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Diversity of Quantitative Characteristics and Growth Hormone Gene of the Local Thin-tailed Sheep in Some Districts at Jambi Province

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Abstract
This study aims to determine the diversity of quantitative characteristics and growth hormone gene of thin-tailed sheep (DET) in several districts/cities in Jambi province. There are two stages of this researchis, the field and laboratory. Data collected in the field was characteristic of quantitative: wither height (WH), body length (BL), chest grid (ChG), chest deep (ChD), the width of the chest (ChW), body weight (BW), Body weight gain (BWG) and blood samples. The total number of samples of 160 tail aged 1-2 years (I1 = a pair of permanent teeth). Research in the laboratory include : DNA isolation, amplification and gelpurification using *Mspl* restriction enzyme by PCR-RFLP.
Data analysis includes, frequency of genes, heterozygosity values, Informative Polymorphic Content and the balance of GH gene genotype. Differences in quantitative characteristics between districs/cities among Genotypes GH gene fragment *MspI* and *AluI* was analyzed using t-test
Analysis of the data include : the frequency of the gene, the value of heterozygosis, Informative Polymorphic Content (PIC) and the balance of GH gene genotype. Quantitative characteristic differences among districts / cities as well as the quantitative characteristic differences between genotypes Fragments *MspI* and AleI GH gene were analyzed using t-test.
The present study concluded that : 1) there is no differences in quantitative characteristics both female male between Sungai Penuh City and Kerinci , as well as between Muaro Juambi dan Batanghari District 2) there is polymorphism of GH gene on the TTS highlands and lowlands in Jambi Province. 3) quantitative characteristics of TTS in the highlands and lowlands associated with genotype frequency of gene GH MspI and AluI.
The conclusion of this study were 1) Quantitative characteristics both male and female between Kerinci with Sungai Penuh City is no different, between Muara Jambi and Batanghari District no different but between Kerinci and Sungai Penuh City and Muara Jambi and Batanghari is different; 2) The marker of -RFLP *MspI* and *AluI* showed there is polymorphism of GH gen of thin-tailed sheep in all selected lodation; 3) Characteristics of quantitative of thin-tailed sheep in all districts/cities associated with genotype frequencies of GH gene *MspI* and *AluI*.
Keywords: thin-tailed sheep, phenotype diversity, growth hormone gene (GH), PCR-RFLP.

1. Introduction

The spread fairly evenly thin tail sheep in the Province of Jambi ranging from lowlands to highlands, so that the development potential because it is quite adaptive to various environmental conditions. High demand for meat is not followed by an increase in population. This leads to a gap between production and demand for meat. during the period 2010 - 2014, the increase in population of only 3.84% per year, while demand (slaughter) increased by an average of 6.93% per year. This conditions would lead to decreasing in population of thin-tailed sheep in Jambi Province. it will be heading for extinction. Approximately the original animal world's estimated 30% have been categorized to extinction.

One effort in order to preserve thin-tailed sheep that is necessary to find the data base through quantitative characterization of the characters that have economic value. However, quantitative characterization of the characters are generally less effective because it requires the number of animals a lot and a long time. Advances in science and technology in the rapidly growing field of molecular genetics complete genome of sheep , so as to characterize the genetic diversity quickly and cheaply

Characterization of genetic diversity associated that the production traits that related to economic traits such as growth can be done through in-depth analysis on the structural genes or other parts which are crucial for the growth of livestock such as GH gene. Evaluation of GH gene polymorphism need to be done to encourage the selection of the growth traits, especially associated with the quantitative characteristics of the TTS in Province of Jambi. Whether the difference is due to differences in diversity of GH gene or of environmental variation. This is necessary in order to study molecular genetics at present and in the future.

This study aims to: 1) investgate the quantitative characteristics and Polymorphisms of growth hormone (GH) gene in several districts /citiesin Jambi province; 2) investigate the relationship between Polymorphisms GH gene with quantitative characteristics thin-talied sheep in several districts cities in Jambi province.

2. Materials and Methods

The research material was locally thin-tailed sheep (TtS) in the highlands and lowlands in Jambi Province. 160 TTS (age 1-2 years old, I1= a pairs of permanent teeth) that divide into 80 head of male and 80 head of female were observed in Muaro Jambi Regency and Batanghari Regency (Lowland), Kerinci Regency and Sungai Penuh City (highland). The data collected is quantitative characteristics including, withers height (WH), the body length (BL), chest grid (ChG), the chest depth(ChD), chest width (ChW), body weight (BW), body weight gain (BWG) and blood sample were collected in all location. Blood sampling of thin-tailed sheep taken through the jugular vein with no heparin venoject vacuum tubes. Blood samples are then preserved with absolute ethanol. Thereafter, blood samples were added absolute ethanol in the ratio 1: 1 and stored at room temperature. Observed variables associated with DNA analysis on GH's gene fragment of *MspI* and *AluI,* included : (1) the frequency of the gene, (2) GH's gene allele obtained from the analysis of PCR-RFLP MspI and AluI (3) the balance of genes in the population, (4) heterozygosity, (5) the value of Polymorphic Informative Content (PIC), and (6) evaluate the relationship between GH's gene and quantitative characteristics.

Purposive sampling in Survey method was applied on field study, meanwhile detection in laboratory to detect polymorphism of GH gene was performed with PCR_RFLP technique. Laboratory studies include DNA isolation, amplification of GH genes, and Gel Purification. DNA isolation of thin-tailed sheep blood was done by using a protocol Genomic DNA Purification Kit from Promega. DNA that has been isolated subsequently clarified by using two pairs of primers. More details primers use dare presented in Table1.

Table 1. The length and location of GH gene and primers used for PCR analysis

The Position of segmen	Length (bp)	Primer Name	Sekuen (5' 3')	*Annealing Degree*
175-774	599	GHY1-Fwd. GHY1-Rev.	$^{5'}$TTG CAT AAA TGT ATA GAG CAC ACA G$^{3'}$ $^{5'}$CCC CAC CTC TAG GAC ACA TC$^{3'}$	60,3^{0}C
671-1361	690	GHY2-Fwd. GHY2-Rev.	$^{5'}$CTG TTT GCC AAC GCT GTG$^{3'}$ $^{5'}$AAG CCA CGA CTG GAT AAG GA$^{3'}$	60,3^{0}C

Amplification begins with denaturation at 94°C for 2 min, followed by 40 amplification cycles, each cycle is programmed for 30 minutes 94°C denaturation, annealing 62^{0}C for 80 seconds, extension 72°C for 90 seconds, the amplification process ends with a final extension at 72°C temperature for 5 minutes , Amplification results can be viewed by performing electrophoresis with agrose 2%, which is colored with Ethidium Bromide. Furthermore, the bands will be visible on gel formed in each groove of wells containing DNA samples of PCR products. Determination of the size of each fragment of GH formed on agrose gel by comparing the position of band formed by positioning the ladder of DNA bands. DNA is visualized then documented by Gel Documentation system (Biometra-German) and then take the picture and stored on Compact Disc.

Polymorphism detection by PCR-RFLP results of the PCR amplification products obtained were then digested with AluI restriction enzyme cutting sites AG*CT and MspI cutting site C*CGG (Promega). Total volume for the digestion consisting of 50 mL of nuclease free water (ddH2O) 17.5 mL, 25 mL PCR product, the enzyme 5 mL buffer, enzyme MspI or AluI 2.5 mL. This mixture is then incubated for approximately 12 hours, then migrated on Agarose gel 2% by Ethidium Bromide. Furthermore, in electrophoresis using electrophoresis with Thermo Scientific models A5, power supply EV 231 Consort, USA, made a 100 Volt, 74 mA for 2 hours. Further examined by Gel documentation system (Biometra-German) and then in the photo and stored on Compact Disc. Frequency Genotype PCR-RFLP expected from the combination of various alleles generated based on the presence or absence of one or more cut sitesin a way if the tape obtained not cut marked (- /-), if cut of fall (+ /+) and if there are all well clipped and truncated (+/-) (Kumari etal., 2014) (https://www.ncbi.nlm.nih.gov/ probe/docs/techrflp/, 2015).

Genotype frequencyis calculated based on the number of alleles of a genotype divided by the number of samples. Allele frequency is calculated by summing all the alleles divided by 2N. GH gene allele frequencies derived from the analysis of PCR-RFLP MspI identifieris calculated using the formula (Nei, 1987). Genetic diversity (genetic variability) frequency estimationis was applied through observed heterozygosity (Ho), heterozygosity expectations (Hi) and standard error (Nei, 1987). An allele informative level is calculated using the value of Polymorphic Informative Content (PIC). Hardy-Weinberg equilibrium was tested with Chi-square (X2). Differences between the quantitative characteristics of the Regency / City as well as the quantitative characteristic differences between genotypes GH gene MspI fragment was analyzed using t-test. Vector average value characteristics were analyzed by quantitative DET-Hotelling T2 test (Gaspersz, 2006). When the T2-Hotelling test results showed significant ($P < 0.05$), then the data processing followed by Principal Component Analysis (AKU) (Gaspersz, 2006).

3. Results and Discussion

3.1. Phenotype characteristics between District

DET average quantitative characteristics of males and females aged I1 in several districts / cities in Jambi province are presented in Table 2. Based on Table 2.

Characteristics of quantitative (BW, BWG, BL, WH, ChG, ChD and ChW) of thin-tailed sheep both males and females in sequence from highest to lowest are Kerinci district of > Sungai Penuh City > Muaro Jambi > Batang Hari District. Results of the analysis showed that the average difference test DET quantitative characteristics of males and females in Kerinci district had no significant (P>0.05) with Sungai Penuh City. Quantitative characteristics of thin-talied sheep in Kerinci district and Sungai Penuh City significantly different (P <0.05) with at Muaro Jambi and Batang Hari regency. Characteristics of the BW,BWG, WH, ChG and ChD of thin-tailed sheep in Jambi Muaro not significant (P>0.05) with Batanghari District, but the BL and ChW in Muaro Jambi significantly different (P <0.05) with Batanghari District,This condition indicates that the quantitative characteristics in Kerinci district and Sungai Penuh City better than Muaro Jambi and Batanghari.

The difference is presumably because of differences in the environment of maintenance where Kerinci and Sungai Penuh City is highland area with a height of ≥1500m above sea level (asl), while Muaro Jambi and Batang Hari is a lowland area with an altitude of 100-500m above sea level. According to Calderon, etal. (2005) states that there is a significant difference between the performance of livestock production in the low lands (hot area) with up land (cold regions). Differences in productivity is closely related to temperature and humidity factors. There is interaction between temperature and humidity or "Temperature Humidity Index" (THI).

Table 2. Mean Characteristics of thin-tailed sheep both Male and Female in Some District / Municipality in Jambi Province

District/ City	Quantitative Characterictics						
	Bw	BWG	BL	WH	ChG	ChD	ChW
Kerinci							
- Male (kg)	20,45±2,54^{a}	76,44±11,34^{a}	56,98±2,55^{a}	53,72±2,75^{a}	62,88±2,28^{a}	24,76±2,58^{a}	14,94±2,46^{a}
- Female (kg)	18,17±2,68^{b}	52,44±14,70^{b}	54,99±2,76^{b}	52,84±2,76^{b}	61,77±2,72^{b}	22,04±2,62^{b}	14,80±2,37^{b}
Sungai Penuh							
- Male (kg)	20,03±2,35^{a}	74,89±14,85^{a}	56,62±2,61^{a}	53,11±1,98^{a}	62,25±2,05^{a}	24,13±2,51^{a}	14,86±2,46^{a}
- Female (kg)	17,98±2,66^{b}	50,11±17,86^{b}	54,02±2,57^{b}	52,30±2,78^{b}	60,93±2,36^{b}	21,73±2,14^{b}	14,51±2.54^{b}
Muaro Jambi							
- Male (kg)	18,69±2,80^{c}	61,44±10.12^{c}	54,44±2,89^{c}	50,48±2,90^{c}	59,32±1,89^{c}	21,06±2,50^{c}	12,81±1,57^{c}
- Female (kg)	16,04±3,93^{d}	36,67±13,53^{d}	51,81±2,74^{d}	49,62±2,76^{d}	58,67±2,05^{d}	19,23±2,41^{d}	12,01±2,05^{d}
Batanghari							
- Male (kg)	18,69±3,35^{c}	60,33±21,96^{c}	52,07±2,66 e	52,02±2,78^{c}	59,27±1,99^{c}	20,06±2,09^{c}	11,40±2,79^{e}
- Female (kg)	15,98±3,46^{d}	36,33±17,82^{d}	50,16±2,45^{f}	49,74±2,20^{d}	58,27±1,74^{d}	18,97±1,82^{d}	10,65±1,81^{f}

Description: different letters in the same column different rows show Significant Difference (P <0.05)

3.2.Hotelling'sT^2-analysis and Principal Component (AKU)

Hotelling'sT^2-analysis showed chest grid (ChG) performed the highest eigen vectors obtained in the equation DET size of males and females in all four districts /cities. That is the ChG is a characteristic measure because it has the largest contribution to the measurement equation. Research results together with the results Amirudin (2008) which states that the primary identifier variables local sheep body size is the circumference of the chest. Furthermore, according to Gunawan et. al. (2011) that in general identifier size is positively correlated with scores that measure chest grid (ChG) in all types of sheep.

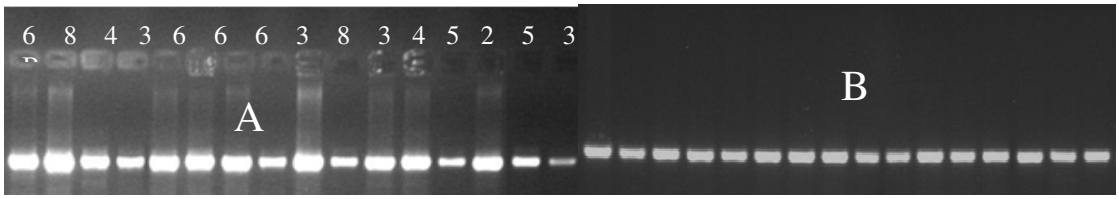
The highest eigen vectors obtained in equation form thin-tail sheep breeding both males and females in all four districts /cities is the chest width (ChW) due to it has the largest contribution to the equation form.

The correlation between the scores and the size of chest circumference was found to be between +0.7639 to+ 0.9308. The positive sign indicates that the increase insize of chest circumference will increase the score of the size of the body or vice versa. While the correlation between the score of the shape and width of the chest was found to be between +0.0910 to +0.1617. This correlation value is the highest value of correlation between the value of the correlation between the scores and the variable linear shape of the body surface were observed. This indicates that the increase in the size of the chest width (ChW) will increase the body form score or otherwise.

3.3. DNA isolation, amplification and analysis of PCR-RFLP MspI Identifier

Isolation of genomic DNA of blood samples of 160 thin-tailed sheep in four districts / cities using genomic DNA Purification Kit (Promega-USA) produce moderate quality and quantity. DNA isolation results obtained were compared with standard DNA (λ DNA 50ng/mL). The results obtaine dare presented in Figure1a

and 1b



Description: B = basic form, the numbers 2, 3, 4 and thereafter = number of times dilution

Figure 1. A = results of DNA isolation using Genomic DNA Purification Kit from Promega. B = The results of electrophoresis of DNA concentration equalization

Figure 1a. Showed that the concentration of DNA derived from the thin-tailed sheep in the Fourth District /cities in Jambi Province. Figure 1b. Pictures dilution to equalize the concentration. High and low concentrations of DNA produced is highly dependent on the ability of lysis of the cell nucleus. This is consistent with the statement Yurnalis et al.(2013) which states that ifa cell nucleus can bein either the lysis with the resulting DNA concentration is high enough and the quality of the DNA will be good or otherwise. Results PCR Primer GHY1 and GHY2 to amplify DNA is presented in Figure 2a and 2b.

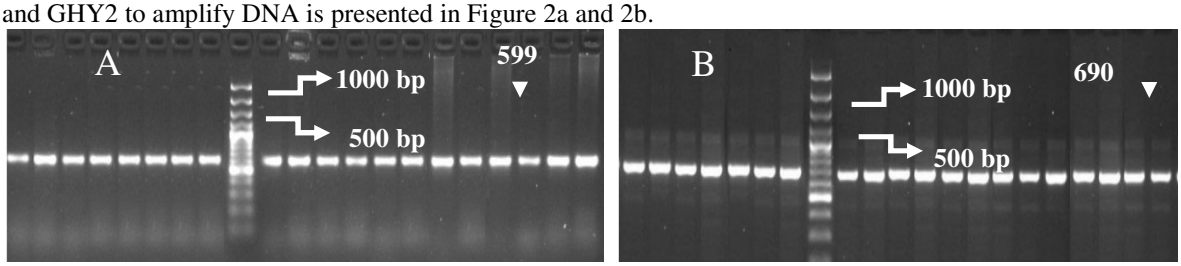


Figure 2.A Primer PCR results GHY=1, B = Results PCR Primer GHY 2

Based on Figure 2a, and 2b results of PCR were performed to amplify DNA using primers GHY 1 and GHY 2 sequentially generate fragment 599bp and 690bp. Results of PCR amplification products were as expected. According Rahayu et. al. (2006) Primer is an essential part of the primer PCR as an initiatorin the synthesis of target DNA, in addition to the PCR results were favorably affected by several factors such as purity DNA extraction result, the accuracy of the primaries are used, as well as the accuracy of PCR conditions. This condition indicates that the PCR reaction conditions and primers used through design with primer 3 program is quite good, because it gives a very specific PCR products as expected.

Results of electrophoresis of PCR-RFLP product growth hormone gene of thin-tailed sheep in the Fourth District / City using restiction enzyme *MspI* primer GHY 1, and GHY 2presented in Figure 3a and 3b.

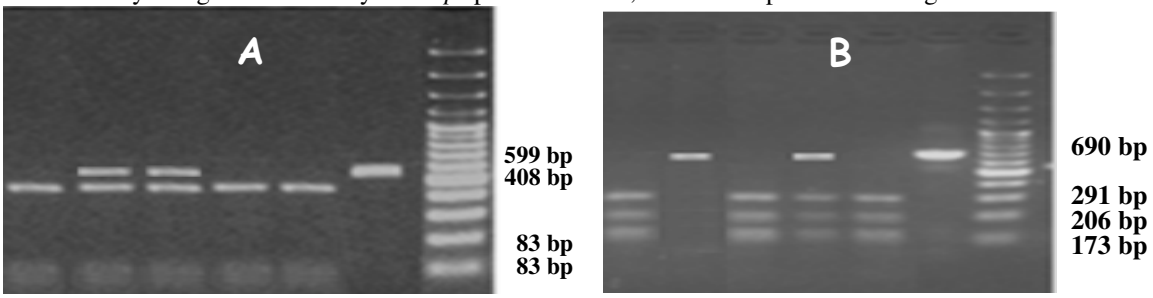


Figure 3. A = results of electrophoresis of PCR-RFLP product growth hormone gene using restriction enzyme *MspI* at Primary GHY 1, B = results of electrophoresis of PCR-RFLP product growth hormone gene using restriction enzyme *MspI* at Primary GHY 2.

Amplification of GH gene for marker of PCR-RFLP *MspI* resulted restricted point C*CGG in the fourth Regency / City in male and female animals there is no difference. Primary GHY 1with a length of 599bp after restricted obtained point three DNA fragments cut point is at 408bp, 83bp and 83bp. Number of bands that should be obtained is four is 408bp, 83bp, 83bp and 25bp. Primary GHY 2with a length of 690bp, obtained three cut point that is 291bp DNA fragment, 205bp and 173bp. Number of bands that should be obtained four is 21bp, 291bp, 173bp and 205bp. Characterization by marker of PCR-RFLP *Msp*I at GHY Primer1in the fourth District / Municipality in Jambi province both in male and female animals are not found 25bp cut points, the Primary GHY2 not found a point 21bp cut. This is presumably because the PCR-RFLP MspI not recognize the restrited sites or because the distance is too small or short.

3.4.Frequency of genotype, allele and Hardy-Weinberg Equilibrium

Polymorphism or genetic diversity can be determined based on the analysis of the frequency of allele and

genotype frequencies (Mariana, 2011). Genotypes were found in studies of thin-tailed sheep for GH gene *MspI* in the fourth District / Cities visualized in Figure4.

Genotype frequencies of GH gene *MspI* of thin-tailed sheep obtained in four district / city are presented in Table 3.

The results showed that the thin-tailed sheep in the four districts / cities are polymorphic. According to the Nei and Kumar (2000) that an alleleis said to be polymorphic if one alleleis less than 99%. Diversity can be indicated by the presence of two alleles in a single population or more. The results also show the population allele frequency of thin-tailed sheep has a (+) higher than the allele (-). This result does not vary much with the results Kumari et al. Research (2014) against 9 nations of sheep in India who obtain the A allele frequency (+) higher than the frequency of allele B (-).

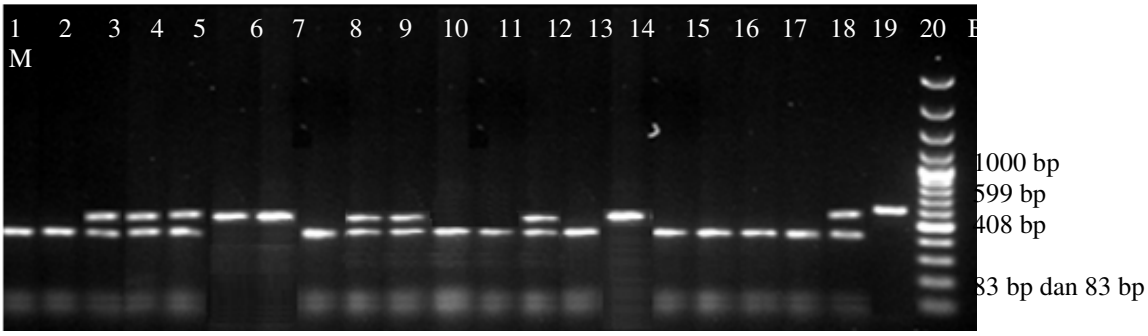


Figure 4 Genotype cutting results GH gene fragment PCR products with the enzyme MspI primer GHY 1 Description: M= Ladder 100bp, B = Blank / control without cut, 1= Individuals homozygote +/+, 2= Individuals homozygote -/- 3= Individual +/- heterozigote

Testing of the Hardy-Weinberg equilibrium law on thin-tailed sheep population in the highlands and lowlands on the GH gene PCR-RFLP MspI and AluI performed using chi-square test. GH gene MspI allele frequency and AluI on thin-tailed sheep in the highlands and lowlands in Hardy-Weinberg imbalance ($P < 0.01$). The condition describes that based on Hardy-Weinberg law, there is the population imbalance where the frequency of genes and genotypes are not fixed from generation to generation. Population imbalance is also possible due to uncontrolled mating system so that there are chances of selection. According Ulupi et al., (2014) imbalance genotype frequencies or allele frequencies in a population occur due to accumulation genotypes, divided population, mutation, selection, migration and mating in the same group (endogamy)

Table 3. Frequency of GH gene MspI genotype DET males and females in four districts / municipalities in the province of Jambi.

Location/ District	Total of Sampel	Genotipe	Genotype frequencies	Allele Frequency
Muaro Jambi	80	**+/+**	40 (0,5000)	+/+ = 0,6500
		+/-	24 (0,3000)	-/- = 0,3500
		-/-	16 (0,2000)	
Batanghari	80	**+/+**	46 (0,5700)	+/+ = 0,7000
		+/-	20 (0,2500)	-/- = 0,3000
		-/-	14 (0,1750)	
Sungai Penuh	80	**+/+**	44 (0,5500)	+/+ = 0,6875
		+/-	22 (0,2750)	-/- = 0,3125
		-/-	14 (0,1750)	
Kerinci	80	**+/+**	42 (0,5250)	+/+ = 0,6625
		+/-	22 (0,2750)	-/- = 0,3375
		-/-	16 (0,2000)	

3.5.Value Estimation of heterozygosis and PIC (Polymorphic InformationContent)

Heterozygosity values is one of the parameters used to measure the level of genetic diversity in a population (Ahmed et al., 2014). Result of heterozygosity estimation with PCR-RFLP marker of MspI and AluI

at all study sites showed that the value of expected heterozygosity (He) higher than observed values (Ho). More details can be seen in Table 4. According to Machado et al. (2003), if the Ho value is lower than the He value indicates a degree of endogamy (mating within the group). Furthermore, Javanmard et al. (2005) found heterozygosity with a value below 50% indicates the low variation of a gene in the population.

PIC value of thin-tailed sheep for GH gene *MspI* fragment in Muaro Jambi, Batang Hari District, Kota sungai Penuh and sequentially Kerinci district is 0.4032; 0.3759; 0.3835 and 0.3972. Based on PIC values obtained can be stated that the marker of PCR-RLFP GH gene *MspI* fragment in all four districts / cities included in the medium category (Moderate), so that these values can be expressed quite informative as GH gene fragment identifier for *MspI*. PIC values can be used as a basis in determining whether an identifier informative and determine whether there is a polymorphic allele in addition based on the value of heterozygosis. Furthermore, Puja et al., (2013) stated that the PIC is high enough to indicate that the sample population is very heterogeneous and indicated little going selection for certain characteristics while the value of PIC little to indicate that the sample population is very homogeneous and indicated their selection for certain characteristics.

3.6.The Relationship Between Polymorphisms of GH Genes in Quantitative Characteristics.

Results of test analysis between genotype (+ /+, +/- and -/-) for quantitative characteristics (BW, BWG, WH, BL, ChG, ChD and ChW of thin-tailed sheep) in the fourth District / City have the same pattern that is significantly different ($P < 0.05$). The results obtained indicate that there is a relationship between genotype with BW, BWG, WH, BL, ChG, ChD and ChW of thin-tailed sheep. This is consistent with the statement of Hua et al., (2009). That diversity haploit Hae III genes on Boers GH effect on birth weight, weaning weight, body weight gain per day before weaning weight and age of 11 months.Hajihussein et al. (2013) stated that in sheep , genotype frequencies have a relationship with the characteristics of livestock. Furthermore, according to Alakili et al. (2012) in goats genotype frequencies can be used as a molecular marker genes growth properties. These results indicate that PCR-RFLP MspI identifier can be use das a selection to olor genotype frequency diversity can be used as the basis of selection and breeding programs are very useful in order to increase the success of preservation local sheep in Jambi Province.

4. Conclusion

Based on the results of the study can be summarized as follows:

1. There is no differences in quantitative characteristics of both male and female of thin-tailed sheep between Kerinci with Sungai Penuh City, as well as between Muara Jambi and Batanghari district, but the is differences between Kerinci District, Sungai Penuh City and Batanghari, Muaro Jambi district.
2. The Marker of PCR-RFLP *MspI* showed there is gene polymorphism of GH gene of thin-tailed sheep in the fourth District / cities in Jambi Province.
3. Quantitative characteristics of thin-tailed sheep in the fourth District / cities associated with genotype frequencies of GH gene *MspI*.

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