Effect of CYP2E1 Gene and Breed on Carcass and Non-carcass Traits of Indonesia Lamb

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Abstract. This study aimed to analyze the effect of CYP2E1 gene polymorphisms and breed sheep on carcass and non-carcass traits. A total of 50 rams used in this study were collected from 20 crossbreed sheep (10 Barbados Cross Sheep (BCS) and 10 Compass Agrinac Sheep (CAS)) and 30 local breed sheep (15 Javanese Thin Tailed (JTT) and 15 Jonggol sheep (JS)). The sheep were slaughtered at 10–12 months old with an average body weight of 20.45 kg. Identification of the CYP2E1 gene polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The effect of the CYP2E1 gene and breed with carcass and non-carcass traits were described using a T-test. The result showed that we found three genotypes: GG (401 bp), GT (138, 263, and 401 bp), and TT (138 and 263 bp) in local sheep and crossbred sheep. The CYP2E1 gene polymorphisms (g.50657948 T $>$ G) were in Hardy-Weinberg Equilibrium (HWE). The CYP2E1 gene polymorphisms had an effect ($P < 0.05$) on carcass traits in local sheep, while in the crossbred sheep not found. The breed has a significant impact ($P < 0.05$) on carcass and non-carcass traits. However, the CYP2E1 gene had no significant association ($P > 0.05$) with non-carcass traits. The TT genotype had the highest value of carcass traits compared to GG and GT genotypes. Therefore, developing sheep with high carcass quality can be produced by selecting CYP2E1 genetic markers in local Indonesian sheep.

Keywords: Breed · carcass and non-carcass characteristic · CYP2E1 gene · PCR-RFLP · sheep

1 Introduction

Animal slaughtering can be classified into two parts, i.e., carcass and non-carcass (offal). The carcass is the main result of animal slaughtering with a high economic value. At the same time, non-carcass components are generally considered a by-product in the slaughter process, and their value is enough to cover the cost during slaughter [1]. The element of non-carcass weight usually affects a carcass characteristic, especially

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weight carcass. The high weight of non-carcass components will decrease the carcass percentage. Hasanah *et al.* [2] research reported that carcass and non-carcass percentage values are 48 – 54% and 36 - 39%, respectively.

The carcass has a variation in composition through genetics and breed [3], age [4], sex of the animal [5], and nutritional and environmental effects [6]. Various species differ considerably regarding carcass weight and fat percentages, muscle, and bone [7]. Carcass and non-carcass traits become more relevant for the sheep breeding program because of their economic value. Several studies have reported that genetics and breed affected carcass and non-carcass traits [8, 9]. The genetic parameter estimates shown by the heritability value between genetic and meat quality, including carcass and non-carcass traits, have a value of 0.22–0.32 [10]. This result indicates that selection based on genetics will be more effective in improving genetic merit for lamb quality, especially carcass and non-carcass traits.

Meat quality is a complex trait that is influenced by a wide variety of genes. One gene has been reported to be associated with meat quality is the CYP2E1 gene. The CYP2E1 gene (Cytochrome P450 2E1) is located on chromosome 22 in sheep and regulates androsterone and skatole in liver tissue. In Indonesian sheep, the CYP2E1 is associated with meat quality [11] and flavor odor [12]. The other family of Cytochrome P450, CYP2A6, has been reported to play a role in fatty acid composition [13] and flavor and odor properties [14, 15]. Our previous study has not studied the relationship between CYP2E1 gene polymorphisms with carcass and non-carcass traits in various breeds of Indonesian sheep. Though the variety of breeds also affects the carcass and non-carcass traits in the lamb quality. Belhaj *et al.* [3] and Baihaqi *et al.* [16] reported an association between breed sheep and carcass and non-carcass traits.

Therefore, this study aimed to analyze the effect of CYP2E1 on carcass and non-carcass traits of four breeds of Indonesia sheep, i.e., two crossbreed sheep (Barbados Cross Sheep (BCS), Compass Agrinac Sheep (CAS)) and two local breed sheep (Javanese Thin Tailed (JTT) and Jonggol sheep (JS)).

2 Materials and Method

2.1 Animals

Totals samples used in this study were collected from 50 rams consisted 20 crossbreed sheep (10 of BCS and 10 of CAS) and 30 local sheep breeds (15 of JTT and 15 of JS). The crossbreed sheep were taken from the Center for Research and Development of Animal Husbandry, Bogor, Indonesia (Latitude/Longitude: 6°35′06.6″S 106°48′24.4″E). The local breed sheep were collected from Sinar Harapan farm (Latitude/Longitude: 7°03′13.0″S 106°49′59.4″E) for JTT and Jonggol Animal Science Teaching and Research Unit (JASTRU) (6°28′24.3″S 107°00′49.7″E) for JS. All rams were kept in group cages and fed ad libitum in the form of forage and concentrates. The carcass and non-carcass traits were measured from the rams with a body weight of 20.45 kg aged between 10–12 months.

2.2 Slaughter Procedure and Sample Collection

Slaughtering was performed according to standard halal methods at a commercial abattoir PT Pramana Pangan Utama (PPU) Slaughter House. First, the rams were skinned after the blood was out from the body. Then, all components of non-carcass, such as blood, skin, head, feet, etc., were weighed. The hot carcass weight was measured before its chilled. The dressing percentage was calculated from slaughter weight. After the carcasses were chilled at four °C for 24 h, the carcass was weighed to get a cold carcass weight, and the carcass was split along the vertebral column in two halves. The right part of the carcass was divided into eight pieces based on commercial cuts (neck, shank, shoulder, breast, rack, loin, flank, and leg), and all cutting parts were divided into meat, bone, and fat. The *longissimus dorsi* samples were taken for DNA purification. All pieces were put on ice and stored at -20 °C.

2.3 DNA Purification and PCR-RFLP Amplification

Genomic DNA was purified from *longissimus dorsi* tissue using a Genomic DNA Mini Kit (Geneaid Biotech, Taiwan) based on the manufacturing protocol. The DNAzol reagent was used for hydrolyzing RNA and allowing the selective precipitation of DNA from the lysate with four steps that is homogenized tissue sample, DNA precipitation, washing DNA, and DNA stabilization.

The SNP g. 50657948 T > G of the CYP2E1 gene used in this study refers to the study of Harahap *et al.* [11, 12]. A pair of primers used for amplification of the 401 bp (base pair) target DNA were (F:5'-CCC AGT CAT CAG AGT CAG TA -3' and R: 3' –GCA TAC AGT GGT TTT CCT GG- 5') to amplify the CYP2E1 gene. The PCR amplifications were performed in a 15 µl consisting 1 µl DNA samples, 0.4 µl of primers (forward and reverse), 7.5 µl of MyTaq Red Mix, and 6.1 µl of deionized water. The PCR amplification using the AB System machine with initial processes was predenaturation for 1 min at 95 °C. The next stage was denaturation for 15 s at a temperature of 95 °C, annealing for 15 s at 60 °C, and extension for 10 s at 72 °C. Then, the process was repeated for 35 cycles. The final step was elongation for 1 min at 72 °C.

The amplicon product was taken as much as 3 µl to detect PCR results by 1.5% agarose gel electrophoresis. The gel electrophoresis was made from 0.45 g agarose gel with 30 mL of 0.5x TBE and heated in a microwave for 3 min. The DNA peqGREEN was added and homogenized with a magnetic stirrer. The samples were run in a bath of electrophoresis with a voltage of 100 V for 37 min. Further, 5 µl amplicon products were taken, and the restriction enzyme (*NlaIII* enzymes) was added for genotyping the CYP2E1 gene using PCR-RFLP for 4 h at 37 °C. The PCR-RFLP product was separated using 2% agarose gel with the procedure described previously. The fragments were visualized under UV Transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA). The genotype of CYP2E1 consisted of TT: 138 bp, 263 bp, GG: 401 bp, and GT: 138 bp, 263 bp, and 401 bp.

2.4 Statistical Methods

2.4.1 Allele and Genotype Frequencies

Allele and genotype frequencies were analyzed using genotyping data of four sheep breeds; BCS, CAS, JTT, and JS. Allele and genotype frequency was calculated with the following formula:

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

where xi is the G and T allele frequency, xii is the ii-genotype frequency, nii is the sample number of ij genotype, nij is the samples number of ij genotype, and N is samples total.

2.4.2 *Hardy-Weinberg Equilibrium Values* [17]*:*

$$X2 = \sum[(O - E)2/\ E]$$

where χ2 is the chi-square value, O is the observed values of the i genotype, and E is the expected values of the i genotype.

2.4.3 Association Analysis

Association between the CYP2E1 with carcass and non-carcass traits using the T-test to compare genotypes and breed (Minitab® 18 Software). The patterns were used below:

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

where X1 and X2 are the average traits for genotype or breed 1 and 2; n1 and n2 are individual numbers of genotype or breed 1 and 2, and δ is the combined standard deviation.

3 Results and Discussion

3.1 CYP2E1 Genotyping

The CYP2E1 gene 401 bp PCR fragments, previously measured by the ladder on a 2% agarose gel, were sequenced following purification. PCR-RFLP results showed a polymorphism of the CYP2E1 gene indicated by three genotypes consisted GG (401 bp), GT (401, 263, and 138 bp), and TT (263, 138 bp) (Fig. 1). Our population's composition of alleles and genotypes is quite balanced (Table 1). However, the GT genotypes still were dominant to GG and TT genotypes. This result is in line with the research conducted by Harahap *et al.* [11, 12]. The chi-square revealed that the 50657948 T > G of the CYP2E1 gene is in Hardy-Weinberg Equilibrium. This result indicates that the population's gene frequency does not change without evolutionary forces, including selection, mutation, gene migration, and genetic drift.

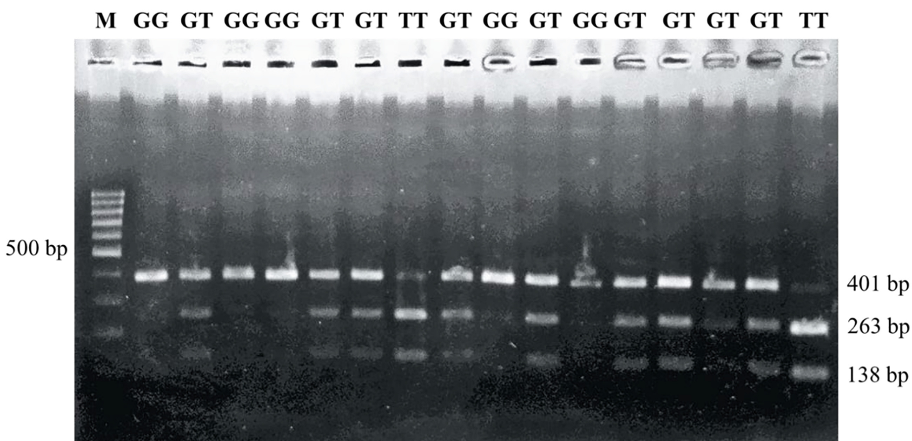

Fig. 1. The CYP2E1 gene polymorphism in Indonesian sheep

3.2 Effect of CYP2E1 Gene and Breed on Carcass Traits

The CYP2E1 gene polymorphisms had a significant effect (P < 0.05) on carcass traits in local sheep, i.e., retail cut carcass parameters including leg, total muscle, and intramuscular fat (Table 2). The T allele of the CYP2E1 gene played a role in increasing carcass characteristics. The TT genotype had a higher carcass characteristic value than GG and GT genotypes. However, the carcass characteristic on crossbreed sheep was not affected by CYP2E1 gene. Haren *et al.* [9] and Listyarini *et al.* [18] also reported that carcass traits were affected by gene polymorphism in Indonesian sheep.

Furthermore, the breed of sheep also had a significant effect (P < 0.05) on carcass traits (Table 3). In this study, the local sheep had a higher carcass characteristic than crossbred sheep. The TT genotype had a higher leg weight than GG and GT genotypes in both local and crossbred sheep. The leg is the main carcass component of lamb beside the shoulder, rib (rack), and loin, which has a high economic value [19]. Dagong *et al.* [20] reported that the leg weight presentation in carcass was 29–31%. However, the crossbreed sheep have less total fat than local sheep. The subcutaneous and intramuscular fat composition was almost identical in the crossbreed sheep, whereas, in local sheep, the percentage of subcutaneous fat was higher than intramuscular fat. This result shows that local sheep accumulate much fat in their subcutaneous. Fat is an important macronutrient and plays a role in overall meat palatability. The amount of subcutaneous and intramuscular fat is often used as a visual cue by consumers to judge meat quality intramuscular fat influences sensory quality traits of meat, including flavor, juiciness, and tenderness. The highest intramuscular fat keeps the meat moist and increases the consumer's palatability [21].

3.3 Effect of CYP2E1 Polymorphisms and Breed on Non-carcass Traits

The CYP2E1 gene polymorphisms had no significant impact (P > 0.05) on non-carcass traits either in local sheep or crossbred sheep (Table 4).

Table 1. The number of sheep per genotype and allele frequency of each breed

Sheep	Breed	N	Frequency of genotype			Frequency Allele		Chi-square (χ^2)
			GG	GT	TT	G	T	
CAS	Crossbreed	10	0.60 (5)	0.60 (5)	0.40 (0)	0.30	0.70	1.83
BCS	Crossbreed	10	0.3 (0)	0.30 (6)	0.30 (4)	0.75	0.25	1.11
JS	Local	15	0.13 (2)	0.20 (3)	0.67 (10)	0.23	0.77	2.91
JTT	Local	15	0.33 (5)	0.60 (9)	0.07 (1)	0.63	0.37	1.28
Totals		50	0.24 (12)	0.46 (23)	0.30 (15)	0.47	0.53	0.29

These findings were consistent with the study by Dagong *et al.* [20], who reported a non-significant difference among CAST polymorphism with non-carcass traits. However, the breed was strongly associated ($P < 0.05$) with non-carcass traits (Table 5). The local sheep had higher non-carcass traits compared to crossbreed sheep. This high level cannot be separated from the difference in body weight between the two, affecting the non-carcass component.

In this study, the local sheep had a higher body weight than crossbreed sheep. Body weight is generally correlated with carcass percentage also non-carcass percentage [22]. The average weight of the non-carcass characteristic in this study was lower than that of Dagong *et al.,* with a body weight range of 21–23 kg. The highest non-carcass component was skin, followed by head and blood. The skin and head values in local sheep are very different, while in cross sheep, they are relatively the same.

This difference is under a study conducted by Yagoubi *et al.* [23], who reported that the Percentage of skin and head were 14.38% and 7.39%, respectively. Baihaqi *et al.* [16] reported that the contribution of total non-carcass components as a percentage of slaughter weight represented between 44.8%-46.4% in Priangan and Javanese Fat-tailed sheep.

4 Conclusion

The CYP2E1 gene was polymorphic in Indonesian sheep. Three genotypes were found in local or crossbred sheep, i.e., GG, GT, and TT. The CYP2E1 gene polymorphisms significantly affected carcass traits in local sheep. The TT genotype sheep had a higher carcass characteristic than other genotypes. Furthermore, the breed of sheep also significantly affected carcass and non-carcass traits. The local sheep had a higher carcass and non-carcass traits than crossbred sheep. Therefore, the development of sheep with high carcass traits could be done by selecting CYP2E1 genetic markers in local Indonesian sheep.

Table 2. The effect of the CYP2EI gene on carcass characteristics in crossbred and local sheep

Parameters	Genotype on crossbred sheep (x ± SE Mean)			Genotype on local sheep (x ± SE Mean)		
	GG (n = 5)	GT (n = 11)	TT (n = 4)	GG (n = 7)	GT (n = 12)	TT (n = 11)
Slaughter Weight (kg)	16.68 ± 1.48	18.64 ± 1.61	20.00 ± 2.35	21.53 ± 0.35	21.81 ± 0.65	23.83 ± 1.12
Dressing Percentage (%)	33.96 ± 0.15	33.66 ± 1.01	33.2 ± 0.72	40.74 ± 1.08	40.54 ± 0.53	42.98 ± 1.24
Hot Carcass (kg)	5.67 ± 0.52	6.28 ± 0.57	6.67 ± 0.89	8.78 ± 0.33	8.85 ± 0.32	10.30 ± 0.70
Cold Carcass (kg)	5.13 ± 0.49	5.80 ± 0.55	6.21 ± 0.83	8.77 ± 0.33	8.75 ± 0.30	10.31 ± 0.70
Half Carcass (kg)	2.56 ± 0.24	2.88 ± 0.26	2.98 ± 0.32	4.23 ± 0.24	4.32 ± 0.18	5.12 ± 0.35
Length Carcass (cm)	106.00 ± 4.44	103.55 ± 3.28	98.75 ± 5.81	66.86 ± 1.56	67.08 ± 1.22	68.64 ± 1.50
Leg (kg)	0.88 ± 0.08	0.98 ± 0.09	1.08 ± 0.09	1.48 ± 0.08^{b}	1.45 ± 0.06^{c}	1.73 ± 0.11^{a}
Loin (kg)	0.17 ± 0.02	0.21 ± 0.03	0.23 ± 0.04	0.36 ± 0.02	0.38 ± 0.02	0.42 ± 0.04
Flank (kg)	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.16 ± 0.01
Shoulder (kg)	0.45 ± 0.05	0.49 ± 0.06	0.53 ± 0.06	0.80 ± 0.06	0.73 ± 0.04	0.74 ± 0.07
Rack (kg)	0.18 ± 0.021	0.23 ± 0.03	0.23 ± 0.04	0.39 ± 0.02	0.39 ± 0.02	0.47 ± 0.04
Breast (kg)	0.22 ± 0.02	0.25 ± 0.03	0.27 ± 0.04	0.45 ± 0.03	0.43 ± 0.02	0.48 ± 0.03
Shank (kg)	0.25 ± 0.03	0.28 ± 0.03	0.29 ± 0.04	0.39 ± 0.03	0.39 ± 0.02	0.39 ± 0.03
Neck (kg)	0.23 ± 0.03	0.23 ± 0.04	0.30 ± 0.07	0.41 ± 0.04	0.46 ± 0.03	0.50 ± 0.03
Totals muscle (kg)	1.25 ± 0.14	1.51 ± 0.18	1.64 ± 0.20	2.32 ± 0.12^{b}	2.31 ± 0.10^{c}	2.83 ± 0.19^{a}
Totals bone (kg)	0.87 ± 0.08	0.93 ± 0.09	0.96 ± 0.11	1.26 ± 0.06	1.25 ± 0.04	1.32 ± 0.07
Totals fat (kg)	97.30 ± 16.60	103.50 ± 13.40	133.50 ± 15.90	404.10 ± 35.30	450.70 ± 36.70	479.50 ± 69.00
Subcutaneous fat (g)	55.80 ± 25.50	52.10 ± 10.30	67.23 ± 8.93	283.70 ± 32.10	320.70 ± 30.10	283.00 ± 44.40
Intramuscular fat (g)	41.60 ± 11.70	51.44 ± 7.95	66.27 ± 8.99	120.40 ± 10.40^{c}	130.00 ± 11.00^{b}	196.50 ± 32.30^{a}

Note: $\bar{x}$ = means of carcass traits; n = number of samples per genotypes; Superscript a, ab, b = * = significantly different at 5%

Table 3. The effect of breed per genotype of the CYP2E1 gene on carcass characteristics

Parameter	P Value of local Vs. Crossbred sheep		
	GG vs. GG	GT vs. GT	TT vs. TT
Slaughter Weight (kg)	0.003*	0.104	0.121
Dressing Percentage (%)	0.000*	0.000*	0.001*
Hot Carcass (kg)	0.000*	0.003*	0.014*
Cold Carcass (kg)	0.000*	0.001*	0.007*
Half Carcass (kg)	0.001*	0.001*	0.004*
Length Carcass (cm)	0.000*	0.001*	0.000*
Leg (kg)	0.000*	0.002*	0.005*
Loin (kg)	0.000*	0.001*	0.008*
Flank (kg)	0.005*	0.000*	0.000*
Shoulder (kg)	0.001*	0.002*	0.102
Rack (kg)	0.000*	0.002*	0.007*
Breast (kg)	0.000*	0.000*	0.005*
Shank (kg)	0.007*	0.016*	0.066
Neck (kg)	0.007*	0.003*	0.009*
Totals muscle (kg)	0.000*	0.004*	0.005*
Totals bone (kg)	0.002*	0.004*	0.017*
Totals fat (kg)	0.000*	0.000*	0.011*
Subcutaneous fat (g)	0.000*	0.000*	0.014*
Intramuscular fat (g)	0.001*	0.001*	0.034*

Table 5. The effect of breed per genotype of the CYP2E1 gene on non-carcass characteristic

Table 5	P Value of local Vs. Crossbred sheep		
	GG vs. GG	GT vs. GT	Table 5
Blood	0.017*	0.004*	0.284
Head	0.004*	0.093	0.035*
Skin	0.000*	0.002*	0.035*
Foot	0.002*	0.001*	0.039*
Tail	0.000*	0.000*	0.001*
Limp + Lug + Heart	0.000*	0.000*	0.008*
Total	0.001*	0.001*	0.041*

Table 4. The effect of CYP2E1 gene on non-carcass *traits* in local and crossbred sheep

Parameters	Genotype on Local Sheep (x ± SE Mean)			Genotype on Crossbred Sheep (x ± SE Mean)		
	GG (n = 7)	GT (n = 12)	TT (n = 11)	GG (n = 5)	GT (n = 11)	TT (n = 4)
Blood	0.72 ± 0.03	0.78 ± 0.02	0.81 ± 0.04	0.54 ± 0.05	0.56 ± 0,06	0.70 ± 0.08
Head	1.97 ± 0.05	1.78 ± 0.05	1.99 ± 0.09	1.34 ± 0.12	1.51 ± 0,14	1.48 ± 0.15
Skin	2.11 ± 0.08	2.13 ± 0.14	2.24 ± 0.12	1.03 ± 0.09	1.37 ± 0,16	1.54 ± 0.21
Foot	0.59 ± 0.02	0.58 ± 0.03	0.61 ± 0.03	0.46 ± 0.02	0.46 ± 0,03	0.48 ± 0.04
Tail	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Limp + Lug + Heart	0.74 ± 0.02	0.75 ± 0.02	0.76 ± 0.03	0.46 ± 0.04	0.44 ± 0,05	0.54 ± 0.04
Total	6.18 ± 0.14	6.08 ± 0.19	6.47 ± 0.27	3.83 ± 0.27	4.35 ± 0.37	4.74 ± 0.52

Note: $\bar{x}$ = means of non-carcass traits; n = number of samples per genotypes; Superscript a, ab, b = * = significantly different at 5%

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