Identification of Pit-1 Gen Using PCR-RFLP of Padjadjaran Sheep and Evaluate of Growth Rate

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Abstract

The objectives of this research were to evaluate variation of Pit-1 gen of Padjadjaran Sheep and evaluate of their growth rate. The data comprised of 15 lambs female and 15 lambs male records of 0 – 12 mouths old lambs. Variation of Padjadjaran Sheep PIT-1 gen was analyzed using PCR-RFLP and used primer by 5'-GAGGGATAATTACAAATGGTCC-3' and 5'-TGTTAACAGCTGTGGGACACAC-3', length fragment analyse using HinfI restriction enzyme. Presence or absence of deference bands in a PCR fragment of the result can be distinguished by differences in the electrophoretic migration of the fragment. The result showed that the variation Pit1 gene showed that there was in the position 345 bp, 137 bp and 115 bp. In this experiment the difference migration did occur in all samples (monomorphic), that was amplificated with reverse and forward primer. The average male birth weight (BW), weaning weight (WN) and Yearling Weight (YW) was 2.29 ±0.42kg ; 8.96 ±1,89 kg and 30.12 ±5,65 kg. The average female birth weight (BW), weaning weight (WN) and Yearling Weight (YW) was 2.33 ±0.49 kg ; 8.23 ±1.99 kg and 26.11 ±5.50 kg. Correlation value between age and body weight was 0,99 for female and 0,97 for male. The highest body weight gain per month was 2.85 kg for female at the age of six months and 3.4 kg for male at the age of one year. The best equition of growth rate was $\hat{Y}$= 1,862 + 0,127X_1 0,076X_2

1. Introduction

Padjadjaran sheep is one of the local sheep, which were developed by researcher with the objective being to rapid growth as a source of meat. Characteristics of the Padjadjaran sheep have white fur, have big earlobe and a horned for male. Padjadjaran Sheep has variation sheep *mt*-DNA of deletion along 75 *bp* at 1447 *bp* position on the d-loop of *mt*-Chromosome. However, PIT-1 is one of the genes that are the family of growth genes in addition to IGF1, GH and GHRH. Although the criteria of performance body weight, body height and body length are variable that needs to be observed in the evaluation of livestock growth.

Growth is weight gain or body measurement gain until they reach adult size or per additional body mass per unit time. Specific growth occurs in young animals which is formed by the large bone, protein, and water networks. Growing character is important for a sheep breeding business whose final goal is meat production.

The study of growth can not be separated by the processes of various fields of physiology, biochemistry, genetic endocrinology and nutrition [1]. Growth starts from the front of the animal (anteriorly) to the back (posterior).

The appearance of a livestock is the result of a continuous growth process, throughout the life of the livestock. Body components have different growth rates [2]. Meanwhile, the size of the body surface and body parts have the utility to estimate body weight and carcass, as an image of the body shape of a livestock according to the character of his breed.

Shoulder height is usually used by researchers to know that a livestock grows perfectly, while the body length used to measure the growth of livestock can also be used as a quality guide of carcass.

Body weight of a livestock at a certain age can serve as a guide for growth in ages (0-12 months), and can also be correlated to form a growth curve. The growth rate is expressed as the weight gain or the length increase of a body part per unit time to adulthood.

Genomic molecular observation can be used as a tool to conduct selection by Marker Assistant Selection method (MAS), because it is expected to associate between gene marker with quantitative trait locus (QTL),

Genetic diversity is important in breeding programs for the genetic optimization of the acquisition of certain properties can be achieved when there is enough opportunity for the selection of genes or the desired properties.

Beside that, genetic diversity plays an important role in the survival of the population. The loss of genetic diversity can reduce the chance of survival of the population.

Through a complicated on differentiation phase of the anterior pituitary and the regulation of prolactin gene (PRL), growth hormone (Growth hormone-GH) and thyroid-stimulating hormone-β (TSH-β), then Gen sheep PIT-1 is recommended as a candidate gene to the nature of the production.

Somatic cells residing on white blood cells can be used as a source for the DNA analysis. In the microsatellite study to obtain superior genes for the purification of Padjadjaran sheep, so that the origin of the nation can be standardized based on Polymerase Chain Reaction (PCR) and PCR product was cut with restriction enzyme Hinf-1 and electrophoresed on agarose gel an stained with ethidium bromide (Etbr). The result showed that there were bandings in the difference position as Restriction Fragment Length Polymorphism (RFLP)

Therefore, based on the availability of the genetic resources, it is essential to form seeds of Padjadjaran sheep, which have criteria in accordance with the will of the breeder, for purposes of standardization and certification. The purpose of this study was to determine the variation of PIT-1 gene in blood samples and to take out Marker Assistant Selection in Padjadjaran sheep base on growth rate.

2. Materials and Methods

2.1. Material and Methods description:

2.1.1. Blood sampling as many as 30 samples, grouped into group female lamb and 15 male lamb.
2.1.2. Pre-treatment sample storage (using vacuum-tainer containing EDTA anticoagulant).
2.1.3. Isolation of DNA samples using the Genomic DNA Purification Kit (fermentage*)* [3]
2.1.4. PCR analysis for each of the isolated DNA with four pairs of primers using Tag Dream Green PCR Master Mix.

2.2. Electrophoresis of PCR Product

PCR product was cut with restriction enzyme Hinf1 and electrophoresis purification used 1.5% agarose gel stained with ethidium bromide (Etbr) staining to see the pattern of band microsatellite, that was amplificated by primer.

2.3. Hardy Weinberg equilibrium

H-W equilibrium was calculated by Chi-quadrat [4]

$$X^2 = \sum \frac{(Oi-Ei)}{Ei}$$

Where:
X^2 = chi square value
O_i = value of observation
E_i = value of expectation

2.4. Growth rate evaluation

Evaluation of growth rate can be done by using correlation and regression

3. Results and Discussion

At the holding cell isolation method optimization with the Genomic DNA Purification Kit (fermentage), used as a comparison sample of whole blood and uppers (serum). It was found that isolation by using whole blood (mixture) at stage 3 there will be no separate phase 3 well (the upper aqueous phase), the middle (cell debris), and the lower (organic phase) as in isolation by using serum. solation using whole blood at stage 3 there will be only two phases, namely solid phase (gel-like) on the top and the organic phase. Because it was later used as an isolated sample is part of the sheep blood serum. Furthermore, by using the PR1 (primer 5'-GAGGGATAATTACAAATGGTCC-3' and 5'-TGTTAACAGCTGTGGGA CACAC-3').

The result showed that Fragment Length of PIT-1 gene was uniform on 345 *bp*, 137 *bp* and 115 *bp* after restricted by Hinft1 Enzyme. According to [5] that the PIT1 gene hasn't heterozygote allele in the population of Padjadjaran Sheep. The PIT-1 gene was not associated with body weight on difference age. It belongs to the gene group to encode the proteins involved with growth. Specific pituitary transcription in code by factor PIT-1 Code by PIT-1's gen [6].

The condition of data during the study can be said uniform because Variation Coefficient is still below 15%, however as it is known that growth will be grouped in a phase acceleration (self-accelerating phase) and then subsequently followed by a slow phase (self-inhibiting phase).

The point where the highest weight gain is the sixth month (2.83 kg), while the height increase shoulder also in the sixth month is 3.13 Cm and the longest increase is also in the sixth month is 2.47 Cm, usually at that time the animal enter puberty phase.

According to former researcher that growth is divided in every quarter, the first quarter of growth is 50 percent, in the second quarter it reaches 25 percent, while in the third quarter the remaining 25 percent . Sheep are said to be adults when they are 18 months old where the growth period is 100 percent and growth for up to 12 months is still included in the 75 percent growth period [3].

Table 1. Result of Electrophoresis After Cutting by Restricted Enzyme Hinf1

No.	Region	Sample Code	Fragment Length as Restriction Result (*bp*)				
			62	115	137	283	345
1	Sempur	Blood sample ♂		√	√		√
2		Blood sample ♂		√	√		√
3		Blood sample ♂		√	√		√
4		Blood sample ♂		√	√		√
5		Blood sample ♂		√	√		√
6		Blood sample ♀		√	√		√
7		Blood sample ♂		√	√		√
8		Blood sample ♂		√	√		√
9		Blood sample ♂		√	√		√
10		Blood sample ♂		√	√		√
11		Blood sample ♂		√	√		√
12		Blood sample ♂		√	√		√
13		Blood sample ♂		√	√		√
14		Blood sample ♂		√	√		√
15		Blood sample ♂		√	√		√
16		Blood sample ♀		√	√		√
17		Blood sample ♀		√	√		√
18		Blood sample ♀		√	√		√
19		Blood sample ♀		√	√		√
20		Blood sample ♀		√	√		√
21		Blood sample ♀		√	√		√
22		Blood sample ♀		√	√		√
23		Blood sample ♀		√	√		√
24		Blood sample ♀		√	√		√
25		Blood sample ♀		√	√		√
26		Blood sample ♀		√	√		√
27		Blood sample ♀		√	√		√
28		Blood sample ♀		√	√		√
29		Blood sample ♀		√	√		√
30		Blood sample ♀		√	√		√

Table 2. Structure Data of Average Body Weight, Shoulder Height and Body Length

N	Age (month)	Average of Body Weight (kg)		Average of body Length (cm)		Average Height of Should (cm)	
		Female	Male	Female	Male	Female	Male
30	0	2.33	2.29				
30	1	5.19	6.67	33.47	39.07	37.53	39.07
30	2	7.06	8.19	41.43	43.33	41.13	43.33
30	3	8.23	8.96	42.47	44.53	42.47	44.53
30	4	8.71	9.80	44.33	45.47	44.33	45.47
30	5	9.83	10.59	46.33	46.27	46.33	46.27
30	6	12.66	12.74	49.47	48.57	49.47	48.57
30	7	14.92	15.51	51.30	50.00	51.30	50.00
30	8	17.10	18.26	52.53	51.53	52.53	51.53
30	9	19.40	20.79	53.47	52.80	53.47	52.80
30	10	21.53	23.65	54.73	54.13	54.73	54.13
30	11	23.65	26.72	55.93	55.20	55.93	55.20
30	12	26.11	30.12	57.53	57.13	57.53	57.13

4. Conclusion

Variation of reverse and forward primers, showed that migration is relatively the same for all the samples and estimated (based on comparison with a marker) that is the size of the fragments between 115 *bp*, 137 *bp* and 345 *bp*. PIT-1 gene can not be used as marker for Marker Assistant Selection (MAS). Pit1 gen has more functions as a coupling candidate (binding factor) in the process of transcription of protein biosynthetic from pituitary, formation of Growth Hormone, GHRH and IGF1.
The highest body weight gain per month was 2.85 kg at the age of six month for female and 3.4 kg for male at the age of one year. The best eqution of growth rate was $\hat{Y}$= 1,862 + 0,127X_1 0,076X_2
The ram that is prepared for fattening can start from the age of 1 (one) year, and for the ewe at the age of 18 months can be mated to ready to become dam.

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