SINGLE NUCLEOTIDE POLYMORPHISMS OF LIPOPROTEIN LIPASE GENE AND ITS ASSOCIATION WITH MARBLING QUALITY IN LOCAL SHEEPS

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ABSTRAK

Lipoprotein lipase (LPL) merupakan enzim kunci dalam metabolisme dan transport lipoprotein yang mempengaruhi level trigliserida darah. LPL mengontrol partisi triasilgliserol antara jaringan lemak dan otot serta dapat meningkatkan penyimpanan lemak atau menyediakan energi dalam bentuk asam lemak untuk pertumbuhan otot. Penelitian ini bertujuan untuk mengeksplorasi keragaman gen LPL pada domba lokal Indonesia dan hubungannya dengan kualitas marbling. Total 66 DNA genom (260 bp) domba lokal Indonesia yang terdiri atas domba ekor tipis sumatera (50 ekor) dan domba garut (16 ekor) digunakan dalam penelitian ini. Amplifikasi DNA genome menggunakan Polymerase Chain Reaction dan metode direct sequencing digunakan untuk mengidentifikasi keragaman sekuens. Hasil sekuens dianalisis menggunakan software Bioedit dan MEGA 5.2. Sekuens kemudian disejajarkan menggunakan metode Clustal W dengan gen bank X.68308.1. Assosiasi keragaman gen LPL dengan kualitas marbling dianalisis menggunakan metode ANOVA satu arah dan uji lanjut menggunakan uji beda nyata terkecil. Hasil penelitian menemukan 3 SNPs baru pada posisi basa g.26>C/G, g.27>G dan c.192 T>C pada domba garut dan insersi g.26>C/G pada domba ekor tipis sumatera. Keragaman gen LPL posisi basa c.192 pada domba garut berasosiasi dengan asam lemak heneikosanoat, dimana genotipe TT (0.04%) memiliki kandungan lebih tinggi dibandingkan genotipe CC (0,03%) dan CT (0,02%).
Kata kunci: gen LPL, SNPs, lamb, asam-asam lemak, henekosanoat

ABSTRACT

Lipoprotein lipase (LPL) is a key enzyme that plays in metabolism and transport lipoprotein and therefore has an influence on blood triglyceride levels. LPL controls triacylglycerol partitioning between adipose tissue and muscle that increases fat storage or provides energy in the form of fatty acids for muscle growth. The research was aimed to explore Single Nucleotide Polymorphisms of LPL gene and to associate SNP with marbling quality. A total of 66 genomic DNAs consisted of sumatera thin-tail ed sheep (50 heads) and garut sheep (16 heads) were used in this study. Polymerase Chain Reaction was used to amplify genomic DNA and direct sequencing method was to identify polymorphism sequences. The sequences were analyzed with Bio Edit and MEGA 5.2. The BLAST sequence was obtained from gene bank X.68308.1. The association between the genotype and marbling quality was analyze by one way ANOVA and further between mean differences were tested using least sgnificant difference. The results showed that 3 novel SNPs i.e. insertion g.26>C; insertion g.27> G and c.192T>C on garut sheep and a SNP insertion g.26>C/G on sumatera thin-tail ed sheep. The diversity of LPL gene at c.192T>C was associated with heneicosanoic acid, whereas TT genotype (0.04%) was higher than CC (0.03%) and CT (0.02%).
Keywords: LPL gene, SNPs, lamb, fatty acids, heneicosanoic acid

INTRODUCTION

Meat quality can be defined as factors that affect consumers' assessment of the quality of the meat where the meat quality is associated with polygenic traits (Dunner *et al.*, 2013). The consumer's decision of cooked meats including tenderness, juiciness and flavor, is closely associated with marbling quality (Hocquette *et al.*, 2006). Marbling or intramuscular fat is the flecks and streaks of fat within the lean sections of meat (Guo *et al.*, 2014).

Depositions of animal body fat were found in the visceral, subcutan, intermuscular and intramuscular. Distribution of fat, fatty acid composition and type of muscle fibers, approximately 35% were influenced by genetic (Williams, 2008) and varieties of environmental factors. Marbling affect consumers' assessment because these fats cannot be separated from the meat, the fat is different from the other parts.

Fats composed of glycerol esters, cholesterol, phospholipids and fatty acids. Fatty acids are grouped into 3 categories: saturated fatty acid (SFA), mono unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA). Nutritional guidelines suggest that fat intake ranged from 15%-30% of total caloric needs, SFA is limited between 0-10%, MUFA 16%, PUFA 7% and cholesterol should not exceed 300 mg/day (Wong, 1989; Chizzolini *et al.*, 1999). SFA consumption that exceeded the needs is suspected as the cause of various diseases such as cardiovascular disease, atherosclerosis and other diseases. Heritability of fatty acid composition of scottish blackface sheep marbling was ranged from moderate to high (Karamichou *et al.*, 2006), which indicates a large genetic influence.

Selection of marbling quality using conventional methods, difficult and expensive relatively, so based on phenotype selection, marbling quality rarely included. Alternative that can be done is through genomic selection. Genomic selection begins with the discovery of candidate genes were associated with desirable traits. One is through the exploration of single nucleotide polymorphisms (SNPs) of the gene and then associated with the desirable traits. SNPs are DNA sequence variations that when arise a single nucleotide (A, T, C or G) is different from the sequence generally. The among the genes influence marbling quality was LPL gene. Sevane *et al.* (2013) reported that LPL gene diversity in some breeds of cattle was associated with increased fatty acid gamma-linolenic acid and dihomo-arachidonic fatty acids.

Lipoprotein lipase (LPL) is a major enzyme that plays in metabolism and transport lipoprotein and therefore it has an influence on blood triglyceride levels (Wang & Eckel, 2009; Crissa *et al.*, 2010; Holmes *et al.*, 2011). Lipoprotein components comprise of cholesterol, triglycerides, phospholipid and apolipoprotein. Triglycerdes in VLDL are hydrolyzed by LPL in the blood capillaries. LPL controls triacylglycerol partitioning between adipose tissue and muscle that increases fat storage or provides energy in the form of fatty acids for muscle growth (Bonnet *et al.*, 2000; Ren *et al.*, 2002; Dunner *et al.*, 2013).

LPL is produced in adipose tissue, heart and skeletal muscle and then transferred to the surface of capillary endothelium. LPL was encoded by the LPL gene consist of 10 exons and 9 introns which were located on chromosome 2 in *Ovis aries* (www.animalgenome.org/cgi-bin/QTLdb/OA).

LPL gene polymorphisms and its associations with marbling quality has no such studies were conducted in Indonesian local sheep. The objectives of this study were to explore SNPs of LPL gene in the coding region and its association with lamb marbling quality of local sheep.

MATERIALS AND METHODS

Materials

Blood samples from 50 heads sumatera thin tail ed sheep (Padang, West Sumatera) and 16 heads garut sheep (Garut, West Java) were collected by using venoject 2-3 mL from jugular vein. The blood samples were preserved in ethanol absolute 96% with ratio of 1:1 and kept in room temperature for laboratory analysis. The intramuscular fat samples were collected from *m. longissimus dorsi* of Garut sheeps to analyze fat, cholesterol and fatty acid composition.

DNA Extraction and Amplification Using PCR Method

Genomic DNA was extracted by using Phenol-chloroform technique (Sambrook *et al.* 1989) and modified by using buffer lysis cell (250 µL 1 x STE, 40 µL SDS and 10 µL proteinase-K). The DNA was purified by adding 40 µL 5M NaCl, 400 µL phenol chloroform and 400 µL CIAA (Chloroform Iso Amyl Alcohol) and precipitated by using 40 µL 5M NaCl and 800 µL ethanol absolute. The precipitation was washed

once by adding 800 µL of 70% ethanol and centrifuged with the speed of 12.000 rpm for 5 minutes. The ethanol was discarded and evaporated, than the precipitated DNA was disolved in 100 µL of 80% TE (Elution buffer).

Polymerase chain reaction (PCR) method was used to amplify the fragment of LPL gene. The primers used were designed using the Primer Blast program from NCBI (http://www.ncbi. nlm.nih.gov/ tools.primer-blast) with reference to GenBank accession number X.68308.1. The forward primer sequence was F'5-AAACCTGCCGCTTCTAGCTC-3' and reverse primer sequence was F'5-TCTTGTAATCCTGTCGGCGG -3'. The primer sequences flanking the base position at 17 to 277 with a length of 260 bp sequence was a small part of the 5'UTR and the first exon of LPL gene (Figure 1).

Each PCR reaction was made with cocktail 50 ng (2-3 µL) DNA template, 0.25 µM primer forward and reverse, 12.5 µL Dream Tag Green Master Mix from Thermo Scientific #K 1081 and dH_2O up to 25 µL. Samples were initially denaturated at 95^0C for five minutes and followed by 35 cycles of denaturation at 95^0C for 45 s, annealing at 58^0C for one min and extension at 72^oC for one min. Final extension was at 72^0C for 5 min. Gene Amp PCR system 9700 and Master Cycler Personal 22331 Eppendorf were used for PCR amplification. PCR products were then separated on 1.5% agarose/0.5 x TBE, stained with 2.5 µL of ethidium bromide (EtBr) and calibrated with 100 bp ladder marker. Electrophoresis chamber was run on 100 volt power supply for thirty minutes. Finally, the gel was visualized under UV transilluminator (Figure 2). The PCR product samples were then subjected to direct sequence analysis by dideoxy sequencing in ABI 3730 XL automated DNA sequencer at the 1st base laboratory Singapore.

Quality of Marbling Analysis

Nine of sixteen of male garut sheeps, had aged 8-10 months and weight at slaughtered 22,46 kg was identified of diversity LPL gene at base c.192, consists of TT (n=4), CT (n=2) and CC (n=3), slaughtered and *m.longissimus dorsi* used to analyze lamb marbling quality. The fat determined with soxhlet method with petroleum eter (AOAC, 2005). The cholesterol content was carried out with dye 10 g $FeCl^36H^2O$ in 100 mL glacial asetat acid (AOAC, 2005) by spectrometer in absorbance with wavelength of 565 nm. The profile of fatty acids was followed AOAC (2005). The lipids after extraction were esterified and methylated with BF3 20%. The methyl esters of fatty acids were separated and quantified in a gas chromatograph (*GC-2010 plus Shimadzu)*.

Data Analysis

The results of sequences fragment of LPL were analyzed with BioEdit (Hall, 2011), MEGA version 5.2 (Kumar *et al.*, 2004) and POP GENE ver.1.31 (Yeh *et al.*, 1999) software. The BLAST sequence was obtained from Gene Bank with accession number of X.68308.1. The analyzed sequence was required to ensure the fragment of LPL gene of sheep and to find out the existence of mutation in LPL gene and polymorphisms in the sequences. Frequency of gene, genotype and Hardy–Weinberg equilibrium were estimated by Nei and Kumar (2000). The observed heterozygosity (H_o) and excepted heterozygosity (H_e) were estimated with POPGENE 32. The association between the genotype and marbing quality was analyze by one way ANOVA and *Least Significant Difference* (LSD) used to know different least squares means. The model mathematics is as follows:

$$Y_{ij} = \mu + G_i + E_{ij}$$

Where; Y_{ij} is the traits measured in animal, μ is the population means, G_i is the effect of genotype

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  1 gaattcgcgg ccgcggaaac ctgccgcttc tagctcccca ccctcccctt taaagggtga
 61 cttgctccgc gccagaccgc tgctccagcc tgctgccgcc tcgggctcag cggctctact
121 gctctgtccg cgctcgcgcc cggtgccccg catctcctac ggagggacat cccccgagat
181 ggagagcaag gtcctgcttc tgctggctct gagcgtgtgg ctgcagagtc tgaccgtctc
241 ccgcggaggg ctggtcgccg ccgacaggat tacaagagga aaagatttta gagacattga
301 aagtaaattt gctctcagga ctcccgaaga cacagctgag gacacttgcc acctcattcc
361 tggagtgacg gaatctgtgg ctaactgtca cttcaaccac agcagcaaaa cctttgtggt
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Figure 1. LPL Amplicon Sequence: Genbank X.68308.1. The primers sites were underlined and bold.
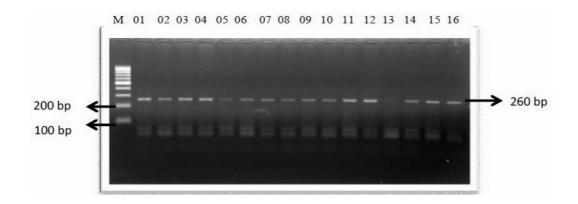
Figure 2. PCR Amplification of LPL Gene (260 bp); 0-16 = individual samples; m = DNA ladder 100 bp.

and E_{ij} the residual random effect associated with animal traits.

RESULTS AND DISCUSSION

Single Nucleotide Polymorphisms of Lipoprotein Gene in Sumatera Thin Tailed and Garut Sheep

Sixty six (66) sequences of sumatera thin-tail ed sheep (50) and garut sheep (16) on LPL gene encoding region were aligned with gene bank X.68308.1 (Figure 3). The results showed that one SNP i.e. insertion at base g.26>G/C (Figure 4b and Figure 4c). The one SNP was reconstructed three diplotypes: diplotype A, D and E (Tabel 1). Three SNPs were identified on Garut i.e. insertion at g.26>C and g.27>G (Figure 4a) and mutation at c.192T>C (Figure 5). These mutations were formed 5 diplotypes i.e. A, B, C, F and (Table 1). Diplotype A was similar to the gene bank sequences X.68308.1. Three novel SNPs in this study has never been reported in other sheep populations even though percentage of LPL gene diversity at base g.26 and g.27 were relatively low in both populations.

Insertion of at base position g.26>C and g.27>G caused arise stop codon at amino acids at 13, 66 and 73 as was found on diplotype A, while insertion at base g.26> G/C, the stop codon arise of 6 and 15 of amino acids sequence (Figure 6). Mutation at base g.26 and g.26 were called frameshift mutation that is a mutation that causes a stop codon is not at place properly.

With regard to mutation at base c.192 on garut sheep, was identified substitution thymine to cytocine was non synonymous mutation, it changed amino acid (Val>Ala) while in sumatera thin-tail ed sheep was monomorfic (Table 2). Frequency of C (0.59) was the predominant than T (0.41) in garut sheep. Frequencies of genotype were CC (0.44), CT (0.31) and TT (0.25) and this genotypes frequencies were in Hardy-Weinberg equilibrium (P>0.05) (Table 2).

Ding *et al.* (2011) reported that one SNP at exon 7 of LPL gene at base 19913 C>T, which changed Phenylalanine>Serine, responsible for carcass traits and lipid deposition in yak. Three SNPs found in 3'UTR of Lipoprotein Lipase gene g.74 T>C, g.130 T>C and g.133 T>A were associated with CLA content on milk sheep (Crissa *et al.*, 2010) and other researchers have been no reports on the effect of these 3 SNPs.

Association of Polymorphisms Lipoprotein Lipase Gene c. 192 with Marbling Quality of Garut Lamb

As shown in Table 3 and Table 4, the polymorphisms of LPL gene at base c.192 T>C did not significantly (P>0.05) was affected fat, cholesterol and fatty acid profiles at *longissimus dorsi* muscle, except on fatty acid profile of saturated fatty acid; one long chain saturated fatty acid heneicosanoic acid (P<0.05). Whereas, TT genotype (0.04%) was higher than CT (0.02%) and CC (0.03%). Heneicosanoic acid is a minor fatty acid in nature, but the presence of heneicosanoic $(_{21:0})$ affect the meat quality. The presence of odd long-chain saturated fatty acids indicates accumulation of propionic acid and biotin deficiency. Propionate is one of the volatile fatty acids of rumen fermentation. Conversion propionate into methyl malonyl CoA requires biotin. The methyl malonyl CoA was required for elongated fatty acid process by providing two
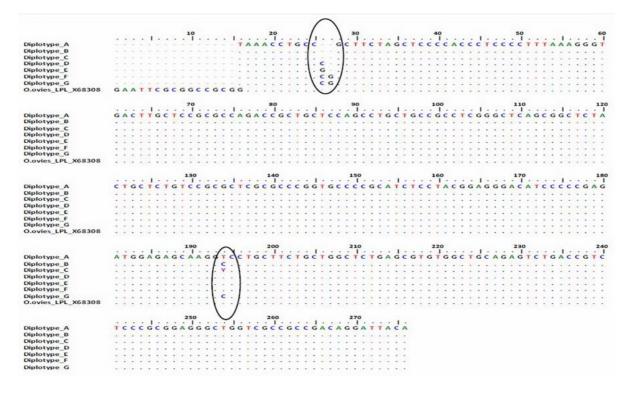
Figure 3. Alignmented of Lipoprotein Lipase Gene in Sumatera Thin-tailed Sheep and Garut Sheep

Figure 4a. Insertion g.26>C and g.27>G

Figure 4b. Insertion g.26>G

Figure 4c. Insertion g.26>C
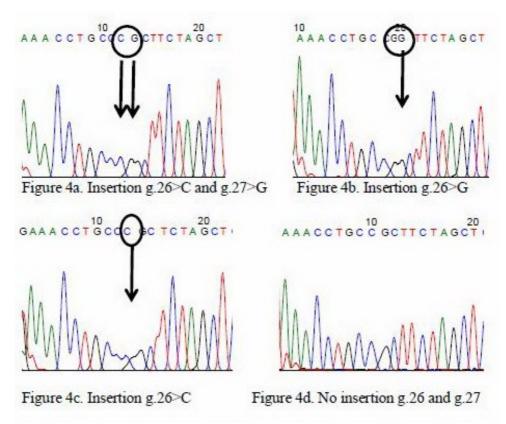
Figure 4d. No insertion g.26 and g.27

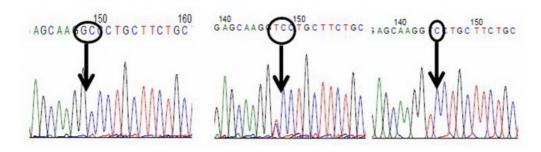Figure 4. Partial Sequences of LPL Gene, which Showed Insertion at g. 26 and g.27

Figure 5. Partial Sequences of LPL Gene with a Mutation at Base c.192 T>C

Table 1. Diplotypes of LPL Gene of Sumatera Thin Tailed Sheep and Garut Sheep at Positions of 17 to 278 Bases

Diplotype	At mutation			Frequency (n)	
	c. 26	g.27	c.192	STT	Garut
Diplotype A	-	-	T	0.840 (42)	0.1875 (3)
Diplotype B	-	-	C	0.000 (0)	0.3750 (6)
Diplotype C	-	-	C/T	0.000 (0)	0.3125 (5)
Diplotype D	C	-	T	0.020(1)	0.0000 (0)
Diplotype E	G	-	T	0.140 (7)	0.0000 (0)
Diplotype F	C	G	T	0.000(0)	0.0625 (1)
Diplotype G	C	G	C	0.000(.0)	0.0625 (1)
Total				50	16

STT (Sumatera Thin Tailed sheep).

Figure 6. Alignmented of Amino Acids Sequences of Seven Dyplotypes of LPL Gene in Sumatera Thin Tailed and Garut Sheep

carbon atoms. Biotin deficiency could inhibit the formation of methyl malonyl CoA and caused odd long-chain fatty acids. In addition, the presence of fatty acids heneicosanoic acid could oxidize omega fatty acids, but the exact mechanism is still unknown.

The results of this study indicate that the marbling fat content of garut sheep was 1.60% -1.73%, lower than other sheep breeds such as south african mutton, merino, dormer (Cloete *et al.*, 2004), moranda nova, santa inez and crosses dorper x santa inez (Costa *et al.,* 2009) as well as cholesterol content (6.10% - 8.91%) was lower than reported Costa *et al.* (2009) and Mas'ud (2012). It is suspected due to the age of the sheep were used relatively young (8-10 months), where the deposition of marbling not yet formed perfectly. Fat deposition was initially formed in the visceral, subcutaneous, intermuscular and last intramuscular. Cholesterol is a component of fat, while a little fat content, cholesterol content is also slightly.

LPL gene expression was influenced by nutrition, fat depot, adipocyte sizes, insulin levels, propionic acid levels (Ren *et al.*, 2002; Lee and Hossner, 2002) and type of tissue where the gene was expressed (Sevane *et al.*, 2013). The gene expression on marbling was lower than intermuscular, subcutaneous and perirenal. In the cattle, LPL was activated on muscles by *peroxisome proliferator-activated receptor gamma* (PPARG), when low glucose levels. PPARG previously activated by the sirtuin 1 (SIRT1) and *peroxisome proliferator-activated receptor gamma coactivator 1 alpha* (PPARGC1A). LPL leads to increased oxidation

Table 2. Frequency of Gene, Genotype, Heterozygosity and χ^2 of Sumatera Thin Tailed Sheep and Garut Sheep at Base c.192 T>C

Population	n	Gene Frequency		Genotype Frequency			Heterozygosity		χ^2 test
		C	T	CC	CT	TT	Ho	He	
Garut	16	0.59	0.41	0.44	0.31	0.25	0.313	0.498	2.379[ns]
STT	50	0.00	1.00	0.00	0.00	1.00	-	-	-

STT = Sumatera Thin Tailed sheep, ns = represent non significantly at P>0.05

Table 3. Effect of Polymorphism of LPL Gene at Base c.192 on Fat, Cholesterol, SFA,MUFA, PUFA and Its Ratio of Lamb Marbling of Garut Sheep

Variable	Genotype						Significance
	TT (n=4)		CC (n=2)		CC(n=3)		
	Mean	CV	Mean	CV	Mean	CV	
Fat (%)	1.730	0.68	1.680	0.21	1.600	0.56	ns
Cholesterol (%)	8.910	0.56	8.050	0.50	6.100	0.39	ns
SFA (%)	37.250	0.08	38.250	0.04	39.450	0.14	ns
MUFA (%)	26.410	0.09	30.650	0.16	29.780	0.21	ns
PUFA (%)	2.360	0.22	2.360	0.05	2.580	0.41	ns
MUFA/SFA ratio	0.709	0.14	0.801	0.11	0.755	0.09	ns
PUFA/SFA ratio	0.063	0.30	0.062	0.01	0.065	0.55	ns

SFA = Saturated Fatty Acid; MUFA = Mono Unsaturated Ffatty Acid; PUFA = Poly Unsaturated Fatty Acid; CV = Coefficient of Variance; n = sample size

Table 4. Association of Diversity of LPL Gene at Base c.192 with Fatty Acid Profile of Lamb Marbling of Garut Sheep (% w/w)

Fatty Acids		Genotype						Sig.
		TT (n=4)		CT (n=2)		CC(n=3)		
		Mean	CV	Mean	CV	Mean	CV	
SFA								
Capric Acid	C 10:0	0.10	0.29	0.09	0.16	0.11	0.16	ns
Lauric Acid	C12:0	0.56	0.40	0.41	0.02	0.35	0.34	ns
Myristic Acid	C14 0	3.93	0.30	3.21	0.03	3.31	0.06	ns
Pentadecanoic Acid	C15:0	0.40	0.21	0.30	0.07	0.29	0.14	ns
Palmitic acid	C16:0	17.68	0.05	18.61	0.15	20.85	0.18	ns
Heptadecanoic Acid	C17:0	0.79	0.08	0.77	0.01	0.70	0.06	ns
Stearic Acid	C18:0	13.59	0.10	14.71	0.07	13.66	0.15	ns
Arachidic Acid	C20:0	0.11	0.20	0.11	0.20	0.09	0.00	ns
Heneicosanoic Acid	C21:0	0.04[a]	0.20	0.02[b]	0.00	0.03[b]	0.22	*
Bahenic Acid	C22:0	0.05	0.29	0.04	0.35	0.06	0.33	ns
MUFA								
Myristoleic Acid	C14:1	0.14	0.28	0.12	0.35	0.11	0.10	ns
Palmitoleic Acid	C16:1	1.77	0.06	1.99	0.27	1.90	0.08	ns
Oleic Acid	C18:1n9c	24.45	0.10	28.50	0.15	27.71	0.22	ns
Cis-11-Eicosenoic Acid	C20:1	0.05	0.28	0.05	0.16	0.05	0.29	ns
PUFA								
Linoleic Acid	C18:2n6c	1.52	0.19	1.51	0.12	1.68	0.41	ns
Linolenic Acid	C18:3n3	0.19	0.65	0.22	0.10	0.24	0.21	ns
Cis-11,14-Eicosedienoic Acid	C20:2	0.05	0.11	0.04	-	0.05	0.29	ns
Cis-8,11,14-Eicosetrienoic Acid	C20:3n6	0.05	0.42	0.06	0.13	0.06	0.37	ns
Arachidonic Acid	C20:4n6	0.49	0.36	0.56	0.10	0.54	0.62	ns

SFA = Saturated Fatty Acid; MUFA = Mono Unsaturated Fatty Acid; PUFA = Poly Unsaturated Fatty Acid; n = samples size; Sig. = significancy; * = significant (P<0.05); ns = non significantly (P>0.05)

of fatty acids for energy utilization. Diversity of LPL gene SNP ss65478732 was associated with two poly unsaturated fatty acids i.e. dihomo-gamma-linolenic acid and arachidonic acid (Sevane *et al.*, 2013)

Oleic was most abundant fatty acid in this study, followed by palmitic, stearic, myristic, palmitoleic and linoleic. Several studies have reported that oleic is the major fatty acid was found in sheep (Cloete *et al.*, 2004; Costa *et al.*, 2009; Gallardo *et al.*, 2011; Borys *et al.*, 2012;

Popova, 2014). Oleic acid is formed by stearic acid by activity of *stearoyl CoA desaturase* (Wood *et al.*, 2008). Oleic play an important role in the enhancement of meat characteristics, cell elasticity, tenderness and palatability of meat (Smith *et al.*, 2009). While Purbowati *et al.* (2005) was reported the most abundant fatty acids of local male sheep were linoleic, attended oleic, palmitic, stearic, linolenic and palmitoleic.

Guo *et al.* (2014) reported that on marbling fat quality in cattle and sheep was determined by

polygenes and there expression influenced by environmental factors such as diseases and feed. According to Sondakh *et al.* (2012), feed has medium chain fatty acids 1.0-1.5% content could be increased unsaturated fatty acids (9.05%-17.96%) and decreased saturated fatty acids (6.59%-11.88%). The major genes which are potential include *fatty acid binding protein 3* (FABP3), *peroxisome proliferator activated receptor gamma* (PPARG), *diacyl glycerol o-acyltransferase* (DGAT1), *acetyl CoA carboxylase alpha* (ACACA), *fatty acid synthase* (FASN), *fatty acid binding protein 4* (FABP4), *carnitine palmitoyltransferase 1 binding* (CPTIB), *steoryl-CoA desaturase* (SCD), *peroxisome proliferator activated receptor gamma coactivated 1 alpha* (PPARGC1A), *activating transcription factor 4* (ATF4), *SREB cleavage activating protein* (SCAP) ((Narukami *et al.* 2011; Sevane *et al.*, 2013; Guo *et al.*, 2014).

CONCLUSION

The results were presented 3 SNPs of LPL gene insertion g.26>C and g.27> G and a mutation c.192T>C on garut sheep and only one SNP insertion g.26>C/G on sumatera thin-tail ed sheep. Polymorphisms of LPL gene at base c.192 on garut sheep was associated with saturated fatty acid *heneicosanoic*, whereas TT genotype (0.04%) had higher percentage than CC (0.03%) and CT (0.02%). This designed primer has the potential as genetic marker of marbling quality of lamb garut sheep on marker assisted selection (MAS).

REFERENCES

AOAC (Association of Official Analytical Chemist). 2005. Official Methods of Analysis of the Association of Official Analitycal Chemist International. 18th ed. Association of Official Analytical Chemists, Arlington.

Bonnet, M., C.Leroux, Y. Faulconnier, J-F Hocquette, F. Bocqueier, P. Martin and Y. Chilliard. 2000. A lipoprotein lipase activity and mRNA are up-regulated by refeeding in adipose tissue and cardiac muscle of sheep. Am. Soc. Nutr. Sci.:749-756

Borys, B., J. Oprzadek, A. Borys and M. Przegalinska-Goraczkowska. 2012. Lipid profile of intramuscular fat in lamb meat. Anim. Sci. Papers and Reports. 30(1):45-56.

Chizzolini, E., Zanardi, V. Dorigoni and S. Ghidini. 1999. Calorific value and cholesterol content of normal and low-fat meat and meat product. Food Sci. Technol. 10:119-128.

Cloete, J.J.E., L.C. Hoffman, S.W.P. Cloete and J.E. Fourie. 2004. A comparison between the body composition, carcass characteristics and retail cuts of south african mutton merino and dormer sheep. S. Afr. J. Anim. Sci. 34 (1):44-51

Costa, R.G., A.S.M. Batista, P.S. Azevedo, R.C.R.E. Queiroga, M.S. Madruga and J.T.A Filho. 2009. Lipid profile of lamb meat from different genotypes submitted to diets with different energy levels. R. Brass. Zootec. 38(3):532-538

Crisa, A., C. Marchitelli, L. Pariset, G. Gontarini, F. Signarelli, R. Napolitano, G. Catillo, A.Valentini and B. Moili. 2010. Exploring polymorphisms and effects of candidate genes on milk fat quality in dairy sheep. J. Dairy Sci. 93:3834-3845.

Ding, X.Z., C.N. Liang, X. Guo, C.F. Xing, P.J. Bao, M. Chu, J. Pei, X.S. Zhu and P. Yan. 2012. A novel single nucleotide polymorphism in exon 7 of LPL gene and its association with carcass traits and visceral fat deposition in yak (*Bos grunniens*) steers. Mol. Biol. Rep. 39:669-673

Dunner, S., N. Sevane, D. Garcia, O. Cortes, A. Valentini, J.L.Williams, B. Mangin, J. Canon, H. Leveziel, and T.G. Consortium. 2013. Association of genes involved in carcass and meat quality traits in 15 European bovine breeds. Livest. Sci. 154: 34-44

Gollardo MA, R Pulido, C Gallo. 2011. Fatty acid composition of longissimus dorsi muscle of suffolk down lambs fed on different dryland forages. *Chileanjar* 71(4):566-571.

Guo, B., K. Kongsuwan, P.L.G. Wood, G. Zhou, W. Zhang and B.P. Dalrympe. 2014. A gene expression estimator of intramuscular fat percentage for use in both cattle and sheep. J. Anim. Sci. Biotechnol. 5(35):1-12.

Hall, T. 2011. BioEdit; An important software for molecular biology. GERF Bull. Biosci. 2(1): 60-61.

Hocquette, J. F., G. Renand, H. Levéziel, B. Picard1, I.C. Malek. 2006. The potential benefits of genetics and genomics to improve beef quality – a review. Anim. Sci. Papers and Reports. 24 3):173-189

Holmes, R.S., J.L. Vandeberg and L.A. Cox. 2011. Comparative studies of vertebrate lipoprotein lipase: a key enzyme of very low density lipoprotein metabolisms . Comp. Biochem. Physiol. Part D. Genomics Proteomics 6(2):224-234.

Karamichou, E., R.I. Richardson, G.R. Nute, K.P. Gibson and S.C. Bishop. 2006. Genetic analysis and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. J. Anim. Sci. 84:3228-3238.

Kumar,S., K. Tamura and M.Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetic analysis and sequence alignment. J. Brief. Bioinform. 5(2):150-163.

Lee, S.H. and K.L. Hossner. 2002. Coordinate regulation of ovine adipose tissue gene expression by propionate. J. Anim Sci 80: 2840-2849.

Mas'ud, M.S. 2012. Produksi, sifat fisik dan sifat kimia daging domba yang diberi ransum mengandung limbah udang. Dissertation. Bogor Agricultural UniversityBogor

Narukami, T., S. Sasazaki, K. Oyama, T. Nogi., M. Taniguchi and H. Mannen. 2011. Effect of DNA polymorphisms related to fatty acid composition in adipose tissue of Holstein cattle. J. Anim. Sci. 82:406-411

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press: New York.

Popova, T. 2014. Fatty acid composition of longissimus dorsi and semi membranosus muscles during storage in lambs reared in doors and on pasture. Emi.J.Food Agric. 26(3):302-308.

Purbowati, E., E. Baliarti, S.P.S. Budhi and W. Lestariana. 2005. Profil asam lemak daging domba lokal jantan yang dipelihara di pedesaan pada bobot potongan dan lokasi otot yang berbeda. Bull. Petern. 29(2):62-70

Ren, M.Q., J. Wegner, O. Bellmann, G.A. Brockmann, F. Scheider, F. Teuscher and K. Ender. 2002. Comparing mRNA levels of genes encoding leptin, leptin receptor and lipoprotein lipase between dairy and beef cattle. Dom. Anim. Endocrin. 23:371-381

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Clonning: A laboratory manual. 2nd Ed. Cold Spring Harbor Laboratory Pr., USA.

Sevane N, E Armstrong, O Cortes, P Wienar, R Pong Wong, S Dunner and GQ Consortium. 2013. Association of bovine meat quality traits with gene included in the *PPARG* and *PPARGC1A* networks. Meat Sci. 94:328-335

Smith S., B. Gill, C.A. Lunt DK and Brooks, M.A. 2009. Regulation of fat and fatty acids composition in beef cattle. Asian-Aust. J. Anim. Sci. 22(9):1225-1233

Sondakh, E.H.B., L.M.Yusiati, H.Hartadi and E.Suryanto. 2012. The effect of methanogenic inhibitor feed on propionic acid and lamb meat chemical quality. J. Indonesian Trop. Anim. Agric. 37(3):183-188

Wang, H and R.H.Eckel. 2009. Lipoprotein lipase: from gene to obesity. Am. J. Physiol. Endocrinol. Metab. 297:271-288

Williams, J.L. 2008. Genetic control of meat quality traits. In: Meat Biotechnology. (F.Toldra, ed.). Springer Sci., LLC.P. 21-60

Wong, D.W.S. 1989. Mechanism and Theory in Food Chemistry. An AVI Book. New York.

Wood, J.D., M. Enser, A.V. Fisher, G.R. Nute, P.R. Sheard, R.I. Richardson, S.I. Hughes and F.M. Whittingto. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343-358.

Yeh, F. C., R. C. Yang and T. Boyle. 1999. POPGENE Version 1.31: Microsoft Window-Based Freeware for Population Genetic Analysis. Canada University of Alberta (CAN). Edmonton, AB.