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Polymorphism of Growth Hormone (*GH*/*Hae*III) Gene and Its Association with the Performance of Merino Cross Rams

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ABSTRACT

Merino cross sheep (75% Merino × 25% Garut) are introduced by the government of Indonesia for meat production purposes. The present study aimed to determine the polymorphism in the exon 2 region of the growth hormone (*GH*) gene (422 bp) in Merino cross rams using the PCR-RFLP technique and to analyze its relationship with body weight and body measurements of the rams. A total of 145 rams aged one-year-old with an average body weight of 29.08 ± 7.96 kg from the breeding station in West Java, Indonesia were considered as the experimental animals. It was indicated that a missense mutation of c.55G > A (p.G19S) was detected in the target sequence of the *GH* gene in Merino cross rams. The PCR-RFLP analysis in the *GH* gene of Merino cross with HaeIII restriction enzyme (*GH*/*Hae*III) was observed in a moderate category with a polymorphic informative content (PIC) value of 0.22. Therefore, the G allele was more frequent than the A allele (0.85 versus 0.15). Furthermore, the genotype AA was not present among the sheep that were part of the study. However, the polymorphism of p.G19S was found to have a significant association with birth, weight, and chest depth measurement in one-year-old Merino cross sheep. However, the *GH*/*Hae*III gene in Merino cross rams exhibited polymorphism, primarily with two genotypes: GG (wildtype) and GA (carrier). The G allele was identified as the dominant allele in the ovine *GH* gene, occurring with a frequency of 0.85. Importantly, the polymorphism of the *GH/Hae*III gene was significantly linked to birth weight and chest depth in one-year-old Merino cross rams. These findings provide preliminary insights that could potentially aid in the early stages of molecular selection for Indonesian Merino cross sheep.

Keywords: Growth hormone gene, Merino cross ram, Performance, Polymorphism

INTRODUCTION

Sheep is an important livestock in Indonesia and can be developed as a potential export commodity. According to the Indonesian Ministry of Agriculture, sheep meat production in Indonesia in the year 2021 was about 50,702.06 tons (Kementan, 2022a). Meanwhile, the projection of sheep meat consumption in Indonesia in the same year was about 41,776 tons (Kementan, 2022b). Hence, there are about 8,926.06 tons of sheep meat surplus for export potency. The sheep meat production can be increased by a genetic improvement program with a selection program. Recently, a molecular selection involving functional genes has been used to obtain the genetic marker for the productivity of sheep (Bowles, 2015). In the year 2000, a grading-up program to produce Merino cross sheep (75% Merino and 25% Garut) was assessed by the government of Indonesia for meat production purposes. Therefore, previous studies recorded that sheep have 1.34±0.51 of estimated breeding value (EBV) for litter size and 0.10 ± 0.03 kg/day of post-weaned daily weight gain (Putra et al., 2023; Margawati et al., 2023).

The ovine growth hormone (*GH*) gene is one of the common candidate genes that are used for molecular selection in sheep (Gebreselassie et al., 2020). This gene is located on chromosome 11 along 1,795 bp with five exons. Previous studies reported a missense mutation (p.G19S) in exon 2 of the *ovine GH* gene that affects the production traits in sheep (Kumari et al., 2014; Susilorini et al., 2017; Rashijane et al., 2022; Muniasamy et al., 2023). Therefore, a mutation p.G19S can be detected using the PCR-RFLP technique with *Hae*III restriction enzyme (Hua et al., 2009). Unfortunately, studies aimed at detecting genetic markers for production traits in Indonesian Merino cross sheep are very limited. Margawati et al. (2023) reported a polymorphism of the BMPR1B/*Ava*II gene in Indonesian Merino cross sheep but its was not associated with the growth traits. Nonetheless, a polymorphism of *CAPN/BseSI* gene was significantly associated with birth weight of Merino cross sheep (Puruhita et al., 2023).

It is crucial to conduct research aimed at identifying genetic markers associated with growth traits of Merino cross sheep, as this will ultimately enhance their production characteristics. The purpose of the present study was to identify the mutation site or single nucleotide polymorphism (SNP) in the *GH* gene (exon 2) of Merino cross rams using PCR-

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RFLP method with *Hae*III restriction enzyme (*GH*/*Hae*III). In addition, the effect of *GH*/*Hae*III gene polymorphism on body weight and body measurements of animals in the current study was investigated.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Animal Ethics Committee of the Indonesian Institute of Science (LIPI) with the following permit number, 002/KKE/UM/VIII/2017.

Animals, research site, and DNA extraction

A total of 145 Merino crossbreed rams (75% Merino, 25% Garut) aged one year old were used in the experiment. The sheep were collected from the Cimanglid research farm at Bogor Regency, West Java, Indonesia. This farm is located at latitude 6° 38' 14" S and longitude 106° 46' 31" East with an altitude of 15-150 m asl; 20-30 °C of air temperature about 70% relative humidity and 2500-5000 mm/year of rainfall. The blood samples (± 3ml) were taken from the jugular vein of the animal using a venoject and vacutainer tube containing EDTA. The genomic DNA extraction was performed using the High Salting method according to Montgomery and Sise (1991). The DNA sample of each animal was stored at -20°C for further analysis.

PCR-RFLP

Amplification of the ovine *GH* gene (GenBank: KP120857.1) was performed using primer pairs of *GH*-F: 5'- CTC TGC CTG CCC TGG ACT -3' and GH-R: 5'- GGA GAA GCA GAA GGC AAC -3' with the target sequence of 422 bp (Hua et al., 2009, Figure 1). Amount 10 µL of PCR reaction consisted of 5 µL of PCR master mix (MyTaq Redmix, Bioline, USA), 1 µL of each primer (10 pmol/µL), 2 µL of free-nuclease water and 1 µL of DNA template (50 ng/µL) were assessed to amplify the target gene. The amplification of the ovine *GH* gene was performed in a PCR program of pre-denaturation at 94^{o}C for 5 minutes, followed by 35 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 65^{o}C for 30 seconds, initial extension at 72^{o}C for 45 seconds and a final extension at 72^{o}C for 7 minutes. Therefore, the amplicons were visualized using 1% agarose gel through electrophoresis analysis at 100 Volt for 45 minutes. Subsequently. The DNA fragments were stained with EtBr and visualization under UV light. In addition, the detection of *GH* gene polymorphism was performed with RFLP analysis using *Hae*III restriction enzyme (GG*CC) at 37°C for 3 hours using the water bath. Therefore, the electrophoresis by 2% agarose gel and UV light was assessed for the visualization of RFLP results.

Management of animal

The sheep were raised in a colony stall (1 male and 30-50 females) with an intensive system at the research site. The feed ration consisted of Elephant grass (*Pennisetum purpureum*) and the commercial concentrate with the composition of 14% crude protein, 4% fat, 7% crude fiber, 8% ash, and 12% TDN with 2,700 kcal/kg of metabolizable energy (ME). The water was given *ad libitum*. The natural mating was managed in the studied farm to produce the lambs. The body weight (BW) of the animal was obtained with a hanging weight scale. Therefore, the animal weighing was performed at birth, weaning age (± 3 months of age), and continued with a regular weighing time every two weeks from weaning to yearling age (one year old).

Body weight

The Merino cross sheep in the current study were kept during the ACIAR project from 1999 to 2002. Hence, the data records of BW in sheep during that period were used in the present study for association analysis. The data correction was performed in the BW to reduce an experimental error according to the method of Hardjosubroto (1994) according to the following formulas (Formula 1-5).

$$BW_c = BW \times CTB \quad \text{(Formula 1)}$$

$$BW_{120} = \left(BW + \left(\frac{WW - BW}{T_w} \right) \times 120 \right) \times CTB \quad \text{(Formula 2)}$$

$$BW_{365} = \left(BW_{120} + \left(\frac{YW - BW_{120}}{T} \right) \times 245 \right) \times CTB \quad \text{(Formula 3)}$$

$$DG_{pre} = (BW_{120} - BW_c) / 120 \quad \text{(Formula 4)}$$

$$DG_{post} = (BW_{365} - BW_{120}) / 245 \quad \text{(Formula 5)}$$

where, BW_c is the corrected birth weight, BW_{120} is the body weight at 120 days of age, BW_{365} is the body weight at 365 days of age, BW is the actual birth weight, WW is the actual weaning weight, YW is the actual yearling weight, T_w is the weaning age, T is the period between weaning to weighing times, DG_{pre} is the pre-weaned daily gain, DG_{post} is the post-weaned daily gain, CTB is the constant for type of birth for example 1.0 (single) and 1.10 (twin).

Body measurements

Body measurements of thirteen parts, including head length (HL), head width (HW), withers height (WH), body length (BL), chest girth (CG), rump length (RL), rump width (RW), chest depth (CD), chest width (CW), front leg length (FLL), back foot length (BFL), front leg circumference (FLC), and back foot circumference (BFC) of yearling rams (365 days of age) for association analysis were recorded. All body measurements were measured according to Alderson (1999) as shown in Figure 2.

Statistical analysis

The association analysis between the genotype of the *GH*/*Hae*III gene and body weight was performed by GLM (General Linear Model) procedure in the SAS software package. The linear model was as Formula 6.

$Y_{ik} = \mu + G_i + \varepsilon_{ik}$ (Formula 6)

where, Y_{ik} was the ik traits observation value; μ was the mean; G_i was the effect of i^{th} genotype and ε_{ik} was the residual error. Therefore, the genetic diversity parameters of genotype frequency, allele frequency, observed heterozygosity (H_o), expected heterozigosity (H_e), number of effective allele (n_e), polymorphic informative content (PIC), and Chi-square (χ^2) values for *GH*/*Hae*III gene were calculated according to the studies of Nei and Tajima (1981), Weir (1990), Hildebrand et al. (1992), Nei and Kumar (2000), and Kaps and Lamberson (2004), respectively. The p value less than 0.05 considered for the significant differences.

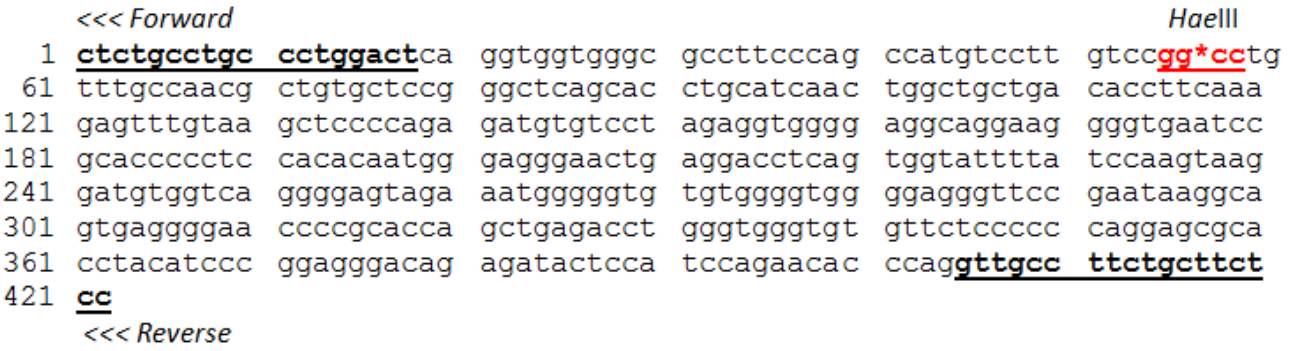

Figure 1. Primer position (black bold underline) and *Hae*III restriction site (red bold underline) in the target sequence of *ovine* Growth Hormone (*GH*) gene (GenBank: KP120857.1) along 422 bp. A transition mutation of c.55G>A (p.G19S) occurred in the exon 2 of the ovine *GH* gene

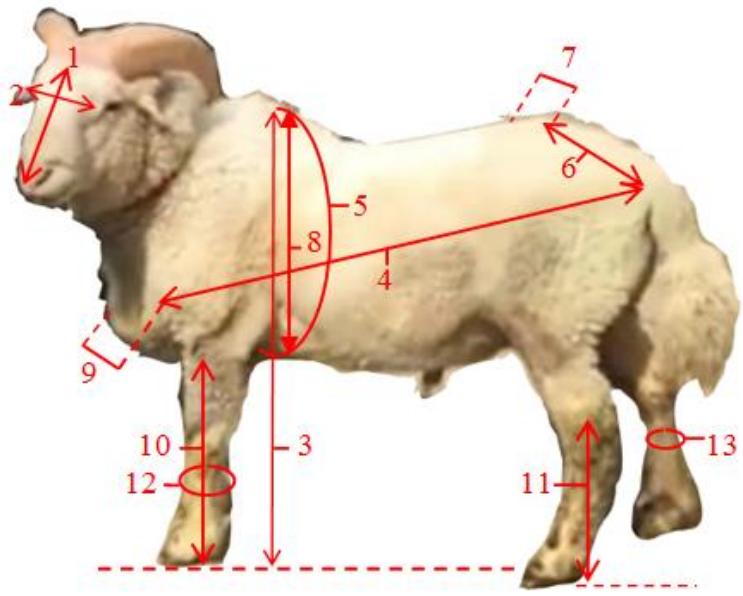

Figure 2. The sheme of body measurements in a Merino cross sheep consisted of head length (1), head width (2), withers height (3), body length (4), chest girth (5), rump length (6), rump width (7), chest depth (8), chest width (9), front leg length (10), back foot length (11), front leg circumference (12), and back foot circumference (13)

RESULTS AND DISCUSSION

The result of PCR-RFLP analysis in *GH*/*Hae*III gene of Merino cross sheep reveals two genotype patterns of GG (366 bp) and GA (422 bp and 366 bp) as illustrated in Figure 3. Nonetheless, the DNA fragment along 56 bp is not observed in the present study. Therefore, the GG genotype (0.70) and G allele (0.85) were observed superior in *GH*/*Hae*III gene of studied animals as presented in Table 1. Subsequently, a mutant AA genotype was absent in the included animals of the current study. Similar findings were observed in the Egyptian sheep breeds the presence of two genotypes of GG and GA in Barki (36% GG and 64% GA), Rahmani (19% GG and 81% GA), and Ossimi (77% GG and 23% GA) as reported by Othman et al. (2015). In Iraqi sheep breeds, two genotypes of GG and GA were observed in Awassi (70% GG and 30% GA) and Karadi (60% GG and 40% GA) as reported by Mahdi et al. (2018). Moreover, Two genotypes of GG and GA were also observed in Kenguri (42% GG and 58% GA) and Kilakarsal (29% GG and 71% GA) sheep breeds of India (Hiremath et al., 2017; Muniasamy et al., 2023). In contrast, the AA genotype was present in the Iraqi Hamdani sheep (50% GG; 10% GA; 40% AA) and Awassi sheep (47% GG; 33% GA and 20% AA) as reported by Mahdi et al. (2018) and El-Mansy et al. (2023), respectively. Moreover, the *GH*/*Hae*III gene was monomorphic in Palu sheep with 100% of the GG genotype (Malewa et al., 2019).

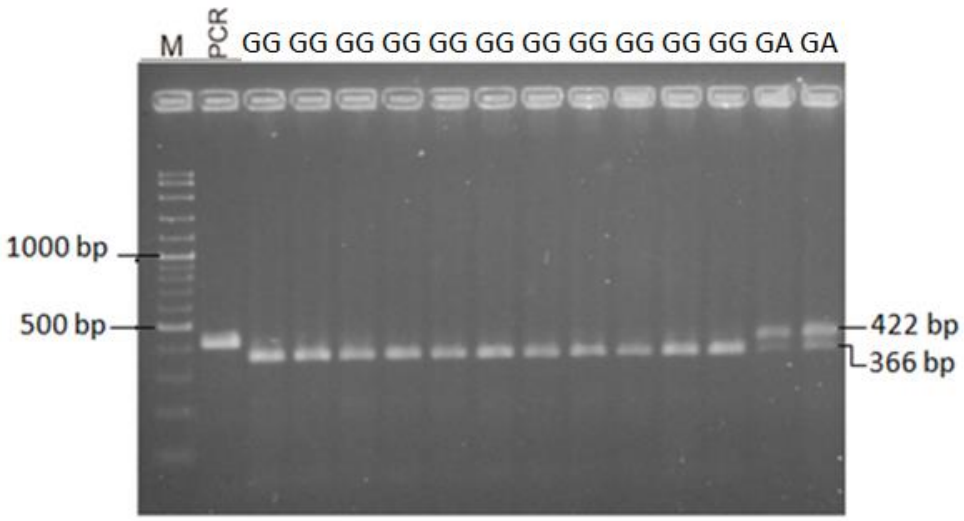

Figure 3. The PCR-RFLP results of the *GH*/*Hae*III gene in backcross rams showed two genotypes of GG (366 bp) and GA (422 bp and 366 bp). A 56 bp DNA fragment in the GA genotype is not visible. M: DNA ladder 100 bp

The observed heterozygosity (H_o) value was higher than the expected heterozygosity (H_e) and suggested that the number of heterozygous animals in the animal population was higher than the homozygous animals. The number of effective allele (n_e) values was 1.34 and suggested that this gene has a common allele of G. The genetic diversity of *GH* gene in animals included in the present study was not in Hardy-Weinberg equilibrium/HWE ($\chi^2 > 3.84$). In livestock animals, the HWE equilibrium could be attributed to selection, migration, cross-breeding, and inbreeding (Falconer and Mackay, 1989). Subsequently, the polymorphic informative content (PIC) value in the *GH* gene of the Merino cross was 0.22 and included in the moderate category. The PIC value can be classified into low (PIC < 0.10), moderate (0.10 < PIC < 0.30) and high (PIC > 0.30) categories (Nei and Kumar, 2000). The association analysis revealed that the polymorphism of *GH*/*Hae*III gene in animals under study is significantly associated with CD measurement (Table 2). Thus, the average CD in the GG genotype was higher than the GA genotype. Additionally, the polymorphism of the *GH*/*Hae*III gene in animals under study was significantly associated with birth weight (Table 3).

Hiremath et al. (2017) reported that the polymorphism of the *GH*/*Hae*III gene was not significantly associated with BW, WH, BL, and CG in the Indian Kenguri sheep which is similar to the findings of the present study. Despite this, the *GH*/*Hae*III gene polymorphism was not significantly associated with CG and BL measurements of Kacang and Boer goats (Ilham et al., 2016; Rashijane et al., 2022). Nonetheless, the polymorphism of *GH*/*Hae*III gene able to affect the CG measurement of Raini Cashmere, Sirohi and Barbari goats (Gooki et al., 2019; Singh et al., 2015). Moreover, Rashijane et al. (2022) reported that the GA genotype in the *GH*/*Hae*III gene of Boer goats had a higher adult weight than the GG genotype. In Salsk and Etawah sheep, carrying an A allele of the *GH*/*Hae*III gene influenced the body weight trait (Gorlov et al., 2017; Susilorini et al., 2017). In contrast, carrying a G allele in the *GH*/*Hae*III gene of Egyptian Awassi sheep can increase the body weight (El-Mansy et al., 2023). In Kalakasar sheep, the polymorphism of GH/*Hae*III gene was associated with yearling weight where the GA was the superior genotype (Muniasamy et al., 2023).

Rashijane et al. (2022) reported that the heterozygous Boer (GA) had higher body weight than wildtype (GG). Subsequently, the GA genotype was detected as the superior genotype for marketing weight and post-weaning weight gain of Egyptian Awassi sheep (El-Mansy et al., 2023).

In this study, the polymorphism in the exon 2 of the *GH* gene in studied animals was associated with birth weight and chest depth measurement at one year old. However, detection of the genetic marker in the other exonic regions of the *GH* gene is important to obtain other potential SNPs that associated with growth traits. Previously, the polymorphism of the *ovine GH* gene was observed in the exon 4 region (p.R121K) and it affected the WH in Dorper sheep (Madikadike et al., 2023) and the body weight in Harri sheep (Abdelmoneim et al., 2016). Furthermore, Cauveri et al. (2016) did not detect SNPs in all exonic regions of the Indian Nilagiri sheep *GH* gene. In addition, a missense SNP of p.G186S was observed in exon 5 of the Italian Sarda sheep *GH* gene (Vacca et al., 2013).

Table 1. The genetic diversity in *GH*/*Hae*III gene of Merino cross rams

Genotype frequency (N)			Allele frequency (%)		H_o	H_e	n_e	PIC	χ^2
GG	GA	AA	G	A					
0.70 (102)	0.30 (43)	0.00 (0)	85.00	15.00	0.30	0.25	1.34	0.22	4.395

N: Number of observation; H_o: Observed heterozygosity; H_e: Expected heterozygosity; n_e: Number of effective allele; PIC: Polymorphic informative content; χ^2: Chi-square value.

Table 2. Association results of *GH*/*Hae*III gene polymorphism with body measurements of Merino cross rams at 365 days of age

Body measurements (cm) \ Genotype	GG (N=101)	GA (N=43)
Head length	18.61 ± 4.53	18.81 ± 4.05
Head width	12.89 ± 2.43	12.99 ± 2.44
Withers height	56.47 ± 7.25	56.58 ± 6.33
Body length	55.36 ± 9.74	55.44 ± 8.42
Chest girth	67.74 ± 9.55	68.09 ± 10.73
Rump length	19.52 ± 2.81	19.14 ± 2.93
Rump width	14.64 ± 3.45	14.85 ± 3.13
Chest depth	24.46 ± 4.43^a	24.30 ± 3.18^b
Chest width	14.19 ± 2.15	14.23 ± 2.07
Front leg length	18.04 ± 1.80	18.33 ± 1.76
Back foot length	21.47 ± 2.37	21.65 ± 1.74
Front leg circumference	7.67 ± 1.24	7.83 ± 1.38

N: Number of observations. Different superscript letters in the similar row differ significantly ($p < 0.05$).

Table 3. Association results of *GH*/*Hae*III gene polymorphism with body weight of Merino cross rams

Corrected weight \ Genotype	GG (N=36)	GA (N=21)
Corrected birth weight (kg)	3.46 ± 0.88^a	3.49 ± 1.36^b
Body weight at 120 days of age (kg)	17.99 ± 4.46	18.30 ± 4.26
Body weight at 365 days of age (kg)	29.57 ± 8.57	29.59 ± 7.00
Pre-weaning weight gain (kg/day)	0.12 ± 0.04	0.12 ± 0.03
Post-weaning weight gain (kg/day)	0.05 ± 0.03	0.05 ± 0.02

N: Number of observations. Different superscript letters in the similar row differ significantly ($p < 0.05$).

CONCLUSION

The *GH*/*Hae*III gene in Merino cross rams was polymorphic with two common genotypes of GG (wildtype) and GA (carrier). Thus, the G allele was detected as the dominant allele in the *ovine GH* gene with frequency of 0.85. The polymorphism of the *GH*/*Hae*III gene in Merino cross rams was significantly associated with birth weight and chest depth at one year old. Furthermore, the obtained results can be used as early information to develop a molecular selection of Indonesian Merino cross sheep.

DECLARATIONS

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Endang Tri Margawati and Herman Willem Raadsma planned the experiment and collected the data records, Widya Pintaka Bayu Putra analyse the data, interpreted and made the write up and Thobela Louis Tyasi evaluated the paper. All authors confirmed the last content of the article before publication.

Competing interests

The authors have declared no conflict of interest.

Ethical considerations

The authors confirm that all authors have reviewed and submitted the manuscript to this journal for the first time.

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