Buletin Peternakan 45 (3): 148-154, August 2021

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v45i3.65604

Association of Cytochrome P450 2A6 (CYP2A6) Gene Polymorphisms with Fatty Acid Traits in Indonesian Native Sheep

Gilang Kurnia Abdillah, Kasita Listyarini, Ratna Sholatia Harahap, Asep Gunawan, and Cece Sumantri*

Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, 16680, Indonesia

Article history
Submitted: 1 May 2021
Accepted: 17 June 2021

*Corresponding author:
Telp. +62 856 8801 86
E-mail: ceces@apps.ipb.ac.id

ABSTRACT

A member of the cytochrome P450 superfamily enzyme that play a role in microsomal fatty acids oxidation process encoded by cytochrome P450 2A6 (CYP2A6). This study aimed to examined the CYP2A6 gene polymorphisms and their association on fatty acid traits in Indonesian native sheep. A total of 299 rams aged 10-12 months with body weights between 20-30 kg used to identify the CYP2A6 gene polymorphism are consisted of 36 samples of barbados cross sheep (BCS), 35 samples of compass agrinak sheep (CAS), 20 samples of javanese fat tailed sheep (JFT), 36 javanese thin tailed sheep (JTT), 20 samples of garut sheep (GS), 45 samples of garut composite sheep (GCS), meanwhile for the fatty acid analysis was carried out by using 107 of loin samples from the total sheep. Identification of the CYP2A6 gene polymorphisms were performed using PCR-RFLP (Polymorphism Chain Reaction-Restriction Fragment Length Polymorphism) with the BSmAI restriction enzymes. The amplification product was 286 bp. Polymorphism were found in JFT, JTT, GCS and JS with GG and GT genotypes, while BCS, CAS, and GS were monomorphic with TT genotype. The CYP2A6 BSmAI polymorphism was in Hardy-Weinberg Equilibrium for JFT, JTT and JS, while BCS, CAS, GS, GCS, and combined was deviated based on chi square. A SNP g.49170107 G>T of the CYP2A6 gene polymorphism was significantly associated ($P < 0.05$) with only erucic acid (C22:1n9). The GT genotypes had a higher value than GG genotypes The CYP2A6 gene could be used as a selection marker to improve fatty acid traits in Indonesian native sheep.

Keywords: CYP2A6 gene, Fatty acid, PCR-RFLP, Sheep

Introduction

Sheep is one of the potential livestock to fulfill animal protein consumption. Lamb meat production in 2018 was 48,674 tons or only contributed 1.35% of the total national meat production in Indonesia (DPKH, 2018). Apart from the low national lamb meat production, the national lamb meat consumption rate is still low compared to other meats. The price of sheep which is relatively more expensive than other animal protein sources is one of the factors contributing to the low consumption of Indonesia. Gunawan *et al*. (2018a) reported that, apart from price, there is an assumption in Indonesian society that lamb meat contains cholesterol and high saturated fatty acid (SFA) content, the odor of meat which is less desirable and difficult to remove are factors that are thought to cause low national consumption of lamb meat. Consumption of SFA, and cholesterol correlates with an increase low density lipoprotein (LDL) in blood. High LDL content can trigger atherosclerosis which is the beginning of the onset of coronary heart disease (Ma'rufi and Rosita, 2014). A genomic selection-based on breeding program to produce lamb meat with high unsaturated fatty acid and low SFA content are potential to be implemented. Fatty acids composition is a quantitative trait that influenced by genetic factors and controlled by more than one gene (Gunawan *et al*., 2018a). Fatty acids trait in sheep has a heritability value ranging from 0.25-0.46 which indicates that increase in genetic quality through an effective selection program to be carried out (Rovadoscki *et al*., 2018).

Several genes were reported to be significantly associated with fatty acid content in sheep including APOA5 (Gunawan *et al*., 2018b), AHSG (Munyaneza *et al*., 2019a), BHMT (Munyaneza *et al*., 2019b), DGAT1 (Gunawan *et al*., 2019), and KIF12 (Gunawan *et al*., 2018a). Cytochrome P450 2A6 (CYP2A6) are genes thought to contribute in the fatty acid traits in sheep. The CYP2A6 gene is located on chromosome 14 in sheep and mainly expressed in the endoplasmic reticulum of the liver and olfactory mucosa (Listyarini *et al*., 2018; Kirby *et*

al., 2011). The CYP2A6 gene encodes a member of cytochrome P450 superfamily enzymes that was found to play a role in in the microsomal monooxygenase system as the terminal oxidase and contribute to metabolizes several important substrates such as biosynthesis of cholesterol, convert cholesterol to bile acids, forming steroid hormones, metabolize vitamin D3 to the active 1,25-dihydroxyvitamin D3, fatty acids metabolism, and biotransformation of exogenous xenobiotics (McDonagh *et al*., 2012; Cederbaum, 2015; Wang *et al*., 2017). The CYP2A6 gene found known to be associated with class 1 obesity in humans (Wang *et al*., 2019), had a significant effect ($P<0.05$) to the off odor and flavor in sheep (Listyarini *et al*., 2018) and associated with C20:3n3 fatty acid (cis11,14,17-Eicosatrienoic acid) in Korean pigs (Roh *et al*., 2011). There was no study investigated CYP2A6 gene polymorphism effects on Indonesian native sheep fatty acid traits. Therefore, this study is needed to examined the CYP2A6 gene polymorphisms and their association on fatty acid traits in Indonesian native sheep.

Materials and Methods

Animals and samples

Identification of CYP2A6 gene polymorphisms sample were obtained from blood and liver of 299 rams from seven breeds, while fatty acid analysis were obtained from loin sample of 110 rams (Figure 1 and Table 1). All sheep had age between 10-12 months old, body weights between 20-30 kg, and slaughtered in a commercial abattoir. The blood and liver sample were taken 100 µl for identification of CYP2A6 gene polymorphisms and the loin samples were taken 100 mg for fatty acids analysis. The samples were put in an ice flask and stored at a temperature of -20ºC.

Fatty acid analysis

A total of 100 mg loin sample were used for fatty acid analysis. The fatty acid analysis was conducted according to the protocol of Association of Official Analytical Chemists (AOAC, 2012). These measurements including fat content, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and fatty acid total.

DNA isolation and PCR-RFLP amplification

The DNA isolation refers to phenol-chloroform standard (Sambrook and Russel, 2001). Previous research from Listyarini *et al.* (2018) was described a single nucleotide polymorphism (SNP) of CYP2A6 g.49170107 (G>T) gene that used in this study. A pair of primers used for amplification of the 286 bp (base pair) target DNA refers to Listyarini *et al.* (2018) were F: 5'-CTT TCT GGT CCT CAT CTT TG-3 'and R: 5'-GGT ATT GAT GAG GAA TGG TG-3'. DNA amplification was initiated by the pre-denaturation stage for 1 minute at a temperature of 95ºC. Then, there is 35 cycles consisted of 15 seconds at 95°C denaturation, 15 seconds at 55°C primary annealing, 10 seconds at 72°C DNA extension and 1 minute at 72ºC final primer elongation. The PCR product then visualized by electrophoresis on 1.5% agarose gel. The PCR product then digested using the RFLP technique using BSmAI enzyme. The digested product then visualized on 2% agarose gel using UV Transilluminator.

Statistical analysis

Gene frequency including genotype and allele were calculated according to the populations of BCS, CAS, JFT, JTT, GS, GCS and JS genotyping data by the formula of Nei and Kumar (2000). Genotype frequency:

$$Xii=\frac{nii}{N}$$

Allele frequencies:

$$Xi=\frac{(2nii+\sum i\neq j\ nij)}{2N}$$

Description:
Xii = frequency of ii genotype,
Xi = frequency of i allele,
nii = number of the ii-genotype sample,
nij = number of the ij-genotype sample,
N = total samples.

Hardy-Weinberg equilibrium (Nei and Kumar, 2000):

$$X^2 = \sum \frac{(O-E)^2}{E}$$

Description:
X^2 = chi squared,
O = total of observations genotype
E = total of genotype to expectations.

Association analysis

The CYP2A6 gene polymorphism association with fatty acid traits was conducted according to the GLM procedure (SAS Inst, Inc., Cary, NC). The mathematics model was:

$$Yij = = \mu + G + Eij$$

Description:
Yij = dependent variable for trait measured in the population,
µ = populations mean for traits,
Gi = genotype fixed effects,
E = random error.

Differences was considered statically significant if p-value <0.05. Pair wise differences between the effects of genotype was tested by performing Duncan Multiple Range Test (DMRT).

Results and Discussion

CYP2A6 gene polymorphism in Indonesian native sheep

The CYP2A6 gene (g.49170107 G>T) was amplified with product length 286 bp (Figure 2). The polymorphism of the CYP2A6 gene (Figure 3) were detected and define as GG (286 bp), GT (286 bp, 217 bp, and 69 bp) and TT (217 bp, and 69 bp) which are a combination of T and G alleles.

The genotype frequencies of GG and GT were 0.900 and 0.100 for JFT, 0.889 and 0.111 for JTT, and 0.991 and 0.009 for JS, respectively. The GG and TT genotype frequencies were 0.978 and 0.022 for GCS, respectively. Samples of BCS, CAS, and GS only has GG genotype. Total combined sample showed that GG genotype was the most frequent (97.3%) followed by GT (2.4%) and TT (0.3%). Statistical result of the CYP2A6 gene analysis results is presented in Table 2. The G allele was dominant in all sheep breeds. Gene polymorphism only occurred in JFT, JTT, GCS, and JS while BCS, CAS, and GS did not vary because the values of genotype frequency and allele frequency were equal to 1.00. The CYP2A6 BSmAI polymorphism is in Hardy-Weinberg Equilibrium for JFT, JTT and JS, while BCS, CAS, GS, GCS and combined is deviated. Polymorphisms of genotyped in this study more polymorphic than Listyarini *et al.* (2018) that found two genotypes in the CYP2A6 gene that is GT and TT genotypes. Listyarini *et al.* (2018) found TT genotypes as dominant genotypes than others in JFT sheep. With a larger population sample compared to Listyarini *et al*. (2018), we found the GG genotypes were common in seven breed of Indonesian native sheep. Beside CYP2A6 gene, there are other families cytochrome that also associated with fatty acid in sheep. Study conducted by Harahap *et al.* (2020) reported that CYP2E1 gene were found three genotypes in Indonesian native sheep is GG, GT, and TT. The mutation in the genotype of guanine to thymine in the CYP2A6 gene is categorized a translation mutation.

Figure 1. Phenotypic of Indonesian native sheep.

Table 1. The samples of study

Samples	N	DNA extraction sample	Fatty acid analysis sample
BCS	36	36	10
CAS	35	35	10
JFT	20	20	20
JTT	36	36	33
GS	20	20	-
GCS	45	45	10
Jonggol sheep (JS)	107	107	27
Totals	299	299	110

N= number of samples

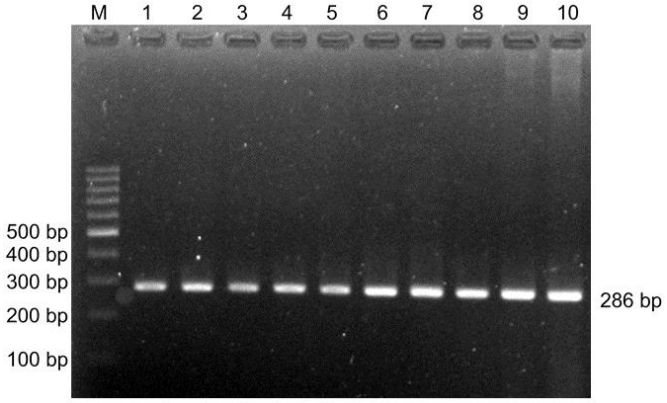

Figure 2. The PCR amplification result for the CYP2A6 gene. M= 100 bp markers; No: 1-10 = a random sample of individual sheep breed.

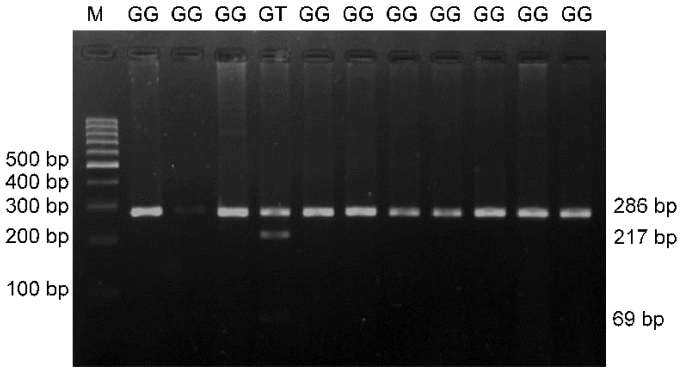

Figure 3. The CYP2A6 gene PCR-RFLP result using BSmAI restriction enzyme in 2.5% agarose; M= 100 bp markers; 1, 2, 3, 5, 6, 7, 8, 9, 10, 11= GG genotype; 4= GT genotype.

Table 2. Frequency of genotype and allele of CYP2A6 gene and chi square test

Samples	N	Genotype Frequency			Allele Frequency		X^2
		GG	GT	TT	G	T	
BCS	36	1.000 (36)	0.000 (0)	0.000 (0)	1.000	0.000	-
CAS	35	1.000 (35)	0.000 (0)	0.000 (0)	1.000	0.000	-
JFT	20	0.900 (18)	0.100 (2)	0.000 (0)	0.950	0.050	2.161^{ns}
JTT	36	0.889 (32)	0.111(4)	0.000 (0)	0.944	0.056	0.125^{ns}
GS	20	1.000 (20)	0.000 (0)	0.000 (0)	1.000	0.000	-
GCS	45	0.978 (44)	0.000 (0)	0.022 (1)	0.978	0.02	45.000^{s}
JS	107	0.991(106)	0.009 (1)	0.000 (0)	0.995	0.005	0.002^{ns}
Total	299	0.973 (291)	0.024 (7)	0.003 (1)	0.985	0.015	13.228^{s}

N= number of samples; ns= not significant at P<0.05, s=significant at P<0.05); $\chi 2$= Chi-square, X_{tab}, 0.05= 3.841 ($\chi^2 < \chi_{tab}$) = significant.

Association of CYP2A6 gene polymorphism and fatty acid traits

The SNP of CYP2A6 (g.49170107 G>T) gene study showed that there is association between CYP2A6 gene polymorphism with fatty acids traits in Indonesian sheep. The CYP2A6 polymorphism is associated (P<0.05) with monounsaturated fatty acid (MUFA), especially erucic acid (C22:1n9). Sheep with the GT genotype contained higher erucic acid (C22: 1n9) than the GG genotype. The effect of the CYP2A6 genotype on fatty acid traits of Indonesian sheep is presented in Table 3. Cis-13-docosenoic acid or 22:1Δ13 was commonly known as erucic acid. Erucic acid is an unbranched long chain monounsaturated fatty acid that formed naturally in the body and metabolized to oleic acid. It contains 22 carbons and a cis-configurated double bond on C-13. Erucic acid also a member of omega 9 fatty acid. Long chain monounsaturated fatty acid is thought to have cardiotoxic effect as the study reported that there is association between high circulating of erucic acid content in the body with congestive heart failure (Imamura *et al*., 2013). High content erucic acid food consumption has negative impact on human health as they classified as natural toxicant (Abbott *et al.,* 2003). Begin with myocardial lipidosis (accumulation of lipid in the heart muscle fibers) as the effect of poor mitochondrial beta-oxidation of erucic acid in the heart, high accumulation of triacylglycerol will lead into reducing heart contractility before finally damage the tissue (Bremer and Norum, 1982). European Food Safety Authority (EFSA) recommends erucic acid Tolerable Daily Intake (TDI) are 7 mg per kg human body weight per day (EFSA, 2016). Overconsumption of SFA, trans MUFA, and

Table 3. The CYP2A6 genotypes association with fatty acid traits (%)

Variables	Genotype		
	GG (n=103)	GT (n=7)	TT (n=0)
Fat content	3.353 ± 3.333	3.003 ± 2.246	0.000 ± 0.000
Saturated Fatty Acid (SFA)	36.350 ± 14.050	35.360 ± 14.730	0.000 ± 0.000
Octanoic acid (C8:0)	0.038 ± 0.111	0.003 ± 0.008	0.000 ± 0.000
Capric acid (C10:0)	0.217 ± 1.372	0.107 ± 0.068	0.000 ± 0.000
Lauric acid (C12:0)	0.430 ± 0.474	0.427 ± 0.508	0.000 ± 0.000
Tridecyl acid (C13:0)	0.012 ± 0.013	0.007 ± 0.013	0.000 ± 0.000
Myristic acid (C14:0)	2.796 ± 1.853	2.681 ± 1.504	0.000 ± 0.000
Pentadecanoic acid (C15:0)	0.466 ± 0.217	0.400 ± 0.210	0.000 ± 0.000
Palmitic acid (C16:0)	16.799 ± 6.586	17.600 ± 7.500	0.000 ± 0.000
Heptadecanoic acid (C17:0)	0.827 ± 0.417	0.786 ± 0.419	0.000 ± 0.000
Stearic acid (C18:0)	14.500 ± 6.949	13.180 ± 6.250	0.000 ± 0.000
Arachidic acid (C20:0)	0.112 ± 0.099	0.087 ± 0.062	0.000 ± 0.000
Heneicosylic acid (C21:0)	0.022 ± 0.025	0.027 ± 0.035	0.000 ± 0.000
Behenic acid (C22:0)	0.059 ± 0.087	0.030 ± 0.024	0.000 ± 0.000
Trycosylic acid (C23:0)	0.030 ± 0.052	0.009 ± 0.015	0.000 ± 0.000
Licnoceric acid (C24:0)	0.045 ± 0.097	0.007 ± 0.011	0.000 ± 0.000
Unsaturated Fatty Acid	27.600 ± 13.020	31.200 ± 14.020	0.000 ± 0.000
Monounsaturated fatty acid (MUFA)	23.950 ± 12.110	27.280 ± 12.330	0.000 ± 0.000
Myristoleic acid (C14:1)	0.130 ± 0.107	0.120 ± 0.081	0.000 ± 0.000
Palmitoleic acid (C16:1)	1.404 ± 0.613	1.349 ± 0.653	0.000 ± 0.000
Heptadecenoic acid (C17:1)	0.307 ± 0.351	0.207 ± 0.259	0.000 ± 0.000
Elaidic acid (C18:1n9t)	2.885 ± 7.048	1.126 ± 1.279	0.000 ± 0.000
Oleic acid (C18:1n9c)	22.070 ± 11.540	25.590 ± 11.570	0.000 ± 0.000
Eicosenoic acid (C20:1)	0.023 ± 0.079	0.000 ± 0.000	0.000 ± 0.000
Erucic acid (C22:1n9)	0.001 ± 0.003b	0.004 ± 0.008a	0.000 ± 0.000
Nevornic acid (C24:1)	0.039 ± 0.089	0.011 ± 0.015	0.000 ± 0.000
Polyunsaturated fatty acid (PUFA)	3.651 ± 2.982	3.916 ± 2.114	0.000 ± 0.000
Linoleic acid (C18:2n6c)	2.133 ± 1.913	2.654 ± 1.531	0.000 ± 0.000
Linolelaidic Acid (C18:2n9t)	0.027 ± 0.072	0.054 ± 0.093	0.000 ± 0.000
α-Linolenic acid (C18:3n3)	0.303 ± 0.266	0.514 ± 0.440	0.000 ± 0.000
γ-Linolenic acid (C18:3n6)	0.028 ± 0.062	0.009 ± 0.011	0.000 ± 0.000
Eicosadienoic acid (C20:2)	0.044 ± 0.052	0.0557 ± 0.034	0.000 ± 0.000
Dihomo-γ-linolenic acid (C20:3n6)	0.066 ± 0.113	0.043 ± 0.032	0.000 ± 0.000
Arachidonic acid (C20:4n6)	0.857 ± 1.316	0.414 ± 0.293	0.000 ± 0.000
Docosadienoic acid (C22:2)	0.006 ± 0.037	0.000 ± 0.000	0.000 ± 0.000
Eicosapentanoic acid (C20:5n3)	0.178 ± 0.206	0.174 ± 0.238	0.000 ± 0.000
Docosahexanoic acid (C22:6n3)	0.043 ± 0.072	0.051 ± 0.044	0.000 ± 0.000
Fatty acid total	66.770 ± 23.840	67.700 ± 28.300	0.000 ± 0.000

a,b= significantly at (P<0.05).

cholesterol and has an overweight body (obesity) prone to fall on cardiovascular disease, atherosclerosis, and other diseases (USDA, 2010). This research result indicated that the CYP2A6 gene might plays important role in fatty acid metabolism. The family of cytochrome known as a gene that plays a role in meat quality, such us flavour and odour (Harahap *et al*., 2020; Listyarini *et al*., 2018; and Gunawan *et al*., 2013), pH, meat tenderness, and fatty acid composition (Harahap, 2021). Besides that, constitutive regulation of CYP2A6 (CYP2A5 in mice) expression in the liver was influenced by hepatic nuclear factor 4 alpha (HNF-4α) (Ulvila *et al*., 2004). HNF-4 transcription factors contribute in the activation genes involved in fatty acid oxidation (Chen *et al*., 2020). Genes contribute to lipid metabolism and transport such as ApoA-I (Malik and Karathanasis, 1996), ApoA-II (Ribeiro *et al*., 1999), ApoA-IV (Ktistaki *et al*., 1994), ApoE (Dang *et al*., 1995), MCAD (Carter *et al*., 1994), microsomal triglyceride transfer protein (MTP) (Hagan *et al*., 1994), and cholesterol 7a-hydroxylase (CYP7A) (Stroup and Chiang, 2000) were reported to be activated by HNF-4α. The disappearance of HNF-4α expression led to the accumulation of lipid in the liver, decrease serum cholesterol and triglyceride level, and excess concentration of serum bile acid (Hayhurst *et al*., 2001).

Conclusions

The CYP2A6 gene was polymorphic in JFT, JTT, GCS, and JS, but monomorphic in BCS, CAS, and GS. The analysis showed that the CYP2A6 gene was significantly associated with erucic acid. The research indicates that SNP g.49170107 G>T of CYP2A6 gene potential to be used as a genetic marker for selection of fatty acid traits in sheep quality.

Acknowledgments

This work was financially supported by PTUPT project from Indonesian Ministry of Research, Technology and Higher Education Fiscal Year 2021 Number: 1/E1/KP.PTNBH/2021 dated 08 March 2021.

References

Abbott, P., J. Baines, P. Fox, L. Graf, L. Kelly, G. Stanley, and L. Tomaska. 2003. Review of the regulations for contaminants and natural toxicants. Food Control 14: 383-389.

AOAC. 2012. Official Methods of Analysis. 19th edn. Association of Official Analytical Chemist, Arlington.

Bremer, J. and K. R. Norum. 1982. Metabolism of very long-chain monounsaturated fatty acids (22: 1) and the adaptation to their presence in the diet. J. Lipid Res. 23: 243-256.

Carter, M. E., T. Gulick, D. D. Moore, and D. P. Kelly. 1994. A pleiotropic element in the medium-chain acyl coenzyme A dehydrogenase gene promoter mediates transcriptional regulation by multiple nuclear receptor transcription factors and defines novel receptor-DNA binding motifs. Mol. Cell. Biol. 14: 4360–4372.

Cederbaum, A. I. 2015. Molecular mechanisms of the microsomal mixed function oxidases and biological and pathological implications. Redox Biol. 4: 60-73.

Chen, L., R. P. Vasoya, N. H. Toke, A. Parthasarathy, S. Luo, E. Chiles, J. Flores, N. Gao, E. M. Bonder, X. Su, and M. P. Verzi. 2020. HNF4 regulates fatty acid oxidation and is required for renewal of intestinal stem cells in mice. Gastroenterology. 158: 985-999.

Dang, Q., D. Walker, S. Taylor, C. Allan, P. Chin, J. Fan, and J. Taylor. 1995. Structure of the hepatic control region of the human apolipoprotein E/C- I gene locus. J. Biol. Chem. 270: 22577–22585.

DPKH. 2018. Statistik Peternakan dan Kesehatan Hewan. Direktorat Jenderal Peternakan dan Kesehatan Hewan, Jakarta.

EFSA Panel on Contaminants in the Food Chain (CONTAM). 2016. Erucic acid in feed and food. EFSA Journal. 14: e04593.

Gunawan, A., S. Sahadevan, M. U. Cinar, C. Neuhoff, C. Große-Brinkhaus, L. Frieden, D. Tesfaye, E. Tholen, C. Looft, D. S. Wondim, and M. Holker. 2013. Identification of the novel candidate genes and variants in boar liver tissues with divergent skatole levels using RNA deep sequencing. PLoS One. 8(8).p.e72298.

Gunawan, A., S. Tazkya, K. Listyarini, M. Yamin, I. Inounu, and C. Sumantri. 2018a. Karakterisasi gen KIF12 (Kinesin Familly 12) serta hubungannya dengan komposisi acid lemak pada domba. JITRO 5: 88-94.

Gunawan, A., D. Anggrela, K. Listyarini, M. A. Abuzahra, Jakaria, M. Yamin, I. Inounu, and C. Sumantri. 2018b. Identification of single nucleotide polymorphism and pathway analysis of Apolipoprotein A5 (APO5) related to fatty acid traits in indonesian sheep. Trop. Anim. Sci. J. 41: 165-173.

Gunawan, A., R. S. Harahap, K. Listyarini, and C. Sumantri. 2019. Identifikasi keragaman gen DGAT1 serta asosiasinya terhadap karakteristik karkas dan sifat perlemakan domba. JITRO 6: 259-266.

Hagan, D. L., B. Kienzle, H. Jamil, and N. Hariharan. 1994. Transcriptional regulation of human and hamster microsomal triglyceride transfer protein genes. Cell type-specific expression and response to metabolic regulators. J. Biol. Chem. 269: 28737–28744.

Harahap, R. S., R. R. Noor, and A. Gunawan. 2020. Effect of CYP2E1 gene polymorphisms on lamb odor and flavour in indonesian sheep. The 3th International Conference of Animal Science and Technology (ICAST) 2020; 2020 Nov 3; Makassar, Indonesia.

Harahap, R. S. 2021. Karakterisasi gen CYP2E1 dan HSD17B13 sebagai kandidat gen untuk perbaikan kualitas daging domba Indonesia. Thesis. IPB University, Bogor.

Hayhurst, G. P., Y. H. Lee, G. Lambert, J. M. Ward, and F. J. Gonzalez. 2001. Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Mol. Cell Biol. 21: 1393-403.

Imamura, F., R. N. Lemaitre, I. B. King, X. Song, L. M. Steffen, A. R. Folsom, D. S. Siscovick, and D. Mozaffarian. 2013. Long-chain monounsaturated fatty acids and incidence of congestive heart failure in 2 prospective cohorts. Circulation. 127: 1512–1521.

Kirby, M., G. D. Nichols, and M. Antenos. 2011. CYP2A5 induction and hepatocellular stress: an adaptive response to perturbations of heme homeostasis. Curr. Drug Metab. 12: 186-197.

Ktistaki, E., J. M. Lacorte, N. Katrakili, V. I. Zannis, and I. Talianidis. 1994. Transcriptional regulation of the apolipoprotein A-IV gene involves synergism between a proximal orphan receptor response element and a distant enhancer located in the upstream promoter region of the apolipoprotein C-III gene. Nucleic Acids Res. 22: 4689–4696.

Listyarini, K., Jakaria, M. J. Uddin, C. Sumantri, and A. Gunawan. 2018. Association and expression of CYP2A6 and KIF12 genes related to lamb flavour and odour. Trop. Anim. Sci. J. 41: 100-107.

Ma'rufi, R. and L. Rosita. 2014. Hubungan dislipidemia dan kejadian penyakit jantung koroner. JKKI 6: 47-53.

Malik, S. and S. K. Karathanasis. 1996. TFIIB-directed transcriptional activation by the orphan nuclear receptor hepatocyte nuclear factor 4. Mol. Cell. Biol. 16: 1824–1831.

McDonagh, E. M., C. Wassenaar, S. P. David, R. F. Tyndale, R. B. Altman, M. Whirl-Carrillo, and T. E. Klein. 2012. PharmGKB summary: Very important pharmacogene information for cytochrome P-450, family 2, subfamily A, polypeptide 6. Pharmacogenet. Genomics. 22: 695–708.

Munyaneza, J. P., A. Gunawan, and R. R. Noor. 2019a. Identification of single nucleotide polymorphism and association analysis of alpha 2-heremans schmid glycoprotein (AHSG) gene related to fatty acid traits in sheep. IJSRST. 6: 351-360.

Munyaneza, J. P., A. Gunawan, and R. R. Noor. 2019b. Exploring effects of betaine-homocysteine methyltransferase (BHMT) gene polymorphisms on fatty acid traits and cholesterol in sheep. JITAA. 44: 243-251.

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

Ribeiro, A., D. Pastier, D. Kardassis, J. Chambaz, and P. Cardot. 1999. Cooperative binding of upstream stimulatory factor and hepatic nuclear factor 4 drives the transcription of the human apolipoprotein A-II gene. J. Biol. Chem. 274: 1216–1225.

Roh, J. G., S. W. Kim, and K. S. Kim. 2011. Effect of the single nucleotide polymorphism from cytochrome P450 (CYP2A6) gene to fatty acid composition traits in Korean native pig crossed progeny. Korean J. Agric. Sci. 38: 689-693.

Rovadoscki, G. A., S. F. N. Pertile, A. B. Alvarenga, A. S. M. Cesar, F. Pértille, J. Petrini, V. Franzo, W. V. B. Soares, G. Morota, and M. L. Spangler.2018. Estimates of genomic heritability and genome-wide association study for fatty acids profile in Santa Inês sheep. BMC Genomics. 19: 375.

Sambrook, J. and D. Russel. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Stroup, D. and J. Y. Chiang. 2000. HNF4 and COUP-TFII interact to modulate transcription of the cholesterol 7alpha-hydroxylase gene (CYP7A1). J. Lipid Res. 41: 1–11.

Ulvila, J., S. Arpiainen, O. Pelkonen, K. Aida, T. Sueyoshi, M. Negishi, and J. Hakkola. 2004. Regulation of CYP2A5 transcription in mouse primary hepatocytes: roles of hepatocyte nuclear factor 4 and nuclear factor I. Biochem. J. 381: 887-894.

USDA. 2010. Dietary Guidelines for Americans 7th edn. Government Printing Off, Washington DC.

Wang, K., X. Chen, S. C. Ward, Y. Liu, Y. Ouedraogo, C. Xu, C. Xu, I. A. Cederbaum, and Y. Lu. 2019. CYP2A6 is associated with obesity: studies in human samples and a high fat diet mouse model. IJO. 43: 475-486.

Wang, X. H., X. X. Cui, X. Q. Sun, X. H. Wang, X. C. Li, Y. Qi, W. Li, M. Y. Han, I. Muhammad, and X. Y. Zhang. 2017. High fat diet-induced hepatic 18-carbon fatty acids accumulation up-regulates CYP2A5/CYP2A6 via NF-E2-related factor 2. Front Pharmacol. 8: 1-12.