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Polymorphism and Expression of HSD17β13 Gene and Its Association with Lamb Quality of Indonesian Sheep

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Abstract. HSD17β13 (17beta 13-Hydroxysteroid dehydrogenases) are important enzymes in steroid metabolism. This study investigated the polymorphisms and expression of the HSD17β13 gene in lamb quality traits in Indonesian sheep. A total of 200 of rams of seven breeds administered in the study were Javanese fat-tailed (JFT), Javanese thin-tailed (JTT), Garut sheep (GS), Jonggol sheep (JS), Garut composite sheep (GCS), Compass agrinac sheep (CAS), Barbados cross sheep (BCS) aged 10-12 months and weighed 20-30 kg. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and MspI enzyme restriction were performed to identify the polymorphism of the HSD17β13 gene. The lamb quality traits were measured for physical quality, flavor, odor, and fatty acid composition. The results showed that the HSD17β13 gene was polymorphic and had three genotypes i.e., CC, CT, and TT. The highest genotype frequency was the CC when compared to the other genotypes. The allele frequency of HSD17β13 gene was not in Hardy Weinberg Equilibrium. The polymorphism of HSD17β13 gene was significantly ($P<0.05$) associated with the fatty acid composition and Saturated Fatty Acid (SFA), i.e., tridecanoic acid (C13:0), arachidic acid (C20:0), henecosanoic acid (C21:0), and tricosanoic acid (C23:0). The polymorphism was also significantly associated with the Monounsaturated Fatty Acid (MUFA) i.e. ginkgoleic acid (C17:1) and nervonoic acid (C24:1), and Polyunsaturated Fatty Acid (PUFA) i.e. eicosedienoic acid (C20:2). The level of expression of the HSD17β13 gene based on quantitative real time-PCR analysis results was not significantly different ($P>0.05$) among genotypes for lamb quality traits. It can be concluded that the polymorphism of HSD17β13 gene was associated with the fatty acid composition of lamb in Indonesian sheep.

Keywords: fatty acid, flavor odor, HSD17β13 gene, lamb, meat quality

Abstrak. HSD17β13 (17beta 13-Hydroxysteroid dehydrogenases) merupakan enzim yang berperan penting dalam metabolisme steroid. Penelitian ini bertujuan menginvestigasi keragaman dan ekspresi gen HSD17β13 pada kualitas daging domba Indonesia. Total ternak yang digunakan sebanyak 200 ekor domba jantan berumur 10-12 bulan dengan bobot badan 20-30 kg yang berasal dari tujuh rumpun berbeda, yaitu domba ekor gemuk (DEG), domba ekor tipis (DET), domba garut (DG), domba jonggol (DJ), domba komposit garut (DKG), domba compas agrinak (DCA), dan domba barbados cross (DBC). Identifikasi keragaman gen HSD17β13 menggunakan metode *Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)* dengan enzim restriksi *MspI*. Parameter kualitas daging yang diukur diantaranya, yaitu sifat fisik daging domba, bau, rasa, dan komposisi asam lemak. Hasil penelitian menunjukkan bahwa gen HSD17β13 memiliki keragaman genotipe yang ditandai dengan adanya tiga genotipe berbeda, yaitu CC, CT, dan TT. Genotipe CC memiliki nilai frekuensi tertinggi dibandingkan genotipe lainnya. Frekuensi alel gen HSD17β13 pada populasi penelitian ini tidak berada dalam kesetimbangan Hardy-Weinberg. Keragaman gen HSD17β13 berasosiasi secara nyata ($P<0.05$) dengan komposisi asam lemak, diantaranya asam lemak jenuh, yaitu asam tridekanoat (C13:0), asam arakidat (C20:0), asam henekosanoat (C21:0), dan asam trikosanoat (C23:0); asam lemak tak jenuh tunggal, yaitu asam ginkgoleat (C17:1) dan asam nervonat (C24:1); asam lemak tak jenuh ganda, yaitu asam eikosadinoat (C20:2). Tingkat ekspresi gen HSD17β13 berdasarkan hasil analisis *quantitative real time-PCR* tidak menunjukkan perbedaan yang nyata ($P>0.05$) antara ketiga genotipenya dalam sifat kualitas daging domba. Keragaman gen HSD17β13 dapat disimpulkan memiliki peranan penting dalam sifat komposisi asam lemak Indonesia.

Kata kunci: asam lemak, rasa dan bau, gen HSD17β13, domba, kualitas daging

Introduction

Lamb consumption in Indonesian is steadily rising from 131.41 thousand tons in 2019 to 132.96 thousand tons by May 2020. It triggers lamb production from 129.17 to 130.20 thousand tons and import from 2.27 to 2.80 thousand tons (OECD-FAO, 2020). Lamb demand has increased in global market since

2003 and lamb consumption is predicted to reach 16,382 thousand tons by 2025. Therefore, it takes prolific measures to overcome the shortage of lamb supply by increasing meat production through selection and crossing programs (Mortimer and Przybylski, 2016).

Molecular-based selection has been conducted through several studies related to the genes that affect meat quality traits. A scientific approach using Quantitative Trait Loci (QTL) has been carried out since 1990 but only 789 studies on QTL in sheep were found up to 2013 (Zhang et al., 2013), implying that these studies are still relatively rare compared to those of cattle (Zlobin et al., 2019). Walling et al. (2004) reported that chromosome 18 in Suffolk and Texel sheep were related to muscle area. QTL for carcass composition in Merino cross sheep found on chromosomes 1-3, 6, 7, 9-11, 14, 16, 18, 19, and 23 (Cavanagh et al., 2010). Karamichou et al. (2006) reported a significantly association of QTL that affected carcass composition and lamb quality of Scottish Blackface sheep and the QTL were found on chromosomes 1, 2, 3 and 5.

Genome Wide Association Study (GWAS) is another approach to identify particular genes candidate (Zhang et al., 2013; Duijvesteijn et al., 2018). Wang et al. (2015) using the GWAS found three genes candidate (ALDOA, STK32B, FAM190A) in OAR6 and OAR24 associated with the quality of lamb marbling. The GWAS could describe the QTL associations and genes candidate that affect lamb quality traits but are unable to describe the expression level and role as well as the mechanism of the gene.

The transcriptome approach was discovered to assess the level of gene expression. Transcriptomics examines the relationship between the genome and cell function. RNA sequencing (RNAseq) is one of several transcriptome scientific approaches (Putranto, 2013). Sun et al. (2016) reported that the expression of the MRFs, GXP1, and STAC3 genes has an important role in muscles growth and the development process that affects meat production. Jo et al. (2016) reported that the HSD17β13 gene was associated with pork quality in Berkshire breed.

Information regarding to the HSD17β13 genes in sheep is still rare, especially related to the lamb quality in Indonesian sheep. The HSD17β13 gene is located on chromosome 6 in sheep. The HSD17β13 gene is one of the 17β-HSD families that mediate the physiological function of androgens and estrogens by catalyzing the inter conversion forms of active and inactive steroids (Baker, 2001). The expression of 17β-HSD protein is regulated by steroids. Induction of 17βHSD causes high androstenone levels in the liver (Moe *et al.,* 2008) and it is a protein related to lipids (Horiguchi *et al.,* 2008). A liver contains a lot of protein that regulates androstenone and plays a role in homeostatic steroids so that the liver has an association with the smell and other properties of meat. Therefore, the aim of this study was to characterize the HSD17β13 gene and describe its association on lamb quality.

Materials and Methods

Animals and sample collection

A total of 200 Indonesian rams were used to identify polymorphisms of the HSD17β13 gene consisted of 20 of Javanese fat-tailed (JFT), 37 of Javanese thin-tailed (JTT), 20 of Garut sheep (GS), 21 of Jonggol sheep (JS), 34 of Garut composite sheep (GCS), 35 of Compass agrinac sheep (CAS), and 33 of Barbados cross sheep (BCS). All sheep were reared on different farms, were fed ad libitum, and maintained under similar rearing conditions. Some of the total rams were slaughtered at around 10-12 months of age with the body weight of 20-30 kg to analyzed lamb quality. The whole blood samples and a small amount of liver tissues were taken and put into 1.5 mL tubes which containing DNA/RNA stabilization solution. After slaughtered, the carcasses were frozen at -20 °C. Twenty-four hours after storing time period,

the carcasses were analyzed for meat quality using the bicep femoris samples, while for fatty acid analysis using the longissimus dorsi muscles. The samples for meat quality and fatty acid analyses were wrapped in plastic and kept frozen at -20 °C prior to the analysis.

Measurement of lamb quality traits

The lamb quality traits measured were pH, tenderness, drip loss, and cooking loss. The pH was measured in the longissimus dorsi muscle using a pH meter. The meat tenderness was assessed by using a sheer force. Drip loss was measured by calculating the amount of water loss after being pressured on filter paper for 5 minutes. The cooking loss was calculated as the difference between the weight before and after cooking and expressed as a percentage of the initial weight.

Measurement of flavor, odor, fatty acid composition

The lamb odor and flavor determination were referring to Listyarini *et al.* (2018) method. The fat content was measured on the longissimus dorsi muscle by using the Soxhlet extraction method. Fatty acid compositions were separated and determined using GC-2010 Plus-Shimadzu based on the AOAC 2005 procedure. The amount saturated fatty acid (SFA), unsaturated fatty acid (UFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), as well as MUFA/SFA and PUFA/ SFA ratios, then were calculated for determining the fatty acid composition.

DNA Extraction, PCR Amplification, and Genotyping using PCR-RFLP

Genomic DNA was extracted from the whole blood samples using a Genomic DNA Mini Kit (Geneaid Biotech, Taiwan). The primers used for PCR amplification were designed by MEGA 7.0 (Table 1). The premix for PCR amplification consisted of 1 µl of DNA samples, 0.4 µl of primers, 6.1 µl of MyTaq HS Red Mix, 7.5 µl of nuclease water. The amplification process of PCR using the AB Systems machine began with the pre-denaturation step at 95 °C for 1 min. The second phase consisted of 35 cycles, each cycle consisting of a denaturation process at 95 °C for 15 s, primer annealing at 59 °C, and DNA extension at 72°C for 15 s. The last step was the final extension at 72 °C for 1 min. The HSD17β13 gene polymorphisms were genotyped by PCR-RFLP technique using MspI enzyme restriction. The amplified and digested DNA fragments were separated on 2% agarose gel electrophoresis and visualized under UV Transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA).

HSD17β13 gene expression analysis

RNA extraction and cDNA reconstruction

Total RNA was isolated from each longissimus dorsi sample using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) based on the manufacturer's protocol. The first stage of the procedures was

Table 1. Primer sequences for PCR-RFLP and qRT-PCR

Gene Name	Primer sequence	Application	Size of primer (bp)	TA (°C)
HSD17β13	>F: 5'-ATA CTT TGG CCA CCT GAT GC-'3 >R: 5'-TTT CAG GAA GCA AGG ACC TG-'3	PCR-RFLP	427	60
HSD17β13 mRNA	>F: 5' - CCC ATC AAC ACC TAG AAT GC -3' >R: 5' - CAG CAG TGA TTC CAA GTA GG -3'	qRT-PCR	178	61
GAPDH	>F: 5' - GAG AAA CCT GCC AAG TAT GA -3' >R: 5' - TAC CAG GAA ATG AGC TTG AC-3'	qRT-PCR	203	62
β-Actin	>F: 5' - GAA AAC GAG ATG AGA TTG GC-3' >R: 5' - CCA TCA TAG AGT GGA GTT CG-3'	qRT-PCR	194	62

total RNA analysis using RNase-Free DNase (Promega) and followed by the quantification of RNA using a spectrophotometer (NanoDrop, ND8000, Thermo Scientific). The quality of RNA was analyzed using Aligent 2100 Bioanalyzer and RNA Nano 6000 Labchip kit (Agilent Technologies). RNA extract was transcribed into complementary DNA (cDNA) using a First Strand cDNA (Thermo Scientific, Lithuanian, EU) Transcriptor Synthesis kit.

The quantitative real-time PCR

Quantification of cDNA was performed using Fast SYBR® Green Master Mix (Roche, Mannheim, Germany) on Applied Biosystems® StepOnePlusTM Real-Time PCR Systems (Analytic Jena Engine, Germany). Gene-specific primers for the qRTPCR (Table 1) were designed using MEGA 7.0. The reactions comprised 10 µL, including 5 µL of SYBR® Green Master Mix, 0.5 µL of each forward and reverse primers, 2 µL of cDNA (50 ng/µl), and 2 µL of nuclease-free water. Each sample was analyzed in duplicate and relative gene expression was calculated according to the efficiency corrected (Pfaffl, 2001). The geometric mean of the Ct values of two housekeeping genes (GAPDH and β-Actin) for normalization of the target genes were further used for mRNA expression profiling (Listyarini et al., 2018).

Data analysis

Allele and genotype frequencies

Genotype and allele frequencies were determined by calculating the number of certain genotypes in each population, while the allele frequency was determined by calculating the ratio of an allele to the total allele at a locus in the population. The genotype and allele frequencies were calculated using Nei and Kumar's (2000) formula and the Hardy-Weinberg equilibrium was determined by using Hartl and Clark (1997) procedures.

Meat quality, flavor, odor, and fatty acid

The association the SNP g.101 689 913 C>T of HSD17β13 gene with lamb quality traits, flavor, odor, and fatty acid were performed using the Student T-test procedure of Minitab® 18 Software (Akers, 2018) with the following formula (Walpole, 1995):

$$t = \frac{(X_1 - X_2)}{\delta^2 \frac{\sqrt{1}}{n_1} + \delta^2 \frac{\sqrt{1}}{n_2}} \delta^2$$

where: X1 and X2= the average traits for genotype 1 and 2; n1 and n2= individual number of genotype 1 and 2; and δ = the combined of standard deviation.

HSD17β13 gene expression analysis

Data from the relative quantification of PCR were transformed by using the difference between target gene and geometric mean of the references gene (ΔCT), as described by Silver *et al.,* (2006) using the following formula:

$$\Delta CT = Ct_{\text{target gene}} - Ct_{\text{reference gene}}$$

Results and Discussion

Variation of genotypes in HSD17β13 gene

The SNP g.101 689 913 C>T of the HSD17β13 gene was successfully genotyped by PCR-RFLP showed polymorphism in Indonesian sheep (Figure 1). The genotype variations found were the CC (279, 148 bp), CT (148, 279, and 427 bp), and TT (427 bp) genotypes. The genotype frequency of CC was higher than those of CT and TT genotypes (0.87 vs 0.10 and 0.03). Based on the X2 analysis the HSD17β13 allele was not in Hardy-Weinberg Equilibrium (X2 > 3.84) (Table 2). It was caused by a higher proportion of the CC genotypes. This result was inconsistent with that by Jo *et al.* (2016) who reported that the allele of the HSD17β13 gene in pig was in Hardy Weinberg Equilibrium. The different results could be due to selection, non-random mating, and the small number of sheep used in this study.

Table 2. The number of animals per genotype and allele frequency of each snp

Sample	N	Genotype Frequency			Allele Frequency		X^2
		CC	CT	TT	C	T	
Indonesian sheep	200	0.87 (174)	0.10 (20)	0.03 (6)	0.92	0.08	20.56*

Note: N = number of samples, (..) = number of genotypes which CC, CT, TT genotype, χ2 table = 3.84

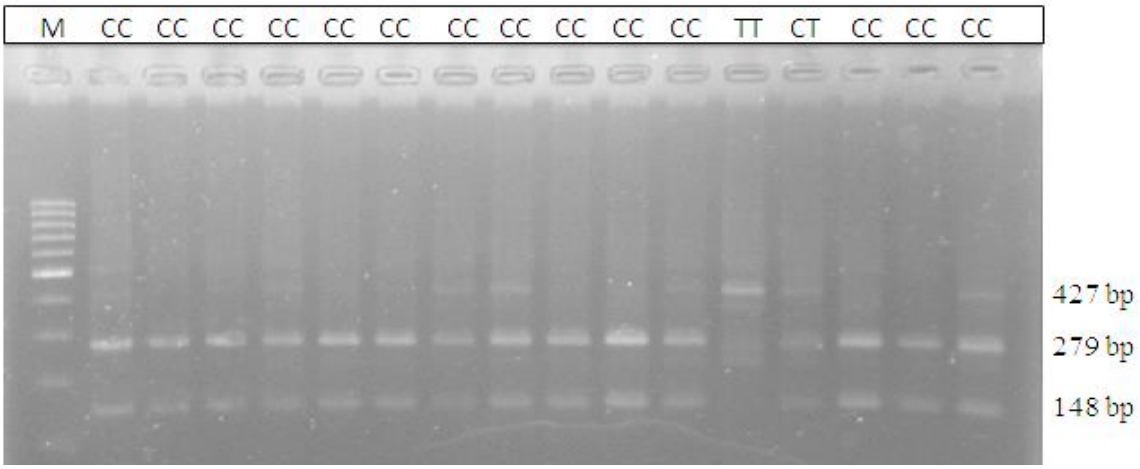

Figure 1. PCR-RFLP product of the SNP g. 101 689 913 C>T in the HSD17β13 gene. M= 100 bp ladder size; CC, CT, and TT= genotypes.

Table 3. Genotype and association analysis of HSD17β13 genes with lamb quality

Parameters	Genotype of HSD17β13 gene ($\bar{x}$ ± Std Dev)			P value		
	CC (n =70)	CT (n = 8)	TT (n = 2)	CC vs CT	CC vs TT	CT vs TT
Shear force (kg/cm^2)	3.57 ± 0.91	4.22 ± 0.98	3.35 ± 1.62	0.111^{ns}	0.761^{ns}	0.599^{ns}
pH value	6.39 ± 0.64	6.10 ± 0.40	6.19 ± 0.71	0.098^{ns}	0.879^{ns}	0.892^{ns}
Drip loss (%)	47.89 ± 6.66	50.30 ± 3.98	38.11 ± 12.95	0.163^{ns}	0.480^{ns}	0.414^{ns}
Water holding capacity (%)	27.08 ± 2.71	27.89 ± 3.00	24.09 ± 3.85	0.489^{ns}	0.472^{ns}	0.418^{ns}

Note: $\bar{x}$ = means of meat quality values; Std Dev= standard deviation; and vs: versus. Ns (not significantly different at 5%. Numbers shown in parentheses are the number of individuals with the specified genotype.

Correlation among genotypes variation and traits

Association with lamb quality

The result showed that the HSD17β13 gene was not significantly associated with lamb quality traits (Table 3). These results were inconsistent with Jo *et al*. (2016) who found families of the HSD17β13 had been associated with meat quality consist of drip loss, backfat thickness, and carcass weight. On the other hand, Karisa *et al*. (2013) reported that the HSD17B12 gene was associated with quality grade and marbling. In this study, the CT genotypes had a lower pH value but higher sheer force when compared to other genotypes. Conversely, the drip loss and water holding capacity parameters of the TT genotypes were lower than other genotypes (Table 3). The lamb quality is affected by polygenes. Some of the genes that affected lamb quality were LEPR (Azizah et al., 2020) and DGAT1 (Gunawan et al., 2019).

Association with flavor, odor, and fatty acid composition

The HSD17β13 SNP g.101 689 913 C>T affected the fatty acid compounds and fat content. The saturated fatty acid (SFA) (tridecanoic acid (C13:0), arachidic acid (C20:0), henecosanoic acid (C21:0), and tricosanoic acid (C23:0)) of the TT genotype was lower than the CC and CT genotypes.

Table 4. Genotype and association analysis of HSD17β13 genes with fatty acid traits

Parameters	Genotype of HSD17β13 gene ($\bar{x}$ ± Std Dev)			P-Value		
	CC (n=85)	CT (n=10)	TT (n=5)	CC vs CT	CC vs TT	CT vs TT
Fat Content (%)	3.82 ± 3.28	3.36 ± 3.38	1.69 ± 1.58	0.697	0.037*	0.217
Saturated Fatty Acid (%)	45.98 ± 17.60	50.25 ± 19.93	27.90 ± 24.2	0.531	0.176	0.125
Oktanoic acid, C8:0	0.04 ± 0.11	0.02 ± 0.05	0.09 ± 0.21	0.416	0.590	0.494
Caprat acid, C10:0	0.26 ± 1.51	0.09 ± 0.05	0.06 ± 0.07	0.320	0.251	0.479
Lauric acid, C12:0	0.47 ± 0.49	0.62 ± 0.38	0.28 ± 0.23	0.244	0.178	0.055
Tridecanoic acid, C13:0	0.01± 0.01	0.01 ± 0.00	0.00 ± 0.01	0.000*	0.057	0.670
Myristic acid, C14:0	3.04 ± 1.71	3.55 ± 1.36	2.22 ± 2.08	0.294	0.437	0.251
Pentadeconoic acid, C15:0	0.51 ± 0.15	0.50 ± 0.16	0.33 ± 0.31	0.727	0.267	0.331
Palmitic acid, C16:0	18.65 ± 3.69	19.72 ± 4.62	12.28±10.34	0.496	0.240	0.199
Heptadecanoic acid, C17:0	0.93 ± 0.33	0.81 ± 0.16	0.55 ± 0.51	0.066	0.174	0.330
Stearic acid, C18:0	15.91 ± 5.37	16.53 ± 3.77	11.95±11.27	0.649	0.477	0.425
Arachidic acid, C20:0	0.13 ± 0.09	0.16 ± 0.11	0.05 ± 0.06	0.435	0.040*	0.031*
Henecosanoic acid, C21:0	0.12 ± 0.27	0.14 ± 0.23	0.01 ± 0.02	0.857	0.000*	0.127
Behenic acid, C22:0	0.06 ± 0.08	0.09 ± 0.11	0.05 ± 0.07	0.356	0.806	0.376
Tricosanoic acid, C23:0	0.09 ± 0.16	0.16 ± 0.29	0.01 ± 0.03	0.455	0.006*	0.148
Tetracosanoic acid, C24:0	0.04 ± 0.09	0.08 ± 0.13	0.02 ± 0.03	0.353	0.319	0.191
Unsaturated Fatty Acid (%)	26.31 ± 13.03	26.80 ± 14.52	14.62±16.59	0.921	0.197	0.206
Monounsaturated Fatty Acid (%)	29.16 ± 11.15	31.53 ± 12.46	12.35±14.94	0.577	0.069	0.048*
Myristoleinic acid, C14:1	0.14 ± 0.08	0.21 ± 0.20	0.08 ± 0.08	0.234	0.223	0.088
Palmitoleic acid, C16:1	1.56 ± 0.39	1.66 ± 0.48	0.87 ± 0.82	0.527	0.136	0.103
Ginkgoleic acid, C17:1	0.35 ± 0.35	0.24 ± 0.25	0.07 ± 0.16	0.230	0.013*	0.141
Oleic acid, C18:1n9c	24.15 ± 9.18	25.46 ± 10.43	18.83±13.83	0.645	0.099	0.082
Elaidic acid, C18:1n9t	2.91 ± 7.38	2.12 ± 4.70	4.47 ± 8.36	0.646	0.696	0.576
Cis-11-Eicosenoic acid, C20:1	0.02 ± 0.08	0.04 ± 0.08	0.00 ± 0.00	0.597	Nt	nt
Nervonoic acid, C24:1	0.09 ± 0.17	0.12± 0.11	0.02 ± 0.03	0.561	0.004*	0.026*
Polyunsaturated Fatty Acid (%)	8.33 ± 10.58	10.56 ± 12.09	2.26 ± 1.98	0.588	0.000*	0.064
Linoleic acid, C18:2n6c	2.42 ± 1.91	2.59 ± 1.50	1.07 ± 1.24	0.740	0.074	0.068
y-linolenic acid, C18:3n6	0.04 ± 0.07	0.07 ± 0.08	0.02 ± 0.04	0.385	0.302	0.154
Linolenic acid, C18:3n3	0.24 ± 0.23	0.23 ± 0.25	0.15 ± 0.28	0.859	0.508	0.618
Eicosedienoic acid, C20:2	0.05 ± 0.05	0.05 ± 0.02	0.02 ± 0.02	0.813	0.025*	0.043*
Homo-y linolenic acid, C20:3n6	0.07 ± 0.11	0.07 ± 0.09	0.03 ± 0.06	0.841	0.316	0.336
Arachidonic acid, C20:4n6	0.81 ± 1.29	1.19 ± 1.88	0.72 ± 0.97	0.545	0.848	0.530
Docosadienoic acid, C22:2	0.01 ± 0.03	0.01 ± 0.04	0.00 ± 0.00	0.685	Nt	nt
Eicosapentanoic acid, C20:5n3	0.13 ± 0.19	0.14 ± 0.14	0.19 ± 0.20	0.940	0.577	0.624
Docosahexanoic acid, C22:6n3	0.04 ± 0.07	0.03 ± 0.03	0.04 ± 0.08	0.592	0.940	0.800
Fatty Acid Total (%)	61.40 ± 27.60	60.50 ± 32.90	47.00±40.00	0.938	0.470	0.535
MUFA/SFA Ratio	0.63	0.62	0.44	-	-	-
PUFA/SFA Ratio	0.18	0.21	0.08	-	-	-

Note: nt= not tested using T-test due to very small amount detected; $\bar{x}$ = means of fatty acid composition; Std Dev= standard deviation; *= significantly different at 5%; and vs: versus. Numbers shown in parentheses are the number of individuals with the specified genotype.

The monounsaturated Fatty Acid (MUFA) (ginkgoleic acid (C17:1) and nervonic acid (C24:1)) and the polyunsaturated fatty acid (PUFA) (eicosadienoic acid (C20:2)) of the CT genotype were higher than CC and TT genotypes (Table 4). In general, the T-test showed that the individuals with heterozygous CT genotype were associated with higher SFA

and UFA when compared to other genotypes. The HSD17β13 gene belongs to a 15-member family that is involved in various metabolic processes including steroid hormones, fatty acids, cholesterol, and bile acids (Su *et al.,* 2019). The previous study result showed that the HSD17β13 was associated with lipid droplets (Su *et al.,* 2014; Abul *et al.,* 2018; Ma *et al*., 2019; Horiguchi *et al.,* 2008). Fatty acids are the major components of triacylglycerols, phospholipids, and other complex lipids. Many beneficial properties have been described for ginkgolic acid (C17:1) such as anti-tumoral (Fukuda *et al.,* 2009; Zhou *et al.,* 2010) and antibacterial (Hua *et al.,* 2017) effects as well as suppression of inflammation along with reduced COX-2 expression and PGE2 levels in human umbilical vein endothelial cells (Li *et al.,* 2018). Nervonic acid (C24:1) is a monounsaturated omega-9 fatty acid important in the biosynthesis of myelin in the brain, can be an indicator of brain maturation (Li *et al.,* 2019). Linoleic acid (LA) and related omega-6 fatty acids have been implicated in contributing to metabolic dysfunction. However, there is some evidence to support that certain omega-6 fatty acids, such as eicosadienoic acid (EDA), may play a beneficial role in inflammation (Weir *et al.,* 2018). The PUFA: SFA ratio is an indicator of healthy and important meat to lower the risk of coronary heart disease. The results of this study showed that the PUFA: SFA ratios for CC and CT genotypes were in health recommendation 0.18 and 0.21, respectively (Table 4). The ratio for PUFA: SFA that categorized as healthy is above 0.40 with a minimum limit of 0.12 (Wood *et al.,* 2008). The HSD17β13 gene of SNP g.101 689 913 C>T had no significant association with flavor and odor (Table 5). In Indonesian lamb quality, several genes that affected flavor odor and fatty acid composition were APOA5 (Gunawan et al., 2018), CYP2A6 (Listyarini et al., 2018), BHMT (Munyaneza et al., 2019), and CYP2E1 (Harahap et al., 2020).

HSD17β13 expression

The expression levels of HSD17β13 in liver tissues were determined by qRT-PCR (Figure 2). The level of expression of the HSD17β13 gene was not significantly different between genotypes because all sample sheep relatively had CC genotypes. A study by Jo *et al.* (2016) found that the families of HSD17β13 were HSD17B4 had a significant difference in mRNA expression for meat quality in pigs. The HSD17β13 gene belongs to the 17-Beta-hydroxysteroid dehydrogenase (17β-HSD) family which mediates the physiological function of androgens and estrogens by catalyzing the interconversion of active and inactive steroid forms (Baker 2001). HSD17β13 gene expression is highest in the liver and is a lipid-related protein (Horiguchi *et al.,* 2008).

Table 5. Genotype and association analysis of HSD17β13 genes with odor and flavor compounds

Flavor and odor compound (μg/g)	Genotype of HSD17β13 gene ($\bar{x}$ ± Std Dev)			P value
	CC (n=23)	CT (n=1)	TT (n=0)	
4-methyloctanoic (MOA)	0.13 ± 0.33	0.36 ± 0.00	0.00 ±.0.00	Nt
4-methylnonanoic (MNA)	0.39 ± 0.13	0.12 ± 0.00	0.00 ±.0.00	Nt
3-methylindole (MI)	0.27 ± 0.06	0.05 ± 0.00	0.00 ±.0.00	Nt
4-methylphenol (MP)	24.31 ± 2.96	3.34 ± 0.00	0.00 ±.0.00	Nt
Ethyloctanoic (EOA)	0.36 ± 0.45	0.47 ± 0.00	0.00 ±.0.00	Nt

Note: nt= not tested using T-test due to very small amount detected; $\bar{x}$ = means of fatty acid composition; Std Dev= standard deviation; and vs: versus. Numbers shown in parentheses are the number of individuals with the specified genotype.

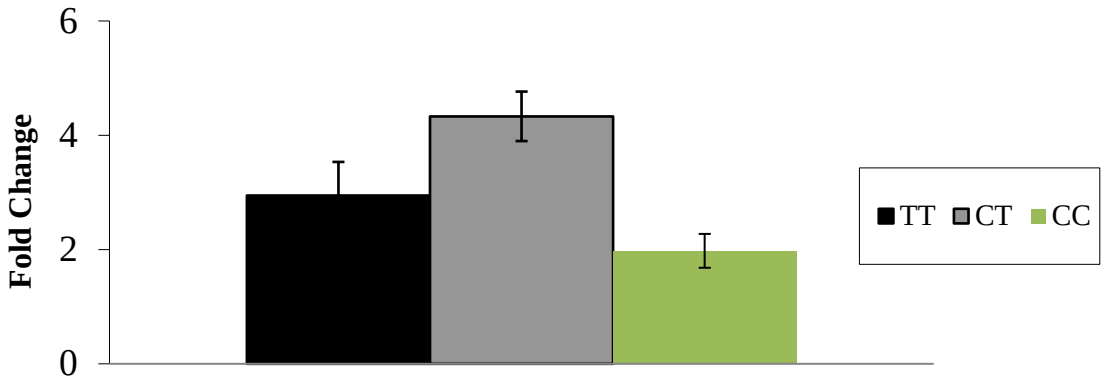

Figure 2. Expression levels of the HSD17β13 gene in liver tissues of Indonesian sheep.

Conclusions

The SNP g.101 689 913 C>T in the HSD17β13 gene was polymorphic in Indonesian sheep. The proportion of the HSD17β13 gene was not in Hardy Weinberg Equilibrium mainly due to the high CC genotype frequency. The HSD17β13 SNP g.101 689 913 C>T affected the fatty acid compounds and fat content. The saturated fatty acid (SFA) (tridecanoic acid (C13:0), arachidic acid (C20:0), henecosanoic acid (C21:0), and tricosanoic acid (C23:0)) of the TT genotype was lower than the CC and CT genotypes. The monounsaturated Fatty Acid (MUFA) (ginkgoleic acid (C17:1) and nervonic acid (C24:1)) and the polyunsaturated fatty acid (PUFA) (eicosadienoic acid (C20:2)) of the CT genotype were higher than CC and TT genotypes. The individuals with heterozygous CT genotypes were associated with higher SFA and UFA when compared to other genotypes. The CC genotype had a ratio PUFA/SFA value that categorized as good for health. The HSD17β13 gene expression was not significantly different among genotypes. It could be concluded that the polymorphism of the HSD17β13 gene might contribute to fatty acid composition in lamb meat quality.

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