J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
49(3):204-214, September 2024
DOI: 10.14710/jitaa.49.3. 204-214

Association of CYP2A6 gene related to the characteristic carcass, commercial cuts, quality of meat and cholesterol of lamb meat

R. F. Istiqlal[1], K. Listyarini[2], J. Jakaria[2], C. Budiman[2], and A. Gunawan[2]*
[1]*Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia*
[2]*Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia*
**Corresponding E-mail: agunawan@apps.ipb.ac.id*

Received December 10, 2023; Accepted June 21, 2024

ABSTRACT

As meat-producing livestock, sheep are a benchmark for communities seeking quality and healthy meat. The CYP2A6 gene has emerged as a potential tool for improving lamb meat quality. This study investigated the genetic variability of the CYP2A6 gene and its association with key determinants of lamb meat quality. The research involved 140 male sheep from five breeds: Javanese thin-tail sheep (JTTS), Garut sheep (GS), Jonggol sheep (JS), Barbados cross sheep (BCS), and Compass agrinak sheep (CAS). PCR-RFLP was employed to identify CYP2A6|BsmAI gene polymorphism and the general linear model (GLM) was used to analyze the gene's association with meat quality. The results showed a polymorphism in the CYP2A6 gene (SNP g.49170107 G>T), presenting two genotypes: GG and GT. The analysis results demonstrated that the CYP2A6 gene ($P<0.05$) significantly correlates with carcass characteristics (live weight, hot carcass weight, carcass percentage, cold carcass weight), commercial cuts, physical meat quality, and cholesterol content. The GT genotype exhibited superior meat quality to the GG genotype, suggesting that the CYP2A6 gene could serve as a valuable genetic marker for enhancing lamb meat quality.

Keywords: Carcass characteristics, Cholesterol, Commercial cuts, CYP2A6, Sheep

INTRODUCTION

The demand for lamb meat exhibits a consistent upward trend each year. The quality of lamb meat is vital in influencing consumer preferences. Alongside quality, the nutritional content of lamb meat is also crucial for enhancing its appeal to the public. However, meeting the growing demand for sheep is challenging due to insufficient breeding stock. Developing sheep farming for fattening is simpler than breeding, leading to an inadequate supply of breeding sheep. As demand and the sheep population grows, it is imperative to enhance the quality of the meat to meet consumer expectations.

Public perception regarding nutritional values, such as high cholesterol and saturated fatty acid content, is also a contributing factor to the limited interest in sheep meat (Suryadi *et al.,* 2016). One strategy to improve the quality and nutritional value of sheep meat is to identify genes that affect cholesterol content and meat quality. Previous RNA-seq findings by Gunawan *et al.* (2018) highlighted the CYP2A6 gene as a potential candidate for enhancing lamb meat

quality. CYP2A6, also known as cytochrome P450, family 2, subfamily A, polypeptide 6, is situated on chromosome 14 and comprises 9 exons. It belongs to the cytochrome P450 group, which catalyzes various reactions involving sulfur-bound iron and produces detectable carbon monoxide complexes at 450 nm. This cytochrome is found in most animal and organelle cells, plants, and microorganisms, mainly acting as a monooxygenase (Cammack *et al.,* 2006). Raunio *et al.* (2020) indicated that this gene is present in several body parts, including the digestive system, lungs, skin, nasal epithelium, and kidneys, with the highest expression observed in the liver.

Limited information exists regarding the CYP2A6 gene in sheep, particularly its influence on meat quality in Indonesian sheep. The CYP (Cytochrome P450) enzyme family plays a crucial role in metabolizing and detoxifying various compounds in the body, including hormones and foreign substances, as well as synthesizing cholesterol, steroids, and other lipids (Xu *et al.,* 2002). Among its functions, CYP is involved in the metabolism of androstenone, a steroid compound produced in the adrenal and gonadal glands during testosterone metabolism. Androstenone, one of the three main androstenones found in humans and animals, can impact meat flavor, often associated with odor (Sahadevan *et al.,* 2014). Its presence, influencing meat flavor and contributing to cholesterol synthesis, links it to cholesterol levels (Whittington *et al.,* 2004). Studies have linked the CYP2A6 gene to flavor, odor, and fatty acid presence in sheep meat (Listyarini *et al.,* 2018; Abdillah *et al.,* 2021). Another gene in the cytochrome family, CYP2E1, is also associated with the flavor and odor of sheep meat (Harahap *et al.,* 2021a). Prior research has suggested that this gene is linked to fatty acids, which can affect cholesterol and fat levels in lamb, collectively influencing meat quality. However, previous studies have not explored the relationship between CYP2A6 gene polymorphisms and cholesterol content or meat quality in various Indonesian sheep breeds. Nonetheless, these factors significantly impact the quality and nutritional value of sheep meat. This study seeked to identify CYP2A6 gene polymorphisms and examine their association with cholesterol content and meat quality.

MATERIALS AND METHODS

Animals and Samples

This study involved various breeds of sheep, including Barbados cross sheep (BCS), Compass agrinak sheep (CAS), Jonggol sheep (JS), Garut sheep (GS), and Javanese thin-tail sheep (JTTS). Rams aged between 10 and 12 months were housed in group pens. The sheep were selected from farmers who maintained high standards of care, including regular cleaning of sheep manure, proper disposal of waste and left-over feed, and ensuring clean bedding, among other practices. Sheep with an average weight of 25 kg and evenly distributed body weight were chosen. During the study period, the sheep were fed a diet consisting of *Pennisetum purpureum* and concentrate. The study involved 140 sheep to investigate the association between the CYP2A6 gene and carcass characteristics, commercial cuts, meat quality, and cholesterol levels. Data for the gene association study with commercial cuts and meat quality included BCS (n=10), CAS (n=10), JS (n=15), GS (n=20), and JTTS (n=85). The gene association study involving carcass characteristics and cholesterol levels included data from BCS (n=7), CAS (n=10), GS (n=16), JS (n=15), GCS (n=10), and JTTS (n=82). Due to limitations in sample size, the data used for the association with cholesterol comprised 83 samples. All animal-related procedures were approved by the Animal Ethics Commission of IPB University (approval no. 117-2018 IPB).

Slaughter Procedures of Sheep and Carcass Characteristics Measurements

The sheep slaughtering procedure adheres to the Indonesian National Standard (2018) for halal slaughtering of ruminant animals SNI 99003:2018, focusing on animal welfare principles. Sheep are slaughtered one by one, ensuring that the halal slaughterer holds a competency certificate as a halal slaughterer and is of the Islamic faith. It is ensured that the halal slaughterer has slaughtered each animal using a sharp knife by severing the digestive tract (trachea), food pipe (esophagus), and two blood vessels (jugular vein and carotid artery). The halal slaughterer performs the slaughtering on the front of the sheep's neck with a single cut or a

maximum of three reciprocal cuts without lifting the knife or breaking the neck bone.

The carcass characteristics are measured through several parameters, namely live weight, hot carcass weight, carcass length, cold carcass weight, and carcass percentage. The measurement begins by recording the hot carcass's weight immediately after slaughter. The carcass is then split into two parts along the backbone, the left and right sides, and the length of each split carcass is measured. Subsequently, the carcass is hung at 4°C for 24 hours. This method follows the previous method in previous research by Amri *et al*. (2023). After the cooling period, the carcass is weighed again, and the right-side carcass is used for cutting into commercial cuts, which will later be analyzed for nutritional content and meat quality. Meanwhile, the left-side carcass is stored for future use.

The commercial cuts from the right-side carcass are divided into eight parts: leg, loin, flank, shoulder, rack, breast, shank, and neck. Each part is then weighed to measure its weight.

Analysis of Meat Quality

Meat quality analysis includes parameters such as pH level, tenderness, cooking loss, and water-holding capacity. Following slaughter, the pH level of the longissimus dorsi muscle was measured using a pH meter after the carcass had been stored for 24 hours. Tenderness was assessed using the Warner-Bratzler Shear Force (WBSF) technique. Cooking loss was determined by measuring the difference in weight between the meat sample before and after cooking in a water bath at 80°C for 1 hour. Water-holding capacity was calculated based on the weight loss of the meat sample (initial weight of 5 grams) after being compressed at a pressure of 2,250 g for 5 minutes.

Cholesterol Content Analysis

The analysis of cholesterol content in lamb meat was performed using the High-Performance Liquid Chromatography (HPLC) method. A total of 10 mg of the sample Longissimus dorsi is analyzed for fat content and transferred to a 10 mL volumetric flask. Then, a few milliliters of 2-Propanol are added, and the mixture is extracted using ultrasonic waves for several minutes. Cholesterol is dissolved in 2-Propanol. After that, the solution is filtered using a 0.45-micron membrane. The sample is then analyzed using HPLC. HPLC has advantages over Gas Chromatography as it can detect non-volatile substances. HPLC can be used for various purposes, such as determining molecular weight, examining substance content, clinical testing, forensics, food analysis, environmental chemistry, and chemical analysis.

DNA Isolation and PCR-RFLP Amplification

The Geneaid gSYNC DNA Extraction Kit was used to isolate DNA from Longissimus dorsi muscle samples. The SNP g. 49170107 G>T from the CYP2A6 gene, as referenced in Gunawan *et al.* (2018), was used in this study. A pair of primers (F: 5'-CTTTCTGGTCCTCATCTTTG-3' and R: 5'-GGTATTGATGAGGAATGGTG-3') was utilized to amplify the CYP2A6 gene, resulting in a 286 base pair PCR product, as mentioned in the study by Listyarini *et al.,* (2018). PCR amplification was performed using a 16 µl reaction mixture containing 2 µl of DNA sample, 0.4 µl of forward and reverse primers, 6.1 µl of distilled water, and 7.5 µl of MyTaq Red Mix. A thermal cycler from ESCO was utilized to prepare the PCR enhancement. This included a starting denaturation step at 95°C for one diminutive, taken after 35 intensification cycles. Each cycle included denaturation at 95°C for 10 seconds, strengthening at 55°C for 15 seconds, and expansion at 72°C for 10 seconds. The method concluded with a last expansion step at 72°C for 10 seconds, taken after by holding at 25°C for 5 minutes. The PCR enhancement items were visualized utilizing electrophoresis with a 1.5% agarose gel.

The PCR-RFLP technique for genotyping utilized the BsmAI restriction enzyme. Five µl of the PCR product was combined with a mixture containing 0.9 µl of distilled water, 0.7 µl of Tango buffer, and 0.4 µl of BsmAI (Thermo Fisher Scientific, USA) restriction enzyme, followed by incubation at 37°C for 4 hours. Subsequently, the PCR-RFLP results were subjected to electrophoresis on a 2% agarose gel. A 100 bp DNA marker was employed to determine the size of the DNA fragments and identify the CYP2A6 genotypes. The genotypes of CYP2A6

were identified as follows: GG= 286 bp; GT= 286, 217, and 69 bp; and TT= 217 and 69 bp.

Statistical Analysis

The calculation of allele frequency and genotype frequency was described by Nei and Kumar (2002). To investigate the effects of genotype, we utilized PROC (General Linear Model) GLM techniques in SAS 9.4 software to determine the associations between the phenotype and the CYP2A6 gene polymorphism (g. 49170107 G>T). The effect of the breed factor was tested first in PROC GLM. As expected, the breed did not effect on the traits, so it was not included in the statistical analysis model. A similar model was used for association analysis by Ekawati *et al.* (2022) and Yudhananda *et al.* (2023). The analysis of the CYP2A6 gene's association with carcass,characteristics commercial cuts, meat quality, and cholesterol followed the methodology outlined in the study conducted by Listyarini *et al.* (2018) using the following formula:

$$Yij = \mu + Gi + Eij$$

Where:

Yij = carcass characteristics, commercial cuts, meat quality, cholesterol

μ = the mean of the population

Gi = the i-th genotype's fixed impact (i = GG and GT)

Eij = the residual error

Statistical significance was determined by a p-value <0.05. To assess pairwise differences between genotype effects, the Duncan's multiple range test (DMRT) was conducted. This test helps identify treatments or groups that produce significantly different outcomes.

RESULTS AND DISCUSSION

Polymorphism of the CYP2A6 Gene

The PCR amplification of the CYP2A6 gene with G>T mutation was successfully performed using primers designed by Listyarini *et al.,* (2018) for the SNP g. 49170107 G>T and validated with Primer Stats (286 bp) from this study was shown in Figure 1. The restriction enzyme BsmAI was used in PCR-RFLP to digest the PCR products. The CYP2A6 revealed two genotypes, GG (286 bp) and GT (286, 217, and 69 bp), according to the RFLP investigation. The outcomes of this research agree with those of Listyarini *et al.* (2018) study, which found two genotypes for the CYP2A6 gene, GG and GT, which stand for allele combinations of the G and T. The GG homozygous genotype is represented by a single band at 286 bp, while the GT heterozygous genotype is represented by three bands at 286, 217, and 69 bp from this study as shown in Figure 2.

CYP2A6 hereditary quality polymorphisms were analyzed using allele recurrence, genotype recurrence and the Hardy-Weinberg balance equation in Table 1. The results showed that the G allele was the dominant allele in the entire sheep population, with a recurrence of 98%, while the recurrence of the T allele was 2%. This indicates that the heterozygous allele represents 2% of the population. The low frequency of certain alleles can be caused by factors such as small population size, closed breeding framework, hereditary drift, selection, and non-random mating (Asmare *et al.,* 2023). The allele frequencies in sheep are also influenced by human-driven selective breeding. When farmers choose to breed sheep with specific traits, such as high meat yield, the prevalence of alleles associated with these traits will increase. The observed genotype frequencies exhibit variation, with the GG genotype frequency being 0.96, and the GT genotype frequency being 0.04. Genetic polymorphism was observed only in the JTTS populations. However, the allele and genotype frequencies of 1.00 indicate that the CYP2A6 gene is monomorphic in the GS, JS, BCS, and CAS populations. In this study, the X^2 analysis of the CYP2A6 allele population demonstrated Hardy-Weinberg equilibrium with a value of 0.046. Additionally, the application of the Hardy-Weinberg equilibrium assumes the absence of the effects of natural selection, migration, mutation, and constant genetic drift from one generation to the next within a population (Kliman, 2016).

Association of CYP2A6 Gene Polymorphism with Carcass Characteristic

The association between the CYP2A6 gene and carcass traits shows a significant association (P<0.05) with live weight, hot carcass weight, carcass percentage, and cold carcass weight.

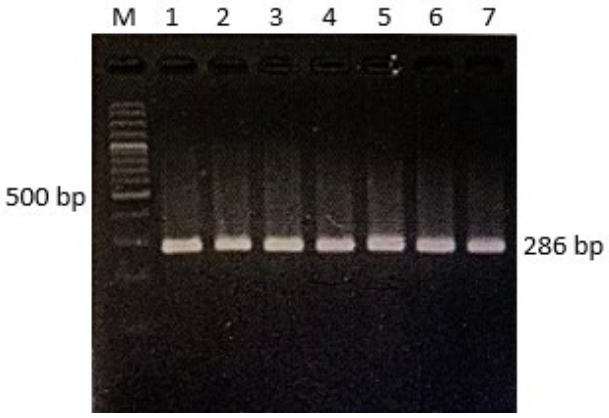

Figure 1. The result of the CYP2A6 gene amplification was displayed on a 1.5% agarose gel. M= 100 bp marker; 1-7= samples from sheep.

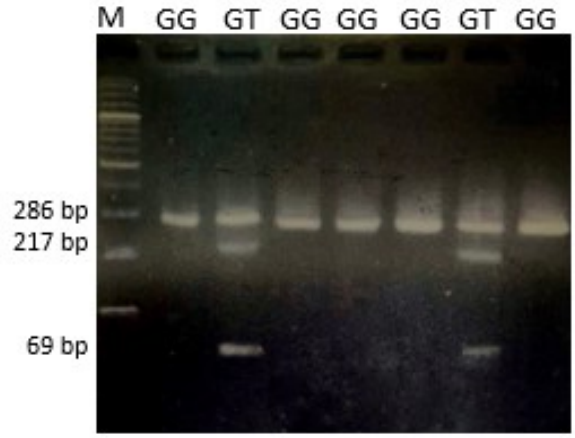

Figure 2. PCR-RFLP result of CYP2A6 gene utilizing the BsmAI enzyme, visualized on a 2% agarose gel; M= 100 bp ladder size standard; GG (286); GT (286, 217, and 69) genotype; bp= base pair.

However, there is no significant association with carcass length. This aligns with the research by Malewa and Awaluddin (2022) on Palu sheep, which used a different gene (IGF-1) and also had an insignificant effect on body weight and size. The recorded live weight signifies the sheep's body weight shortly before slaughter. The live weight shows a positive association with carcass percentage, which is also elevated. Sheep carrying the GT genotype exhibit greater live weight, hot carcass weight, carcass percentage, and cold carcass weight than those with the GG genotype, as outlined in Table 2. The T allele is determined to have a positive impact on meat quality attributes. The study recorded an average live weight ranging from 24.44 to 35.28 kg, hot carcass weight between 9.82 and 15.87 kg, and cold carcass weight spanning 9.63 to 15.79 kg. A separate study by (Dagong *et al.,* 2012) revealed a carcass weight of 9.88 kg for sheep with a cutting weight of 22.44 kg.

The findings of this research highlight a positive association between live weight and carcass weight, meaning that an increase in cutting weight results in a higher carcass weight. However, the presence of organ components in the abdomen and a significant skin weight contribute to a reduction in the produced carcass weight

(Kirton *et al.,* 1995). This is attributed to the classification of organ components in the abdomen and sheepskin as non-carcass. Additionally, in this study, carcass length exhibits a negative association with the resulting carcass weight, differing from the results (Cloete *et al.,* 2004), which suggested that longer sheep carcasses are associated with higher carcass weights. Other influencing factors on carcass weight include the age, breed type, and gender of the animal (Cloete *et al.,* 2004).

Association of CYP2A6 Gene Polymorphism with Commercial Cuts

The polymorphism of the CYP2A6 gene showed significant results (P<0.05) in all parameters of commercial cuts, namely leg, shoulder, flank, rack, breast, loin, and neck. The association of the CYP2A6 gene with each parameter of the commercial cuts in this study is detailed in Table 3. The GT genotype yields more significant carcass cuts compared to the GG genotype. The commercial cuts with the sequentially highest weights are leg, shoulder, breast, loin, rack, neck, shank, and flank. However, Wang *et al.* (2012) propose a different ranking, suggesting that lamb carcass cuts with the highest values are shoulder, rack, loin, and leg, whereas cuts with lower values include neck, shank, breast, and flank. The overall percentage distribution of meat, bone, and fat is 58–66%, 19–23%, and 4–16%, respectively. Increased cutting weight is anticipated to influence carcass composition, which is evident in the elevated fat ratio, particularly subcutaneous fat, in the carcass (Hasanah *et al.,* 2019).

Association of CYP2A6 Gene Polymorphism with Physical Quality of Meat

The CYP2A6 gene association research showed that the CYP2A6 gene had no significant (P>0.05) effect on pH, tenderness, cooking loss percentage, water holding capacity (WHC), and drip loss in Table 4.

The GG genotype has a lower pH value, tenderness, and cooking loss than the GT genotype. The muscle pH value in animals is approximately seven and decreases after the animal is slaughtered. This is due to the breakdown of glycogen into lactic acid as the muscle converts into

Table 1. Frequency of genotype and allele of the CYP2A6 gene

Sheep Breed	N	Genotype frequency			Allele frequency		χ^2
		GG	GT	TT	G	T	
JTTS	85	0.94 (80)	0.06 (5)	-	0.97	0.03	0.08
GS	20	1.00 (20)	0.00 (0)	-	1.00	0.00	0.00
JS	15	1.00 (15)	0.00 (0)	-	1.00	0.00	0.00
BCS	10	1.00 (10)	0.00 (0)	-	1.00	0.00	0.00
CAS	10	1.00 (10)	0.00 (0)	-	1.00	0.00	0.00
Totals	140	0.96 (135)	0.04 (5)	-	0.98	0.02	0.046*

N= number of samples; (..)= number of samples with genotypes GG, GT, TT, *= significantly different (χ^2 0.05 = 3.84).

Table 2. Association of CYP2A6 gene with sheep carcass characteristic

Parameter	Genotype ($\bar{x}$ ± SE Mean)		p-value
	GG (n=135)	GT (n=5)	
Live weight (kg)	24.84 ± 0.40	32.73 ± 2.55	0.0003*
Hot carcass weight (kg)	10.02 ± 0.23	14.68 ± 1.19	0.0001*
Carcass percentage (%)	41.48 ± 0.43	46.93 ± 0.93	0.0168*
Carcass length (cm)	68.70 ± 1.36	61.50 ± 4.07	0.3156ns
Cold carcass weight (kg)	9.86 ± 0.23	14.61 ± 1.18	0.0002*

$\bar{x}$= means of carcass characteristic; SE= standard error; *= significantly at (P<0.05); ns= not significantly at (P<0.05). Numbers shown in parentheses are the number of individuals with specified genotypes.

meat (Moreno *et al.,* 2020). The final pH of the meat at 24 hours post-mortem ranges from 5 to 6 (Hamoen *et al.,* 2013). The rate of post-mortem pH decline will affect the characteristics of meat quality. Low pH results in a decreased water-holding capacity of meat proteins, while higher pH values lead to less cooking loss (Suliman *et al.,* 2021).

According to the study by Harahap *et al.* (2021b), the CYP2E1 gene, which belongs to the same family as CYP, does show a significant association with pH value and meat tenderness. The research indicated that various pathways, namely the PPAR signaling pathway, xenobiotic metabolism through cytochrome P450, chemical carcinogenesis, cGMP-PKG signaling pathway, and drug metabolism via cytochrome P450, played significant roles in determining tenderness differences in lamb meat. The PPAR signaling pathway, which plays a crucial role in lipid metabolism, has been widely acknowledged as a crucial biological pathway that governs animal meat quality. The genes associated with the PPAR signaling pathway directly impact muscle tenderness by influencing the marbling characteristics in livestock (Listyarini *et al.,* 2023). In this study, the tenderness value of sheep meat is relatively lower compared to the findings of Dagong *et al.* (2012), which reported tenderness values ranging from 2.63 to 3.13 kg/cm2. Tender meat had values below 4.37 kg/cm2, while tough meat had values above 5.37 kg/cm2 (Destefanis *et al.,* 2008). The factors contributing to meat tenderness include sarcomere length, degradation of myofibrils due to protease activity, collagen and its cross-linking, intramuscular fat (IMF), and protein denaturation during the cooking process (Warner *et al.,* 2021).

Table 3. Association of CYP2A6 gene with sheep commercial cuts

Parameter (g)	Genotype ($\bar{x}$ ± SE Mean)		p-value
	GG (135)	GT (5)	
Leg	1506.2 ± 34.3	2108.0 ± 152.0	0.0014*
Loin	358.3 ± 12.5	640.0 ± 95.7	0.0001*
Flank	132.1 ± 7.25	232.0 ± 35.4	0.0101*
Shoulder	813.2 ± 25.4	1114.0 ± 97.3	0.0261*
Rack	375.0 ± 13.5	580.0 ± 88.7	0.0051*
Breast	429.6 ± 13.8	726.0 ± 107.0	0.0001*
Shank	390.5 ± 9.4	482.0 ± 42.2	0.0683ns
Neck	391.3 ± 19.6	515.0 ± 67.5	0.0173*

$\bar{x}$= means of commercial cuts; SE= standard error; *= significantly at (P<0.05); ns= not significantly at (P<0.05). Numbers shown in parentheses are the number of individuals with specified genotypes.

Table 4. Association of CYP2A6 gene with physical quality of meat

Parameter	Genotype ($\bar{x}$ ± SE Mean)		p-value
	GG (135)	GT (5)	
pH	6.02 ± 0.05	5.73 ± 0.09	0.4245ns
Tenderness (kg/cm^2)	3.63 ± 0.07	3.57 ± 0.34	0.9884ns
Cooking loss (%)	46.26 ± 0.71	48.89 ± 1.20	0.4026ns
WHC (mgH_2O)	84.82 ± 0.83	80.31 ± 2.62	0.5686ns
WHC (drip loss)	28.27 ± 0.28	26.77 ± 0.87	0.4028ns

$\bar{x}$= means of commercial cuts; SE= standard error; ns= not significantly at (P<0.05). Numbers shown in parentheses are the number of individuals with specified genotypes.

Table 5. Association of CYP2A6 gene with cholesterol

Parameter	Genotipe ($\bar{x}$ ± SE Mean)		p-value
	GG (79)	GT (4)	
Cholesterol (mg/100 g)	72.82 ± 1.83	90.30 ± 11.40	0.0431*

$\bar{x}$ = means of cholesterol; SE= standard error; *= significantly at (P<0.05). Numbers shown in parentheses are the number of individuals with specified genotypes.

Association of CYP2A6 Gene Polymorphism with Cholesterol

The analysis of the association between the CYP2A6 gene and cholesterol levels showed a significant association (P<0.05). Sheep with the GT genotype had higher cholesterol levels than those with the GG genotype in Table 5. Based on previous research, mRNA expression of CYP2A6 in the liver from high MI or skatole at the GT genotype (Listyarini *et al.,* 2018). A complete understanding of the relationship between skatole production and its accumulation in adipose tissue remains elusive. However, there is evidence highlighting the important role of hepatic clearance via P (450) CYP in reducing skatole concentrations in the bloodstream (Marro *et al.,* 2024). The calculation of cholesterol values in this study comes from the total cholesterol, which includes low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides. The CYP2A6 is a protein-encoding gene that consists of 494 amino acids. The monooxygenase cytochrome P 450 protein is involved in the synthesis of cholesterol, steroids, and other lipids, as well as several processes involved in drug metabolism (Zanger and Schwab, 2013). Cholesterol homeostasis requires the collaboration of various tissues to balance cholesterol absorption, cholesterol biosynthesis, and its distribution in the bloodstream, where the body subsequently takes it up. In the bloodstream, most of the transported cholesterol is in the form of LDL. Excess cholesterol in the blood is eliminated through cells that reside within macrophages. The release of cholesterol from peripheral cells to apoA-l plasma generates HDL, which is then transported back to the liver. The cholesterol released from the liver can either be absorbed or excreted from the body (Luo *et al.,* 2020).

CYP2A6 was reported as a candidate gene related to the flavor and odor of lamb meat (Listyarini *et al.,* 2018). The flavor and odor of lamb meat can affect the generation of particular enzymes that convert fats into fatty acids subsequently absorbed by the body (Liu *et al.,* 2020). Cholesterol is related to fatty acids associated with the level of LDL in the blood (Zhu *et al.,* 2018). Abdillah *et al*. (2021), showed a significant association (P<0.05) between the GG genotype and higher amounts of palmitoleic acid (C16:1) in the blood. Palmitoleic acid has been found to positively reduce LDL cholesterol levels. Bernstein *et al.* (2014) stated that purified palmitoleic acid has anti-inflammatory effects and strong lipid modulation properties, which can alter blood lipids. Palmitoleic acid also reduces LDL levels and increases HDL levels. It could be speculated that CYP2A6 has a potential to be a candidate gene for the selection of lamb meat with different cholesterol levels.

CONCLUSION

The SNP g.90317673 C>T of the CYP2A6 gene was polymorphic in Javanese thin-tail sheep (JTTS). Two genotypes, GG and GT, demonstrate the polymorphism. The CYP2A6 gene was significantly associated with cholesterol, carcass characteristics, and commercial cuts. The GT genotype had higher values than the GG genotype for all parameters. This study indicated that the CYP2A6 gene has the potential to be a candidate genetic marker for carcass characteristics, commercial cuts, and cholesterol.

ACKNOWLEDGMENTS

This research was financially supported by Penelitian Tesis Magister (PTM) at the Directorate of Research, Technology and Community Services Ministry of Education, Culture, Research, and Technology Year 2023 Number: 18877/IT3.D10/PT.01.02/M/T/2023 dated 20 June 2023.

REFERENCES

Abdillah, G. K., K. Listyarini, R. S. Harahap, A. Gunawan and C. Sumantri. 2021. Association of cytochrome P450 2A6 (CYP2A6) gene polymorphisms with fatty acid traits in Indonesian native sheep. Bulletin of Animal Science. 45(3):148–154. https://doi.org/10.21059/buletinpeternak.v45i3.65604.(in Indonesian)

Amri, F., A. Gunawan and C. Sumantri. 2023. Effect of DGAT1 gene on hot and cold carcass, neck, and non-carcass traits in Indonesian sheep. JIPTHP. 11(1):48-53 https://doi.org/10.29244/jipthp.11.1.48-53. (in Indonesian)

Asmare, S., K. Alemayehu, J. Mwacharo, A. Haile, S. Abegaz and A. Ahbara. 2023. Genetic diversity and within-breed variation in three indigenous Ethiopian sheep based on whole-genome analysis. Heliyon. 9 (4):e14863. https://doi.org/10.1016/j.heliyon.2023.e14863.

Bernstein, A. M., M. F. Roizen and L. Martinez. 2014. Purified palmitoleic acid for the reduction of high-sensitivity C-reactive protein and serum lipids: A double-blinded, randomized, placebo-controlled study. J. Clin. Lipidol. 8(6):612–617. https://doi.org/10.1016/j.jacl.2014.08.001.

Cammack, R., T. Atwood, P. Campbell, H. Parish, A. Smith, F. Vella and J. Stirling. 2006. Oxford Dictionary of Biochemistry and Molecular Biology. 2nd Ed. Oxford University Press. New York. P. 227.

Cloete, J. J. E., L. C. Hoffman, S. W. P. Cloete and J. E. Fourie. 2004. A comparison between the body composition, carcass characteristics and retail cuts of South African mutton merino and dormer sheep. J. Anim. Sci. 34(1):44–51. https://doi.org/10.4314/sajas.v34i1.4040.

Dagong, M. I. A., R. Herman, C. Sumantri, R. R Noor and M. Yamin. 2012. Carcass and physical meat characteristics of thin tail sheep (JTTS) based on calpastatin gene (CAST) (Locus intron 5 – exon 6) genotypes variation. IJAVS. 17(1):13–24.

Destefanis, G., A. Brugiapaglia, M. T. Barge and E. D. Molin. 2008. Relationship between beef consumer tenderness perception and Warner-Bratzler shear force. J. Meat Sci. 78 (3):153–156. https://doi.org/10.1016/j.meatsci.2007.05.031.

Ekawati, R. P., R. S. Harahap, K. Listyarini, C. Sumantri and A. Gunawan. 2022. Polymorphisms KIF12 gene|BfaI and its association on carcass characteristics and lamb meat quality. Bulletin of Animal Science. 46 (4):257–262. https://doi.org/10.21059/buletinpeternak.v46i4.77029. (in Indonesian)

Gunawan, A., Jakaria, K. Listyarini, A. Furqon, C. Sumantri, S. H. Akter, and M. J. Uddin. 2018. Transcriptome signature of liver tissue with divergent mutton odor and flavor using RNA deep sequencing. Gene. 676 (2018):86–94. https://doi.org/10.1016/j.gene.2018.06.086.

Hamoen, J. R., H. M. Vollebregt and R. G. M. V. D. Sman. 2013. Prediction of the time evolution of pH in meat. J. Food Chem. 141 (2013):2363-72. https://doi.org/10.1016/j.foodchem. 2013.04.127.

Harahap, R. S., R. R. Noor and A. Gunawan. 2021a. Effect of CYP2E1 gene polymorphisms on lamb odor and flavor in Indonesian sheep. IOP Conf. Ser.: Earth Environ. Sci. 788(1):012022. https://doi.org/10.1088/1755-1315/788/1/012022.

Harahap, R.S., R. R. Noor and A. Gunawan. 2021b. The polymorphism and expression of CYP2E1 gene and its relation to carcass and meat quality of Indonesian lamb. Trop. Anim. Sci. J. 44(4):377–385. https://doi.org/10.5398/ tasj.2021.44.4.377.

Hasanah, U., Hasnudi, E. Mirwandhono, N. Ginting, P. Patriani and M. Baihaqi. 2019. Carcass composition of Fat-tailed sheep at different slaughter weights. IOP Conf. Ser.: Earth Environ. Sci 2019; 260:012055. https://doi.org/10.1088/1755-1315/260/1/012055.

Kirton, A. H., A. H. Carter, J. N. Clarke, D. P. Sinclair, G. J. K. Mercer and D. M. Duganzich. 1995. A comparison between 15 ram breeds for export lamb production 1. Live weights, body composition, carcass measurements, and composition. N. Z. J. Agric. Res. 38(3):347–360. https://doi.org/10.1080/00288233.1995.9513136.

Kliman, R. M. 2016. Encyclopedia of Evolutionary Biology. 1st Ed. Academic Press. New York. P. 208-211.

Listyarini, K., C. Sumantri, S. Rahayu, M. A. Islam, S. H. Akter, M. J. Uddin and A. Gunawan. 2023. Hepatic transcriptome analysis reveals genes, polymorphisms, and molecules related to lamb tenderness. Animals. 13(4):674. https://doi.org/10.3390/ani13040674.

Listyarini, K., Jakaria, M. J. Uddin, C. Sumantri and A. Gunawan. 2018. Association and expression of CYP2A6 and KIF12 genes related to lamb flavor and odor. Trop. Anim. Sci. J. 41(2):100–107. https://doi.org/10.5398/tasj.2018.41.2.100.

Liu, T. T., X. T. Liu, Q. X. Chen and Y. Shi. 2020. Lipase inhibitors for obesity: a review. Biomed Pharmacother. 128:110314. https://doi.org/10.1016/j.biopha.2020.110314.

Luo, J., H. Yang and B. L. Song. 2020. Mechanisms and regulation of cholesterol homeostasis. Nat. Rev. Mol. Cell. Biol. 21(4):225–245. https://doi.org/10.1038/s41580-019-0190-7.

Malewa, A. D. and Awaluddin. 2022. Polymorphisms of Palu sheep IGF-1 gene and their relationship with skeletal growth. Trop. Anim. Sci. J. 45(1):9-15. https://doi.org/10.5398/tasj.2022.45.1.9.

Marro, P., R. Wesoly, and V. Stefanski. 2024. Influence of different plant extracts on CYP-mediated skatole and indole degradation in pigs. Animals. 14 (6): 888. https://doi.org/10.3390/ani14060888.

Moreno, I., P. Lipová, L. Ladero, J. L. Fernández -García and R. Cava. 2020. Glycogen and lactate contents, pH and meat quality and gene expression in muscle Longissimus dorsi from Iberian pigs under different rearing conditions. Livest. Sci. 240(2020):104167. https://doi.org/10.1016/j.livsci.2020.104167.

Nei, M. and S. Kumar. 2022. Molecular Evaluation and Phylogenetics. Oxford University Press. New York.

Raunio, H., O. Pentikäinen and R. O. Juvonen. 2020. Coumarin-based profluorescent and fluorescent substrates for determining xenobiotic-metabolizing enzyme activities in vitro. Int. J. Mol. Sci. 21(13):4708. https://doi.org/10.3390/ijms21134708.

Sahadevan, S., A. Gunawan, E. Tholen, C. GroBe-Brinkhaus, D. Tesfaye, K. Schellander, M. Hofmann-Apitius, M. U. Cinar and M. J. Uddin. 2014. Pathway-based analysis of genes and interactions influencing porcine testis samples from boars with divergent androstenone content in back fat. PLoS One. 9(3): e91077. https://doi.org/10.1371/journal.pone.0091077.

[SNI] Indonesia National Standard. 2018. Halal slaughtering on ruminants. SNI 99003: 2018. ICS 03.120.10. National Standardization Agency. Jakarta.

Suliman, G. M., A. N. Al-Owaimer, A. M. El-Waziry, E. O. S. Hussein, K. Abuelfatah and A. A. Swelum. 2021. A comparative study of sheep breeds: fattening performance, carcass characteristics, meat chemical composition and quality attributes. Front. Vet. Sci. 8: 647192. https://doi.org/10.3389/ fvets.2021.647192.

Suryadi, D., S. Rahayu, C. Firmansyah and S. Kuswaryan. 2016. Consumer preferences of meat lamb in West Java. Sosiohumaniora. 18(1):27–33. https://doi.org/10.24198/Sosiohumaniora.V18I1.9353.

Wang, J. and S. Shete. 2012. Testing departure from hardy-weinberg proportions. Methods Mol. Biol. 850: 77-102. https://doi.org/10.1007/978-1-61779-555-8_6.

Warner, R., R. Miller, M. Ha, T. Wheeler, F. Dunshea, X. Li, R. Vaskoska and P. Purslow. 2021. Meat tenderness: underlying mechanisms, instrumental measurement, and sensory assessment. Meat Muscle Biol. 4(2):1-25. https://doi.org/10.22175/mmb.10489.

Whittington, F.M., G.R. Nute, S. I. Hughes, J. D. McGivan, I. J. Lean, J. D. Wood and E. Doran. 2004. Relationships between skatole and androstenone accumulation, and cytochrome P4502E1 expression in Meishan x Large White pigs. J. Meat Sci. 67(4): 569-576. https://doi.org/100.1016/j.meatsci.2003.12.010.

Xu, C., S. Goodz, E. M. Sellers and R. F. Tyndale. 2002. CYP2A6 genetic variation and potential consequences. Adv. Drug Deliv. Rev. 54(10):1245-1256. https://doi.org/10.1016/s0169-409x(02)00065-0.

Yudhananda, A., K. Listyarini, C. Sumantri and A. Gunawan. 2023. Characterization and association of CFHR5 gene polymorphism with fatty acid composition and cholesterol in sheep. Bulletin of Animal Science. 47 (3):172-183. https://doi.org/10.21059/buletinpeternak.v47i3.84177. (in Indonesian)

Zanger, U. M. and M. Schwab. 2013. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 138(1):103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007.

Zhu, Z., F. Wu, Y. Lu, Z. Wang, J. Zang, H. Yu, C. Guo, X. Jia, X. Shen and G. Ding. 2018.