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PERBEDAAN MORFOMETRI DAN QUANTITATIVE POLYMERASE CHAIN REACTION BERDASARKAN GEN GDF9 ANTARA DOMBA SAPUDI DAN GARUT

Phenotype and Genotype Expression with the GDF9 Gene in the Sapudi and Garut Sheeps

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ABSTRACT

Penelitian ini bertujuan untuk mengetahui ekspresi fenotipe dan genetipe pada domba Sapudi dan Garut, serta perbedaan antara ekspresi gen GDF9 dengan sifat fenotipe pada domba Sapudi dan Garut. Metode penelitian yang digunakan adalah metode deskriptif kuantitatif. Materi yang digunakan adalah 48 ekor domba pejantan yang dipeliharan di UPT Pembibitan ternak dan HMT di Jember dan Peternakan Anak Seribu Pulau Farm Kangploso Malang. Sedangkan untuk pengamatan ekspresi gen GDF9 ekson 1 dengan analisa Quantitative Polymerase Chain Reaction (*qPCR)* menggunakan primer gen GDF9 dengan sampel sebanyak 8 ekor. Analisa data menggunakan analisa uji-t tidak berpasangan dan dilanjutkan dengan uji BNT. Pengamatan sifat fenotipe meliputi panjang badan (PB), lingkar dada (LD) dan tinggi badan (TB). Sifat genotipe yang diamati adalah ekspresi gen GDF9. Hasil penelitian menunjukkan bahwa karakteristik sifat fenotipe domba Sapudi dan Garut terdapat perbedaan sangat nyata ($P<0,01$). Berdasarkan penelitian terdapat perbedaan ekspresi sifat fenotipe domba Sapudi dan Garut pada panjang badan (Sapudi $63,21^{a}\pm3,39$; Garut$77,71^{b}\pm5,66$) cm, lingkar dada domba (Sapudi $76,15^{a}\pm3,81$; Garut $83,00^{b}\pm8,75$) cm dan tinggi badan (Sapudi $64,96^{a}\pm3,97$; Garut $73,50^{b}\pm5,60$) cm. Terdapat perbedaan sangat nyata ($P<0,01$) sifat genotipe dari hasil qPCR gen GDF9 antara bangsa domba yaitu domba Sapudi 31,66±5,61 dan Garut 23,70±2,40.

Kata Kunci : GDF9; ukuran tubuh; Domba

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ABSTRACT

This study aims to determine the expression of phenotypes and genotypes in Sapudi and Garut sheep, as well as differences between GDF9 exon 1 gene expression and phenotypic traits in Sapudi and Garut sheep. The research method used is a quantitative descriptive method. The material used was 48 rams kept at UPT Breeding Cattle and Forages in Jember and Seribu Pulau Children's Farm, Kangploso Farm, Malang. As for the observation of GDF9 gene expression by qPCR analysis using GDF9 gene primers with a sample of 8 tails. Data analysis used unpaired t-test analysis and continued with the BNT test. Observation of phenotypic traits included body length (PB), chest circumference (LD), and body height (TB). The genotype observed was GDF9 gene expression. The results showed very significant differences in the phenotypic characteristics of the Sapudi and Garut sheep (P<0.01). Based on the study, there were differences in the expression of the phenotypic traits of Sapudi and Garut sheep on body length (Sapudi 63.21^{a} ± 3.39; Garut 77.71^{b} ± 5.66) cm, chest circumference (Sapudi 76.15^{a} ± 3.81; Garut 83, 00^{b} ± 8.75) cm and height (Sapudi 64.96^{a} ± 3.97; Garut 73.50^{b} ± 5.60) cm. There was a very significant difference (P<0.01) in genotypic traits from the qPCR results of the GDF9 gene between sheep breeds, namely Sapudi sheep 31.66 ± 5.61 and Garut sheep 23.70 ± 2.40.

Keywords: *GDF9; body size; beef lamb; qPCR.*

INTRODUCTION

Sheep is one of the livestock that is in great demand by the Indonesian people to keep because it can reproduce quickly, the capital spent is relatively tiny, maintenance is more manageable, and able to adapt well to the environment, and it is resistant to disease. Many small breeders keep sheep There are several breeds of sheep in Indonesia, including Sapudi and Garut.

Garut and Sapudi sheep are local sheep in Indonesia that are kept as meat producers and play an essential role in the local community, so their population must be increased (Heriyadi, 2005; Mirella et al., 2022; Mudawamah et al., 2022; Nasich et al.,). In order to increase the sheep population and reproduction through detecting the genetic variation of these sheep in terms of reproduction. A large amount of phenotypic and genetic diversity will provide possibilities for genetic improvement. So it is necessary to improve the genetic reproduction of sheep in Indonesia based on breeding using molecular techniques for genetic improvement. Livestock has provided a better advantage by allowing the identification of the primary genes associated with the reproduction of traits that can be further utilized as the markers-assisted selection in breeding sheep. Productive and reproductive traits are influenced by many minor genes scattered throughout the genome. The selection of these genes required special techniques, and the lineage of their parents assisted in the selection. This essential gene was found to participate in determining sheep fertility. Fertility is one of the traits inherited from parents related to fertility which is controlled by the Bone morphogenetic protein receptor IB (BMPR-1B), bone morphogenetic protein 15 (BMP15), and Growth Differentiation Factor 9 (GDF9) genes. The differential growth factor gene (GDF9) is one of the promising marker genes for economically beneficial traits, which is recommended for use as a marker of the reproductive productivity of sheep.

Based on the description above, it is necessary to research the diversity of characteristics in Garut and Sapudi sheep by observing phenotypic traits, namely, morphometrics based on the GDF9 gene, so that quantitative aspects and genotypes can be identified based on GDF9 gene expression.

MATERIAL AND METHOD

Material

The material used was 48 ewes consisting of 34 Sapudi sheep and 14 Garut sheep. The sheep were reared at UPT Cattle and Forage Breeding in Jember and Pulau Seribu Anak Farm Karangploso Malang with Natural Service Management. There were two types of management mating in small ruminants included AI (Artificial Insemination) and NS (Natural Service) (Mudawamah et al., 2014 and 2019[a]). The GDF9 primer exon 1 (Mudawamah et al., 2019[b]) as a sequence below:

Forward 5'GGAAGAAGACTGGTATGGGGAAATG 3'
Reverse 5' CTGCTCCTACACACCTGCCGC 3'.

The number of samples for the GDF9 gene quantification test was eight, consisting of 4 Sapudi sheep and 4 Garut sheep. The materials were ddH_2O, cyber green, DNA extraction (PW Buffer, GA Buffer, TE Buffer, GD Buffer and GB Buffer), and GDF9 gene primer.

The following research procedure was data collection on phenotype and genotype characteristics at UPT Cattle Breeding and Foraged in Jember and Seribu Pulau Farm, Kangploso Malang. The analysis of GDF9 gene expression using the qPCR method in the Integrated Laboratory and Halal Center at the University Islam of Malang.

Method

This study used a quantitative descriptive method by taking case studies at Seribu Pulau Farm Kangploso Malang, UPT Livestock Breeding, and HMT Jember. Data analysis with unpaired t-test continued with the Least Significant Difference Test (LSD). The variables observed in this study include body length (PB), chest circumference (LD) and height (TB). The genotypic trait observed was GDF9 gene expression.

RESULTS AND DISCUSSION

Phenotypic traits expression of Sapudi and Garut sheeps

Body length

Based on the unpaired t-test analysis results, the body length of Sapudi and Garut sheep differed significantly ($P<0.01$). The average body length phenotypic measurements of each breed of sheep are in Figure 1.

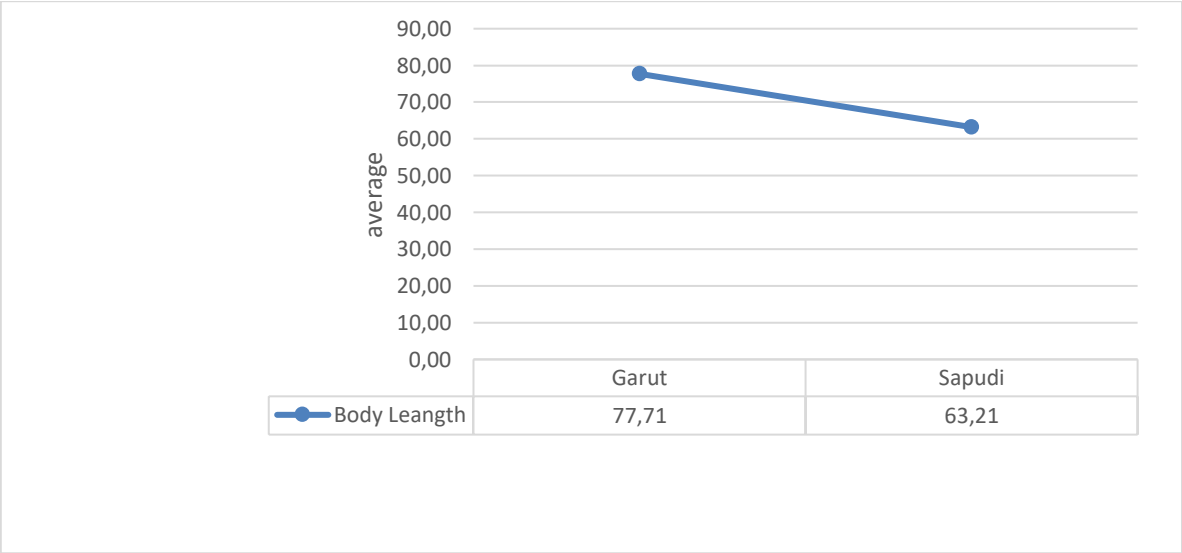

Figure 1. The body length of Sapudi and Garut Sheep

Figure 1 showed that the average body length of Sapudi was 63.21 ± 3.39 cm, and Garut was 77.71 ± 5.66 cm. Sapudi sheep were lower than the Indonesia Standard

National (ISN), whose body length was 70 ± 5.1 cm, while higher than Tanziila's study (2018) body length of ram Sapudi was 55.57 ± 5.99 cm. Garut sheep was lower than the ISN for body length of ram Garut aged 18 months around 64 cm. Heriyadi (2012) body length of ram Garut was 63.41 cm long.

Chest circumference

Based on the results of the unpaired t-test analysis, there is a significant difference (P<0.01) in the chest circumference between Sapudi sheep and Garut sheep. The mean measurements of the phenotypic trait of chest circumference for each sheep breed can be seen in Figure 2.

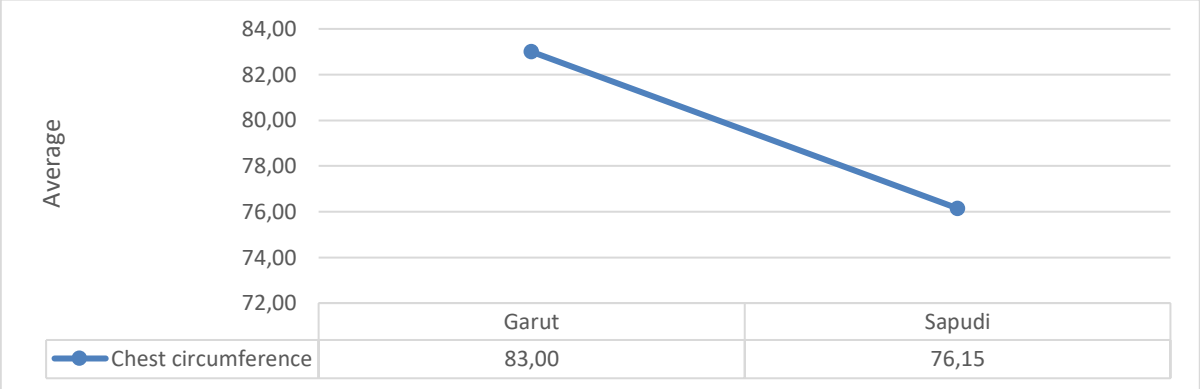

Figure 2. The chest circumference of Sapudi and Garut Sheep

Figure 2 showed that the average chest circumference in Sapudi sheep was 76.15 ± 3.81 cm and 83.00 ± 8.75 cm in Garut sheep. The chest circumference of Sapudi was lower than INS 2012 (84.8 ± 4.3 cm) and higher than Tanziila's study (2018) was 63.47 ± 5.18 cm. Meanwhile, the SNI for the Garut ram on the chest circumference was 89.0 cm, compared to Heriyadi's (2012) study which showed that the chest circumference of the Garut ram was 88.73 cm. The chest circumference is a very important trait s a selection basic (Malewa & Salmin,2008) because it indicates the speed of livestock growth. Komariah et al. (2015) that chest circumference is the part of the body that is enlarged towards the side. The increased body weight in sheep causes an increase in size and is followed by material and muscle development in the chest area so that the size of the chest circumference increases. Basbeth et al. (2015) added that the increase in the chest circumference is due to the size of the chest circumference following the growth and development of muscle tissue in the chest area.

Body Height

Based on the unpaired t-test analysis results, the body height of Sapudi and Garut sheep was significantly different (P<0.01). The average height phenotypic measurements of each breed of sheep are in Figure 3. Based on Figure 3, body height increased with the increasing age of livestock. According to Haryanti et al. (2015) sheep aged <6-12 months, chest circumference is better-used body height as a selection criterion.

The results of the study in Table 4.3 mean height in Garut and Sapudi sheep showed that there was a very significant difference (P <0.01), the average value of Garut height was 73.50 ± 5.60 cm according to the INS provisions that for sheep Garut height is 74 cm. and Sapudi's height is 64.96 ± 3.97 cm, lower than the Minister of Agriculture Decree no. 2389 (2012) that for male Sapudi sheep 70.4 ± 4.2 cm and higher than Tanziila's study (2018) chest circumference in male Sapudi sheep 57.04 ± 3.15 cm.

Figure 3. The body height of Sapudi and Garut Sheep

GDF9 gene expression with genotypic traits in Sapudi and Garut sheep

Based on the unpaired t-test analysis results, the term of the GDF9 gene in Sapudi and Garut sheep was significantly different ($P<0.01$). The average expression of the GDF9 gene from each sheep breed can be seen in Figure 4.

Figure 4 shows an increase in GDF9 gene expression in Sapudi sheep 23.70 ± 2.40 and Garut sheep 31.66 ± 5.61. According to Altieri et al,. (1999) the GDF9 gene is expressed in oocytes and ovarian granulosa cells from the primary follicle stage until the oocyte is ovulated. GDF9 protein is a polypeptide molecule of the TGF-β Growth Factor family. The protein is used for oocyte maturation and maturation. The GDF9 protein stimulates granulosa cell differentiation, including inducing LH receptors and steroidogenesis. In contrast, the GDF9 gene was not expressed in rams. Mudawamah, M. et al. (2018) one of the genes influencing prolific properties is the GDF9 gene. Afifi Inayah. (2011) the GDF9 gene also reduces its binding capacity with other proteins, which can affect the phenotype.

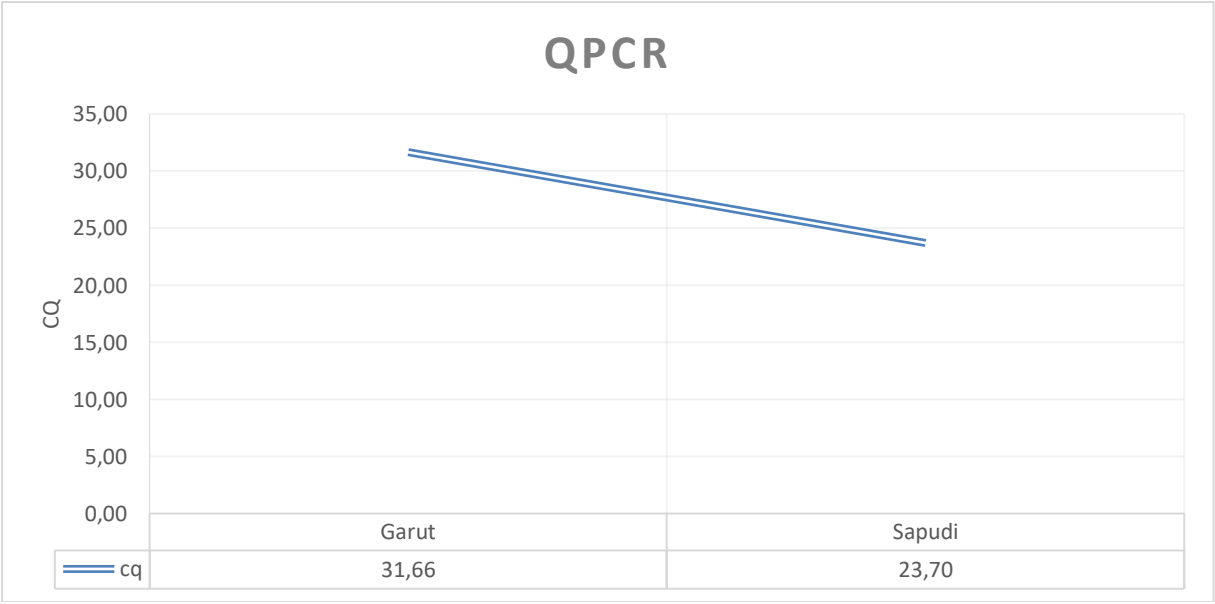

Figure 4. GDF9 Gene qPCR Expression of Sapudi and Garut Sheep

Alterations caused by the GDF9 gene and mRNA are present in germinal cells during follicular opening, in primodinal oocytes, and at all subsequent follicular growth stages in sheep.

Type 3 protein gene that inhibits insulin-like growth factor (IDFBP-3) is a potential marker gene related to animal growth and development, referring to the structural genes responsible for the manifestation of insulin-like growth factor activity. As is well known, IGF-I and IGF-II are hormones responsible for growth and regeneration processes in mammals and are involved in the development of the mammary glands (Yu.A, et l., 2018).

Differences in the expression of phenotypes and genotypes for Sapudi sheep compared to Garut sheep based on the GDF9 gene

The analysis results in Figure 4 show that the percentage of sheep based on phenotypic and genotypic traits in Garut sheep is higher than in Sapudi sheep.

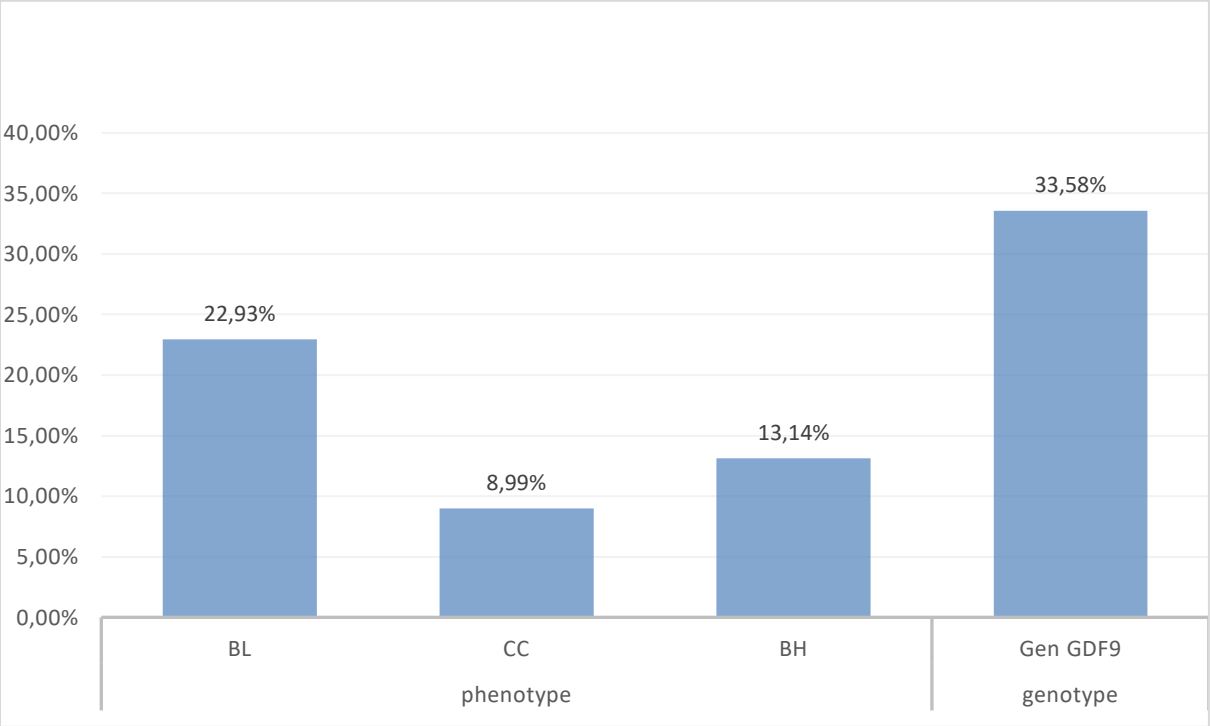

Figure 4. Differences in phenotype and genotype expression for Sapudi sheep compared to Garut sheep based on the GDF9 gene

Figure 4 shows the differences in the expression of the phenotypic traits of the two sheep breeds, including body length (22.93%), chest circumference (8.99%), and body height (13.14%). Genotypic traits based on the GDF9 gene in Sapudi and Garut sheep were 33.58%. Genetic and environmental factors and the interaction between the two influence sheep performance. Genetic factors are important because they are inherited, meaning that the version expressed by livestock will be passed on to their offspring. Genetic factors in question, for example, the ability of animals to survive tropical conditions and resistance to parasites (Ilham, 2015). However, environmental factors also affect sheep performance, for example, maintenance management, quality feed, disease prevention, and the livestock environment. So this is the reason for getting the availability of superior ram seeds.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that there is an effect of phenotypic expression of Sapudi sheep which is lower than that of

Garut sheep, as well as a genotypic expression based on the GDF9 gene. And the effect of differences in phenotype expression was 15.03%, and the genotype was 33.58% based on the GDF9 gene, namely that Garut sheep were higher than Sapudi sheep.

THANK-YOU NOTE

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