversity. There are many nations of both animal and plant species, as well as many nations within the same species. This high biodiversity is caused by the high rainfall and the variety of plant species. Within the diverse population there are also groupings of native, local and imported livestock, for cattle, goats and sheep. Imported sheep that have arrived in Indonesia, such as Dorper and Texel sheep.

Texel sheep is a breed of local sheep from the Netherlands. The sheep came from the island of Texel, the largest of Wadden Island off the north coast of the Netherlands. Its original breed is unknown, but it is thought to have been the result of a cross between several breeds of English sheep. Texel This is a very muscular breed of lamb, and produces lean meat (Anonymous, 2021). Dorper sheep bred in South Africa, were first bred as a combination of Blackhead Persian and Dorset Horn. Is a sheep meat, wool and milk. Dorper sheep are unique in that they are raised as a substitute for goat meat. Because they have a long breeding season, herders with little experience can often breed several times each year. This makes the Dorper breed one of the most economical in terms of meat production. Dorper sheep can give birth up to 3 times every 2 years, with an interval of about 8 months. Dorpers are known for their fertility and good nurturing instincts. With quite adaptive traits, Texel and Dorper sheep are very suitable to be developed in areas in Indonesia with various types of unique ecosystems, some are hilly, have cool temperatures and some are dry and hot and Dorper are very suitable to be kept in the Pujon area, Malang Regency, which has cool air. The research location is relatively the same, namely in the Districts of Pujon and Karangkates, there are also Fat Tailed Sheep (DEG). As relatively new imported livestock, it is necessary to conduct an assessment of Texel and Dorper sheep regarding their suitability with local environmental conditions, and when compared to DEG, Dorper sheep are also Fat Tailed type sheep. Observations on qualitative and quantitative characters need to be done to analyze their adaptability, especially quantitative characters. On the other hand, qualitative characteristics are also important to see to what extent the authenticity of the sheep breeds is by comparing them with their original characteristics. In West Java, Garut sheep are crossed with Dorper sheep to produce sheep that can produce lean meat. The results of crosses with local sheep showed satisfactory results, especially because of the high body weight gain. Genetic variation can be observed by observing the ability to inherit quantitative characters (known as heritability). Heritability is also known as genetic parameter together with repeatability and genetic correlation. Selection of livestock based on heritability, especially for growth characters, has been widely carried out, but the results still vary, because the performance seen in the phenotype is the sum of genetic variation and environmental variation and their interactions. By observing through genomic analysis, it is possible to know the effect of potential genes (candidate genes) which are thought to have a strong influence on livestock performance. By utilizing advances in the field of molecular biology, it is possible to make selection efforts that can be carried out at the DNA level, namely by looking for genes that control natural biodiversity, especially production or reproductive characters. Simple DNA analysis can be applied to detect alleles that have a positive effect on high economic value loci (ETL / Economic Trait Loci) (Sumantri, Jakaria, Yamin, Nuraini and Andreas, 2011). Selection using marker genes is an alternative to livestock carrier biotechnology to produce the desired trait (according to the marker gene).

Mapping of genes in the sheep genome marks the start of new horizons in the field of livestock breeding. Genes that can be used as genetic markers to select production traits and have high economic value are known as auxiliary selection markers or Marker Assisted Selection (MAS). Identification

of genetic markers is the first step and is quite accurate in selecting livestock production. The genes used as genetic markers are generally genes that code for physiological characters that do affect the growth of sheep, these are also called candidate genes. Candidate gene marker approach. The candidate gene marker approach is based on existing supporting theoretical knowledge such as physiological and biochemical causative evidence showing that the selected gene is involved in the desired trait (Sutarno, 2016). It is necessary to investigate the genetic variation in Texel and Dorper sheep in areas different from their original conditions, compared to DEG as local sheep in rearing locations such as Pujon and Karangkates areas which are known for their hilly areas with sufficient rainfall and food availability. sufficient, and the air temperature is cool.

Objectives of the research was to analyze the phenotypic and genetic diversity (genetic polymorphism) at the Myostatin locus which is thought to have a relationship with good growth in Pujon (Fat Tail sheep), Texel sheep and Dorper sheep in Sebaluh-Pandesari Village, Pujon and Karangkates Districts, Malang Regency. Problems of the research, In line with the increase in demand for meat, especially beef, it is felt that the fulfillment of this demand is getting harder, because demand is increasing but the speed of supply is not fast enough. To meet the meat shortage, the government is trying to import 502, 000 head of feeder cattle, equivalent to 112, 503 tons of beef, 85, 500 tons of beef imports, and 100, 000 tons of Brazilian beef and Indian buffalo meat in certain circumstances. The stock at the end of 2021 is estimated at 58, 725 tons which is also expected to be able to meet the needs of January 2022 (Anonymous, 2021). Therefore we need an alternative supply of meat other than beef such as mutton, lamb or other commodities. As a substitute for beef, a commodity is needed that can provide good quality and low-fat meat. In accordance with the existence of livestock in Indonesia, besides local livestock, there are also imported livestock and their crosses with local livestock which are able to produce meat of the desired quality.

Texel and Dorper sheep are raised on smallholder farms in Pujon, together with local sheep (Fat Tail Lamb). Because these cattle are also prepared as improver sheep which will be crossed with local sheep, it is necessary to study their qualitative and quantitative characteristics. For this reason, it is necessary to study the appearance of the phenotype in the area to assess whether it is able to adapt well or not.

This research is expected to be able to produce innovations based on quality research in order to improve academic quality and can contribute significantly to increasing the nation's competitiveness.

2. Material and Method

2.1 Research Location

The research was conducted at CV. Dwi Tunggal Mandiri (DTM) with at Pujonsub district, Malang Regency, East Java Indonesia. Material of the research consisted of 37 Fat Tailed Sheep, 35 Texel Sheep and 19 Dorper Sheep. Quantitative variables that were measured were body weight aged 2-3 years (kg), chest circumference, body length, height and tail circumference.

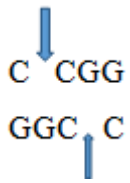
The research was conducted through 2 stages, namely field research and laboratory research. (1) Field Research. This study was conducted to observe the growth-related characters in sheep such as weaning weight, sex adult weight, body weight gain, and taking blood samples. Blood samples were taken as much as 400 ul in the jugular vein area using a Vacuutainer tube. This research was conducted on 50 sheep of each nation. (2) Laboratory research: DNA analysis is carried out using the PCR-RFLP (Polymerase Chain reaction-Restricted Fragment length Polymorphism) method which includes:

1) Extraction of DNA using 200 ul of whole blood sample. To analyze the exon 3 region of the MSTN gene, specific primers were designed using Primer3 software. The 25 l volume contained 25 ng of genomic DNA, 12.5 l 2x the reaction mixture of each primer.
2) PCR (Polymerase Chain Reaction) was performed. The protocol for PCR was carried out with a Thermal Cycler machine for 35 cycles with the following steps: 5 min at 95°C perdenaturation, denaturation at 94°C for 45 seconds, then annealing step at 73.9°C for 45 seconds, extended at 72°C for 40 seconds, with final extension at 72°C for 10 minutes. PCR was performed using the following primers (Haren et al., 2020).

Primer:

-forward
5-TCGGGTAGGAGTGTTTTGG-3

-reverse
5-AAAATTGTTGAGGGGAAGACC-3
(3) After the PCR is completed, the fragment is cut by a restriction enzyme, namely Msp1 (Sahu, Jeichitra, Rajendran and Raja, 2016) with sequence as below:



Data analysis
Analysing polymorphism of the Myostatin gene in Fat Tail sheep, Texel sheep and Dorper sheep using % PIC (Polymorphic Information Content) (Budak et al, 2003) as follows:

1) PICi = $1-p^2_{i\ j}$; whereas PICi is the polymorphic information content at the i^{th} locus, p_{ij} is the frequency of the j-th allele and the i-th locus
2) Quantitative data were analysed with One Way Annova method assisted by Minitab software version 17 (2017).

3. Result and Discussion

Results were divided into two parts: I. Observation of quantitative data (body weight, chest circumference, body length and height, tail circumference). II. Molecular data

derived from RFLP analysis of Locus Myostatin exon 3 regions.

Quantitative performance of Texel, Dorper and Fat Tailed Sheep

Results of quantitative variables can be seen in Table 1. The results show that the Texel sheep have the lowest tail circumference among the three breeds, this is because the Texel sheep are not a Fat Tailed type like Dorper and DEG.

Table 1: Performance means of Texel, Dorper and Fat Tailed Sheep at the location

Texel sheep	Performances		
Variabels	Mean ± SD *	N **	CV *** (%)
Chest Girth	66.64 ± 7.37	35	11.06
Body length	53.94 ± 6.02		11.16
Body height	56.14 ± 6.95		12.38
Tail circumference	17.53 ± 6.51		37.14
Dorper sheep			
Variabels	Mean ± SD *	N **	CV *** (%)
Chest Girth	85.68 ± 9.36	19	10.9
Body length	69.73 ± 7.92		11.36
Body height	61.58 ± 5.56		9.03
Tail circumference	25.29 ± 8.66		33.44
Fat Tailed Sheep			
Variabels	Mean ± SD *	N **	CV *** (%)
Chest Girth	71.73 ± 7.31	37	10.19
Body length	58.05 ± 6.23		10.73
Body height	61.68 ± 6.49		10.52
Tail circumference	21.23 ± 5.76		27.13

* Standard of deviation
** Number of observations
*** Coefficient of variation

Table 1 showed that Dorper sheep has a best performance, and followed by FatTailed Sheep (see the chest girth). Dorper sheep showed good growth, perhaps that's caused of the adaptability of Dorper sheep.

GENETIC POLYMORPHISM AT MYOSTATIN LOCUS USING RFLP

In the RFLP process, several stages are carried out, namely:
1. DNA isolation, which was then followed by PCR (Polymerase Chain Reaction). The results of PCR-RFLP for each breed of sheep can be seen in the image below. The primers used are as follows:

Primers:
- forward 5′-TGCGGTAGGAGAGTGTTTGG-3′
- reverse 5′-AAAATTGTTGAGGGGAAGACC-3′

DNA analysis using the PCR-RFLP (Polymerase Chain reaction-Restricted Fragment Length Polymorphism) method which includes the results of the PCR-RFLP of each sheep breed, and it turns out that the results obtained are *monomorphic (uniform*). This can be proven in the image below. These results are in line with studies reported by other studies targeting the Myostatin gene, the results are the same, namely *monomorphic*. With its monomorphism in the intron 1, 2 and exon 3 regions, it shows that there are no mutations in these regions at all. The results of the cuts by the restriction enzyme MSp1 in the amplified area of each sheep can be seen in Figure 1 (Texel sheep), Figure 2 (Dorper sheep) and Figue 3 (Fat Tailed Sheep).

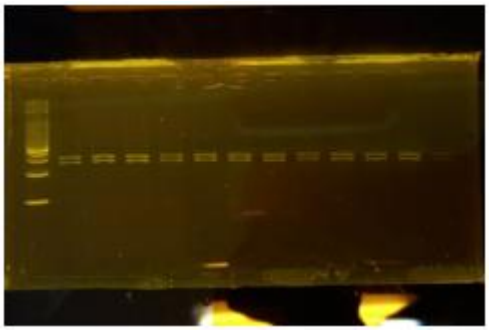


Figure 1: PCR-RFLP result with agarose gel electrophoresis in Texel sheep (Cut off by the restriction enzyme MSp1. Two band are produced. Concluded as *monomorphic*)

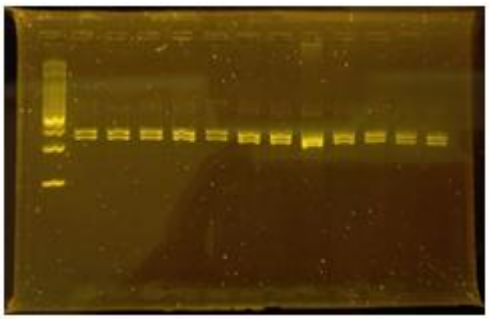


Figure 2: PCR-RFLP result with agarose gel electrophoresis in Dorpersheep (Cut off by the restriction enzyme MSp1. Two bands are produced. Concluded as *monomorphic*)

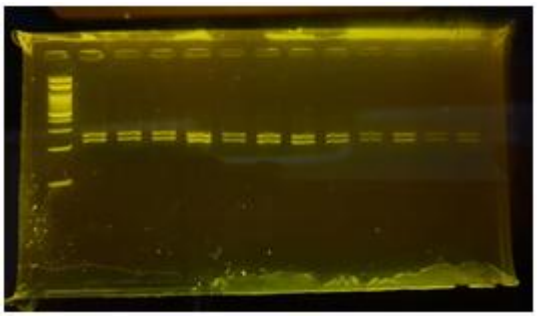
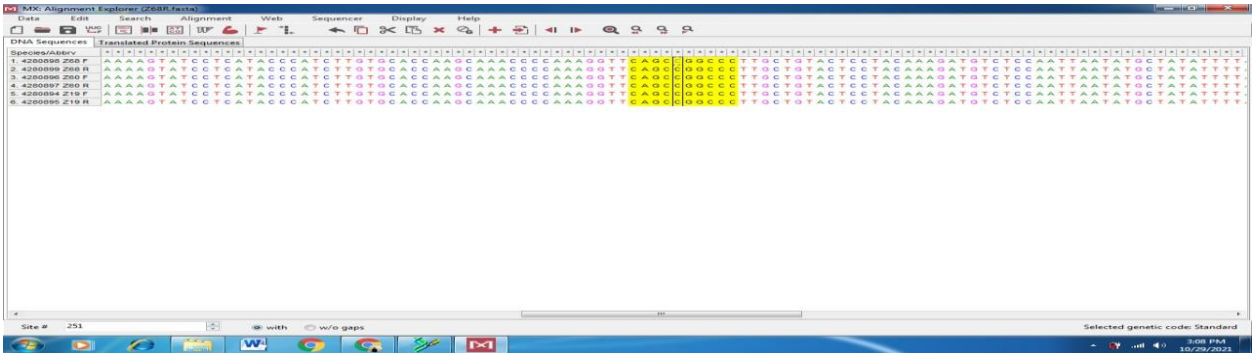


Figure 3: PCR-RFLP result with agarose gel electrophoresis in Fat Tailed Sheep (Cut off by the restriction enzyme MSp1. Two bands are produced. Concluded as *monomorphic*)

Sequencing Result

Sequenced samples show monomorphic, with some bands that appear to be 1 line but in fact there is a cut (2 bands) but the image is overlapped so that it looks like one band. The results of the sequence show that the point of cutting by MSp1 occurs at point 251.



The results of the study in the exon 3 area, the results of cutting by the MSp1 enzyme both in Texel, Dorper and DEG sheep, all the results were monomorphic. This result is not different from the results of Sakova, Dimitrova, Teneva and Petrov (2016) studies on Karakachan sheep in intron 2 and exon 3 regions using the PCR-RFLP method with HAEIII enzyme digestion, all of which were monomorphic. Likewise, research by Dehnavi, Azari, Hasani, Nassiry, Mohajer, Ahmadi Shahmohamadi and Yousefi (2012) who conducted research using the PCR-RFLP method on Zel sheep in intron 1, 2 and exon 3 regions, it also obtained monomorphic results, except for in indtron 2 which is polymorphic. The results of the study of Sahu1, Jeichitra, Rajendran and Raja (2016) in a study of polymorphisms in the Myostatin gene in intron 1 and exon 1 were also monomorphic, this indicates that the MSTN gene in the intron 1, 2 and exon 1, 2 and 3 regions of the gene Myostatin using MSP1 and HAEIII enzymes is a conserve region (conserve region) that is not easy to mutate, and is easy to target for evolutionary research.

4. Conclusion

It was concluded that the genetic polymorphism at the Myostatin locus in Texel, Dorper and DEG sheep at the study site was monomorphic, indicating that the Myostatin locus is a conserved region that is not prone to mutations in DNA bases. On the other hand, the results of the measurement of the qualitative variables show a fairly high coefficient of variance. It is recommended that further research be carried out with different target areas and different restriction enzymes.

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References

[1] Anonymous, 2007. Better sheep ideal for lamb market. Farmers Weekly-Farming & Agricultural news from Farmers Weekly Interactive (fwi. co. uk)
[2] Anonymous.2021. Texel Sheep Society. https: //texel. uk/
[3] Anonymous.2016. Permentan 47-2016 tentang Pedoman Penyusunan Programa Penyuluhan Pertanian.
[4] Budak H**,** Pedraza F, Cregan PB, Baenziger PS, Dweikat I (2003). Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of Pearl Millet germplasm. Crop Sci. Ame., 43: 2284-2290.
[5] Bozhilova-Sakova, M., I. Dimitrova, A. Teneva and N. Petrov, 2016. PCR-RFLP analysis of MSTN gene in Karakachan sheep breed. Bulg. J. Agric. Sci.22 (Suppl.1): 115–117
[6] Dehnavi, E, Azari, MA, Hasani, S, Nassiry, M. R., Mohajer, M, Ahmadi, A. K., Shahmohamadi, L, Yousefi, S.2012. Polymorphism of Mysotatin Gene in Intron 1 and 2 and Exon 3, and their Association withYearling Weight, using PCR-RFLP and PCR-SSCP Technique in Zel Sheep. Hindawi Publishing Corporation Biotechnology Research International Volume 2012, Article ID 472307, 5 pages doi: 10.1155/2012/472307.
[7] Nugroho, H. W. Busono. S. Maylinda.2017. Polymorphisms of the Myostatin gene (MSTN) and its associationwith growth traits in Bali cattle. Indian Journal of Animal Research (51): 817-820.
[8] Haren, H. I. H., D. Purwantini, P. Prayitno, and M. Y. Sumaryadi.2020. Polymorphism of Myostatin Gene (MSTN) Coding Region in Batur Sheep. Animal Production.21 (1): 10-15, 2019 Accredited by Kemenristek Dikti No 32a/E/KPT/2017. ISSN 1411-2027 10
[9] Hidayat, R.2020. Regulasi Impor Indukan Domba dan Kambing. Majalah Trobos, 1 Februari 2020.
[10] Indra, I. (2020). Manajemen Pemeliharaan Domba Jantan Garut Di PT Agro Investama Garut Jawa Barat.
[11] Osei, MK., J. A. Danquah. And J. A. Opoku.2018. Marker-Assisted Selection (MAS): A Fast-Track Tool in Tomato Breeding. DOI: 10.5772/intechopen.76007. In book: Recent Advances in Tomato Breeding and Production, Publisher: IntechOpen
[12] Ruzty, R. H.2014. Identifikasi Sifat-sifat Kualitatif Domba Wonosobo Betina (Kasus pada Peternak di Kecamatan Kejajar Kabupaten Wonosobo). Students e-Journal, 3 (2).
[13] Sutarno.2016. Rekayasa Genetik Dan Perkembangan Bioteknologi Di Bidang Peternakan. Dalam: Proceeding Biology Education Conference (ISSN: 2528-5742), Vol 13 (1) 2016: 23-27
[14] Bozhilova-Sakova, M., I. Dimitrova, A. Teneva and N. Petrov, 2016. PCR-RFLP analysis of MSTN gene in Karakachan sheep breed. Bulg. J. Agric. Sci.22 (Suppl.1): 115–117