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Association of growth hormone gene polymorphism with quantitative characteristics of thin-tailed sheep using PCR-RFLP in Jambi province

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The objective of this research was to obtain quantitative growth hormone gene polymorphism association between growth hormone gene genotype with quantitative characteristics of the thin-tail sheep in the highlands and lowlands of Jambi Province. Two phases of research were done on the field and in the laboratory. Field research which was conducted to obtain the quantitative characteristics data includes; withers height (WH), body length (BL), chest grid (ChG), chest depth (ChD), chest width (ChW), body weight (BW) and body weight gain (BWG). Laboratory research included: DNA isolation, amplification and gel purification, characterization and identification using PCR-RFLP with the MspI, and AluI. Quantitative characteristics data was collected from 240 heads of thin-tailed sheep. Blood samples were collected from 160 of them and all were between the ages of 1 and 2 years (I1 = pair of permanent teeth). Field research was conducted in the Kerinci District and Sungai Penuh City (Highlands) and Muara Jambi Districts and Batanghari Districts (lowlands). The purposive sampling technique used in this research revealed that: 1) quantitative characteristics (BW, BWG, BL, ChG, ChD, and ChW) of thin-tailed sheep both male and female in highland were better than in the lowlands; 2) studied locus was polymorphic on the highlands and lowlands; 3) the highest of genotype and allele frequencies of both highlands and lowlands were related to the genotype (+/+) and allele (+), respectively; 4) the highest quantitative characteristic (BW BW, BWG, BL, ChG, ChD, and ChW) of thin-tailed sheep was genotype (+/+); and 5) the diversity of GH genes MspI and AluI were associated with BW, BWG, BL, ChG, ChD, and ChW of thin-tailed sheep both on the highlands and lowlands of Jambi Province.

Key words: Characterization, local sheep, thin-tailed sheep, diversity, growth hormone (GH) gene, lowlands, highlands, PCR-RFLP.

INTRODUCTION

Small ruminants, especially native breed types, play an important role in the livelihoods of a considerable part of human population in the tropics from socio-economic aspects. Thus, integrated attempt in terms of management and genetic improvement to enhance production is of crucial importance (Mohammadabadi and Sattayimokhtari, 2013). Economical and biological efficiency of sheep production enterprises generally

improves by increasing productivity and reproductive performance of ewes. There is a fairly even spread of thin-tailed sheep in the Jambi Province ranging from lowlands to highlands. They have development potential because they adapt to various environmental conditions. High demand for meat is not followed by an increase in population, this thus leads to a gap between production and demand for meat between 2010 to 2014. There was population increase of only 3.84% per year, while demand (slaughter) increased by an average of 6.93% per year (Department of Animal Husbandry and Animal Health, Jambi Province, 2014). These conditions led to decrease in population of TTS in Jambi Province and consequently extinction. Approximately 30% of the original animal world is categorized as tending to extinction.

One effort to preserve TTS is to find the database through quantitative characterization of the characters that have economic value. However, quantitative characterization of the characters are generally less effective because it requires a large number of animals, take a long time and recording must be complete. Advances in science and technology fields of molecular genetics and molecular biology with genome sheep from time to time (Crawford et al., 1995) are expected to contribute significantly to the progress and development of animal husbandry in particular breeding program.

Applications of molecular genetics have many important advantages. One such significant advantage is the genotyping of individuals for specific genetic loci (Javanmard et al., 2008; Mousavizadeh et al., 2009). The genes that affect a polygenic trait are not exactly known, however a number of candidate genes with major effects have been recognized. In candidate gene approach to identify genes responsible for variation in a polygenic trait, the process is selection of candidate genes based on the relationship between physiological or biochemical processes involved in the expression of the phenotype then testing the selected genes as putative quantitative trait loci (QTL) (Mousavizadeh et al., 2009). Furthermore, the study of native breeds is necessary for conservation of genetic resource in livestock (Mohammadi et al., 2009) and a species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites. In addition, the ability of a population to respond adaptively to environmental changes depends on its level of genetic variability or diversity (Khodabakhshzadeh et al., 2016a, b).

Characterization of genetic diversity associated that the production traits that is related to economic traits such as growth can be done through in-depth analysis of the structural genes or other parts which are crucial for the growth of livestock such as growth hormone (GH) gene (Di Stasio et al., 2005; Kumari et al., 2014). GH gene has been used as a candidate gene in finding a link between genotype with phenotype in several species, including Bos taurus and Bos indicus (Ge et al., 2003; Beauchemin et al., 2006; Mohammadabadi et al., 2010), goat (Mousavizadeh et al., 2009; Mohammadabadi, 2012) and sheep (Honarvar et al., 2012; Ghazi et al., 2014). The presence of polymorphisms in the GH gene is also associated with production properties (Pereira et al., 2005), and carcass weight (Beauchemin et al., 2006).

GH gene is the controller of the existence and growth properties polymorphism important in supporting selection of the nature of the growth. It is interesting to study the existence and diversity of GH gene associated with quantitative characteristics of the thin-tailed sheep in the province of Jambi. Is diversity a difference that is due to differences in GH gene owned or variety of environmental variation? The answer to this question is necessary in order to study molecular genetics at present and in the future.

Evaluation of GH gene polymorphism need to be done to encourage selection of the growth traits, especially associated with the quantitative characteristics of the thin tail sheep (TTS) in Jambi Province, whether the difference is due to differences in diversity of GH gene or of environmental variation.

The objectives of this research were to investigate the quantitative characteristics of local TTS, TTS growth hormone gene polymorphism and linkage relationship between polymorphism of growth hormone and quantitative characteristics of TTS at highlands and lowlands in Jambi Province.

MATERIALS AND METHODS

The research material was local thin tail sheep (TTS) in the highlands and lowlands in Jambi Province. Quantitative characteristics data were collected from 240 heads of TTS. Blood samples were collected from 160 sheep between age 1 and 2 years old (I1 = a pair of permanent teeth). Research was done in the Kerinci District, Sungai Penuh City (Highlands), Muara Jambi Districts and Batanghari Districs (lowland).

Data were collected quantitatively using the following characteristics : 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 TTS was taken through the jugular vein with no heparin venoject vacuum tubes. The Blood samples were then preserved with absolute ethanol. Thereafter, absolute ethanol in the ratio 1:1 was added to the blood samples and stored at room temperature. Thereafter, blood samples were added to absolute ethanol in the ratio 1: 1 and stored at room temperature. The observed variables associated with DNA analysis of GH 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, (4) the value of Polymorphic Informative Content (PIC), (5) the presence or absence of mutations, (6) homology of GH's gene

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

Position of the segment	Long (Bp)	Primers	Sequence (5 '3')	Annealing temperature (°C)
175-774	579	GHY1-Fwd. GHY1-Rev.	5 'CAT TTG AAA ATA TGT GAG CAC ACA G 3' 5 'CCC CAC CTC TAG GAC ACA TC 3'	60.3
671-1361	690	GHY2-Fwd. GHY2-Rev.	5 'CTG TTT GCT GTG GCC AAC 3' 5 'CCA AAG AAG CGA CTG GAT GA 3'	60.3
1248-1927	679	GHY3-Fwd. GHY3-Rev.	5 'CCG AGG CAG CAG ACA TTG 3' 5 'GAA CAT GCG GCG CTT ACA	62.5

sequence, and (7) linkage of GH's gene genotype and quantitative trait (8) the relationship between GH's gene and quantitative characteristics.

Purposive sampling in survey method was applied on field study; in the laboratory,the polymorphism of GH gene was detected with PCR-RFLP technique.GH gene amplification, which has further isolated DNA was amplified using 3 pairs of primer: the length of each primer was 579 , 690 and 679 bp. They were located in E1-E5 and I1-I5. More details of the primers used are presented in Table 1.

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

For the polymorphism detected by PCR-RFLP, 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 consists of 50 ml of nuclease free water (ddH_2O) (17.5 ml), 25 ml PCR product, 5 ml enzyme buffer, enzyme MspI or AluI (2.5 ml). This mixture was then incubated for approximately 12 h, and then migrated to agarose gel (2%) by ethidium bromide. Furthermore, it went through electrophoresis, which was done with Thermo Scientific models A5, power supply of EV 231 Consort, USA, of 100 V, 74 mA for 2 h. It was also further examined by gel documentation system (Biometra-German), photographed and stored on Compact Disc.

In the results of the sequence of GH's gene fragment of thin-tailed sheep, its similarity (homology) was analyzed with the sequence contained in GenBank using software (software) computer programs Basic Local Alignment Search Tool (BLAST) (https: //blast.ncbi.nlm.nih. Gov / BLAST. cgi, 2015),which ensures that GH's gene fragment sequence is analyzed. BLAST results had the highest similarity of at least 97%. Furthermore, alignment is done to look for mutations, deletions and insertions in order to obtain similarity (homology) by using software (software) ClustalW computer (http://www.ebi.ac.uk/Tools/ MSA / clustalw2, 2015). Genotype frequencies from PCR-RFLP assumed from the combination of different alleles produced based on the presence or absence of one or more sites if the ribbon is cut, marked (-/-), if cut off all (+ /+) and if the number of truncated and not cut (+/-) (Kumari et al., 2014) (https://www.ncbi.nlm.nih.gov/probe/docs/, 2015).

Genotype frequency was calculated based on the number of alleles of a genotype divided by the number of samples. Allele frequency is calculated by summing all alleles divided by 2N. GH gene allele frequencies derived from the analysis of PCR-RFLP MspI were calculated using the formula (Nei, 1987). The genetic diversity (genetic variability) is done through observation of the estimated frequency of heterozygosity (Ho), heterozygosity expectations (Hi) and standard error heterozigous expectations (Weir, 1996; Nei, 1987). An allele informative level is calculated using a value approach informative polymorphic content (PIC) (Botstein et al., 1980). Hardy-Weinberg Equilibrium was tested with *Chi-square* ($X^{2)}$ (Hartl and Clark, 1997). Analysis of the influence of the GH gene genotype weight gain, body weight and quantitative characteristics was performed by t-test (Gasper, 2006). PCR products were used for sequencing amplification product nukleotid, using the method of Sanger et al. (1977), with the recommended procedure of Sambrook and Russel (2001). Results sequences were obtained and the variability between the highlands and lowlands was tested using DNA Star program.

RESULTS

Phenotype characteristics

Average quantitative characteristics of DET (both males and females) in the highlands and lowlands of Jambi Province are presented in Table 2. In Table 2, the average of all the quantitative traits of both males and females in the highlands (Kerinci distric and Sungai Penuh city) was better than that of the lowlands (district Muaro Jambi and Batanghari). T-test showed that quantitative characteristics of thin-tailed sheep in the highlands were significantly different ($P < 0.05$) from those of the lowlands in Jambi Province.

DNA isolation and amplification

The concentration of DNA isolation makes highlands and lowlands vary from 100 to 400 ng/ml. High and low concentrations of DNA produced are highly dependent on the ability of the cell nucleus to lyse. Yurnalis et al. (2013) state that if the nucleus of the cell lysis process can run well, then the resulting DNA concentration is high enough and the quality of the DNA will be good or otherwise. PCR results performed to amplify DNA in the highlands and lowlands showed that the length of the fragments was similar in each primary GHY1, GHY2 and GHY3 that are 599, 690 and 679 bp, respectively. More detail is shown in Figure 1.

Table 2. Mean quantitative characteristics of the thin tail Local sheep males and females at highlands and lowlands in Province of Jambi.

Site	Karakteristik Kuantitatif						
	BW	BWG	BL	WH	ChG	ChD	ChW
Highland							
Male	20.24±2.44	75.67±13.12	56.80±2.56	53.41±2.40	62.57±2.17	24.45±2.54	14.90±2.44
Female	18.08±2.65	51.28±16.26	54.50±2.69	52.57±2.76	61.35±2.56	21.89±2.38	14.65±2.44
Lowland							
Male	18.69±3.06	60.89±16.96	53.25±3.00	50.25±2.82	59.30±1.92	20.56±2.34	12.11±2.36
Female	16.01±3.67	36.50±15.69	51.00±2.71	49.68±2.48	58.46±1.82	19.10±2.12	11.33±2.04

BWG = body weight gain; BW = weight; BL = body length; WH = wither high; ChG = chest circumference; ChD = chestdeep and the ChW = chest width.

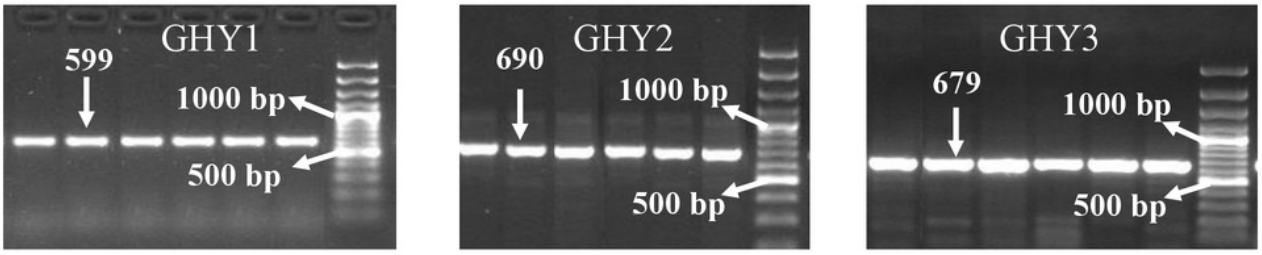

Figure 1. Result of PCR using primer GHY1, GHY2 and GHY3.

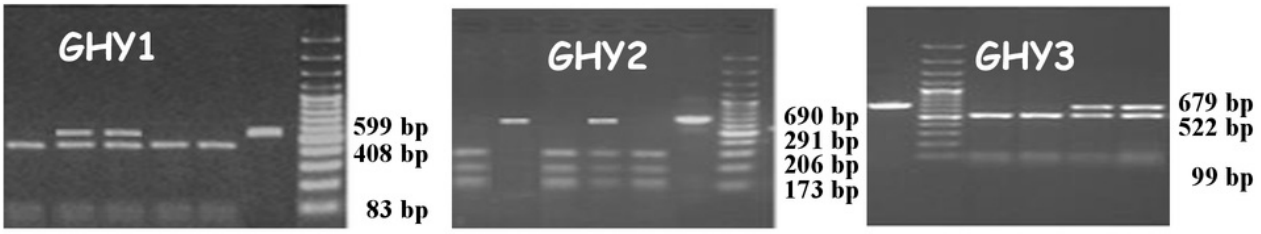

Figure 2. Results of electrophoresis of PCR-RFLP on growth hormone gene of thin-tailed sheep using enzyme MspI at Primary GHY1, GHY2 and GHY3 in the highlands and lowlands.

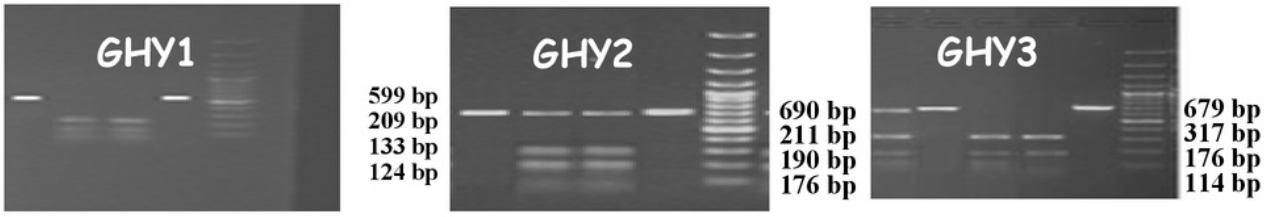

Figure 3. Results of electrophoresis of PCR-RFLP on growth hormone gene of thin-tailed sheep using enzyme *AluI* at Primary GHY1, GHY2 and GHY3 in the highlands and lowlands.

Results of electrophoresis of PCR-RFLP on growth hormone gene of thin-tailed sheep in the highlands and the lowlands using MspI, and AluI enzymes showed the same results in each primary GHY1, GHY2 and GHY3. More details are presented in Figures 2 and 3.This result showed that there was no difference in band number of GH

Table 3. GH gene MspI genotype frequencies and AluI TTS at a lowland and highland in Province of Jambi.

Loction/Enzyme	No. of sample	Genotype	Genotype frequency	Alelle frequency
Highlands		+/+	132 (0.5500)	+= 0.6938
*Msp*I	240	+/-	69 (0.2875)	-= 0.3063
		-/-	39 (0.1625)	
Lowlands		+/+	126 (0.5250)	+= 0.6625
*Msp*I	240	+/-	66 (0.2750)	-= 0.3375
		-/-	48 (0.2000)	
Highlands		+/+	168 (0.7000)	+ = 0.8125
*Alu*I	240	+/-	54 (0.2250)	- = 0.1875
		-/-	18 (0.0750)	
Lowlands		+/+	147 (0.6125)	+ = 0.7125
*Alu*I	240	+/-	48 (0.2000)	- = 0.2875
		-/-	45 (0.1875)	

Table 4. Values of observed heterozygosity (Ho) and expectation heterozygosity (He) of GH gene of PCR-RFLP *Msp*I dan *Alu*I.

Site	n	PCR-RFLP marker	Heterozygosity	
			Ho	He
Highlands	240	*MspI*	0.2875	0.4257
Lowlands	240	*MspI*	0.2750	0.4481
Highlands	240	*AluI*	0.2250	0.3053
Lowlands	240	*AluI*	0.3250	0.4015

gene on restriction point CC*GG and AG*CT in both locations.

Genotype frequencies, allele, Hard-Weinberg equilibrium, estimation of heterozygosity and PIC

Polymorphism or genetic diversity can be determined by analysis of the frequency of genotype and allele frequencies. Results of the cutting with the enzyme MspI at GH gene fragment in both the highlands and lowlands of genotype frequency (+/+) were higher than genotype frequency (+/-) and (-/-). In the highlands the obtained genotype frequencies were (+/+) 0.5500, (+/-) 0.2875 and (-/-) 0.1625 to the frequency of allele (+) 69.38 and (-) 30.63%. In the lowlands, obtained genotype frequencies were (+/+) 0.5250, (+/-) 0.2750 and (-/-) 0.2000 to the frequency of allele (+) 66.25 and (-) 33.75%.

Results cutting with the enzyme AluI for the GH gene fragment in both the highlands and the lowlands genotype frequency (+/+) were higher than the genotype frequency (+/-) and (-/-). In the highlands, obtained genotype frequencies were (+/+) 0.7000, (+/-) 0.2250 and (-/-) 0.0750 to the frequency of allele (+) 81.25% and (-) 18.75%. In the lowlands, the obtained genotype frequencies were (+/+) 0.6125, (+/-) 0.2000 and (-/-) 0.1875 to the frequency of allele (+) 71.25% and (-) 28.75%. More details can be seen in Table 3.

Test 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 was performed using chi-square test. GH gene MspI allele frequency and AluI were tested on thin-tailed sheep in the highlands and lowlands in Hardy-Weinberg imbalance ($P < 0.01$).

Result of heterozygosity estimation with PCR-RFLP marker of MspI and AluI at all study sites showed that the value of expected heterozygosity (He) was higher than observed values (Ho). More details can be seen in Table 4.

Local sheep PIC values thin tail to fragment GH gene MspI and AluI in the highlands and lowlands are presented in Table 5.

Based on the PIC value, it can be stated that marker of PCR-RLFP of gene fragment GH AluI is not different with PCR-RFLP of gene fragment GH MspI in the lowlands and highlands. PIC values obtained are categorised in the medium (moderate), so that these values can be expressed quite informatively as a GH gene fragment marker on MspI and AluI.

Relationship between polymorphisms of GH genes with quantitative characteristics

The average quantitative characteristics of DET in the

Table 5. Estimation of the value of polymorphic information content (PIC) DET gene fragment of GH MspI and AluI in lowland and highland province of Jambi.

Site	n	*Polymorphic Informative Content* (PIC)	
		GH Gene Fragment MspI	GH Gene Fragment AluI
Highlands	240	0.3797	0.2815
Lowlands	240	0.3975	0.3677

Table 6. Means of characteristic GH gene fragment of thin-tailed sheep using *Msp*I and *Alu*I in highlands and lowlands.

Site/characteristics	Genotype		
	+/+	+/-	-/-
Highlands MspI			
BWG	0.0899±0.0114[a]	0.0759±0.0064[b]	0.0645±0.0075[c]
BW	22.2519±2.5286[a]	21.1458±1.7935[b]	20.5311±2.6702[c]
LB	58.8688±2.2773[a]	58.1198±2.0864[b]	56.8361±3.0573[c]
WH	55.4084±2.6777[a]	54.9001±1.9705[b]	54.4013±2.9319[c]
ChG	64.4261±2.4150[a]	63.9291±1.8306[b]	63.0101±2.5642[c]
ChD	25.5693±1.6429[a]	25.0791±1.1344[b]	24.7515±1.8240[c]
ChW	15.4803±1.1689[a]	15.1133±0.8910[b]	14.9986±1.0956[c]
Lowlands MspI			
BWG	0.0764±0.0158 [a]	0.0591±0.0047 [b]	0.0481±0.0058 [c]
BW	21.1274±2.2441 [a]	18.5504±1.6586 [b]	16.6256±1.1799 [c]
LB	56.5173±2.3176 [a]	54.0838±1.6564 [b]	53.1163±1.3145 [c]
WH	52.3217±2.2593 [a]	50.1349±1.5269 [b]	49.1647±0.7901 [c]
ChG	60.9372±1.9024 [a]	59.4086±1.0630 [b]	58.7667±0.4279 [c]
ChD	22.9243±1.3990 [a]	21.0286±1.5550 [b]	21.1440±0.8657 [c]
ChW	13.8673±1.6257 [a]	12.1718±1.4473 [b]	11.7451±0.8958 [c]
Highlands MspI			
BWG	0.0861±0.0131 [a]	0.0741±0.0069 [b]	0.0625±0.0095 [c]
BW	21.9588±2.4528 [a]	21.0263±2.2706 [b]	19.1183±0.6954 [c]
LB	58.6303±2.3587 [a]	57.9232±2.1549 [b]	54.0423±1.9300 [c]
WH	55.3233±2.5692 [a]	55.1358±1.9046 [b]	52.3763±1.8106 [c]
ChG	64.1657±2.3955 [a]	63.9673±2.0305 [b]	61.1494±1.9163 [c]
ChD	25.3835±1.6619 [a]	24.9335±1.6760 [b]	23.2856±2.1599 [c]
ChW	15.4032±1.1677 [a]	15.0934±0.7866 [b]	14.4004±0.6543 [c]
Lowlands AluI			
BWG	0.0717±0.0160 [a]	0.0583±0.0092 [b]	0.0478±0.0101 [c]
BW	20.5331±2.3714 [a]	17.7270±1.6610 [b]	17.0133±1.9803 [c]
LB	55.9954±2.3897 [a]	53.8307±1.4541 [b]	52.7504±1.3055 [c]
WH	51.8231±2.3417 [a]	49.7834±1.1692 [b]	49.2165±0.7234 [c]
ChG	60.6445±1.7416 [a]	58.9798±1.3423 [b]	58.7706±0.6189 [c]
ChD	22.4275±1.6705 [a]	21.3825±1.1932 [b]	21.0119±1.4642 [c]
ChW	13.4447±1.8072 [a]	12.1186±1.1632 [b]	11.8340±0.6611 [c]

Different letter in same row showed significant difference, db = n -2 t_{tabel} α 5% = 1.645.

highlands and lowlands of GH gene fragments using PCR-RFLP MspI, and AluI, is presented in Table 6.

TTS average quantitative characteristics, in the highlands and lowlands of GH genes using PCR - RFLP MspI identifier and AluI genotype were +/+ > +/- > - /-. Results of t test analysis between genotype (+/+ , +/- and -/-) on the quantitative characteristics of DET in the highlands and lowlands have the same pattern that is

significantly different ($P < 0.05$). The results obtained indicate that there is a relationship between genotype GH genes with quantitative characteristics as analyzed by TTS.

Growth hormone gene sequences

Results of alignment analysis of GH gene fragment primer GHY1, GHY2, GHY3 and its combined with BLAST good primer on the highland and lowland showed high similarity (homology) with the gene sequences of ovis aries growth hormone. Homology analysis of primer GHY1, GHY2 and GHY3, and combined with the same accession number are EF077162.1-1973bp, DQ461677, 1-2987bp, DQ461669.1-bp, DQ461667.1-4804 bp, and X12546.1- 2162 bp obtained at 99, 99, 98 and 98%, respectively. These results indicate that the GH gene amplified fragment was GH gene fragment of sheep (Ovis aries).

Multiple alignment is used to predict the structure and function of proteins. One method used is ClustalW. Primary clustalW results for GHY1, GHY2, GHY3 and combined sequentially obtained similarities were 98.57, 98.19, 98.30 and 98.86%.

Based on analysis of the sequences obtained that purportedly mutations occur at both sites is a mutation between the bases cytosine (C) to thymine (T) and the base cytosine (C) to guanine (G). Based on the results analysis, mutation sequences GH gene PCR - RFLP MspI (C*CGG) is C➡T at position 319 bp in exon 1, while the GH gene PCR- RFLP AluI (AG*CT) is C➡G at a position 983 bp in exon 3.

DISCUSSION

Effect of altitude

Kerinci district and Sungai Penuh City are located in the highlands with an altitude of $l \geq 1500$ m above sea level and affected by environmental factor, while Muaro and Batanghari district are located in lowlands with an altitude of 100-500 m above sea level. The difference of altitude will certainly lead to differences in temperature, humidity, rainfall, wind speed and irradiation time. According to Calderon et al. (2005), there is a significant difference between the performance of livestock production in the lowlands (hot area) and upland (cold area). Here in after, referred environmental factors will affect the productivity of sheep (Popoola et al., 2014; Idris et al., 2014; NseAbasi et al., 2014).

Isolation and amplification

Isolated DNA of thin-tailed sheep in highland and lowlands in jambi province range from 100 to 400. Differences in concentrations of DNA produced is highly dependent on the ability of the cell nucleus lysis. If the cell nucleus could be well lysed, then the resulting DNA concentration will be high enough in both quality and quantity. However, if the cell nucleus is not lysed perfectly, DNA concentration will be low and sometimes contaminated with other materials. This indicates that the use of genomic DNA Purification Kit (Promega - USA) is a powerful enough to isolate the genomic DNA DET.

The results of DNA amplification using primers GHY1 DET, GHY2 and GHY3 sequentially generate specific fragments because electrophoresis results produced only one band with a length in accordance with the expected sequence which were 599, 690 and 679 bp. According to Rahayu et al. (2006), primer is an essential part of the primer PCR as an initiator to the target DNA synthesis; in addition to the PCR, results were favorably affected by several factors such as purity DNA extraction result, the accuracy of the primaries used, as well as the accuracy of PCR conditions. This condition indicates that the PCR reaction conditions and primers used through design with primer3 program are quite good because it gives a very specific PCR product as expected.

Allele frequencies, heterozygosity and PIC *(Polymorphic Content Informative)*

A locus would be polymorphic when the frequency of the largest allele is equal to or less than 0.95. Conversely, a locus would be monomorphic when the frequency of the greatest allele exceeds 0.95. Proportion of polymorphic loci in a population is often used as one of the indices of genetic diversity. Other grades which are also often used as an index of genetic diversity in a population are heterozygosity average or the frequency of heterozygotes (H) on average.

PIC can be used as a basis in determining an identifier information, and determining whether there is a polymorphic allele in addition based on the value of heterozygosity. Based on this measure of a good third allele frequency, heterozygosity and PIC values, it can be stated that the population DET in lowland and high in Jambi province have genetic diversity or are polymorphic. This result does not vary much with the results of Kumari et al. (2014) against 9 nations of sheep in India who obtain the A allele frequency (+) higher than the frequency of allele (-). According to Machado et al. (2003), if the Ho value is lower than the He value, it indicates a degree of endogamy (mating within the group). Furthermore, Javanmard et al. (2005) found that heterozygosity with a value below 50% indicates the low variation of a gene in the population. Heterozigosyty value is one of parameter used to determine population polymorphism (Ahmaed et al., 2014). Botstein et al. (1980) stated that the PIC can be used as a basis in determining

whether an identifier information, and determine whether there is a polymorphic allele in addition based on the value of heterozygosity, then Puja et al. (2013) stated that the PIC is high enough to give an indication that the sample population is very heterogeneous and indicated little going selection for certain characteristics while PIC small value indicates that the sample population is very homogeneous and indicated their selection for certain characteristics. Diversity or variation in a species would be very useful in the field of genetics or for the benefit of selection. These variations can be used to identify and locate the origin of a particular type of animal, knowing the kinship between species to the preparation of the gene map. Genetic variation information can be used as the basis for selection to improve livestock production and conservation goals.

Relationship between polymorphisms of GH genes with quantitative characteristics

The result shows that there is a relationship between genotype of GH gene with quantitative characteristics of thin-tailed sheep. This is consistent with the statement of Hajihosseinlo et al. (2013) that stated that genotype frequencies have a relationship with the characteristics of the sheep makooe. Research of Hua et al. (2009) explained that the haplotip diversity of GH's gene of HaeIII on Boer goats had effect on birth weight, weaning weight, body weight gain per day before weaning and body weight till 11 months. Furthermore, according to Alakilli et al. (2012), genotype frequencies can be used as molecular marker genes growth properties on goats. Marker of PCR-RFLP could be used as a selection tool. Polymorphisms of genotype frequency could be used as a basis of selection and breeding programs that very are useful in order to increase the population of thin-tailed sheep in Jambi Province.

Growth hormone gene sequences

There was a change in the base using the restriction enzyme MspI or AluI, although there is a chance of change (mutation) with other bases. This is due to the limited number of samples in sequence analysis. However, these results are in accordance with those reported by Malewa et al. (2014). Mahrous et al. (2014) reported no change between the cytosine (C) to thymine (T) and the cytosine (C) to guanine (G) in the Ovis aries, fat-tailed sheep, Egypt sheep and Saudi Arabia sheep. Furthermore, according to Jakaria (2008), the base nucleotide changes that occur are in the site MspI (CT) and AluI (CG). Ge et al. (2003) suggest changes in the AluI site which can alter the amino acid leucine (L) (CTG) to valine (V) (GTG) on growth hormone.

Mutations obtained in this study were categorized as substitution mutation, where mutation transition type for restriction enzymes MspI and transversion mutation types for restriction enzyme AluI. This is consistent with the statement of Li and Gaur (1991) that the mutations occur as a result of the substitution of adenine to guanine bases (purines) or between cytosine and thymine (pyrimidine). Transversion mutation occured due to the exchange between purine bases (A, G) with a pyrimidine (C, T). It is important to study the mutation as causes of genetic diversity, which can be used to determine the genetic distance (Mahrous et al., 2014), the characteristic of breed (Elkorshy et al., 2013), the relationship with growth traits (Moradian et al., 2013; Jia et al., 2014) and the ability of reproduction (Moradband et al., 2011).

Conclusion

Based on the research, it can be concluded that quantitative characteristics (BW, BWG, BL, ChG, ChD, and ChW) of thin-tailed sheep in both male and female in highland was better than in the lowlands. The marker of PCR-RFLP MspI and AluI GH gene were polymorphic on the highlands and lowlands. The highest genotype frequency and frequencies of allele in both the highlands and the lowlands were the genotype frequency (+/+) and the frequency of allele (+). The highest quantitative characteristic were BW BW, BWG, BL, ChG, ChD, and ChW of thin-tailed sheep was genotype (+/+). The diversity of GH genes MspI and AluI are associated with BW, BWG, BL, ChG, ChD, and ChW of thin-tailed sheep both on the highlands and the lowlands of Jambi Province.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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