Polymorphism of PPM1K Gene and The Association Related to Retail Indonesian Sheep Meat Cuts

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Abstract. The PPM1K (Protein Phosphatase, Mg2+/Mn2+ Dependent 1K) gene is assumed to associate with retail meat cuts. This study aims to determine the association of the PPM1K gene polymorphisms with retail meat cuts among various breeds of Indonesian sheep. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) approach was used to determine the polymorphisms of the PPM1K gene in 130 Indonesian rams aged 10-12 months with body weights ranging from 20-25 kg. The association study between PPM1K and retail meat cuts was analyzed using *General Linear Model* (GLM). The research showed that the PPM1K gene was polymorphic, with three genotypes: AA (458 bp), GG (259 bp and 199 bp), and AG (458 bp, 259 bp, and 199 bp). The PPM1K gene polymorphisms were significantly (P<0.05) associated with breast cut, which is the breast muscle and bone. The GG genotype of the PPM1K gene had a higher value on breast lamb. This result implies that the PPM1K gene could be a potential candidate for marker-assisted selection for improving the lamb quality of Indonesian sheep.

Keywords: Carcass Quality, PCR-RFLP, PPM1K Gene, Retail Meat Cuts, Sheep

Abstrak. Gen PPM1K (Protein Phosphatase, Mg2+/Mn2+ Dependent 1K) diduga berasosiasi dengan potongan daging komersil. Penelitian ini bertujuan mengetahui asosiasi polimorfisme gen PPM1K dengan potongan daging komersil diantara berbagai breed domba Indonesia. Pendekatan *Polymerase Chain Reaction-Restriction Fragment Length Polymorphism* (PCR-RFLP) digunakan untuk menentukan polimorfisme gen PPM1K pada 130 ekor domba jantan Indonesia yang memiliki umur berkisar 10-12 bulan dengan bobot badan mencapai 20-25 kg. Kajian asosiasi antara gen PPM1K dengan potongan daging komersil dianalisis menggunakan metode *General Linear Model* (GLM). Hasil penelitian menunjukkan bahwa gen PPM1K bersifat polimorfik, dengan tiga genotipe: AA (458 bp), GG (259 bp dan 199 bp), dan AG (458 bp, 259 bp, dan 199 bp). Polimorfisme gen PPM1K secara signifikan (P<0,05) berhubungan dengan potongan brisket (*breast cut)*, yaitu otot dan tulang dada. Genotipe GG dari gen PPM1K memiliki nilai lebih tinggi pada bagian dada domba. Hasil ini menunjukkan bahwa gen PPM1K dapat menjadi kandidat potensial untuk seleksi penanda marker sebagai upaya meningkatkan kualitas domba Indonesia.

Kata kunci: Kualitas karkas, PCR-RFLP, Gen PPM1K, Potongan Daging Komersil, Domba

Introduction

Animal products such as meat are known to be the principal consumables to support human nutritional needs. Sheep are one type of livestock widely developed in Indonesia, especially in the West Java region. Various types of sheep are meat-producing livestock identified with multiple benefits. For human nutrition, lamb meat is a crucial source of animal goods. It offers a great source of essential amino acids, fatty acids known as omega-3, B vitamins (6 and 12), and micronutrients in addition to high-quality protein (Willimas, 2007). Improving their composition and nutritional content is crucial to raising retail meat cuts' selling price. Consumers have traditionally valued a premium cut of meat at a fair price, but these criteria vary depending on the stakes. When selecting meat, a specific retail cut is essential (Scozzafava et al., 2016). The expanding need for premium cuts of meat results from the increasing income of consumers in most developed countries. Improvement in the value of retail cut carcasses can increase the

sale value of sheep. The lamb carcass's type and size can affect the cut's commercial value.

Selecting animals to meet the ideal parameters for increasing the lamb quality traits takes time and effort. One of the most practical methods for improving sheep genetics that uses candidate genes through identification variation analysis is breeding programs (Gunawan et al., 2016). According to Matika et al., (2016) and Ladeira et al., (2022), The identification of polymorphisms associated with carcass traits in sheep has been made possible by studies of genome-wide association (GWAS). Hasan et al., (2014) reported that increased genetic advantage will result from high and favorable genetic correlation between all growth traits. Rahmat et al., (2017) analyzed the diversity of genes that regulated the economic characteristics of carcass parts and the quality of meat and offered a novel method for animal selection at the DNA level. The candidate gene that is thought to increase the value of meat cuts is the PPM1K gene. The PPM1K genes in sheep are still inadequately characterized, with the primary use being to increase the value of retail meat cuts of Indonesian sheep. In sheep, the PPM1K gene is found on chromosome 6. The PPM1K gene identified by RNA sequencing will be applied as a biomarker for improving sheep production in Indonesia (Listyarini et al., 2023). Several prospective QTL areas, genes, and polymorphisms were identified by Sahadevan et al., (2014) as potential candidate biomarkers for more in-depth research.

According to McClure et al., (2010), the PPM1K gene has been related to higher midpoint metabolism and carcass weights as well as lower residual feed intake, feed effectiveness, conversion ratio, and fat score. Shao et al., (2020) found that the PPM1K gene is a member of the PDGF (platelet-derived growth factor) gene family that can increase the added value of commercial cuts. The PPM1K gene has not been studied in relationship with retail meat cuts, particularly in lamb. Therefore, the aim of this research was to analyze the PPM1K gene and explain its function as it relates to improving the retail meat cuts of Indonesian sheep.

Materials and Methods

Animals

This research used 130 Indonesian rams between the ages of 10 and 12 months, weighing 20 to 25 kg. The rams in this research consisted of several breeds, including 75 Javanese thin-tail sheep (JTTS), 15 Jonggol sheep (JS), 20 Garut sheep (GS), 10 Compass agrinac sheep (CAS), 10 Barbados cross sheep (BCS), and 10 Garut composite sheep (GCS). The sheep used in the study were confined in a collective cage and fed as much fattening food as they liked. The samples of *longissimus dorsi* and their phenotypes were gathered (Listyarini et al., 2018). The Indonesian Center for Animal Husbandry Research and Development created a variety of sheep breeds, including GCS, BCS, and CAS, employed in this study.

Slaughtering process

The Indonesian National Standard performance test criteria (SNI, 2018) are followed during the slaughtering procedure, including the implementation of animal welfare to support the achievement of safe, healthy, whole, and halal food products. The sheep, with an average body weight of 20–25 kg, experienced slaughter at PT Pramana Pangan Utama (PPU) slaughterhouse's industrial slaughterhouse when they were around ten to twelve months old. Following the slaughtering process, all carcasses are aged for a full day at 4 degrees Celsius before being divided into left and right halves. The right carcass was split into eight commercially cut pieces, which were separated into meat, bone, and fats (subcutaneous, intramuscular, and pelvic). The removed components included the neck, breast, shank, rack, shoulder area, loin, flank, and leg pieces. DNA was extracted from the longissimus

dorsi muscles. Each muscle segment was placed on ice and kept at a temperature of -20°C.

Measurement of retail meat cut

The retail meat cut consists of eight parts: leg pieces, rack, breast, neck area, shank, loins, flanks, and shoulder. Retail cuts of meat are measured per piece and weighed using a digital meat scale. After weighed, the carcass pieces are separated into subcutaneous fat, intramuscular fat, meat, and bones. Separation process is called deboning, each part is weighed again, and then the weight of each part is recorded.

Data analysis

Allelic and genotypic frequencies

Frequencies of alleles and genotypes of the analyzed PPM1K gene polymorphism (g.363 50643 G>A) were calculated. The Hardy-Weinberg equilibrium test results and the observed and expected heterozygosity were computed by Lachance (2016). The genotype and allele frequencies were acquired by Nei and Kumar (2000), and the Hardy-Weinberg equilibrium was computed using the methods of Hartl and Clark (1997).

Statistical analysis

Applying PROC GLM techniques to examine the impact of genotype (Minitab® 18 Software), the relationships between the PPM1K gene polymorphism (g.36350643 G>A) and phenotype were calculated. The following formula model was applied (Abdillah et al., 2021):

$Y_{ij} = \mu + genotype_i + e_{ij}$, where:

Y_{ij} = the performance of the individual lamb for retail meat cut

μ = the population mean for each trait

$genotype_i$ = the fixed effect of i-th PPM1K genotype

e_{ij} = the random error

DNA extraction and PCR-RFLP amplification for PPM1K gene polymorphism

Following the manufacturing process, The Geneaid gSYNC Extraction of DNA Kit (List ID: The General Services Administration050/100/300) was used to extract the longissimus dorsi's whole genome. High-throughput sequencing in sheep was used to find a brand-new PPM1K gene polymorphism (g.36350643 G>A). After generating a pair of particular primers with MEGA 7.0 Programs, the suitability of PCR was assessed using Primer Stat (Table 1).

In a 15 µL reaction volume, 1 µL of DNA from genomic samples, 0.2 µL of reverse and forward primers, 7.5 µL of MyTaq Red Mix, and 6.1 µL of nucleus water were added together for PCR amplification. The PCR amplification technique started with the beginning denaturation of DNA at 95 degrees Celsius for sixty seconds using an AB Systems instrument. The PCR followed by 35 amplification cycles of the primer annealing process at 60 °C for fifteen seconds, followed by elongation at 72 degrees Celsius over 15 seconds, and final extension at 72 degrees Celsius for one minute. Then samples were subjected to the PCR RFLP method to determine polymorphisms using *Tail* enzymes for 4 hours at 37 °C. The pieces from the digested products have been separated using a 2 percent agarose gel using an ultraviolet transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA).

Table 1. Primer sequences of the PPM1K gene for PCR-RFLP analysis

Gene	SNP	Accession Number	Primer	PCR Product	Restriction enzyme
PPM1K	g.363 506 43 G > A	NC_01946 3.2	>F: 5'-GGC ATC TGG AAT GAG ACT GC -'3 >R: 5'-ATC AGT GTT CAC AAG GCC AC -'3	458 bp	*Tail* (ACGT)

Note: Designed using MEGA 7 software.

Results and Discussion

Polymorphisms of PPM1K Gene

PCR-RFLP analysis at the cutting point (G>A) showed the PPM1K gene diversity (SNP g.363 506 43 G>A). *Tail* enzyme is used in the PPM1K gene amplification process. The PPM1K gene was polymorphic as indicated by the founding three genotypes consisting of AG (458 bp, 259 bp, and 199 bp); GG (259 bp and 199 bp); AA (458 bp) (Figure 2). Khasanah et al., (2016) also found polymorphic from her research represented by different allele frequencies. In this research, three genotypes were discovered in all breeds. These results were in line with that of Listyarini and colleague's (2018) study on sheep.), which reported different genes. The types of sheep used in this study include 75 Javanese thin-tail sheep (JTTS), 15 Jonggol sheep (JS), 20 Garut sheep (GS), 10 Compass agrinac sheep (CAS), 10 Barbados cross sheep (BCS), and 10 Garut composite sheep (GCS).

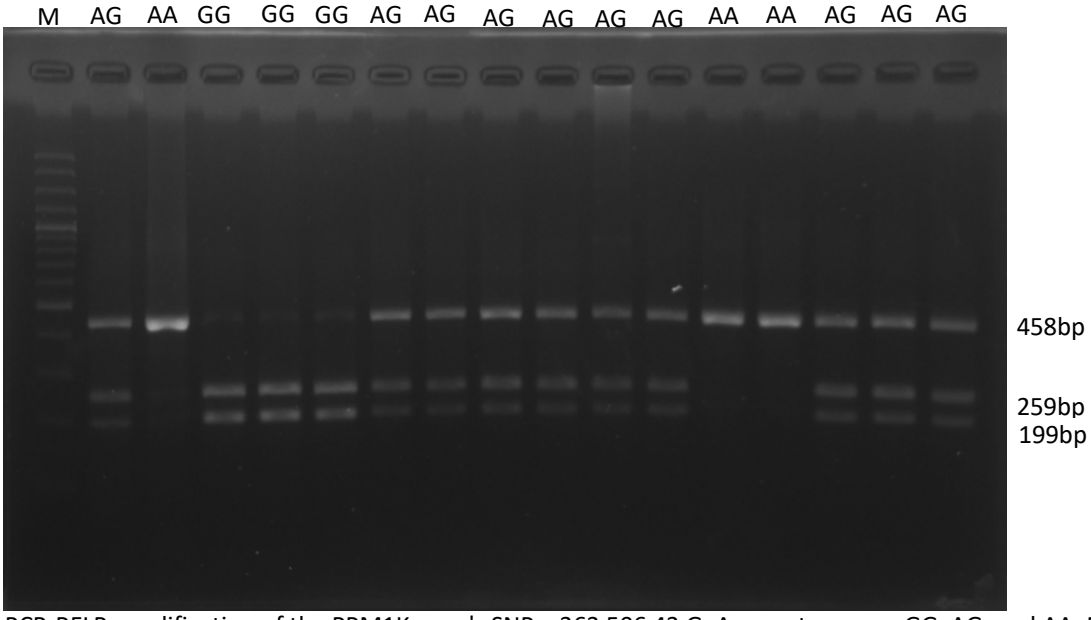

Figure 1. PCR-RFLP amplification of the PPM1K gene's SNP g.363 506 43 G>A. genotypes are GG, AG, and AA; M stands for 100-bp ladder size.

The G allele was the most frequent (0.71). As a result, the analyzed flock's GG homozygotes had the highest frequency (0.53) of individuals. The AA genotype was found in all breeds (0.11). Gunawan et al. (2018) state that an allele frequency is polymorphic if the measured allele frequency is lower than 99%. The observed heterozygosity (Ho) value of 1.85 was equal to the estimated heterozygosity (He = 3.84), indicating that the population was in the Hardy-Weinberg equilibrium (HWE). The proportion of each genotype is evenly distributed in each population. Table 2 reported that the frequency of genotype and the frequency of alleles.

Table 2. The number of allele frequencies of each SNP from animals per genotype

Sample	N	Genotype Frequency			Allele Frequency		X^2
		GG	AG	AA	G	A	
Indonesian Sheep	130	0.53	0.36	0.11	0.71	0.29	1.85

Note: N = number of populations, (..) = number of genotypes which GG, AG, AA genotype, χ2 table = 3.84

Table 3. Relationship between retail meat cuts and PPM1K gene polymorphisms

Parameters (g)	The PPM1K gene genotype ($\bar{x}$ ± SE Mean)		
	AA (n=14)	AG (n=47)	GG (n=69)
Leg	1440 ± 0.15	1450 ± 00.66	1570 ± 0.04
Muscle	934.00 ± 104.0	959.70 ± 47.20	10327.00 ± 28.10
Bone	397.10 ± 31.60	389.20 ± 13.9	419.00 ± 10.10
Subcutaneous Fats	53.10 ± 15.40	60.28 ± 8.47	69.86 ± 7.40
Intramuscular Fats	41.80 ± 16.90	27.51 ± 3.68	41.77 ± 5.89
Loin	326.00 ± 0.05	343.00 ± 0.02	388.00 ± 0.01
Muscle	183.00 ± 26.9	198.00 ± 12.30	213.65 ± 8.72
Bone	101.80 ± 16.4	97.27 ± 6.64	104.62 ± 5.89
Subcutaneous Fats	22.71 ± 7.52	26.32 ± 4.82	37.43 ± 4.16
Intramuscular Fats	17.68 ± 7.58	9.66 ± 2.63	18.29 ± 3.19
Pelvic Fats	39.10 ± 15.8	34.19 ± 7.73	43.98 ± 6.50
Flank	130.00 ± 0.03	113.00 ± 0.01	146.00 ± 0.01
Muscle	93.90 ± 18.60	90.60 ± 11.10	106.36 ± 6.24
Bone	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02
Subcutaneous Fats	19.84 ± 3.90	19.73 ± 3.61	25.50 ± 2.33
Intramuscular Fats	17.20 ± 14.30	8.65 ± 2.42	11.18 ± 2.00
Shoulder	797.00 ± 0.10	795.60 ± 0.04	829.00 ± 0.03
Muscle	493.50 ± 63.8	499.10 ± 29.40	531.90 ± 19.30
Bone	204.70 ± 24.3	209.00 ± 11.50	206.59 ± 9.05
Subcutaneous Fats	27.45 ± 8.47	29.55 ± 4.17	35.69 ± 3.14
Intramuscular Fats:	57.70 ± 17.00	40.79 ± 6.91	59.32 ± 6.09
Rack	340.00 ± 0.04	340.00 ± 0.02	400.00 ± 0.02
Muscle	199.60 ± 30.00	188.90 ± 12.90	221.26 ± 9.94
Bone	124.60 ± 10.10	115.59 ± 5.33	132.74 ± 5.20
Subcutaneous Fats	17.84 ± 6.64	22.70 ± 4.29	32.48 ± 4.36
Intramuscular Fats	21.44 ± 8.49	11.56 ± 2.12	16.50 ± 2.40
Breast	390.00 ± 0.05^{ab}	390.00 ± 0.02^{b}	470.00 ± 0.01^{a}
Muscle	201.20 ± 30.60^{ab}	198.30 ± 11.70^{b}	243.02 ± 9.32^{a}
Bone	118.60 ± 10.10^{ab}	120.37 ± 5.96^{b}	137.18 ± 4.25^{a}
Subcutaneous Fats	32.08 ± 7.80	36.49 ± 5.22	42.31 ± 4.18
Intramuscular Fats	29.39 ± 9.93	31.72 ± 5.64	41.58 ± 4.77
Shank	351.00 ± 0.04	375.00 ± 0.01	409.00 ± 0.01
Muscle	234.60 ± 25.20	227.70 ± 12.40	253.78 ± 7.19
Bone	110.08 ± 7.52	119.11 ± 4.92	121.46 ± 3.23
Subcutaneous Fats	15.98 ± 4.33	13.03 ± 1.46	16.80 ± 1.56
Intramuscular Fats	8.01 ± 1.73	7.44 ± 1.12	8.38 ± 0.83
Neck	396.00 ± 0.06	350.00 ± 0.03	422.00 ± 0.02
Muscle	241.10 ± 40.10	200.40 ± 21.40	256.80 ± 15.30
Bone	132.30 ± 24.40	110.5 ± 11.8	122.40 ± 8.68
Subcutaneous Fats	33.40 ± 16.5	15.49 ± 3.38	19.81 ± 2.17
Intramuscular Fats	16.87 ± 6.28	13.07 ± 2.86	17.59 ± 2.89
Totals	4263 ± 535	4155 ± 229	4665 ± 163

Note: $\bar{x}$= means of the retail cut carcass; SE= standard error; ^{a,b} Mean in the same row with different superscripts differ significantly (P<0.05). The numbers shown in parentheses are the number of individuals with the specified genotype.

The PPM1K gene variants have been associated (P<0.05) with retail meat cuts, particularly breast cuts (Table 3). The breast cut included breast bone, breast meat, and the total weight of the breast. The GG genotypes had a higher value in the breast cut carcass when

compared with AG genotypes in the retail meat cut. The breast meat in the GG genotype has a greater weight when compared to the weight of the other genotypes. This is in line with the body weight of GG genotypes that have the highest value than others. The breast cut is one part of the carcass with a low economic value (Rodriguez et al., 2011) besides the neck, shank, and flank. However, the shoulder area, rib (rack), loins, and leg pieces are the main cuts in lamb carcasses that have the most value. The overall carcass weight is the best indicator of both primal and retail meat cuts, according to Hopkins et al., (2004), Lambe et al., (2009), and Siddell et al., (2012).

The retail meat cut has a close relationship with carcass composition. Soeparno (2005) describes the factors influencing carcass composition, including sex and slaughter weight. Generally, the ram had a higher carcass composition than ewe lamb of the same age. According to Yagoubi et al. (2018), there is a general correlation between slaughter weight and both carcass and non-carcass percentages. The highest non-carcass component will decrease the carcass component. The non-carcass component that generally affected the carcass was the skin, followed by the head and blood. On the other hand, the value of subcutaneous fat is higher than the value of intramuscular fat. This research is comparable to that done in 2022 by Harahap et al. on the value of commercial cuts made from Indonesian sheep that carry the DDC gene. The subcutaneous and intramuscular fat is almost found in all retail meat cut components, while the pelvic fats are only found in leg and loin cuts. Fat is an important nutrient that plays a role in overall lamb palatability. Customers frequently utilise the quantity of subcutaneous and intramuscular fat as a visual indication to assess the flavour, juiciness, and tenderness of meat. The maximum amount of intramuscular fat will maintain the meat's moisture content and improve consumer palatability (Frank et al., 2016).

Conclusions

There were three genotypes of the polymorphic PPM1K gene: AA, AG, and GG. The GG genotype had a greater value for the breast cut of the carcass when compared to the AG genotype. The PPM1K gene polymorphisms were substantially connected with breast cut. The results of this research indicated that the PPM1K gene's SNP g.36350643 G>A might be a useful marker for improving the retail meat cut of Indonesian sheep.

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