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The Polymorphism and Expression of *CYP2E1* Gene and Its Relation to Carcass and Meat Quality of Indonesian Lamb

R. S. Harahap[a], R. R. Noor[b], & A. Gunawan[b,*]
[a]Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University
[b]Department of Animal Production and Technology, Faculty of Animal Science, IPB University
Jalan Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia
*Corresponding author: agunawan@apps.ipb.ac.id
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ABSTRACT

The aim of this study was to conduct the genotyping of the single nucleotide polymorphism (SNP) in g.50657948 T>G of the *CYP2E1* gene and its relation to the carcass and meat quality. A total of 200 Indonesian lambs consisted of 20 Javanese fat-tail sheep (JFTS), 37 Javanese thin-tail sheep (JTTS), 20 Garut sheep (GS), 21 Jonggol sheep (JS), 34 Garut composite sheep (GCS), 35 compass agrinac sheep (CAS), and 33 Barbados cross sheep (BCS) aged between 10-12 months old were used in this study. The polymorphism of the *CYP2E1* gene was characterized using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with NlaIII enzyme restriction. The *CYP2E1* gene found the three genotypes (GG, GT, and TT) and the allele frequencies were in Hardy Weinberg equilibrium. The *CYP2E1* gene was significantly associated with meat quality, i.e., pH value and tenderness, as well as fatty acid composition ($p<0.05$), i.e., saturated fatty acid (SFA): caprylic acid (C8:0), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C:22:0), and tricosylic acid (C23:0), monounsaturated fatty acid (MUFA): elaidic acid (C18:1n9t) and paullinic acid (C20:1); polyunsaturated fatty acid (PUFA): linoleic acid (C18:2n6c) and y-linolenic acid (C20:3n6). A gene expression analysis indicated that the GG genotype had the highest expression level. It could be concluded that the SNP g.50657948 T>G of the *CYP2E1* gene could be used for candidate marker-assisted selection to improve the carcass and meat quality of Indonesian lamb.

Keywords: CYP2E1 gene; carcass quality; meat quality; lamb

INTRODUCTION

The meat quality of lamb is very complex, involving many factors such as tenderness, fatty acid composition, odor, and flavor (Shija *et al.,* 2013; Listyarini *et al.,* 2018). Meat tenderness is determined by the amount and solubility of conective tissue, sarcomere, shortening during rigor development, and post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Koohmaraie & Geesink, 2006). On the other hand, intramuscular fat also indirectly influences meat tenderness. The amount of intramuscular fat (IMF) and fatty acid composition play major roles in the quality of meats, including sensory properties, healthy considerations, and flavor compounds (Hocquette *et al.,* 2010; Frank *et al.,* 2016).

The amount of saturated fatty acids (SFA) in the human diet contribute to sudden cardiac arrest (Chiuve *et al.,* 2012). Fatty acid content does not always have a negative impact on meat quality or consumer health. Unsaturated fatty acids have a good effect on health because they are more reactive and as antioxidants in the body. Unsaturated fatty acids are hydrocarbon chains containing at least one carbon–carbon double bond; monounsaturated fatty acids (MUFA) contain one double bond, and polyunsaturated fatty acids (PUFAs) contain many double bonds. The reduction of SFA sources and an increase in the consumption of MUFA and PUFA were associated with cardiovascular protection and reduce atherosclerosis (Blair & Dhilhon, 2014; Crandell *et al.,* 2016; Ooi *et al.,* 2015). The American Heart Association recommended human diets should include high levels of n-6 PUFAs that comprise at least 5%–10% of the energy intake (Harris *et al.,* 2009). Consumption of n-3 PUFAs could reduce the risk of cardiovascular disease (Mori *et al.,* 2014; Merino *et al.,* 2014; Auger *et al.,* 2016), and improves skin microvascular reactivity (Stupin *et al.,* 2018). In addition, both PUFA's play important roles in human health, including brain development (Li *et al.,* 2019), preventing metabolic dysfunction and inflammation (Weir *et al.,* 2018).

Selection programs for the improvement of meat quality based on phenotypic performance is difficult and expensive. However, the used of genetic markers could be combined with the phenotypic selection for improving the meat quality of lamb. From our previous

study, the *CYP2E1* gene showed a significant association with lamb odor and flavor in meat quality of lamb (Harahap *et al.,* 2020). The enzymes activities can be affected by mutations in coding regions of the genes and expressions of mRNA. This idea is supported by various studies involving polymorphisms of family of *CYP2E1* gene that has been reported to be related to the levels of androstenone in boar taint (Zadinova *et al.,* 2017), odor and flavor compounds in sheep (Listyarini *et al.,* 2018), and levels of skatole in boar taint (Morlein *et al.,* 2012). Furthermore, Lin *et al.* (2006) identified a G>A substitution in nucleotide c.1423 in pig which causes the substitution of amino acid alanine (Ala) with threonine (Thr). This mutation was shown to be responsible for a significant decrease in the expression of the *CYP2E1* protein and lower catalytic activity for the metabolism of skatole. However, no study related to the investigation of the association and expression of *CYP2E1* gene in Indonesian lamb. Therefore, the aim of this study was to identify the association and expression of *CYP2E1* genes with meat quality in Indonesian lamb.

MATERIALS AND METHODS

Animals

This experimental procedure was approved by the 'Institutional Animal Care and Use Committee (IACUC)" issued by IPB University (approval ID: 117-2018 IPB)**.** Association analyses of SNP in the *CYP2E1* gene with meat quality of lamb used a total of 200 lambs consisted of 20 Javanese fat-tail sheep (JFTS), 37 Javanese thin-tail sheep (JTTS), 20 Garut sheep (GS), 21 Jonggol sheep (JS), 34 Garut composite sheep (GCS), 35 compass agrinac sheep (CAS), and 33 Barbados cross sheep (BCS). All sheep were maintained in a group caged and were fed *ad libitum* with fattening feed. The rams were slaughtered in a commercial abattoir at 10–12 months old with an average body weight of 20–25 kg. All rams were slaughtered using the normal commercial procedure as described by Dagong *et al.* (2012). The whole blood samples were taken for the identification of polymorphisms in *CYP2E1* gene and the *longissimus dorsi* samples were taken for fatty acid analysis, liver tissues for gene expression, and *bicep femoris* for lamb quality. The samples were put in an ice flask and were stored at a temperature of -20 ^{o}C.

Carcass and Meat Quality Measurements

The carcass traits measured were live weight, weight of hot carcass, carcass length, and percentage of carcass. Meat quality variables were analyzed, including pH, cooking loss (CL), tenderness, and water holding capacity (WHC). The meat quality measurements refer to the method used by Dagong *et al*. (2012). The pH was measured 24 hours post mortem using pH meter, while cooking loss and tenderness were analyzed using *bicep femoris* sample according to the fiber orientation. The samples were boiled in a waterbath at 80 °C for approximately 20 min. Brickshaped cuts, each at the size of 2.5 × 2.5 × 3.0 cm, were made to measure tenderness using the Warner Bratzler Shear Force (WBSF) by measuring the amount of strength (kg/cm^2) needed to cut the meat cores. Cooking loss measurements (in %) were calculated as the differences in weights of the samples before and after cooking. The water holding capacity of meat was determined by the filter paper press method. A total of 200 mg meat of lamb were put on the Whatman paper and then placed between two slides where a weight of 100 g was placed on the top slide for 5 min to exert downward force and release water from the meat. The water released from the meat was wetting the paper, and the boundary of that wetted area was marked using a sharp pencil and was measured and reported in a percentage ratio of the diameter of the meat to the diameter of the water wetted paper.

Fatty Acid Analysis

The loin samples were used for fatty acid (FA) analyses. The FA composition was quantified using gas chromatography GC-2010 Plus-Shimadzu according to AOAC 2005. GC-MS-based analytical methods for fatty acid analysis consisted of three steps: (1) extraction of the fatty acids from the sample matrix. Total lipids in each sample were extracted using chloroform-methanol at a ratio of 2:1 (v/v); (2) derivatization of the fatty acid using BF3-methanol to be the fatty acid methyl esters (FAMEs); and (3) GC-MS analysis for FAMEs. The fatness traits were expressed as a proportion of the total FAs included fat content, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA).

Genomic DNA Extraction, PCR Amplification, and PCR-RFLP

Genomic DNA was isolated from whole blood samples using a Genomic DNA Mini Kit (Geneaid Biotech, Taiwan). A pair of primers (F:5'-CCCAGTCATCAGAGTCAGTA -3' and R: 3' -GCATACAGTGGTTTTCCTGG- 5') were designed by using MEGA 7 *Software* and used to amplify the *CYP2E1* gene based on the ovine genome sequence (NCBI accession NC_019479.2). The SNP g. 50657948 T>G of *CYP2E1* used in this study refers to Harahap *et al.* (2020). The final 401-bp amplicon was added in 15 μL PCR mixture containing 1 μL of DNA samples, 0.4 μL of primers, 6.1 μL of MyTaq HS Red Mix, and 7.5 μL of nuclease water. The PCR amplification using AB System machine with five processes consisted of predenaturation for 1 min at 95 ^{o}C, followed by 35 cycles of denaturation for 15 s at 95 ^{o}C, annealing for 15 s at 60 ^{o}C, and extension for 10 s at 72 ^{o}C, and a final extension for 1 min at 72 ^{o}C. The PCR products were detected by 1.5% agarose gel electrophoresis. Therefore, the *CYP2E1* gene was genotyped using NlaIII enzymes by PCR-RFLP for 4 h at 37 ^{o}C. The digested product was separated using 2% agarose gel. The fragments were visualized under UV Transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA). The PCR-RFLP product consisted of TT: 138 bp, 263 bp, GG: 401 bp, and GT: 138 bp, 263 bp, and 401 bp.

RNA Extraction and Reverse Transcriptase PCR

The liver tissue was used for RNA extraction. The collected tissue was extracted using the Rneasy Mini Kit (Qiagen) reagent based on the manufacturing protocol. Reverse transcriptase PCR was performed by transcribing RNA extract into complementary DNA (cDNA) using a First Strand cDNA (Thermo Scientific, Lithuanian, EU) Trancriptor Synthesis kit.

Expression Analysis by Quantitative Real Time PCR (qRT-PCR)

Since the association study showed significantly with lamb tenderness, we measured mRNA expression of *CYP2E1* using qRT-PCR**.** The primers were designed based on the sequences of ovine *CYP2E1*, GAPDH, and β-actin (Table 1). qRT-PCR was performed using the SYBR Green Premix. The reaction volume was 10 μL, including 5 μL of SYBR Green Premix, 0.5 μL each of forward and reverse primers, 2 μL of cDNA, and 2 μL of RNase-free ddH_2O. The reaction procedure was as follows: predenaturation at 95 ˚C for 30 s, PCR (analysis mode: quantitative) at 95 ˚C for 5 s and at 60 ˚C for 30 s as much 35 cycles, melting (analysis mode: melting curve) at 95 ˚C for 5 s, at 60 ˚C for 1 min, and cooling at 50 ˚C for 30 s. Fold change value were analyzed by calculating the difference between the cycle threshold (CT) value of target and the CT value of reference CT from each sample. The means of fold change value per genotype were analyzed by using Minitab® 18 Software.

Statistical Analysis

Analysis for allele and genotype frequencies. The allele, genotype frequencies, and Hardy–Weinberg equilibrium status in all sheep populations (JFTS, JTTS, GS, JS, GCS, CAS, and BCS) were calculated by the following formula:

Allele and genotype frequencies (Nei & Kumar 2000):

$Xii = nii / N$ $Xi = (2nii + \sum nij) / 2N$

where Xii was the frequency of ii genotype (GG, GT, and TT); Xi was the frequency of i allele (G and T); nii was the number of the sample of ii genotype; nij was the number of the sample of ij genotype, and N was the population size.

Hardy Weinberg Equlibrium (HWE) (Hartl & Clark, 1997):

$x^2 = \sum[(O - E)2 / E]$

where: χ2 = chi-square; O= total of observations genotype to-I; E= total of genotype to expectations to-i.

Analysis for carcass, meat quality, and fatty acid. The association between the SNP of the *CYP2E1* gene with the carcass and meat quality and fatty acid was performed using T-test procedures to compare genotypes (Minitab® 18 Software). The model of mathematics was:

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

where X1 and X2 were the average traits for genotype 1 and 2; n1 and n2 were an individual number of genotype 1 and 2; and was the combined standard deviation.

Analysis for gene expression related to lamb quality. The relative expression levels of *CYP2E1* mRNA were calculated by the difference between the target gene and geometric mean of the references gene (ΔCT), according to the following formula (Silver *et al.,* 2006):

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

RESULTS

Polymorphisms of *CYP2E1* Gene in Indonesian Lamb

The SNP of *CYP2E1* gene in g. 50657948 T>G was polymorphic in Indonesian lamb (Figure 1). Based on analysis of genotype and allele frequencies (Table 2), we found that three genotypes in *CYP2E1* consisted of the GG (401 bp), GT (401, 263, and 138 bp), and TT (263, 138 bp). The GT genotype was the most frequent (53%) followed by TT (30%) and GG (17%). The G allele frequency was 0.44 and the T allele frequency was 0.56. The allele frequencies of the *CYP2E1* gene in all sheep populations were in Hardy-Weinberg equilibrium ($p>0.05$).

Phenotypic of Carcass and Meat Quality of Lamb and Fatty Acid Composition

Carcass and meat qualities of lamb were detected from six breeds of sheep consisted JTTS, GCS, CAS, BCS, JS, and GS, while fatty acid composition of loin muscle from JFTS, JTTS, GCS, CAS, BCS, and JS (Table 3 & Table 4). The data of meat quality were live weight, hot carcass weight, carcass length, carcass percentage, pH value, tenderness, cooking loss, and drip loss from eighty samples of sheep. Fatty acid composition from

Table 1. Primers sequences for quantitative-real time PCR (qRT-PCR)

Gene	Primer sequence	Size (bp)	Ta (°C)
CYP2E1	F: 5′ - ATT CCC AAG TCC TTC ACC AG -3′	180	60
	R: 5′ - GTT GTT TTT GTG CAC CTG GA -3′		
GAPDH	F: 5′ - GAG AAA CCT GCC AAG TAT GA -3′	203	62
	R: 5′ - TAC CAG GAA ATG AGC TTG AC-3′		
β-Actin	F: 5′ - GAA AAC GAG ATG AGA TTG GC-3′	194	62
	R: 5′ - CCA TCA TAG AGT GGA GTT CG-3′		

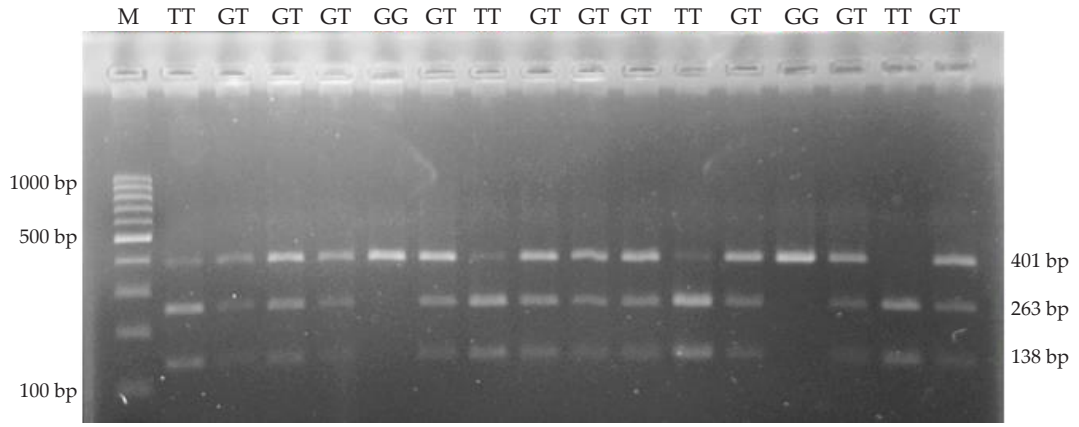

Figure 1. PCR-RFLP genotyping result for the *CYP2E1* gene using NlaIII enzyme restriction in 2.5% agarose; M= 100 bp ladder size; GG, GT, and TT= genotypes.

Table 2. The number of animals per genotype and allele frequencies and $\chi 2$= Chi-square test result

Sample	N	Genotype frequency			Allele frequency		$\chi 2$
		GG	GT	TT	G	T	
JFT	20	0.15 (3)	0.40 (8)	0.45 (9)	0.35	0.65	0.29
JTT	37	0.11 (4)	0.35 (13)	0.54 (20)	0.28	0.72	0.68
GS	20	0.05 (1)	0.40 (8)	0.55 (11)	0.25	0.75	0.08
JS	21	0.24 (5)	0.57 (12)	0.19 (4)	0.52	0.48	0.44
GCS	34	0.21 (7)	0.71 (24)	0.08 (3)	0.56	0.44	6.33
CAS	35	0.17 (6)	0.63 (22)	0.20 (7)	0.49	0.51	2.33
BCS	33	0.24 (8)	0.61 (20)	0.15 (5)	0.54	0.45	1.63
Totals	200	0.17 (34)	0.53 (107)	0.30 (59)	0.44	0.56	1.52

Note: JFT= Javanese fat-tail sheep; JTT= Javanese thin-tail sheep; GCS= Garut composite sheep; CAS= compass agrinac sheep; BCS= Barbados cross sheep; JS= Jonggol sheep; GS= Garut sheep n= Number of samples, $\chi 2$= Chi-square, χ_{tab}, 0.05= 3.841 ($\chi 2 < \chi_{tab}$)= significant.

Table 3. Phenotypic of carcass and meat quality of Indonesian lamb

Carcass and meat quality traits	Breed (μ ± SE Mean)					
	JTTS (n=15)	GCS (n=10)	CAS (n=10)	BCS (n=10)	JS (n=15)	GS (n=20)
Live weight (kg)	25.91 ± 0.75	27.44 ± 0.34	21.00 ± 1.56	15.84 ± 0.87	23.04 ± 0.41	24.91 ± 0.99
Hot carcass weight (kg)	10.32 ± 0.50	9.78 ± 0.21	7.16 ± 0.53	5.25 ± 0.32	8.41 ± 0.20	6.56 ± 0.37
Carcass length (cm)	70.33 ± 0.82	74.50 ± 0.96	106.30 ± 3.48	100.10 ± 3.02	64.86 ± 0.97	64.60 ± 0.26
Carcass percentage (%)	43.10 ± 0.91	35.65 ± 0.66	34.22 ± 0.94	33.07 ± 0.61	39.85 ± 0.43	26.10 ± 0.77
pH value	6.03 ± 0.05	6.03 ± 0.05	6.24 ± 0.11	6.39 ± 0.09	5.70 ± 0.03	7.30 ± 0.04
Tenderness (kg/cm²)	3.92 ± 0.19	3.92 ± 0.29	2.81 ± 0.19	4.55 ± 0.31	4.11 ± 0.07	2.86 ± 0.15
Cooking loss (%)	47.23 ± 1.52	48.65 ± 1.48	38.14 ± 2.75	48.61 ± 2.20	50.58 ± 0.81	50.52 ± 1.06
Drip loss (%)	27.62 ± 0.48	26.45 ± 0.79	25.98 ± 1.49	28.06 ± 1.29	25.82 ± 0.44	28.05 ± 0.32

Note: JTTS= Javanese thin-tail sheep; GCS= Garut composite sheep; CAS= compass agrinac sheep; BCS= Barbados cross sheep; JS= Jonggol sheep; GS= Garut sheep; μ= means of carcass and meat quality values; SE = standard eror.

110 samples of sheep were grouping into saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA).

CYP2E1 Gene Affecting Carcass and Meat Quality

The association analysis of *CYP2E1* gene polymorphisms with carcass characteristics and meat quality were presented in Table 5. The *CYP2E1* gene was significantly associated ($p<0.05$) with meat quality, i.e., pH value and lamb tenderness. In general, the T-test showed that the lambs with homozygous GG genotype were associated with a lower pH value and lamb tenderness than GT and TT genotypes.

CYP2E1 Gene Affecting Fatty Acid Composition

The parameters of fatty acid (FA) compositions were associated ($p<0.05$) with *CYP2E1* gene polymorphisms consisted of saturated fatty acid (SFA): caprylic acid (C8:0), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C:22:0), and tricosylic acid (C23:0), monounsaturated fatty acid (MUFA): elaidic acid (C18:1n9t) and paullinic acid (C20:1); polyun-

Table 4. Phenotypic of the fatty acid composition of Indonesian lamb

Fatty acid	Breed (μ ± SE Mean)					
	JFTS (n=20)	JTTS (n=33)	GCS (n=10)	CAS (n=10)	BCS (n=10)	JS (n=17)
Fat content (%)	7.08 ± 0.89	3.98 ± 0.52	1.95 ± 0.28	1.85 ± 0.38	1.68 ± 0.59	2.24 ± 0.25
Saturated fatty acid (%)	35.28 ± 1.49	41.43 ± 1.04	41.78 ± 1.75	47.85 ± 1.95	33.30 ± 2.99	39.46 ± 3.53
Caprylic acid, C8:0	0.00 ± 0.00	3.26 ± 2.58	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.04
Capric acid, C10:0	0.00 ± 0.00	0.48 ± 0.42	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.04
Lauric acid, C12:0	0.10 ± 0.01	0.27 ± 0.04	0.05 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.10 ± 0.01
Tridecylic acid, C13:0	0.68 ± 0.20	0.16 ± 0.03	0.35 ± 0.03	0.54 ± 0.06	0.47 ± 0.12	0.46 ± 0.08
Myristic acid, C14:0	0.02 ± 0.00	1.37 ± 0.28	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Pentadecylic acid, C15:0	0.16 ± 0.02	0.36 ± 0.04	0.07 ± 0.00	0.12 ± 0.02	0.27 ± 0.07	0.13 ± 0.01
Palmitic acid, C16:0	0.45± 0.04	9.79 ± 1.80	0.49 ± 0.03	0.51 ± 0.03	0.38 ± 0.05	0.49 ± 0.05
Margaric acid, C17:0	1.90 ± 0.05	1.28 ± 0.08	1.17 ± 0.06	1.25 ± 0.08	0.98 ± 0.06	1.51 ± 0.14
Stearic acid, C18:0	0.76 ± 0.07	8.43 ± 1.59	0.28 ± 0.01	0.39 ± 0.04	0.52 ± 0.06	0.00 ± 0.00
Arachidic acid, C20:0	2.26 ± 0.20	1.05 ± 0.17	3.05 ± 0.22	0.25 ± 0.01	5.58 ± 1.21	2.11 ± 0.25
Heneicosylic acid, C21:0	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.02 ± 0.00
Behenic acid, C22:0	0.01 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.13 ± 0.02	0.26 ± 0.03	0.02 ± 0.00
Tricosylic acid, C23:0	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.07 ± 0.01	0.15 ± 0.01	0.01 ± 0.00
Lignoceric acid, C24:0	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.11 ± 0.02	0.28 ± 0.04	0.01 ± 0.00
Unsaturated fatty acid (%)	35.89 ± 0.64	34.40 ± 0.54	27.24 ± 0.59	8.14 ± 1.10	31.36 ± 3.22	31.71 ± 2.95
Monounsaturated fatty acid (%)	32.95 ± 0.71	31.17 ± 0.50	22.73 ± 0.90	5.08 ± 0.51	20.94 ± 2.17	28.38 ± 2.64
Myristoleic acid, C14:1	3.55 ± 0.59	2.05 ± 0.38	2.14 ± 0.12	3.32 ± 0.34	1.97 ± 0.40	2.86 ± 0.31
Palmitoleic acid, C16:1	18.73 ± 0.59	11.61 ± 1.61	16.26 ± 0.75	18.36 ± 0.95	13.15 ± 1.92	18.98 ± 1.71
Ginkgoleic acid, C17:1	1.21 ± 0.10	0.61 ± 0.09	0.80 ± 0.02	0.80 ± 0.03	0.60 ± 0.06	0.68 ± 0.06
Oleic acid, C18:1n9c	0.06 ± 0.01	13.83 ± 2.65	0.00 ± 0.00	23.74 ± 1.23	0.00 ± 0.00	0.14 ± 0.09
Elaidic acid, C18:1n9t	10.43 ± 0.62	8.24 ± 0.93	21.33 ± 0.99	23.86 ± 0.82	15.60 ± 1.77	15.44 ± 1.37
Paullinic acid, C20:1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.03	0.00 ± 0.00
Nervonic acid, C24:1	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.08 ± 0.01	0.23 ± 0.05	0.02 ± 0.00
Polyunsaturated fatty acid (%)	2.94 ± 0.29	3.22 ± 0.21	4.51 ± 0.45	3.06 ± 0.65	10.42 ± 1.30	3.33 ± 0.35
Linoleic acid, C18:2n6c	30.11 ± 0.66	16.50 ± 2.32	21.18 ± 0.85	3.22 ± 0.47	18.94 ± 2.21	26.70 ± 2.49
y-linolenic acid, C18:3n6	0.06 ± 0.00	0.11 ± 0.09	0.16 ± 0.01	0.00 ± 0.00	0.33 ± 0.02	0.11 ± 0.01
α-linolenic acid, C18:3n3	0.23 ± 0.02	0.49 ± 0.05	0.13 ± 0.00	0.14 ± 0.01	0.21 ± 0.04	0.52 ± 0.05
Eicosedienoic acid, C20:2	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.06 ± 0.01	0.10 ± 0.04	0.02 ± 0.00
Dihomo-y-linolenic acid, C20:3n6	0.02 ± 0.01	0.02 ± 0.00	0.08 ± 0.02	0.17 ± 0.02	0.31 ± 0.06	0.03 ± 0.00
Arachidonic acid, C20:4n6	0.32 ± 0.05	0.36 ± 0.04	1.07 ± 0.19	2.18 ± 0.54	3.49 ± 0.59	0.29 ± 0.02
Docosadienoic acid, C22:2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Eicosapentaenoic acid, C20:5n3	0.02 ± 0.01	0.28 ± 0.03	0.07 ± 0.01	0.15 ± 0.05	0.47 ± 0.09	0.28 ± 0.02
Cervonic acid, C22:6n3	0.01 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.15 ± 0.05	0.03 ± 0.00
Total fatty acid (%)	71.26 ± 1.36	77.01 ± 1.40	69.08 ± 1.88	79.79 ± 2.32	64.90 ± 4.91	71.31 ± 6.36

Note: JFTS= Javanese thin-tail sheep; JTTS= Javanese thin-tail sheep; GCS= Garut composite sheep; CAS= compass agrinac sheep; BCS= Barbados cross sheep; JS= Jonggol sheep; μ= means of carcass and meat quality values; SE = standard eror.

saturated fatty acid (PUFA): linoleic acid (C18:2n6c) and y-linolenic acid (C20:3n6). The associations of *CYP2E1* gene with fatty acid compositions are presented in Table 6. The GG genotype was associated with lower SFA but higher UFA followed by GT and TT genotypes. These results indicated that the *CYP2E1* gene might play an important role in lipid metabolism.

Expression of *CYP2E1* Gene

The expression levels of *CYP2E1* were differently regulated ($p<0.05$) among the sheep with a higher effect of GG genotype than the GT and TT genotypes (Figure 2). Quantitative real-time PCR analysis showed the abundance of *CYP2E1* transcripts in animals with divergent lamb qualities in the liver. The GG genotype expression had a low total of saturated fatty acid and high totals of unsaturated fatty acid than others genotype, which is indicates that sheep with GG genotype of *CYP2E1* will produce a healthy lamb meat cause have a high totals of unsaturated fatty acid (Table 6).

DISCUSSION

The polymorphisms of *CYP2E1* gene in this study had three genotypes in Indonesian lamb. However, Listyarini *et al.* (2018) found that only two genotypes in Javanese fat-tail sheep, namely GT and TT genotypes. In this study, GT genotype was common, but Listyarini *et al.* (2018) found that the TT genotype was more frequent

Table 5. The association analysis of *CYP2E1* gene polymorphisms with carcass characteristic and lamb quality

Carcass and meat quality traits	Genotype of *CYP2E1* (μ ± Std Dev)			p-Value		
	GG (n=17)	GT (n=34)	TT (n=29)	GG vs GT	GG vs TT	GT vs TT
Live weight (kg)	22.48 ± 4.61	22.74 ± 4.75	24.81 ± 4.60	0.853	0.108	0.085
Hot carcass (kg)	8.04 ± 1.92	7.57 ± 1.90	8.27 ± 2.70	0.417	0.741	0.252
Carcass length (cm)	80.00 ± 18.68	77.62 ± 19.64	70.28 ± 13.61	0.676	0.072	0.087
Carcass percentage (%)	36.86 ± 4.43	34.54 ± 5.95	34.31 ± 8.49	0.120	0.184	0.902
pH value	6.10 ± 0.46^{b}	6.36 ± 0.64	6.50 ± 0.64^{a}	0.115	0.020*	0.382
Tenderness (kg/cm^2)	4.09 ± 1.02^{a}	3.66 ± 0.80	3.32 ± 0.94^{b}	0.144	0.018*	0.143
Cooking loss (%)	49.47 ± 5.37	48.91 ± 5.82	45.77 ± 7.99	0.736	0.068	0.086
Drip loss (%)	27.47 ± 3.21	26.95 ± 2.78	27.03 ± 2.56	0.578	0.638	0.908

Note: μ= means of carcass and meat quality values; Std Dev= standard deviation; *= significantly at p<0.05; a,b,c= superscript to show a signifanctly different variables. Numbers shown in parentheses are the number of individuals with the specified genotype.

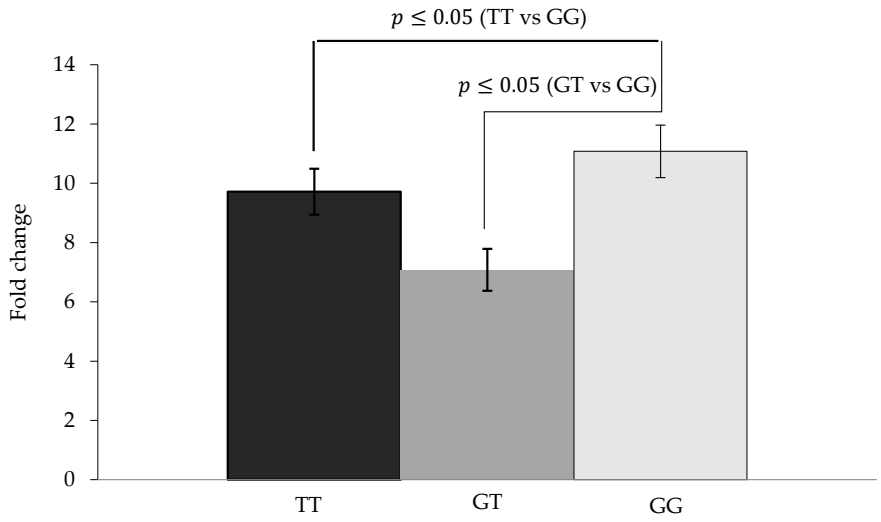

Figure 2. Expression levels of meat quality concerning for *CYP2E1* genotype in Indonesian sheep from high meat quality (GG genotype) and low meat quality (GT and TT genotypes).

than GT genotype. The mutation in the genotype of thymine to guanine in the *CYP2E1* gene is categorized as a translation mutation. Translation mutation is a mutation in purine bases (A,G) to pyrimidine bases (C,T).

In the association analysis, the SNP of *CYP2E1* gene (g.50657948 T>G) was associated with pH value and lamb tenderness (Table 4). GG genotypes were associated with low pH value and meat tenderness in Indonesian lamb than GT and TT genotypes. The pH values found in this study were not categorized in normal pH. The pH values of a fresh slaughtered sheep's carcass should be around 7.0 to 7.3. The ultimate pH values vary between 5.5 and 5.8, which are influenced by several factors, such as gender, slaughter age, production system, genetics, and pre-slaughter management (Zimerman *et al.,* 2011; Gallo *et al.,* 2019). The pH value is related to lamb tenderness. The higher final pH could produce a dark firm dry (DFD) meat that caused a low tenderness (Santos *et al.,* 2019). Measurement of meat tenderness value was conducted using the Warner Bratzler Shear Force (WBSF) tools. Meat tenderness of CAS and GS in this study were in the range of tenderness reported by Dagong *et al.* (2012) that the JTT had a 2.62-3.13 kg/cm^2 for WBSF, while JTT, GCS, BCS, and JS in this study had a higher when compared to their study. The results showed that the breed of sheep could influence a variant of meat tenderness. Meat tenderness is determined by the amount and solubility of conective tissue, sarcomere, shortening during rigor development, and post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Koohmaraie & Geesink 2006). Other parameters of meat quality related to *CYP2E1* gene polymorphisms were studied by Listyarini *et al.* (2018), reporting that *CYP2E1* gene polymorphisms associated with flavor and odor in Indonesian lamb. The *CYP2E1* gene plays an important role in the metabolism of skatole and androstenone (Listyarini *et al.,* 2018; Gunawan *et al.,* 2013a; Gunawan *et al.,* 2013b; Morlein *et al.,* 2012; Neuhoff *et al.,* 2015; Zadinova *et al.,* 2016). The activity of *CYP2E1* in the liver significantly influences skatole concentrations in the fat. The skatole levels in the fat of boars increase during puberty and are correlated with the fat androstenone levels (Robic *et al.,* 2008; Wiercinska *et al.,* 2012). Consequently, male pigs were routinely castrated for meat production. The *CYP2E1* gene affected levels of skatole on lamb meat (Harahap *et al.* 2020). Skatole is produced in the intestine by bacterial degradation and absorbed into the blood. If it passes through the liver without being metabolized, it accumulates in adipose tissue, the liver, and the kidneys. The

Table 6. The association of *CYP2E1* gene with fatty acid composition

Fatness traits	Genotype of *CYP2E1* (μ ± Std Dev)			p-Value		
	GG (n=20)	GT (n=43)	TT (n=37)	GG vs GT	GG vs TT	GT vs TT
Fat content (%)	3.32 ± 3.05	3.26 ± 2.58	4.32 ± 3.94	0.937	0.297	0.169
Saturated fatty acid (%)	38.98± 8.64	40.52 ± 9.32	39.22 ± 9.49	0.524	0.925	0.539
Caprylic acid, C8:0	0.07 ± 0.17	0.05 ± 0.11[a]	0.01 ± 0.05[b]	0.647	0.129	0.031*
Capric acid, C10:0	0.08 ± 0.05	0.40 ± 2.12	0.10 ± 0.00	0.319	0.067	0.363
Lauric acid, C12:0	0.51 ± 0.49	0.52 ± 0.57	0.39 ± 0.33	0.96	0.357	0.244
Tridecylic acid, C13:0	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.577	0.449	0.098
Myristic acid, C14:0	2.72 ± 1.73	3.08 ± 1.69	3.19 ± 1.72	0.442	0.333	0.778
Pentadecylic acid, C15:0	0.48 ± 0.13	0,49 ± 0.17	0.54 ± 0.16	0.748	0.131	0.199
Palmitic acid, C16:0	17.30± 5.09	18.64 ± 4.54	18.83 ± 3.98	0.319	0.253	0.849
Margaric acid, C17:0	0.81 ± 0.29	0.89 ± 0.34	0.98 ± 0.35	0.318	0.059	0.285
Stearic acid, C18:0	16.53 ± 4.26	16.13 ± 5.74	14.97 ± 6.11	0.761	0.266	0.386
Arachidic acid, C20:0	0.18 ±0.12[a]	0.12±0.09	0.09 ± 0.06[b]	0.065	0.016*	0.335
Heneicosylic acid, C21:0	0.04±0.02[a]	0.02±0.02[b]	0.02 ± 0.02[b]	0.019*	0.021*	0.858
Behenic acid, C22:0	0.04±0.17[a]	0.06±0.08	0.04 ± 0.05[b]	0.179	0.028*	0.064
Tricosylic acid, C23:0	0.05±0.07[a]	0.03±0.05	0.02 ± 0.03[b]	0.25	0.028*	0.063
Lignoceric acid, C24:0	0.09 ± 0.15	0.05 ± 0.08	0.02 ± 0.04	0.286	0.058	0.071
Unsaturated fatty acid (%)	32.45 ± 5.03	29.83 ± 11.86	30.59 ± 10.08	0.262	0.413	0.759
Monounsaturated fatty acid (%)	26.96 ± 6.80	25.60 ± 10.90	27.50 ± 9.96	0.548	0.809	0.417
Myristoleic acid, C14:1	0.14 ± 0.07	0.15 ± 0.13	0.13 ± 0.08	0.530	0.882	0.419
Palmitoleic acid, C16:1	1.42 ± 0.43	1.51 ± 0.44	1.64 ± 0.43	0.416	0.071	0.211
Ginkgoleic acid, C17:1	0.31 ± 0.32	0.31 ± 0.31	0.36 ± 0.40	0.979	0.611	0.519
Oleic acid, C18:1n9c	25.01± 6.62	23.58 ±10.6	25.35 ± 9.64	0.518	0.875	0.437
Elaidic acid, C18:1n9t	0.31 ± 0.82[b]	3.61 ± 8.43[a]	3.51 ± 7.27[b]	0.015*	0.012*	0.955
Paullinic acid, C20:1	0.07±0.14[a]	0.03±0.07	0.01 ± 0.01[b]	0.127	0.035*	0.060
Nervonic acid, C24:1	0.08±0.15	0.04±0.08	0.01 ± 0.03	0.246	0.058	0.063
Polyunsaturated fatty acid (%)	5.28±2.81[a]	4.23±3.49	3.08 ± 1.40[b]	0.209	0.003*	0.053
Linoleic acid, C18:2n6c	3.16±1.24[a]	2.46±2.49	1.82 ± 0.93[b]	0.144	0.000*	0.127
y-linolenic acid, C18:3n6	0.03 ± 0.07	0.08 ± 0.14	0.01 ± 0.04	0.806	0.946	0.679
α-linolenic acid, C18:3n3	0.32 ± 0.28	0.35 ± 0.25	0.36 ± 0.30	0.759	0.637	0.809
Eicosedienoic acid, C20:2	0.04 ± 0.02	0.05 ± 0.07	0.04 ± 0.03	0.301	0.900	0.281
Dihomo-y-linolenic acid, C20:3n6	0.10 ± 0.11[a]	0.06 ± 0.09[b]	0.04 ± 0.05[c]	0.609	0.025*	0.050*
Arachidonic acid, C20:4n6	1.32 ± 1.57	0.99 ± 1.48	0.59 ± 0.81	0.451	0.065	0.125
Docosadienoic acid, C22:2	0.00 ± 0.00	0.01 ± 0.03	0.01 ± 0.05	nt	nt	0.945
Eicosapentaenoic acid, C20:5n3	0.25 ± 0.22	0.19 ± 0.21	0.17 ± 0.20	0.333	0.215	0.712
Cervonic acid, C22:6n3	0.05 ± 0.03	0.05 ± 0.10	0.04 ± 0.05	0.711	0.200	0.231
Total fatty acid (%)	71.63 ± 12.09	73.70 ± 14.12	73.41 ± 14.17	0.553	0.627	0.922

Note: nt= not tested using T-test due to no amount detected; μ= means of fatty acid composition; Std Dev= standard deviation; *= significantly at p<0.05; a,b,c= superscript to show a signifanctly different variables. Numbers shown in parentheses are the number of individuals with the specified genotype.

high expression of the *CYP2E1* gene causes low levels of skatole in the adipose tissue that affects the flavor and odor of meat quality of lamb. The *CYP2E1* gene plays a role in metabolism carcinogenesis (Leung *et al.,* 2013). Its effect on carcinogens metabolism was studied not only in humans but also in mice (Konstandi *et al.,* 2013).

The expression of *CYP2E1* gene was the highest in GG genotype than GT and TT genotypes (Figure 2). The expression of GG genotypes was associated with low totals of SFA and high totals of UFA (Table 6.), and the high levels of FA caused an undesirable flavour and odor. Two chemical compounds that determine the lamb's odor and flavor consisted of branched-chain fatty acid and skatole (BCFA). The BCFAs consisted of 4-methyloctanoic acid (4-MeO) and 4-methylnonanoic acid (4-MeN), which were dominated by fatty acids stearic and oleic that were esterified in body fats, but released to an extent as free acids on cooking and thus contributing to flavor (Lu *et al.,* 2014; Listyarini *et al.,* 2018).

Overall, the result showed the average value of SFA in lamb meat was higher than UFA (PUFA+MUFA) in all genotypes. This result agrees with the current study by Munyaneza *et al.* (2019) that generally fatty composition of lamb meat is dominated by SFA. MUFA could reduce LDL cholesterol (low-density lipoprotein) and increase HDL cholesterol (high-density lipoprotein) levels. Oleic acid (C18:1n9c) was the dominant fatty acid in total

MUFA. This result is consistent with the researches by Gunawan *et al.* (2018, 2019), who found that oleic acid is most commonly found in muscle. PUFAs have been identified to have benefits in the physiological system of blood pressure, heart rate, inflammation, and reduce the risk of coronary heart disease.

The *CYP2E1* gene in this study was significantly different in mRNA levels (Figure 2). This result is consistent with the study of Kubesova *et al.* (2019) reported a significant difference in mRNA levels of *CYP2E1* in the pig. The expression of the *CYP2E1* gene is affected by sex. Female pigs showed higher expression of CYP1A2 and *CYP2E1* than the boar (Rasmussen *et al.*, 2011). Ma *et al.* (2019) reported the expression of the *CYP2E1* gene in lipid metabolite with other genes such apolipoprotein C3 (APOC3), cytochrome P4502C34 (CYP2C34), and sulfate transferase 2A1 (SULT2A1) were not influenced by restricted feed intake.

CONCLUSION

The SNP g.50657948 T>G of *CYP2E1* gene was polymorphic in seven breeds of Indonesian lambs. The variant genotypes of *CYP2E1* gene was significantly associated with lamb quality, i.e, pH value, lamb tenderness, and fatty acid composition. The expressions of GG genotypes was higher than GT and TT genotypes. Based on the finding, SNP g.50657948 T>G of the *CYP2E1* gene with GG genotype could be used as a candidate marker for selecting sheep with high lamb quality.

CONFLICT OF INTEREST

Asep Gunawan serves as an editor of the Tropical Animal Science Journal, but has no role in the decision to publish this article. The authors also declare that there is no conflict of interest.

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