Expression of CYP2A6, KIF12, and SULT1C1 in liver of sheep with divergent sheepmeat flavour and odour

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Abstract. The aim of this study was to investigate the expression of some of the key enzymes involved in liver sample of sheep with high and low sheepmeat flavour and odour. The study was conducted with Indonesian Javanese fat tailed sheep. Sheep having a fat branched chain fatty acids 4-methylnonanoic (MNA) greater and less than 215 μg g⁻¹ and 229 will be defined as low and sheep meat odour, respectively. For the flavour, sheep having a fat skatole level less than 0.25 μg g⁻¹ and greater than 0.25 μg g⁻¹ will be defined as low and high flavour samples, respectively. The enzymes investigated were cytochrome P450 2A13 (CYP2A6), kinesin-like protein KIF12 (KIF12), and sulfotransferase 1C1 (SULT1C1). Expression of CYP2A6 in liver had differ between animals with high and low sheep meat flavour. Expression of CYP2A6, which catalyses the first stage of oxidation degradation, was increased in high sheep meat flavour and odour (P > 0.05). Similar pattern, the expression of SULT1C1, which catalyse the second stage of conjugation steroid catabolism, was increase in high sheep meat flavour and odour (P > 0.05). In contrast, the expression of KIF12 was decreased in high sheep meat flavour and odour animals. It is suggested that accumulation sheep meat flavour and odour in liver tissue of Indonesian Javanese fat tailed might be related to a high rate of oxidation in metabolic stage I and conjugation degradation in metabolic stage II.

1. Introduction
Sheep meat consumption is uncommon in many Asian countries including Indonesia, whose consumers often complain about the unpleasant flavour or odour of the sheep meat [1]. The main causes of sheepmeat odour are the two compounds (branched chain fatty acid and skatole): branched chain fatty acid (BCFA), present in all fatty tissue, have been implicated as cause of such flavours as, has indole, which originated from pastoral diets [2, 3]. Branched chain fatty acids (BCFAs; 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic acids) are the chemical compounds that are accepted as the main contributors for odour and flavour [3]. 3-methyldindole (skatole) also involved with ‘boar’ taint in pigs, and to a lesser extent 4-methylphenol (p cresol) are the main compounds implicated as contributors to ‘pastoral’ flavour [4].

A number of candidate genes have been identified for reducing sheepmeat flavour and odour compound with the final goal to utilize this genetic information in the breeding scheme [5]. A recent study using RNA deep sequencing technology identified a number of genes including cytochrome P450 2A13 (CYP2A6), kinesin-like protein KIF12 (KIF12), and sulfotransferase 1C1 (SULT1C1) that were expressed differentially in sheepmeat with divergent levels of flavour and odour compounds [5].
CYP2A6 is one of the enzymes involved in the hepatic metabolism of a naturally produced compound, skatole, in the pig. Low CYP2A6 activity has been linked to excessive accumulation of skatole in pig adipose tissue and development of the phenomenon “boar taint” [6]. KIF12 is the gene encoding a novel kinesin, underlie the Chr 4 main effect on the renal and biliary phenotypes. KIF12 is transcriptionally downregulated by chronic exposure to fatty acids treatment in beta cells [7]. Sulfotransferase enzymes including SULT2A1, SULT2B1 and SULT1A1 are associated with metabolic clearance of androsterone and skatole since they are known to conjugate steroid hormones and drugs into more water soluble compounds that facilitates excretion.

Notably, no study investigated the expression of these genes in sheepmeat flavour and odour. However, functional and positional studies suggested that these genes could be important candidate genes for sheepmeat flavour and odour compounds. Therefore, the present study was aimed to investigate the expression of some of the key enzymes (CYP2A6, KIF12, and SULT1C1) involved in liver sample of sheep with high and low sheepmeat flavour and odour in Indonesian Javanese fat tailed sheep. The results of this study might highlight the selected genes for further study with regard to the sheepmeat flavour and odour.

2. Material and Methods

2.1. Animals

Tissue samples were collected from the Indonesian Javanese fat tailed sheep animals. All the sheep (n=30) were slaughtered in a commercial abattoir. The carcass and meat quality data were collected according to guidelines of the Indonesian performance test. Tissue samples from liver were frozen in liquid nitrogen immediately after slaughter and stored at -20°C until used for isolation of total RNA.

2.2. Isolation of Total RNA and Quantitative Real Time PCR

Total RNA was isolated from the liver using RNeasy Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. DNase treated total RNA was reverse-transcribed with the High-Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA) to produce cDNA. For qRT-PCR, total RNA and cDNA synthesis from liver tissues of two divergent groups of animals (sheepmeat flavour and odour) were done as described above. Gene specific primers for CYP2A6, KIF12, and SULT1C1 for the qRT-PCR were designed by using the Primer3 software [8] (table 1). In each run, the 96-well microtiter plate contained each cDNA sample and no-template control. The qRT-PCR was conducted with the following program: 95 °C for 1 min and 40 cycles; 95 °C for 15 s; 55 and 62 °C for 30 s; and 72 °C for 30 sec on the Analytikjena qTower. For each PCR reaction, 2 μl iTaq™ SYBR® Green Supermix with Rox PCR core reagents (Bio-Rad), 2 μL of cDNA (50 ng/μL) and an optimized amount of primers were mixed with ddH₂O to a final reaction volume of 10 μL per well. All samples were analysed twice (technical replication) and the geometric mean of the Ct values was further used for mRNA expression profiling. The geometric mean of two housekeeping genes GAPDH and Beta Actin was used for the normalization of the target genes. The delta Ct (ΔCt) values were calculated as the difference between target gene and geometric mean of the reference genes: (ΔCt = Ct_target - Ct_housekeeping genes) as described previously [9]. The final results were reported as fold change calculated from delta Ct-values. The differences in CYP2A6, KIF12, and SULT1C1 gene expressions were analysed with the paired t tests. The values of P < 0.05 were considered to indicate statistically significant differences.
Table 1. GenBank accession numbers and primer sequences

| Gene name  | Accession number | Primer sequence                        | Size (bp) | Tm (°C) |
|------------|------------------|----------------------------------------|-----------|---------|
| CYP2A6     | XP_004015316.1   | F: 5’- CAAGGATACCAAGTTCGAG-3’          | 246       | 55      |
|            |                  | R: 5’- ATGATGTTGGTGAAGAAGAG-3’         |           |         |
| KIF12      | XP_011967829.1   | F: 5’- CTGGATGGTCCCTGACTCT-3’          | 196       | 55      |
|            |                  | R: 5’- TAAGGACCCATT TCAGAAGGT-3        |           |         |
| SULT1C1    | XP_004005969.1   | F: 5’- ATCATCCATCACACATCCT-3’          | 190       | 55      |
|            |                  | R: 5’- CAGCCCATCTCTCCTCCATAG-3         |           |         |
| GAPDH      | NC_019460.2      | F: 5’- GAGAAACCTGCCAAGTATGA-3’         | 203       | 62      |
|            |                  | R: 5’- TACCAGGAAATGAGCTTGAC-3          |           |         |
| Beta Actin | NC_019471.2      | F: 5’- GAAAACGAGATGAGATTGGGC-3’        | 194       | 62      |
|            |                  | R: 5’- CCATCATAGAGTGAGTTTCG-3          |           |         |

3. Result and Discussion

3.1. mRNA Expression by Quantitative Real Time PCR

Quantitative real-time PCR analysis showed the abundance of CYP2A6, KIF12, and SULT1C1 transcript in animals with divergent sheepmeat flavour and odour. The results showed that the CYP2A6, KIF12, and SULT1C1 mRNA was differentially regulated between animals with high and low sheepmeat flavour and odour values (P > 0.05) in the liver. Expression of CYP2A6, which catalyses the first stage of oxidation degradation, was increased in high-sheepmeat flavour and odour (P > 0.05) (figure 1). The mRNA expression of CYP2A6 which were up-regulated in high sheepmeat flavour and odour (figure 1) might indicate its function with regard to a high rate of oxidation in metabolic stage I. The expression pattern of the CYP2A6 gene was consistent with the previous study of sheepmeat flavour [10]. Babol et al. [11] Diaz and Squires [12] reported that the first stage of hepatic skatole metabolism involves the P450 system, in particular the isoforms CYP2E1 and CYP2A6. Lin et al. [13] established that CYP2A6 mRNA was only present in liver and kidney. The expression of CYP2A6 mRNA was much higher in liver compared with the other tissues. Cytochrome P450 isozymes are the main enzymes playing roles in phase 1 skatole metabolism, where skatole is degraded to several intermediate products including such as indole-3-carbinol (I3C), 2-aminoacetophenone (2AAP) and 3-metoxiyindole (3MOI) (details reviewed by [14, 15]). The main enzymes of phase 2 skatole metabolism are UDPGT (uridine 5'-diphospho-glucuronosyltransferase) and SULTs (sulfotransferase) [16].

In contrast, the expression of KIF12 was decreased in high sheep meat flavour and odour animals (figure 2). It is suggested that accumulation sheep meat flavour and odour in liver tissue of Indonesian Javanese fat tailed might be related to a high rate of oxidation in metabolic stage I and conjugation degradation in metabolic stage II.

![Figure 1. mRNA expression of CYP2A6 in liver tissues from high and low sheepmeat flavour and odour](image-url)
The expression of KIF12 was decreased in high sheepmeat flavour and odour animals (figure 2). Yang et al. [7] reported that KIF12 is transcriptionally downregulated by chronic exposure to fatty acids, this antioxidant cascade involving KIF12 and Hsc70 is proposed to be a critical target of nutritional excess in beta cells in diabetes. Similar pattern with CYP2A6, the expression of SULT1C1, which catalyze the second stage of conjugation steroid catabolism, was increase in high sheep meat flavour and odour ($P > 0.05$) (figure 3). The expression pattern of the SULT1C1 gene was consistent with the previous study of rats [17]. Klaassen et al. [17] reported that SULT1C1 mRNA increase in males. Hypophysectomy abolished SULT1C1 expression in male rats. It is suggested that accumulation sheepmeat flavour and odour in liver of Indonesian Javanese fat tailed might be related to a high rate of oxidation in metabolic stage I and conjugation degradation in metabolic stage II that CYP2A6, KIF12 and SULT1C1 were involved.

4. Conclusion
Expression of CYP2A6 and SULT1C1 which catalyses the first stage of oxidation degradation and the second stage of conjugation steroid catabolism was increased in high sheepmeat flavour and odour ($P > 0.05$). In contrast the expression of KIF12 was decreased in high sheepmeat flavour and odour animals. This study proposed candidate genes such as CYP2A6, KIF12, and SULT1C1, that might be involved in the liver for sheepmeat flavour and odour metabolism.
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