

Original Article

Quantitative characteristics and growth hormone gene diversity of thin-tailed sheep in Sitinjau Laut, Kerinci Regency

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Abstract

Objective: This study aims to evaluate the quantitative characteristics and growth hormone gene diversity of thin-tailed sheep in Sitinjau Laut, Kerinci Regency.

Methods: The research method used 60 thin-tailed sheep and 60 samples of thin-tailed sheep's blood. The phenotypes observed included: body weight, weight gain, body measurements, and thin-tailed sheep blood samples. The GH gene was identified using the PCR-RFLP method with the Msp1 restriction enzyme. Data analysis included t-test, t²-hotelling, principal component analysis, and allele genotype frequencies.

Results: The results showed that body weight, body weight gain, and sizes of male thin-tailed sheep were significantly different ($P < 0.05$) higher than females. The analysis results on the GH|Msp1 gene locus of thin-tailed sheep were monomorphic with one type of allele, namely ++.

Conclusions: The average body weight, weight gain, and body measurements of male thin-tail sheep were higher than that of females. The body size characteristic of male and female thin-tailed sheep is the chest circumference, while the body shape characteristic of male and female thin-tailed sheep is the chest depth. The fragmentation of the GH|Msp1 gene in thin-tailed sheep is monomorphic.

Keywords: Growth hormone gene; Thin-tailed sheep; Quantitative characteristics

INTRODUCTION

Indonesia is a country blessed with a rich diversity of local livestock, and among them is the thin-tailed Sheep. The thin-tailed sheep is primarily found in rural areas and holds the potential to meet our animal protein needs. Thin-tailed sheep are ruminant livestock with an easy rearing system and a remarkable ability to adapt to different environments. Their robustness is further highlighted by their resistance to diseases and their prolific nature, as they are capable of giving birth to more than one offspring at a time [1].

Despite these advantages, thin-tailed sheep's productivity is relatively lower than other local sheep breeds like the Garut sheep [2]. To have a better understanding of thin-tailed sheep productivity level, an evaluation of the performance should be conducted. It involves evaluating livestock performance based on body weight, weight gain, and body measurements [3,4]. However, selection based on appearance alone is not accurate because environmental factors can influence it, while this influence cannot be passed on to the next generation.

Advances in the latest technology in the molecular field have made characterization

more accurate, enabling us to analyze the structural genes directly. One of the economically valuable genes that play a role in livestock growth is the growth hormone gene (GH) [3,5]. The GH gene regulates growth and metabolism in livestock and acts as a growth controller that can be used to select livestock [3,6]. The Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RFLP) identifier is one of the methods used to identify the GH gene.

Polymerase Chain Reaction (PCR) is a molecular biology technique that aims to amplify or multiply the desired DNA segment *in vitro* [7]. On the other hand, Restriction Fragment Length Polymorphism (RFLP) is a method for viewing DNA sequences that can be detected using different DNA fragments cut using specific restriction enzymes to describe a polymorphism gene [8]. PCR-RFLP molecular technique is cost-effective, takes less time, and produces results that can be obtained directly (Real-Time) compared to other methods [9].

Despite the potential of thin-tailed sheep, we still lack information on the diversity of GH genes and their impact on thin-tailed sheep performance, such as body weight, weight gain and body size. However, the combination of quantitative and molecular characteristics can be used as the main reference for selecting thin-tailed sheep in the future. Given the lack of information related to the Growth Hormone Gene in thin-tailed sheep, it is very important to conduct research aiming to know the diversity of quantitative characteristics and the Growth hormone (GH) gene in thin-tailed sheep.

MATERIALS AND METHODS

Materials

This research was conducted in two stages of research in the area and the laboratory. Area research was carried out in Sitinjau Laut District, Kerinci Regency. Research in the Laboratory was carried out at the Animal Molecular Genetics Laboratory, Division of Breeding and Genetics, Department of Animal Production Science and Technology, Faculty of Animal of Science, IPB University. The study lasted from August

28, 2021, to January 28, 2022. The materials used in this research were thin-tailed sheep aged 11 and 12 months consisting of 30 males and 30 females, and blood samples for each thin-tailed sheep (60 samples). The materials used in this study included sheep blood samples, Geneaid DNA kit for DNA isolation, MyTaq Red Mix, Aquades, agarose powder, TBE Buffer solution, Florosafe, loading dye, DNA ladder, forward and reversed primers, Nuclease Free Water, and restriction enzyme *Msp1*.

Methods

This study utilized several methods, including blood sampling from thin-tailed sheep, DNA extraction, PCR amplification, and PCR-RFLP. The blood samples were obtained using a 3 ml syringe from the jugular venous in the neck, and the collected blood was put into a 4 ml EDTA tube to prevent clotting. After being temporarily stored in a cool box, the samples were then freeze at -20°C before further processing.

The research was conducted in two stages, field and laboratory stages. The first stage involved collecting quantitative data, such as body weight and body weight gain, as well as taking measurements of body length, wither height, chest circumference, chest depth, and chest width. The second stage was carried out in the laboratory, which included DNA extraction, PCR amplification, and restriction with *Msp1* enzymes.

For DNA extraction, the Geneaid DNA Kit was used with a modified working protocol. The extracted DNA was amplified using one pair of primers with an estimated product of 694 bp, designed using the Primer3plus program based on GenBank with access no. X12546. The GH gene amplification was carried out using an ESCO PCR machine, and the amplification results were observed by electrophoresis of PCR products using 3µL of PCR products on 1.5% agarose gel for 35 minutes. More details on the primers used, and the GH gene sequence of thin-tailed sheep are presented in Table 1.

Furthermore, the PCR product was cut using the PCR-RFLP method with the restriction enzyme *Msp1* (↓CCGG), followed by incubation for 4 hours at 37°C. After being

Table 1. The length and location of the GH and Primer genes used for PCR analysis

Segment position	Lenght (bp)	Primer name	Sequence primary (5' to 3')	Annealing temperature	Cuts position
711-1404	694	GH D3	5'AACTGGCTGCTGACACCTTC 3'	60,5	272
		GH D4	5' ACCAGGCTGTTGGTGAAGAC 3'		173
					249

cut with restriction enzymes, the DNA samples were electrophoresed in 2% agarose with a voltage of 100 V for 40 minutes, and the final results were visualized under a UV Transilluminator machine.

Data analysis

The data analysis consisted of t-test, T2-Hotelling test, Principal Component Analysis (PCA), and Genotype and allele frequency. Differences between body weight, body weight gain, and body measurements of male and female thin-tail sheep were analyzed by t-test based on the instructions of Gaspersz [10].

$$t = \frac{X_1 - X_2}{\sqrt{\frac{\sum (X_{j1} - X_1)^2}{n_1(n_1-1)} + \frac{\sum (X_{j2} - X_2)^2}{n_2(n_2-1)}}} \dots\dots(1)$$

Notation:

- t = value of t be count
- X₁ = pattern imply in the first organization,
- X₂ = pattern mean in the second institution,
- X_{j1} = the cost of the J- remark within the first institution
- X_{j2} = the value of the J- statement in the second organization
- N₁ = wide variety of samples in the first organization, and
- N₂ = variety of samples within the second organization

Vector mean values for the body measurements of male and female thin-tailed sheep were analyzed using the T2-Hotelling test [10].

$$T^2 = (X_1 - X_2)S_{G-1}(X_1 - X_2) \dots\dots(2)$$

Next:

$$F = \frac{n_1 + n_2 - p - 1}{(n_1 + n_2 - 2)p} T^2 \dots\dots(3)$$

can be allotted F with levels of freedom V₁ = p and V₂ = N₁ + N₂ - p - 1

Statment:

- T² = belief of T²-Hotelling statistic
 - F = calculated belief for T²-Hotelling
 - n₁ = the variety of observation facts inside the first institution of Sheep
 - n₂ = the range of observation records inside the 2d institution of Sheep
 - X₁ = vector suggest value of a random variable inside the first institution of Sheep
 - X₂ = vector represents a belief of a random variable in the 2nd organization of Sheep
 - SG₋₁ = the inverse of the composite diversification matrix (the inverse of the SG matrix)
 - P = variety of measuring variables.
- Two organizations are declared identical if T² and stated different if T²

$$T \leq \frac{(n_1 + n_2 - 2)p}{n_1 + n_2 - p - 1} F_{\alpha; v_1, v_2} \dots\dots(4)$$

And stated different if T²

$$T \geq \frac{(n_1 + n_2 - 2)p}{n_1 + n_2 - p - 1} F_{\alpha; v_1, v_2} \dots\dots(5)$$

If the T2-Hotelling test shows a significant difference (P < 0.05), then we proceed to PCA. Principal Component Analysis was used to determine the body size and body shape of thin-tail sheep with the following formula [10].

$$Y_j = a_1jX_1 + a_2jX_2 + a_3jX_3 + \dots\dots + a_19jX \dots\dots(6)$$

Note:

- Y_j = j-th main issue (j = 1, 2; 1 = size, 2 = form)
- X_{1,2,3...} = variable to at least one, 2, 3, ... 18
- a_{ij, 2j, 3j, ...} = i-th variable eigenvector (1, 2, 3, ... 18) and j-th main aspect

The proportion of genotype frequencies or the proportion of a particular genotype in a

population, calculated based on the number of alleles divided by the total sample.

$$F_1 = \frac{\sum X_i}{N} \dots\dots(7)$$

Note:

x_i = found genotype

N = overall sample

The allele frequency of the GH gene, namely the proportion of a particular allele compared to all the alleles that occupy the locus, obtained from the analysis of PCR-RFLP characteristics was analyzed using the Nei and Kumar [11] formula.

$$X_i = \frac{(2n_{ii} + \sum_{j \neq i} n_{ij})}{2N} \dots\dots(8)$$

Note:

X_i = frequency of the i-th allele,

N_{ii} = variety of Sheep of genotype ii,

N_{ij} = wide variety of Sheep of genotype ij,

N = general range of samples.

RESULTS

Average body weight and body weight gain for thin-tailed sheep

Table 2 displays the average body weight and body weight gain of male and female thin-tailed sheep at 11 and 12 months. Based on t-test analysis, the results indicated a significant difference ($P < 0.05$) in the mean body weight and body weight gain between male and female thin-tailed sheep. Specifically, the average body weight and body weight gain of

male thin-tailed sheep at 11-12 months were found to be significantly higher than those of their female counterparts.

Average body sizes of thin-tailed sheep

In this study, the researchers investigated the body measurements of male and female thin-tailed sheep aged 11-12 months. The study found that the average body size of male thin-tailed sheep was greater than that of female thin-tailed sheep. Specifically, measurements such as body length (BoL), withers height (WiH), chest circumference (Chc), chest depth (ChD), and chest width (chW) were significantly ($P < 0.05$) higher in male thin-tailed sheep compared to their female counterparts. These findings suggested that there are notable gender differences in body size among thin-tailed sheep at 11 months of age.

The body measurements of male and female thin-tailed sheep aged 11 months were analyzed using T2-hotelling analysis. The findings of the T2-hotelling analysis revealed that the male thin-tailed sheep had significantly ($P < 0.01$) higher body measurements than the female Thin-tailed sheep.

Table 3 presents a comparison of body size and shape, total diversity, and eigenvectors of male and female thin-tailed sheep aged 11 months. The table shows that the total variation of body size values for male and female thin-tailed sheep was 75.3% and 80.9%, respectively. This percentage represents the highest proportion of variance

Table 2. Average body weight at 11 and 12 months of age and body weight gain at 11-12 months of male and female Thin-tailed sheep

Parameter	Male	Female
Body weight 11 month (Kg)	13.65±1.65 ^a	12.48±1.87 ^b
Body weight 12 month (Kg)	15.38±1.87 ^a	13.65±2.00 ^b
Body weight gain 11-12 month (g)	57.7±7.9 ^a	39±11.2 ^b

Different lowercase superscripts on the same line were significantly different ($P < 0.05$).

Body size	Male	Female
Body length (BoL) (cm)	42.76±1.90 ^a	41.26±2.06 ^b
Withers height (WiH) (cm)	40.67±1.81 ^a	39.64±2.06 ^b
Chest circumference (cm)	47.39±1.57 ^a	46.35±2.04 ^b
Chest depth (ChD) (cm)	18.60±1.93 ^a	16.57±1.94 ^b
Chest width (cm)	12.14±1.81 ^a	11.11±1.79 ^b

Different lowercase superscripts on the same line were significantly Body Length (BoL), Withers Height (WiH), Chest Circumference (ChC), CZhest Depth (ChD), Chest Width (ChW)

Table 3. Equation of body size and body shape with total diversity and eigenvectors of male and female Thin-tailed sheep.

Thin-Tailed sheep		Equation	TD (%)	λ
Male	Body size	= 0.486 BoL + 0.490 WiH + 0.496 ChC + 0.333 ChD + 0.409 ChW	75.3	3.76
	Body shape	= -0.294 BoL + -0.227 WiH + -0.197 ChC + 0.898 ChD + 0.130 ChW	13.6	0.68
Female	Body size	= 0.463 BoL + 0.479 WiH + 0.480 ChC + 0.398 ChD + 0.410 ChW	80.9	4.04
	Body shape	= -357 BoL + -0.286 WiH + -0.325 ChC + 0.706 ChD + 0.432 ChW	9.3	0.46

Body Length (BoL), Withers Height (WiH), Chest Circumference (ChC), Chest Depth (ChD), Chest Width (ChW).

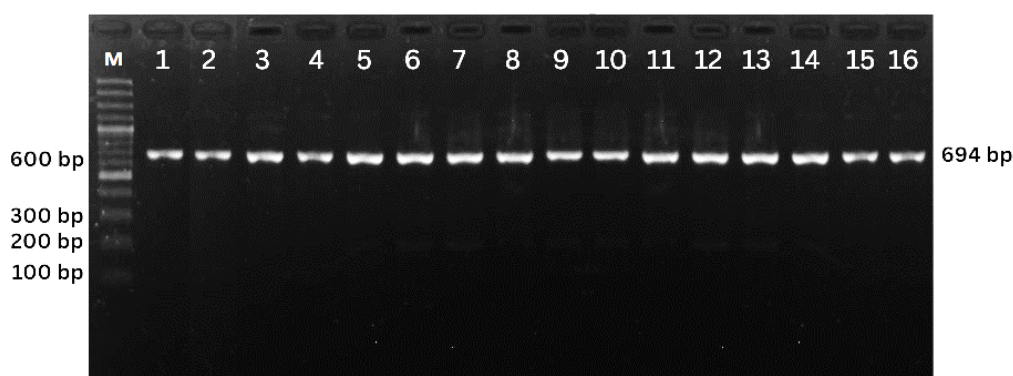


Figure 1. Results of exon 3 GH gene amplification in thin-tailed sheep

among the main components obtained. Additionally, the highest eigenvector in the body size equation for both male and female thin-tailed sheep was chest circumference (ChC).

Furthermore, the table indicates that the similarity of body shape between male and female thin-tailed sheep aged 11 months had a total variation of 13.6% and 9.3%, respectively. This value represents the highest proportion of variance among the main components obtained. Additionally, the highest eigenvector in the body shape equation for both male and female thin-tailed sheep was chest depth (ChD).

The exon 3 region of the GH gene in Thin-tailed sheep was successfully amplified using the Esco PCR machine, producing a PCR product with a length of 694bp. The amplification process followed a PCR protocol with a pre-denaturation stage at 95°C for 1 minute, denaturation at 95°C for 15 seconds, annealing at 61°C for 15 seconds, extension at 72°C for 10 seconds, and a final extension at 72°C for 1 minute. The electrophoresis results

of the GH gene amplification using 1.5% agarose gel and florasafe are displayed in Figure 1, showing the formation of distinct DNA bands with an annealing temperature of 61°C.

Figure 1 presents a clear visualization of the amplified GH gene product in Thin-tailed sheep, indicating the success of the PCR process at the chosen annealing temperature. The thick DNA bands suggest high-quality amplification, which is crucial in genetic studies.

Genotype and allele frequency

The genetic diversity of the GH gene in thin-tailed sheep was analyzed using the RFLP method with the MspI enzyme, which recognizes the cutting site ↓CCGG. Despite the success of the enzyme in cutting the DNA bands, the genotyping results revealed only one genotype, namely the (+/+) genotype with 3 bands at 279 bp, 249 bp, and 173 bp. This finding suggests that the thin-tailed sheep population studied is monomorphic in the GH|MspI gene fragment. Figure 2 illustrates the results of electrophoresis using 2.5%

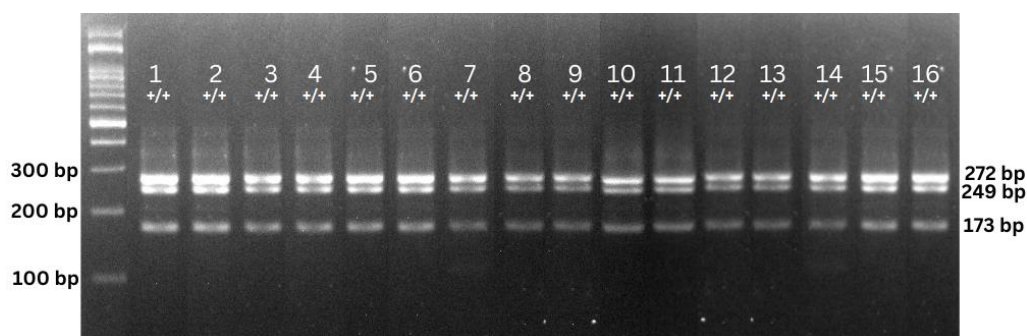


Figure 2. RFLP results of exon 3 GH gene in Thin-tailed sheep

Table 4. Genotyping analysis of the GH|Msp1 gene

Locus	N	Genotype	Genotype frequency	Allele frequency
GH Msp1	60	(+/+)	1	1
		(+/-)	0	
		(-/-)	0	0

agarose and FluorSAFE. Detailed information on genotype and allele frequencies can be found in Table 4.

DISCUSSION

In this study, we investigated the mean body weight and body weight gain of male and female thin-tailed Sheep aged 11 and 12 months. Our results showed that the mean body weight of male Thin-tailed Sheep was 13.65 ± 1.65 kg and 15.38 ± 1.87 kg, while female thin-tailed Sheep was 12.48 ± 1.87 kg and 13.65 ± 2.00 kg, respectively. These values were lower than the body weights reported in some other local sheep Wonosobo sheep aged 6-12 months for males at 40.62 ± 7.55 kg and females at 35.72 ± 3.58 kg [12]. Local Lamongan sheep aged 6-12 months were as large as males, 16.25 and females, 17.53 ± 5.19 [13]. Kisar sheep aged 6 months-12 months were 17.09 ± 4.68 kg [14], Garut sheep aged 6-12 months was 29.32 ± 3.61 [2], Dorper Suffolk (DS) composite sheep aged 10-12 months was 27 kg [15]. Differences in body weight of thin-tailed sheep are thought to be influenced by genetics and the environment. This follows the opinion of Depison *et al.* [4], stating that differences in sheep body weight can be caused by genetic (breed), management, and environmental factors. These findings provide valuable information on the GH gene in thin-tailed sheep and may contribute to future genetic research in this species.

The average body weight gain of male and female thin-tailed sheep aged 11-12 months was 57.7 ± 7.9 g and 39 ± 11.2 g, respectively. While these results are lower compared to some other local sheep breeds, such as Garut sheep (81.17 ± 24.88 g) [16], Lamongan local sheep (150g) [17], male Dorper sheep (47.2g) and female Dorper sheep (30.6g) [18], it's important to note that the body weight gain can be influenced by various factors such as sex, rearing system, genetics, and environment. It's believed that the differences in body weight gain observed in thin-tailed sheep could be attributed to genetic factors, management practices, and environmental conditions [19]. So, it's crucial to carefully manage these factors to ensure optimal growth and weight gain in thin-tailed sheep.

According to the mean difference test analysis, the average body weight and body weight gain of male thin-tailed sheep aged 11-12 months were significantly higher ($P < 0.05$) than that of their female counterparts. This finding is consistent with the study by Depison *et al.* [4], which revealed a significant difference ($P < 0.05$) in the average body weight between male and female Jambi thin-tailed sheep. This is possibly due to rams having a higher feed consumption capacity compared to ewes [20]. Furthermore, male livestock produce testosterone, an androgen steroid hormone that regulates growth and is produced by interstitial cells and adrenal

glands. This hormone can increase the metabolic rate in the body, resulting in faster growth compared to female livestock [21]. These factors may explain the observed differences in body weight and weight gain between male and female thin-tailed sheep. Therefore, it's essential to consider these factors when managing thin-tailed sheep to ensure optimal growth and weight gain.

The findings of this study revealed that male thin-tailed sheep aged 11 months had a larger body size compared to their female counterparts. This result is consistent with the study conducted by Depison *et al.* [4], which found that male Jambi thin-tailed sheep had a significantly greater body size compared to females. Similarly, male Wonosobo sheep, Purwakarta local sheep, and local Lamongan sheep were also reported to have a greater body size than their female counterparts [12,13,22]. These findings suggest that sexual dimorphism is a common characteristic in sheep breeds, where males tend to have a larger body size compared to females. Understanding the differences in body size between male and female sheep can help farmers and breeders manage their flocks more effectively, and ensure optimal growth and productivity. Therefore, it's crucial to consider these factors when selecting breeding stock and developing breeding programs for sheep.

The observed difference in body size between male and female thin-tailed sheep can be attributed to the larger body frame of males compared to females. This difference may be influenced by genetic factors, sex, and hormones. Ananda *et al.* [21] suggested that increasing the size of male livestock results in androgen hormones that stimulate growth. These androgen hormones can activate the production of anabolic proteins, leading to increased growth in male livestock and resulting in larger body size compared to female livestock [22]. Understanding the factors that influence body size differences between male and female sheep is essential for developing effective breeding programs and improving livestock management practices. By considering genetic, hormonal, and sex-related factors, farmers and breeders can optimize the growth and productivity of their flocks.

Ultimately, this can lead to improved profitability and sustainability in the sheep farming industry. To identify the body size and shape characteristics in livestock, principal component analysis was employed [23].

The T2-hotelling analysis conducted in this study clearly demonstrated that male thin-tailed sheep have significantly ($P < 0.01$) larger body measurements compared to their female counterparts. Livestock body sizes can be influenced by various factors such as genetics, origin, management, and mating systems employed [24]. Interestingly, the analysis revealed that the chest circumference (ChC) was the most significant contributor to body size equations in both male and female thin-tailed sheep. Thus, ChC could be used as an indicator of body size, as suggested by Rohayati and Herawati [25], who emphasized the importance of chest circumference, body length, and withers height as key characteristics of sheep body size, commonly used to determine the quality of Garut sheep breeds. Selecting rams with higher chest circumference could potentially increase their body size scores [26]. Moreover, linear body measurements such as body length, chest circumference, shoulder height, and hip height could be used to estimate livestock body weight, particularly in areas or locations where scales are not available to measure body weight [27]. Understanding the significant body measurements that contribute to body size equations can facilitate selective breeding programs and livestock management practices. By selecting rams with higher chest circumference and incorporating linear body measurements in the estimation of body weight, farmers and breeders can optimize their flock's growth and productivity. This can result in increased profitability and sustainability in the sheep farming industry.

According to the findings of this study, the most significant factor that determines the body shape of male and female thin-tailed sheep is the chest depth (ChD). This indicates that ChD is an essential identifier of body shape, as it contributes the most to the shape equation. These results align with the research of Wattimena *et al.* [14], which highlights chest circumference, chest width, chest depth, body

length, and shoulder height as the quantitative characteristics of Kisar sheep that can be used to standardize the quality of lamb breeds. Furthermore, a wider chest depth indicates that the respiratory organs and heart are developing well, which supports the formation of anaerobic energy that is essential for the body's functions [15]. Therefore, chest depth can serve as a crucial parameter in breeding programs for improving the body shape of thin-tailed sheep. Additionally, these findings provide a valuable alternative for estimating the body weight of livestock in areas where scales are unavailable for measurement, as linear body measurements such as chest depth, body length, shoulder height, and hip height can be used as an accurate predictor of body weight [27].

The process of PCR amplification is a crucial step in genetic research, and various factors can influence its accuracy, including annealing temperature, DNA concentration, primer concentration, and magnesium chloride [28]. In this study, we found that the GH|Msp1 gene in the thin-tailed sheep population was monomorphic, with an allele frequency of 1.00. As described by previous research [11], a gene is considered polymorphic if its allele frequencies are less than 0.99. Interestingly, these results are similar to a study by [29], which showed that the GH gene in Palu sheep was also monomorphic. However, other studies at the same gene locus have shown polymorphism, such as Depison *et al.* [3] research on thin-tailed sheep in Jambi province. Their findings showed non-uniform (polymorphic) conditions in both highlands and lowlands, with a higher (+/+) genotype frequency compared to (+/-) and (-/-) genotype frequencies. These differences in gene frequencies can result from various factors, such as selection, mutation, population mixing, inbreeding, outbreeding, and genetic drift [30].

The Kerinci thin-tailed sheep population's uniform condition is believed to be a result of high inbreeding. Unfortunately, one of the issues with breeders is that they only keep a few males in each cage, leading to each female having the opportunity to mate with the same male. The sheep are raised in a semi-intensive manner where they roam freely during the day and return to their pens

in the afternoon. Thin-tail Sheep to mate in an undirected manner without any records kept, ultimately causing the population to become highly inbreeding. Recording is essential for sustainable breeding activities. Through recording, breeders can gather information about their livestock, which will be useful for managing and breeding activities [29].

CONCLUSION

The study results indicate that the average body weight, weight gain, and body measurements of male Thin-tailed sheep were higher than those of females. The body size characteristic of male and female Thin-tailed sheep is chest circumference, while the body shape characteristic of male and female Thin-tailed sheep is chest depth. The fragmentation of the GH|Msp1 gene in Thin-tailed sheep is monomorphic, with only one genotype, namely ++.

CONFLICT OF INTEREST

The author of this manuscript confirms that they have no conflict of interest with any party or organization in relation to the material discussed.

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