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Association and Expression of CYP2A6 and KIF12 Genes Related to Lamb Flavour and Odour

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ABSTRACT

Cytochrome P450, family 2, subfamily A, polypeptide 6 (CYP2A6) and kinesin-like protein KIF12 (KIF12) genes are predicted as candidate genes which play important roles in lamb flavour and odour. The aim of this study was to analyse the genotype polymorphism of CYP2A6 and KIF12 genes, to study association and expression of these genes with lamb flavour and odour. Identification of genes polymorphism and associations of CYP2A6 and KIF12 genes were performed using PCR-RFLP method and GLM analysis. The PCR-RFLP products of CYP2A6 and KIF12 were digested by restriction enzyme B*smA*I and B*fa*I, respectively. The expression of CYP2A6 gene was performed using qRT-PCR. The results showed that the CYP2A6 and KIF12 genes were polymorphics. The CYP2A6 gene found to have two genotypes (TT and GT), whereas the KIF12 gene found to have three genotypes (CC, CT, and TT). The CYP2A6 and KIF12 genes were in Hardy Weinberg Equilibrium (HWE). Association analysis showed that CYP2A6 (g.49170107 G>T) was significantly (P<0.05) associated with 3-methylindole (MI) or skatole, while KIF12 (g.9617965 C>T) was not significantly associated with lamb flavour and odour. The GT genotype exhibited a greater 3-methylindole (MI) or skatole than the TT genotype (P<0.05). The mRNA expression analysis showed that CYP2A6 mRNA expression was higher (P<0.01) in animals with the TT genotype. These results will improve the understanding of the functions of the CYP2A6 in lamb flavour and odour, especially in term of 3-methylindole (MI) or skatole compound within the liver and will shed light on CYP2A6 as a candidate in the selection of sheep with low lamb flavour and odour.

Keywords: CYP2A6 gene, flavour, javanese fat tailed sheep, KIF12 gene, odour

INTRODUCTION

Improving meat quality including flavour and odour is a critical factor in the protection and development of markets for lamb. Improving meat quality can be done with nutritional (Bain *et al*., 2016) and genetic approach. Lamb consumption is uncommon in many Asian countries including Indonesia, whose consumers often complain about the unpleasant flavour or odour of the lamb (Schiller *et al.,* 2015; Hoffman *et al.,* 2015). Lamb flavour and odour is influenced by several factors, such as breed (Alberti *et al.,* 2008; Hopkins *et al.,* 2011), slaughter weight and age (Tejeda *et al*., 2008; Awan *et al.,* 2014), sex (Jayasena *et al.,* 2014), diet (Wood *et al*., 2008), and chemical compound (Watkins *et al.,* 2014). The main cause of lamb odour and flavour are two chemical compound: branch chain fatty acid (BCFA) and skatole (Watkins *et al.,* 2014; Ran-Ressler *et al.,* 2014; Henseler *et al*., 2014). Branched chain fatty acids (BCFAs: 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), and 4-methylnonanoic (MNA) acids) are chemical compounds that are accepted as the main contributors for flavour and odour and may play a role in lamb liking (Young *et al*., 2003; McRae *et al.,* 2013). 3-methylindole (MI or skatole) also involves in flavour and odour in pigs (Bonneau & Chevillon, 2012; Whittington *et al.,* 2011), and to a lesser extent 4-methylphenol (MP) are the main compounds implicated as contributors to 'pastoral' flavour (Young *et al*., 2003; Blanch *et al.,* 2012).

Genetic and breeding is recommended as one of the most realistic approaches for reducing the levels of lamb flavour and odour (SFO). The compounds of MNA, MI, MP, MOA, and EOA are the well defined compounds describing the phenotypic trait (lamb flavour and odour) which is possible to be improved through genetic selection. Skatole level showed a high heritability (0.41) indicating that they could be improved genetically (Windig *et al.,* 2012). Reduction of lamb fla-

vour and odour is of utmost interest for sheep breeders. Identification of genetic factors controlling lamb flavour and odour will contribute in breeding programmes to select animals that produce low levels of lamb flavour and odour.

From the result of our previous RNA seq analysis in liver tissue, cytochrome P450 2A6 (CYP2A6) and kinesin-like protein KIF12 (KIF12) genes showed higher expression level in low lamb flavour and odour than high lamb flavour and odour (Gunawan *et al*., 2018). CYP2A6 and KIF12 genes are reported to be functionally involved in flavour and odour in pig (boar taint) and meat quality trait (Chen *et al.,* 2008; Fan *et al*., 2010).

CYP2A6 is located in chromosome 14. CYP2A6 is one of the key enzymes involved in the skatole metabolism (Wiercinska *et al.,* 2012). Cytochrome P450 isoenzymes are the main enzymes playing roles in phase 1 skatole metabolism, where skatole is degraded to several intermediate products including indole-3-carbinol (I3C), 2-aminoacetophenone (2AAP) and 3-metyloxyindole (3MOI) (Robic *et al*., 2011). High activity of CYP2A6 contributes to lower levels of skatole (Lin *et al.,* 2004). Conversely low CYP2A6 activity is associated with an excessive acccumulation of skatole in the fat tissue of pigs (Chen *et al.,* 2008). KIF12 is located in chromosome 2. KIF12 has been proven related to pig fat and loin muscle (Fan *et al.,* 2010) and related to fatty acids metabolism (Yang *et al.,* 2014). Yang *et al.* (2014) demonstrates that the microtubule motor KIF12 mediates an antioxidant cascade in the beta cells as an intracellular target of excess fat intake or lipotoxicity. However, there was no study investigating the association and expression of CYP2A6 and KIF12 with meat flavour and odour in sheep especially in Javanese fat tailed sheep (DEG). Functional and positional studies suggested that these genes could be important candidate genes for lamb flavour and odour compounds. The aims of this study were to analyse the polymorphism of CYP2A6 and KIF12 genes, to unravel the association and expression of these genes with lamb flavour and odour.

MATERIALS AND METHODS

Animals and Phenotypes

The sheep used in this study were Javanese fat tailed sheep (DEG) (n=25). The sheep were bred at Mitra Tani Farm, Ciampea, Bogor (Latitude/Longitude : 6º 33′ 17″ S/ 106º 42′ 3″ E) and were maintained under the same management systems and was not siblings between each other. The sheep were caged in group and were given ad libitum fattening feed. Samples were taken from loin and liver tissues and phenotypes were collected from the male Javanese fat tailed sheep (DEG) (n=25)with body weight between 25-30 kg and age between 8-12 months. All experimental sheep (n=25) were slaughtered in a commercial abattoir. Carcass and meat quality data were collected according to guidelines of the Indonesian performance test. The loin was taken as much as 500 g for flavour and odour analysis and 30 mg of loin for DNA extraction. The liver was taken as much as 30 mg for RNA extraction. The loin and liver tissues were collected. Then these samples were put in an ice flask and were stored at a temperature of -20 ºC.

Flavour and Odour Analyses

Analysis of flavour and odour compounds were carried out on loin samples. The volatile flavours and odour compounds were extracted using Likens-Nicerson method which is a combination of distillation and extraction with solvent simultaneously using Gas Chromatography Mass Spectophotometry (GC-MS) tool. The phenotypics of lamb flavour and odour measured were MNA, MP, MI or skatole, MOA, and EOA. Lamb having a fat BCFA (MNA and MP) greater than 215 µg/g and less than 229 µg/g were defined as low and high lamb odours, respectively (Watkin *et al.,* 2014). For the flavour, sheep having a fat skatole level less than 0.25 µg/g and greater than 0.25 µg/g were defined as low and high skatole samples, respectively (Gunawan *et al.,* 2013a; Strathe *et al.,* 2013).

DNA Extraction and PCR-RFLP Amplification

DNA extraction was started with the removal of loin tissue. Then, the collected tissue was extracted according to Sambrook & Russel (2001) protocols. Amplification of CYP2A6 and KIF12 genes fragments were conducted using the GeneAmp PCR system ESCO. A total of two pairs of primers (Table 1) were designed in MEGA 6.0 and the profile was checked using Primer Stat. DNA samples were taken as much as 1 µL (50 ng) of extraction and were mixed with the premix with a volume of 14 µL. The premix was made by mixing0.3 µL of primer (5 pmol), 0.4 µL dNTPs (0.16 mM), 1 µL $MgCl_2$ (1.5 mM), 1.5 µL of 1×buffer, 0.05 µL Taq polymerase enzyme (1 U), and 10.75 µL distilled water. The mixture of premix DNA samples were incubated using PCR thermocycler machine. Amplification process began denaturation step at 94 °C, a five-minute dive. The second phase consisted of 35 cycles, each cycle consisted of denaturation process at 94 ºC for 10 seconds, primer annealing at temperatures 55 ºC for CYP2A6 gene, 62 ºC for KIF12 gene for 20 seconds, and DNA extension at 72 °C for 30 seconds. The final stage was the primer extension at 72 °C for ten minutes. The DNA amplification products (286 bp for CYP2A6 gene and 553 bp for KIF12 gene) were visualized by gel electrophoresis agarose 1.5%.

PCR-RFLP were used for validation of SNP genotyping. PCR product and restriction enzymes (B*smA*I for CYP2A6 and B*fa*I for KIF12 gene) were incubated at 37 ºC for 4 hours (Thermo Fisher Scientific, EU, Lithuana). An aliquot of the PCR product of each reaction were checked on 1.5% agarose gel (Fisher Scientific Ltd.) before digestion using different endonucleases (B*smA*I for CYP2A6 and B*fa*I for KIF12 gene). The digested products were separated using 2.0% agarose gel which was stained with FluoroSafe. The fragments were visualized under UV Transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA).

Table 1. GenBank accession numbers and primer sequences

Gene name	Accession number	Primer sequence	Application	Size (bp)	Ta (°C)	Enzyme
CYP2A6	NC_019471.2	F: 5′-CTT TCT GGT CCT CAT CTT TG-3′ R: 5′-GGT ATT GAT GAG GAA TGG TG-3′	Genotyping	286	55	B*sm*AI (5′-GTCGCT-3)
KIF12	NC_019459.2	F: 5′-CAC AGT GAG TGG ACT CAG AC-3′ R: 5′-GCT GCT ACG CCA TTG AAC AG-3′	Genotyping	553	62	B*fa*I (5′-CTAG-3′)
CYP2A6	XM_004015267.3	F: 5′-CAA GGA TAC CAA GTT TCG AG -3′ R: 5′-ATG ATG GTG GTG AAG AAG AG -3′	qRT-PCR	246	55	
GAPDH	NC_019460.2	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	NC_019471.2	F: 5′-GAA AAC GAG ATG AGA TTG GC -3′ R: 5′-CCA TCA TAG AGT GGA GTT CG-3	qRT-PCR	194	62	

Selection of mRNA for Gene Expression

Liver tissues from 6 sheeps with divergent lamb flavour and odour based on different genotype of CYP2A6 were selected for mRNA expression study. The KIF12 genes were not selected for mRNA expression study because they were not significant based on the association study. For this purpose, 3 DEG sheep with TT genotype and 3 DEG sheep with GT genotype were selected. The significant difference between the two groups were calculated using GLM test in SAS ver 9.2 (SAS Institute Inc., Cary, USA).

RNA Extraction and Reverse Transcriptase PCR

RNA extraction began with the removal of liver tissue. The collected tissue was then 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 based on the manufacturing protocol.

Quantification of cDNA

Quantification of cDNA were done by qRT-PCR method with AG qTower 4 channel Analytic Jena Engine, Germany. Gene specific primers for the qRT-PCR (Table 1) were designed in MEGA 6.0 and checked the profile using Primer Stat. In each run, the 96-well microtiter plate contained each cDNA sample and no template control. All samples were analysed twice (technical replication) and the geometric mean of the Ct values were further used for mRNA expression profiling (Gunawan *et al*., 2013b). The geometric mean of two housekeeping genes GAPDH and β-Actin were used for normalization of the target genes. Final results were reported as the relative abundance level after normalizing with mRNA expression level of the housekeeping gene (Gunawan *et al*., 2011).

Data Analyses

Genotyping study. After obtaining genotype through PCR-RFLP method, the value of the genotype and allele frequencies, and Hardy Weinberg Equilibrium (HWE) value were calculated by the following formula:
Genotype and Allele Frequencies (Nei & Kumar, 2000)

$x_{ii} = n_{ii} / N$ $\quad x_i = (2n_{ii} + \sum n_{ij}) / 2N$

Where :
x_{ii} = frequency of genotype ii
x_i = frequency of alel i
n_{ii} = total individuals with genotype ii
n_{ij} = total individuals with genotype ij
N = population size

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

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

Where :
χ^2 = chi-square
O = total of observations genotype to-i
E = total of genotype to expectations to-i

Assosiation study. Association of CYP2A6 and KIF12 genes related to flavour and odour compounds analyses were performed using SAS ver 9.2 (SAS Institute Inc., Cary, USA). The effects of genotype lamb flavour and odour compounds were assessed by the fixed effect model (ANOVA) using PROC GLM. Least square mean values for the loci genotypes were compared by t-test and p-values were adjusted by the Tukey-Kramer correction (Kayan *et al*., 2011; Cinar *et al*., 2012).

$$Y_{ijk} = \mu + genotype_i + e_{ij}$$

Where :
Y_{ijk} = the lamb compounds (MNA, MI, MP, MOA, and EOA)
μ = the population mean
$genotipe_i$= the fixed effect of i-th genotype (i= 1, 2, and 3)
e_{ij} = the residual error

Expression study. Differences in CYP2A6 mRNA expression were analysed with the simple t-test in SAS software (SAS Institute Inc., ver. 9.2) based on delta Ct (ΔCt). Values of $P<0.05$ indicate statistically significant differences. The delta Ct (ΔCt) values were calculated as the difference between target gene and geometric mean of the reference genes: $\Delta Ct = C_{ttarget} - C_{thousekeeping}$ genes (Silver *et al*., 2006).

RESULTS

Phenotype of Lamb Flavour and Odour

The descriptive statistics for lamb flavour and odour in Javanese fat tailed sheep were presented in Table 2. The average concentration of lamb flavour and odour including MNA, MI, MP, MOA, and EOA were 0.373±0.123, 0.264±0.062, 24.030±2.910, 0.138±0.065, and 0.362±0.087 μg/g, respectively (Table 2).

Polymorphism of CYP2A6 and KIF12 Genes

A SNP was genotyped in CYP2A6 (g.49170107 G>T) and KIF12 (g.9617965 C>T). The SNPs of these genes were genotyped in a population of Javanese fat tailed sheep (n=25). The SNP was confirmed by PCR-RFLP. The DNA restriction fragments obtained for g.49170107 G>T of CYP2A6 polymorphism were: 286 bp for the TT genotype and 286, 217, and 69 bp for the GT genotype (Figure 1). The DNA restriction fragments obtained for g.9617965 C>T of KIF12 polymorphism were: 553 and 143 bp for the TT genotype and 553, 413, 143, and 120 bp for the CT genotype, and 413, 143, and 120 bp for the CC genotype (Figure 1). In this study, two genotypes (TT and GT) were found for SNPs at g.49170107 G>T in our populations (Table 3) and three genotypes (CC, CT, and TT) were found for SNPs at g.9617965 C>T in our populations (Table 3). The genotype and allele frequencies of CYP2A6 and KIF12 genes in Javanese fat tailed sheep are presented in Table 4. In case of CYP2A6, homozygote TT and heterozygote GT were more frequent, and homozygote GG was not found in our populations. For KIF12, heterozygote CT and homozygote TT were more frequent, and homozygote CC was rare in our populations. Both genes were detected in Hardy Weinberg Equlibrium (HWE) (P<0.05).

Association of CYP2A6 and KIF12 Genes Polymorphisms with Lamb Flavour and Odour

Association analysis showed that the CYP2A6 gene (g.49170107 G>T) was associated with MI or skatole (Table 4). Individuals with the GT genotype had higher value than the TT genotype among traits (P<0.05) in CYP2A6 gene. This result indicated that the CYP2A6 gene identified by SNP was associated with an increase in MI or skatole in heterozygote but decrease in homozygote animals. However, the KIF12 gene (g.9617965 C>T) had no significant effect on lamb flavour and odour compounds (Table 4). This gene was not associated with all the phenotypes.

Expression of CYP2A6 Gene

In order to determine whether the RNA expressions of the associated CYP2A6 genes were differed between phenotypically divergent sheep livers, quantitative real-time PCR were performed. Quantitative real-time PCR showed that the CYP2A6 mRNA was differentially regulated (P<0.01) between animals with GT genotype (high MI or skatole) and TT genotype (low MI or skatole) in the liver. Higher transcript abundance was detected in animals with TT genotype in the liver (P<0.01) compared to animals with GT genotype in the liver (Figure 2).

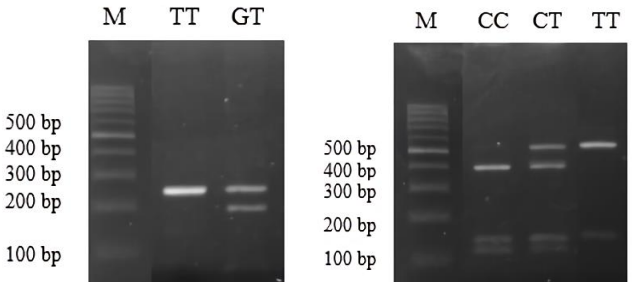
DISCUSSION

In phenotype of lamb flavour and odour study, MNA was the most abundant of the BCFAs while MOA was the least abundant and EOA was intermediate between these two compounds. These results were inconsistent with the previous study by Watkins *et al.* (2014)

Table 2. Descriptive statistics of sheepmeat flavour and odour

Flavour and odour compound (μg/g)	n	Mean	S.E.	Minimum	Maximum
MNA	25	0.373	0.123	0.000	2.836
MI	25	0.264	0.062	0.017	1.379
MP	25	24.03	2.910	3.340	53.08
MOA	25	0.138	0.065	0.000	1.627
EOA	25	0.362	0.087	0.000	1.611

Note: n= Number of samples, MNA= 4-methylnonanoic, MI= 3-methylindole, MP= 4-methylphenol, MOA= 4-methyloctanoic, EOA= 4-ethyloctanoic



A. CYP2A6 B. KIF12

Figure 1. PCR-RFLP genotyping results for the CYP2A6 gene (A) and KIF12 gene (B). M= 100 bp ladder size standard; TT, GT, CC, CT= genotype.

Table 3. The number of animals per genotype and allele frequency

Polymorphism	n	Genotype frequency			Allele frequency		χ2
		TT	GT	GG	G	T	
CYP2A6	25	0.88(22)	0.12(3)	0.00(0)	0.94	0.06	0.101*
		CC	CT	TT	C	T	
KIF12	25	0.12(3)	0.56(14)	0.32(8)	0.4	0.6	0.694*

Note: n = Number of samples, * = Significantly different (χ2 0.05= 3.841), χ2 = Chi-square

reported that MOA was the most abundant among the BCFAs while MNA was the least abundant and EOA was intermediate between these two compounds. The mean MOA concentration for sheep of Terminal sire type of the samples taken at Katanning was 230 µg/kg while, for EOA and MNA, the mean concentrations were 51 and 50 µg/kg, respectively. Watkins *et al.* (2010) reported that MOA, MNA, and EOA were detected to be influenced by sex and age. The MP and MI compounds of the lamb flavour and odour were also measured for these samples. The mean values of MI compound measured in this study were similar to the values measured in Neuhoff *et al.* (2013) and Morlein *et al.* (2012). Neuhoff *et al.* (2013) reported that the mean value of skatole was 0.201 µg/g.

The polymorphism study of CYP2A6 and KIF12 gene fragments were succesfully amplified using PCR for all samples. There were two genotypes for CYP2A6 gene (TT and GT) and three genotypes for KIF12 gene (CC, CT, and TT). In case of CYP2A6, homozygote GG was not found in our populations and this result was similar to another study reported with different genes (CAST-Msp1 locus) that only found two types of genotypes (MN and NN) in Indonesian local sheep (Sumantri *et al.*, 2008). GG genotype was not found probably due to a non-random mating system or because of direct selection (Bourdon 2000). Previous study conducted by Gunawan *et al.* (2013a) reported in animals with different families of cytochrome CYP4A25 that genotype AC had higher value in skatole. Neuhoff *et al.* (2015) reported in animal with different families of cytochrome CYP21 (g.3911T<C) were found that homozygous CC genotype had higher value in skatole. Both genes were detected in Hardy Weinberg Equlibrium (HWE) (P<0.05). The Hardy Weinberg is in equilibrium state if the genetic variation, allele and genotype frequencies in a population remain constant from one generation to the next in the absence of disturbing factors (Allendorf *et al.*, 2013).

In association study, the KIF12 gene (g.9617965 C>T) had no significant effect on lamb flavour and odour compounds. This result was in agreement with the results of previous studies with different genes that there was no significant association of the studied genes to levels of skatole or indole (Moe *et al.*, 2009). Yang *et al.* (2014) demonstrated that the microtubule motor KIF12 mediated an antioxidant cascade in beta cells as an intracellular target of excess fat intake or lipotoxicity. Kinesin-superfamily proteins (KIFs) consists of an emerging superfamily of molecular motors with functions largely relate to the microtubule cytoskeleton (Hirokawa *et al.*, 2009). The KIFs share a common motor domain that translocates along microtubules and serves to convey cargos relevant to developmental signaling (Ueno *et al.*, 2011; Zhou *et al.*, 2009). Our results showed that the CYP2A6 gene polymorphism was associated with MI or skatole. The results of previous studies suggest that CYP2A6 and CYP1A2 are the two main genes of the CYP family which have the most significant effect on the phase I of skatole metabolism (Matal *et al.*, 2009). Gunawan *et al.* (2013a) have identified several polymorphisms in CYPA25, CYP4A24, and CYP4B24 but a variant in CYP4A25 (ANCg.152197351) failed to be associated with skatole level. Several QTL for androstenone and skatole are reported in the region on SSC7 which embraces the candidate gene CYP21 (Grindflek *et al.* 2011). According to Duijvesteijn *et al.* (2010) and

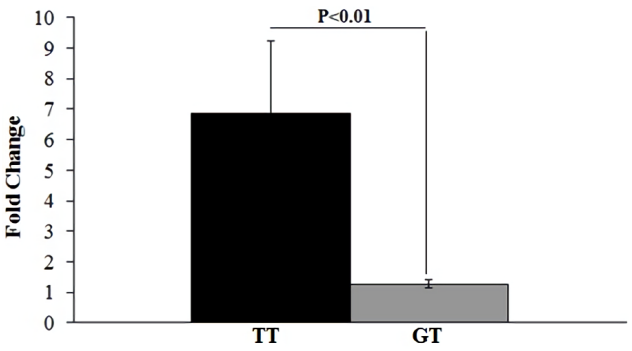


Figure 2. mRNA expression of CYP2A6 in liver from high MI or skatole (GT genotype) and low MI or skatole (TT genotype)

Table 4. Genotype and association analysis of candidate genes with flavour and odour compounds

Polymorphism	Flavour and odour compound (ug/g)	Genotype (µ ± S.E)		
		TT (n = 22)	GT (n=3)	GG (n=0)
CYP2A6	MNA	0.362 ± 0.134	0.591 ± 0.569	-
	MI	0.215 ± 0.046a	0.825 ± 0.554[b]	-
	MP	23.370 ± 3.160	24.200 ± 11.400	-
	MOA	0.141 ± 0.073	0.165 ± 0.135	-
	EOA	0.325 ± 0.077	0.805 ± 0.805	-
		CC (n=4)	CT (n=13)	TT (n=8)
KIF12	MNA	0.026 ± 0.005	0.520 ± 0.214	0.289 ± 0.146
	MI	0.183 ± 0.047	0.229 ± 0.060	0.357 ± 0.169
	MP	22.801 ± 8.650	26.667 ± 4.410	18.422 ± 4.320
	MOA	0.162 ± 0.076	0.067 ± 0.027	0.260 ± 0.198
	EOA	0.570 ± 0.520	0.356 ± 0.110	0.306 ± 0.122

Note: MNA= 4-methylnonanoic, MI= 3-methylindole, MP= 4-methylphenol, MOA= 4-methyloctanoic, EOA= 4-ethyloctanoic. Means in the same row with different superscripts differ significantly (P<0.05).

Chen *et al.* (2008), the gene known in the human genome as CYP2A6 is identical with the pig CYP2A19 gene, although in a number of studies concerning boar taint this gene is being referred to as CYP2A6. Diaz & Squires (2000) and Lin *et al*. (2004) also confirmed its significant role in skatole metabolism and assumed that a close monitoring of CYP2A6 levels and activity could help to regulate the levels of skatole. Lee *et al.* (2005) reported that skatole is formed from the amino acid L-tryptophan during the degradation of proteins. It is produced by the bacteria commonly found in the large intestine of monogastric animals and it is responsible for giving the meat a faecal-like odour.

The mRNA expressions of CYP2A6 gene were up-regulated in the liver. Zamaratskaia & Squies (2009) reported that metabolic processes in the liver play an essential role in skatole or MI accumulation. The expression of CYP2A6 mRNA was much higher in liver compared with the other tissues. The degradation of skatole consists of two phases, i.e., oxidative, followed by conjugation reactions. Phase I involves mainly the cytochrome P450 (CYP) family. Cytochrome P450 isoenzymes are the main enzymes playing roles in phase 1 skatole metabolism, where skatole is degraded to several intermediate products including indole-3-carbinol (I3C), 2-aminoacetophenone (2AAP) and 3-metyloxyindole (3MOI) (Robic *et al.* 2011). The main enzymes are considered to be CYP2A as well as aldehyde oxidase (AO) (Lanthier *et al*., 2007). Phase I of skatole metabolism results in an attachment of a hydroxyl group, which is then used to create a conjugate in phase II (Zamaratskaia & Squires, 2009).

In this study, the mRNA expression of CYP2A6 was up-regulated in high lamb flavour and odour (GT genotype). High activity of CYP2A6 contributed to the lower levels of skatole (Lin *et al.,* 2004). Conversely low CYP2A6 activity is associated with an excessive acccumulation of skatole in the fat tissue of pigs (Chen *et al.,* 2008). Lin *et al.* (2004) demonstrated that low CYP2A6 activity in some pigs was related to a functional polymorphism in the coding region of the corresponding gene and suggested that this polymorphism contributed to a high skatole accumulation. The higher expression of CYP2A6 in sheep with lower skatole (TT genotype) suggested that CYP2A6 might be involved in regulating flavour and odour in sheeps.

CONCLUSION

The CYP2A6 and KIF12 genes were polymorphic in Javanese fat tailed sheep. An association analysis indicated that CYP2A6 gene was associated with lamb flavour and odour compound (MI or skatole). The expressions of CYP2A6 was higher in low MI or skatole (TT genotype) compared to the high MI or skatole (GT genotype). This study demonstrated that polymorphisms of CYP2A6 gene (TT genotype) might contribute to the selection for reducing lamb flavour and odour in Javanese fat tailed sheep.

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