Polymorphism of the SLC25A30 gene in Indonesian sheep and its association with carcass characteristics

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Abstract. Carcass characteristics are one of the factors that significantly impact the quality of meat. Genetic improvement in carcass characteristics is required to encourage interest among consumers. The objective of this study was to discover the presence of the SLC25A30 gene polymorphism at SNP g.15912281 A>T and its potential association with carcass characteristics in Indonesian sheep. The study used 153 rams, consisting of 87 Javanese thin-tail sheep, 20 Javanese fat-tail sheep, 10 Garut composite sheep, 10 Compass Agrinak sheep, 10 Barbadoss cross sheep and 16 jonggol sheep. The PCR-RFLP method was used to investigate the SLC25A30|SspI polymorphism. The association between the SLC25A30 gene and carcass characteristics was analyzed using the T-Test method. The results showed that the SLC25A30 (g.15912281 A>T) gene polymorphism was polymorphic in all breeds. The genotypes identified in the polymorphism were AA, AT and TT. The SLC25A30 gene was significantly associated ($P<0.05$) with carcass characteristics (slaughter weight, hot carcass, carcass percentage, carcass length and cold carcass). In conclusion, the genotype that can be recommended for selecting good meat quality is the TT genotype respectively than the AT and AA genotype The SLC25A30 (g.15912281 A>T) gene indicates as a prospective gene for the purpose of identifying carcass characteristics in lamb meat.

1 Introduction

Lamb meat can be one of the alternative sources to meet the animal protein needs of the Indonesian population. Sheep meat is a good source of dietary protein (20 g/100 g of lean) that is rich in highly digestible essential amino acids in balance to human requirements;

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it is also a good source of nutritionally valuable micronutrients (iron, zinc, selenium, vitamins B3, B6 and especially B12) [1]. Badan Pusat Statistik (BPS) reported that 54,650.53 tonnes of lamb meat were produced in Indonesia in 2022. When compared to the prior year, when it was 50,702.06 tonnes, this amount climbed by 7.79%. Sheep carcass is a key criterion of a sheep's marketability and economic worth. The conformation, dressing (yield), fatness condition, meat colour, carcass linear measurements and fat cover quality (colour and consistency) are the major factors that determine it [2]. Multiple factors, including breed [3], slaughter age [4,5] and weight at slaughter [6,7] affect the quality of lambs' meat. As a result, selection objectives in sheep breeding now emphasise growth and carcass attributes more than in the past. In order to maintain consumer acceptability of lamb meat, producers must provide high-quality meat, which is determined by carcass measurements [8,9]. However, it happens frequently that consumers have expressed uncertainty regarding the higher quantities of cholesterol and saturated fatty acids contained in lamb meat. Both of these factors have been linked to the developing of cardiovascular disease and cancer [10]. Consumers also express displeasure with the taste or odor of lamb meat [11]. Thus, genetic enhancement is essential, and this can be achieved through different molecular techniques like selection and crossbreeding programs.

One candidate gene related to the nutritional composition of meat is the Solute Carrier Family 25 Member 30 (SLC25A30 gene). The SLC gene superfamily currently consists of 65 families with a total of 458 transport proteins found throughout the cell's organelles, with the most prominent family being SLC25, which has 53 members [12] SLC25A30 is a member of the SLC25 family and provides a function in the movement of various chemicals through the mitochondrial membrane [13]. Several key genes related to fatty acid production, adipogenesis, fat accumulation, and lipid metabolism were discovered, including LEPR, APOA5, GFPT1, CYP17A, FABP7, TGFBR2, GSTCD, and SLC25A30 [14].

According to the research results of Listyarini [15] there appears to be a correlation between the SLC25A30 gene and meat tenderness, as shown by RNA sequencing data. Nevertheless, the association between the SLC25A30 gene and sheep remains unexplored. Given this, exploring the association between carcass characteristics and the SLC25A30 gene is necessary. This research aims to examine the variations in the SLC25A30 gene and establish its correlation with various quality parameters in lambs.

2 Materials and methods

2.1 Ethical approval and Animal collected

All procedures involving animals in this study were ethically examined, and authorized IPB University's Animal Ethics Commission has given their clearance, and it is documented as 117-2018 IPB**.** In this study, 153 rams were examined to detect polymorphisms in the SLC25A30 gene. The sample consisted of 10 Barbados cross sheep (BCS), 20 Javanese fat-tailed sheep (GS), 87 Javanese thin-tailed sheep (JTTS), 10 Garut composite sheep (GCS), 16 Jonggol sheep (JS) and 10 Compass agrinac sheep (CAS). This research involved the slaughtering of sheep at the Slaughter House (PT Pramana Pangan Utama (PPU), a commercial abattoir. The slaughterhouse employee followed the halal guidelines and fulfilled animal welfare criteria guidelines in sluthering the animals. The sheep were raised on separate farms, provided unrestricted food access (ad libitum), and kept under comparable rearing conditions.

2.2 Carcass characteristics Analyses

Before to slaughter, the sheep must complete a 17-hour fasting period in order to settle the sheep and minimise the amount of undigested feed in their digestive system [15]. The sheep were slaughtered by cutting their jugular vein, oesophagus, and trachea in three conduits. The sheep blood is collected, quantified, and subsequently suspended to ensure optimal blood drainage. Slaughtered lamb is divided into non-carcass and carcass. All carcass and non-carcass were separated and weighed. In addition, carcass characteristics were measured. The warm carcasses were weighed and withered at 4°for 24 hours. The weight of the cold carcass was calculated and the spinal column was divided into two separate sections, one on the left and one on the right. The right carcass was divided into eight commercial cuts: shoulder, neck, rack, leg, shank, rib, flank and loin, while the left carcass is stored. The commercial pieces are separated between meat, bone, and fat (intermuscular fat and subcutaneous), then each part is weighed to determine the weight and percentage of the part. Carcass characteristics were observed by looking at the hot carcass, slaughter weight, cold carcass, carcass percentage, and carcass length.

2.3 DNA Extraction, PCR-RFLP Amplification and Genotyping

The Single Nucleotide Polymorphism (SNP) of the SLC25A30 gene was identified at position SNP g.15912281 A>T. The SNP was detected through RNA sequencing [16]. Muscle samples of the longissimus dorsi were processed with the Geneaid gSYNC DNA Extraction Kit (GS050/100/300) to isolate genomic DNA following the provided protocol. Amplification of SLC25A30 gene fragments was carried out using the GeneAmp ESCO PCR system. The DNA amplification procedure began with denaturation for one minute at 95 °C, then included 35 cycles consisting of denaturation at 95 °C for 15 seconds, annealing at 62 °C for 15 seconds, extension at 72 °C for 15 seconds, and final extension at 72 °C for 1 minute. The reaction was then cooled to 15 °C for 5 minutes. The PCR samples were verified by electrophoresis on an agarose gel containing 1.5% agarose, and the DNA bands were observed using a thermocycler and a UV transilluminator. The PCR products were subjected to PCR-RFLP (Restriction Fragment Length Polymorphism) using the SspI enzyme and incubated at 37 °C for 4 hours (Table 1). The resulting data were also electrophoresed on a 2% agarose gel to determine the genotype: TT (393 bp), AA (206, 187 bp), and AT (393, 206, and 187 bp).

Table 1. Primer sequence and size of PCR of gene SLC25A30

Gene	Accession number	Size of PCR	TA°C	Enzyme	Primer sequence
SLC25A30	NC_019467.2	393 bp	62	SspI	F: 5'-GCC CTG TGA GAG TAA CAG TA-3'
					R: 5"-TGG TTT AGT ACG AGG GAC GA-3'

3 Statistical Analysis

3.1 Genotype and allele frequencies

The genotype and allele frequencies were estimated using the calculation method of Nei and Kumar [17] and the Hardy-Weinberg equilibrium was identified through the methods of Hartl and Clark [18].

$$X_i = \frac{(2n_{ii}+\sum j\neq j\ n_{ij})}{2N} \qquad X_{ii} = \frac{n_{ii}}{N}$$

Description:
Xi = frequency of allele i;
Xii = frequency of genotype ii;
nij = number of individuals with genotype ij;
ni = number of individuals of genotype ii; and
N = total sample

3.2 Hardy-Weinberg equilibrium

Procedures were used to determine the Hardy-Weinberg equilibrium [18].

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

Description :
X^2 = chi-square
O = total the number of observations of the i-th genotype; and
E = total number of expected of the i-th genotype

3.3 Association of SLC25A30 gene with carcass characteristics

The effect of genotype (SPSS Statistics 26 software) on the phenotype was assessed by conducting T-Test procedures to test the differences value of carcass characteristics between breed on the same genotype. The data for carcass characteristics parameters in crossbred and local sheep were analyzed separately to determine if crossbreeding influenced the genotype associations obtained for the gene with carcass characteristics (slaughtered weight, dressing percentage, cold carcass, hot carcass and carcass length).

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$$

$$S = \sqrt{\frac{\sum_{i=1}^{n}(\bar{X}_i - \bar{X}_1)^2 + \sum_{i=1}^{n}(\bar{X}_i - \bar{X}_2)^2}{n_1 + n_2 - 2}}$$

Description :
X1 and X2= the average traits for genotypes 1 and 2;
n1 and n2= individual numbers of genotypes 1 and 2; and
S = the combined standard deviation

Data analysis is completed by completing both the Kolmogorov-Smirnov and Shapiro-Wilk tests for normality test. To figure out how to interpet the results of the normality test, it is essential to determine the significance value. If the results of the normality tests indicate the data is not normal, a check for data outliers is done. Any data outliers found in each value are then taken out of the data. As a result of deleting outlier data, each parameter can have a different number of samples. Data transformation becomes necessary when a data check has been done and the distribution remains non-normal. After confirming that the data follows a normal distribution, a t-test is used to analyse it.

4 Results and Discussion

4.1 Polymorphism of the SLC25A30 gene

PCR-RFLP amplified a candidate marker for the SLC25A30 gene using the restriction enzyme SspI. PCR amplification with a length of 393 bp was obtained, as expected from the SLC25A30 gene primers. The SNP g. 15912281 A>T SLC25A30 of the SLC25A30 gene exhibits genotype polymorphism characterized by the occurrence of allele variations A and T, namely TT= 393 bp; AA= 206, 187 bp; AT= 393, 206, and 187 bp. The polymorphism of the SLC25A30 gene visualized in Figure 1 represents only a few randomly selected sheep samples.

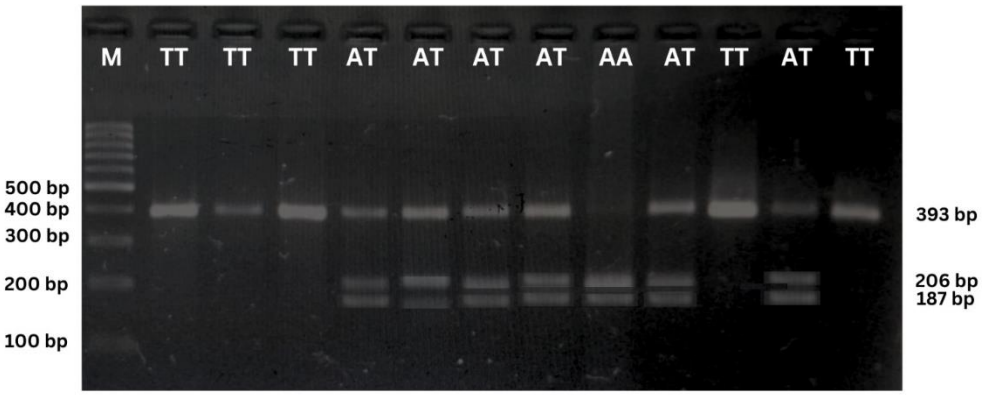

Fig. 1. PCR-RFLP result of SLC25A30 gene; M = marker 100 bp ladder size standard; TT (393 bp); AA (206, 187 bp); AT (393, 206, and 187 bp) genotype; bp = base pair

The SLC25A30 gene has three genotypes (AA, AT, and TT). All genotypes were found in every sheep breed except for Jonggol sheep, Barbados cross sheep, Compass Agrinac sheep, Javanese fat-tailed sheep, and Garut composite sheep, where the AT genotype was undetected (Table 2). In the Indonesian sheep population, at the SNP g. 15912281 A>T locus of the SLC25A30 gene, AA was the most common genotype in this population, while AT was just behind it. Crossbreeding may influence this, such as in Compass Agrinac sheep, where the AT genotype has been selectively bred out of the population. The SNP g. 15912281

A>T of the SLC25A30 gene in crossbred sheep, such as Garut Composite sheep, were found in Hardy Weinberg Equilibrium (HWE). However, in other local sheep groups, such as Garut Sheep, Jonggol Sheep in the SLC25A30 gene was not in Hardy Weinberg equilibrium. The non-equilibrium results from the unequal distribution of genotypes within these varieties. Multiple factors, including mutation, non-random reproduction, natural selection, gene flow, and genetic drift, can lead to deviations from HWE [19] or possibly as a result of these sheep's efforts to adapt to challenging ecological circumstances [20].

Table 2. Frequency of genotype and allele of the SLC25A30 gene

Breed of sheep	N	Genotype Frequency			Allele Frequency		X^2
		AA (98)	AT (8)	TT (47)	A	T	
Javanese thin-tailed sheep	87	0.57 (61)	0.37 (6)	0.06 (20)	0.75	0.25	55.81
Jonggol sheep (JS)	16	0.73 (12)	0.00 (0)	0.26 (4)	0.73	0.26	15.00
Barbados cross sheep (BSCS)	10	0.80 (8)	0.00 (0)	0.20 (2)	0.80	0.20	10.00
Compass agrinac sheep (CAS)	10	0.40 (8)	0.00 (0)	0.60 (6)	0.40	0.60	10.00
Javanese fat-tailed Sheep (GS)	20	0.40(8)	0.00 (0)	0.60 (12)	0.40	0.60	20.00
Garut composite sheep (GCS)	10	0.36 (5)	0.48 (2)	0.16 (3)	0.60	0.40	3.40
Total	153	0.64	0.05	0.30	0.67	0.33	115.83

N=number of samples
(..)=number of samples with genotypes AA, AT, TT
X^2 Table = 3.84

4.2 Association of SLC25A30 gene polymorphism with carcass characteristics

Considering the potential impact of crossbreeding and local sheep on the genotype associations with carcass characteristics (slaughtered weight, dressing percentage, cold carcass, hot carcass and carcass length). Data for carcass traits parameters in crossbred and local sheep were analyzed separately (Table 3).

The association between the SLC25A30 gene and carcass characteristics was analysed, showing significant differences ($P<0.05$) in relation to slaughtered weight, hot carcass, carcass percentage, carcass length and cold carcass (Table 3). Significant differences in dressing percentage carcass and carcass length are detected in each genotype in both local and crossbred breeds. The slaughter weight has an impact on the carcass proportion [21]. The growth rate directly impacts the rise in slaughter weight. Furthermore, the improvement in carcass percentage was additionally impacted by the increase in live weight and age. [22]. Slaughter weight and carcass percentage are directly correlated with the length of the carcass [23]. The body size of livestock will have an impact on the length of the carcass. The research data indicated that the carcass length of sheep with the TT genotype in crossbreed was longest (95,72 cm) ($P<0.05$) than local breed (63,22 cm; 59 cm; 62,62 cm). In the slaughter weight parameter, it is noticeable that the TT genotype has a higher slaughter weight (27,33 kg)

(P<0.05) in the local breed than other genotype, whereas the AT genotype has a greater amount of weight (27,70 kg) in the crossbreed.

Table 3. Association of the SLC25A30 gene with carcass characteristics

Parameter	Type of breed	Genotype of SLC25A30 ($\bar{x}$ ± Standard Deviation)		
		AA	AT	TT
Slaughter weight (kg) (n=153)	Local breed (n=123)	25.65±4.31 (82)	24.35±4.20 (6)	27.33±5.00 (35)
	Crossbreed (n=30)	20.51±5.85 (17)	27.70±2.12 (2)	21.69±5.71 (11)
	P value	0.00*	0.31	0.00*
Hot carcass (kg) (n=153)	Local breed (n=123)	10.69±2.55 (82)	10.10±1.97 (6)	11.88±2.84 (35)
	Crossbreed (n=30)	7.22±2.36 (17)	9.65±0.49 (2)	7.24±2.04 (11)
	P value	0.00*	0.81	0.00
Dressing percentage (%) (n=153)	Local breed (n=123)	43.25±3.86 (82)	41.97±2.01(6)	44.26±4.30 (35)
	Crossbreed (n=30)	34.91±3.02 (17)	34.87±0.88 (2)	33.29±1.47 (11)
	P value	0.00*	0.00*	0.00*
Carcass length (cm) (n=153)	Local breed (n=123)	63.22±6.81 (82)	59.00±4.19 (6)	62.62±7.43 (35)
	Crossbreed (n=30)	94.70±16.76 (17)	73.00±4.24 (2)	95.72±14.98 (11)
	P value	0.00*	0.00*	0.00*
Cold Carcass (kg) (n=146)	Local breed (n=116)	10.46±2.54 (80)	10.06±1.97 (6)	11.81±2.96 (30)
	Crossbreed (n=30)	6.70±2.31 (17)	9.25±0.35 (2)	6.80±2.03 (11)
	P value	0.00*	0.62	0.00*

N=number of samples
$\bar{x}$ = means of the meat quality values
*=significant

The SLC gene family regulates as transporters and has been relating to the ribeye area (REA) in Nelore cattle [24] the ribeye is a popular regarded cut of beef known for its tenderness, rich marbling and flavorful taste. Genes encoding solute carriers belonging to the SLC superfamily, including SLC11A1 and SLC12A4, were identified and explored as prospective candidate genes for determining meat colour in cattle [25]. Candidate genes from the solute carrier family (SLC), consists SLC37A4 and SLC3A2, have been identified as potential genes that regulate the drip loss characteristic in pigs [26]. The SLC gene's control over drip loss can be applied to lamb meat, as drip loss can impact the final cooked product. There is an expectation that the application of the SLC gene may have the potential to enhance the carcass quality in sheep.

5 Conclusion

The SLC25A30 gene SNP g. 15912281 A>T was discovered polymorphism among Indonesian sheep. The distribution of the SLC25A30 gene was not in Hardy-Weinberg equilibrium, increasing the percentage of the AA genotype. The carcass characteristics parameters (slaughter weight, hot carcass, dressing percentage, carcass length and cold carcass) were impacted by the SLC25A30 SNP g.15912281. In conclusion, the genotype that

can be recommended for selecting good meat quality is the TT genotype respectively than the AT and AA genotype and variability in the SLC25A30 gene can affect the quality of carcass characteristics

References

1. Prache, S., Schreurs, N., Guillier, L. Animal. **16**. (2022)
2. Belhaj, Kamal. Farid Mansouri, Aziz Tikent, Yassin Taaifi, Mohamed Boukharta, Hana Caid Serghini, and Ahmed Elamrani. The Scientific World Journal (2021).
3. J.-B. Liu, J. Guo, F. Wang. Small Ruminant Research (2015).
4. Sañudo. Journal of the Science of Food and Agriculture (2019).
5. A. G. D'Alessandro, G. Maiorano, M. Ragni, D. Casamassima, G. Marsico, and G. Martemucci, Small Ruminant Research, (2021).
6. A. Sabbioni, V. Beretti, E. M. Zambini, and P. Superchi. Italian Journal of Animal Science (2016).
7. A. Camacho, A. Torres, J. Capote et al. Journal of Applied Animal Research, vol. **45**. 400–408, (2016).
8. Abubakar, A, CA Frima, V Silvia, CA Fitri, Z Hanum, and E Mariana. IOP Conference Series: Earth and Environmental Science (2023).
9. Ayeb, N, A Ghrab, A Barmat, and T Khorchani. Turkish Journal of Veterinary and Animal Sciences (2016).
10. Maharani, D, D-W Seo, N-R Choi, S Jin, M Cahyadi, C Jo, and J-H Lee. Korean Journal of Agricultural Science. (2013).
11. Gunawan, A, Jakaria, Listyarini K, Furqon A, Sumantri C, Akter SH, Uddin MJ.. *Gene*. **676** :86-94 (2018).
12. Pizzagalli MD, Bensimon A, Furga GS. *The Febs Journal*. 288 : 2784-2835 (2020)
13. Rochette L, Meloux A, Zeller M, Malka G, Cottin Y, Vergely C. 25:2417 (2020).
14. Gunawan A, Listyarini K, Harahap RS, Jakaria, Roosita K, Sumantri C, Inounu I, Akter SH, Islam, MA, Uddin MJ. PlosOne. 16(12) (2021).
15. Dagong MIA, Herman R, Sumantri C, Noor RR, Yamin M. IJAVS. 17:13-24 (2012).
16. Listyarini K, Sumantri C, Rahayu S, Islam MA, Akter SH, Uddin MJ, Gunawan A. Animals. **15** ;13(4) :674. (2023).
17. Nei, Kumar. New York (US): Oxford University Pr (2000).
18. Hartl DL, Clark AG. Sunderland (UK): Sinauer Associates (1997).
19. Graffelman, J, D Jain, and B Weir. Human Genetics. **136**(6):727–741 (2017).
20. Khasanah, H, A Gunawan, R Priyanto, MF Ulum, and Jakaria. Media Peternakan. **39**(2):95–103 (2016).
21. Lei X, Tao QJ, Lin R, Shuai CD, Xi T, Jun XS, Yan ZZ, Sheng HL. **22** :5 (2023).
22. Purslow P,P. Lawrie's meat science. 51-103 (2023)
23. Rajkumar V, Dass G, Verma AK, Das AK. Indian Journal of Animal Sciences. **84** (5) : 569-574 (2014).
24. Silva DBS, Fonseca L,FS, Pinheiro DG, Magalhaes AFB, Muniz MMM, Ferro JA, Baldi F, Chardulo L,AL, Schable RD, Taylor JF, Albuquerque LG. Sci Rep. **10** :14701 (2020).
25. Garzon NAM, Magalhaes AFB, Mota LFM, Fonseca LFS, Chardulo LAL, Alburquerque. Meat Science. **171** (2021)**.**
26. Zhao X, Wang C, Wang Y, Lin H, Wang H, Hu H, Wang J. **20**:89 (2019).