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Polymorphisms KIF12 GENE|BfaI and its Association on Carcass Characteristics and Lamb Meat Quality

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ABSTRACT

One of gene which involves on mediation an antioxidant cascade in beta cells as an intracellular target of excess fat intake is Kinesin Family 12 (KIF12). This study aims to identify the diversity of SNP g. 9617965 C>T KIF12 and association with carcass characteristic and meat quality. The total sample used were 102 ram DNA samples consisted of 10 barbados cross sheep (BCS), 52 javanese thin tail (JTT), 15 jonggol sheep (JS), 15 garut sheep (GS), and 10 compass agrinak sheep (CAS). Identification of the diversity of KIF12|BfaI was conducted using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. The results showed that the amplification product was 696 bp. The diversity of the KIF12 gene was found three genotypes namely CC, CT, and TT. The allele frequency in all types of sheep was in Hardy-Weinberg equilibrium. A SNP of the KIF12 was associated ($P<0.05$) with characteristic of carcass, including length of carcass. The association of KIF12 with meat quality was significant ($P<0.05$) too associated with pH and water holding capacity (WHC). It could be concluded that KIF12 gene is a potential candidate gene for carcass characteristic and meat quality traits in sheep.

Keywords: Carcass characteristic, KIF12 gene, Meat quality, Sheep

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Introduction

Meat is one of the main livestock products that are very useful for humans. Lamb has a big role in meeting the animal protein needs of people in Indonesia. According to the Organization for Economic Co-operation and Development (2021), lamb has the lowest consumption level in Indonesia compared to other red meats, which is 0.4 kg/capita. The low consumption of lamb is due to people's assumptions about lamb which has high cholesterol and a sharp stinging smell (Suryadi *et al.*, 2016). According to Suryadi *et al.* (2016), 85.32% of the total respondents in West Java chose the smell of lamb as a consideration in buying products. Carcass characteristics and meat quality are important factors considered by consumers in considering the feasibility of consumption. Advances in molecular biology have enabled selection efforts to be carried out at the DNA level, namely by looking for gene diversity that controls the economic properties of carcass components and meat quality (Rahmat *et al.*, 2017). The parameters contained in the measurement of carcass characteristics are the results of growth belonging to the characteristics of quantitative, where this trait is influenced by many gene pairs (Hilmia *et al.*, 2022). One of the

genes thought to affect carcass characteristics, and meat quality is Kinesin Family 12 (KIF12).

Kinesin is a protein found in eukaryotic cells. Yang *et al.* (2014) reported Kinesin Family 12 (KIF12) is one of the genes involved in the delivery of antioxidant cascades in beta cells as intracellular targets for excessive fat uptake or lipotoxins. Kinesin has a relationship with the control of triglyceride secretion from the liver (Rai *et al.*, 2017). Genetic diversity in the KIF12 gene is reported to be associated with the quality of the fatness of lamb meat (Gunawan *et al.*, 2018). Gunawan *et al.* (2018) reported that the Single Nucleotide Polymorphism (SNP) gene KIF12 in lamb was found at position g.9617965 C>T and had a significant association with monounsaturated fatty acids, including myristolenic acid (C14:1), oleic acid (C18:1n9c), and fatty acids. Saturated fats are lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0). However, the KIF12 gene did not have a significant effect on flavor and odor in lamb (Listyarini *et al.*, 2018). Besides being reported to be associated with fatty acids, flavor, and odor, the KIF12 gene in swine has a positive association with meat quality (Chen *et al.*, 2008; Fan *et al.*, 2010). However, research related to KIF12 on carcass characteristics and quality of

lamb meat is still limited. Therefore, this study was conducted to identify variations in SNP g.9617965 C>T KIF12 on carcass characteristics and meat quality in sheep.

Materials and Methods

Animals and samples

The samples used in this study were 102 rams aged 10-12 months with live weights ranging from 20-35 kg. The types of sheep used consisted of 10 barbados cross sheep (BCS), 52 javanese thin tail (JTT), 15 jonggol sheep (JS), 15 garut sheep (GS), and 10 compass agrinac sheep (CAS). DNA sample were obtained from *longissimus dorsi* (LD) muscle were taken from the loin sample of sheep. Sheep slaughter at the commercial RPH PT Pramana Pangan Utama (PPU) RPH. The stages of experimental procedures including animal care have been approved by the Institutional Animal Care and Use Committee (IACUC) issued by [IPB University (approval ID: 117-2018 IPB)].

Measurement of carcass characteristics and meat quality

Parameters observed to determine meat quality in lamb were pH value, tenderness, cooking loss, and water holding capacity (WHC). The pH of the meat was measured using a pH meter after the meat had withered for 24 hours postmortem (ultimate pH). The level of tenderness of the meat was measured in the M. biceps femoris muscle using a warner bratzler shear force (WBSF) needle to cut the meat core (kg/cm^2). Cooking loss was measured by subtracting the initial weight of the meat from the weight after being boiled at 80°C. The water holding capacity was measured by calculating the amount of water that came out (mgH_2O) after being pressured for 5 minutes. Meanwhile, the carcass characteristics observed were live weight, warm carcass, carcass percentage, carcass length, and cold carcass. According to Dagong *et al.* (2012), live weight was measured using a scale by weighing the sheep before slaughter. Measurement of a warm carcass by weighing the carcass before withering. Carcass percentage was calculated by comparing carcass weight with live weight and then multiplied by 100% (Anwar *et al.*, 2019). Carcass length was calculated by measuring the carcass using a tape measure. Furthermore, cold carcass was calculated by weighing after the carcass withered at 4°C for 24 hours.

DNA isolation and PCR-RFLP amplification

DNA was extracted using the extraction method of Sambrook and Russell (2001). The primer KIF12 gene with SNP g.9617965 C>T used to amplify the target DNA at 696 bp (base pair) refers to Listyarini *et al.* (2018) was F: 5'-CAC AGT GAG TGG ACT CAG AC-3' and R: 5'-GCT GCT ACG CCA TTG AAC AG-3' which is designed using the MEGA 6.0 program. DNA

amplification was initiated by denaturation for 1 minute using a temperature of 95°C. The second stage consisted of 35 cycles consisting of denaturation for 15 seconds using a temperature of 95°C, primary annealing for 15 seconds at a temperature of 61°C, and primary extension using a temperature of 72°C for 1 minute. The last stage is cooling at 15°C for 5 minutes. After that, the PCR results were electrophoresed to see DNA bands, using 1.5% agarose gel and visualized with the help of UV light using a UV transilluminator. Then the PCR product was cut with the help of the Bfal enzyme with the C|TAG cutting site at 37°C for 4 hours. After cutting, DNA was then re-electrophoresed with 2% agarose gel and observed under UV light to see the resulting genotypes (CC= 413, 143, and 20 bp; TT= 553 and 143 bp; CT= 553, 413, 143, and 120 bp).

Electrophoresis and genotyping

Genotyping was performed using the PCR-RFLP technique using Bfal restriction enzymes incubated at 37°C for 4 hours (Thermo Fisher Scientific, EU, Lithuania). A total of 5 μL of the DNA was electrophoresed at a voltage of 100 Volts for 35-37 minutes using 2% agarose gel. The 100 bp marker was used as a comparison of the resulting DNA fragments. Electrophoresis DNA samples were visualized through UV light (Listyarini *et al.*, 2018). DNA fragments that arise from the results of electrophoresis are compared with markers to determine the length of the fragment and its genotype.

Statistical analysis

Genotype frequency. Genotype frequency is the ratio of the number of a genotype to the total population. Genotype frequency was calculated by comparing the number of genotypes to the population. The formula for calculating the genotype frequency is as follows.

$$X_{ii} = \frac{\sum_{i=1}^n n_i}{N}$$

Description:

X_{ii} = frequency of genotype ii;
 n_i = number of individuals of genotype ii;
 N = total sample.

Allele frequency. Allele frequency is the ratio of the number of alleles to all alleles in a population. Allele frequency was calculated using procedures:

$$X_i = \frac{(2n_{ii} + \sum_{j \neq i} n_{ij})}{(2N)}$$

Description:

X_i = the frequency of the i-th allele;
 n_{ii} = number of individuals of genotype ii;
 n_{ij} = number of individuals with genotype ij;
 N = total sample.

Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium is the balance value between genotype frequency and allele frequency. The suitability test between the expected genotype values and the calculated observations was calculated by chi-square. The Hardy-Weinberg equilibrium was determined by using procedures:

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

Description:

χ^2 = chi-square value;

O = the number of observations of the i-th genotype;

E = number of expected genotypes i

Association of KIF12 gene diversity with meat quality and carcass characteristics. The relationship between the genotype of the KIF12 gene with carcass characteristics and meat quality was analyzed using the General Linear Model (GLM) method with Minitab software, then the Tukey test was performed if it was significantly different ($\alpha < 0.05$). Pair wise differences between the effects of genotype was tested by performing Tukey Model. The mathematics model was:

$$Y_{ij} = \mu + G_i + E_{ij}$$

Description:

Y_{ij} = The composition of carcass characteristics and meat quality is the overall average of genotype i (j=1, 2, and 3);

μ = the mean of carcass characteristics and meat quality;

G_i = Genotype;

E_{ij} = Residual error.

Results and Discussion

KIF12 gene polymorphism in sheep

Amplification of the KIF12 gene in samples of barbados cross (BCS), thin tailed sheep (JTT), jonggol sheep (JS), garut sheep (GS), and compass agrinak sheep (CAS) using PCR technique at 60°C for 35 cycles. The target SNP that was successfully amplified was found on chromosome 2 at position g.9617965 with a C>T change. The length of the KIF12 gene PCR product produced was 696 base pair (bp). The results of the amplification of the KIF12 gene

visualized using UV light are presented in (Figure 1).

The polymorphism of the KIF12 gene (SNP g. 9617965 C>T) identified by the PCR-RFLP method at the cutting site (C|TAG) showed a diversity of genotypes consisting of CC (413, 143, and 120 bp), CT (553, 413, 143, and 120 bp), and TT (553 and 143 bp) (Figure 2). The difference in fragment length in this study indicates that there is variation in the KIF12 gene sequence. RFLP (restriction fragment length polymorphism) method as a method to determine the presence of mutations due to changes in the type of nitrogen base cytosine (C) base changes to thymine (T) in the KIF12 gene are called transition mutations. The transition is referred to as the change of one pyrimidine base (C,T) to another pyrimidine base (C,T). Changes in nitrogen bases cause differences in the size of the restriction fragments that are digested by certain restriction enzymes (Bardakci, 2001).

Genotype frequency, allele frequency, and Hardy-Weinberg equilibrium in a population

The results of the analysis showed that the KIF12 gene had 3 genotypes, namely CC, CT, and TT with frequencies of 0.30, 0.50, and 0.20 respectively. The frequencies of the C and T alleles were 0.55 and 0.45, based on the results obtained indicating that the KIF12 gene in the SNP g. 9617965 C>T is polymorphic (varies). This is in accordance with the research of Gunawan *et al.* (2018) that it is polymorphic (various) because the allele frequency obtained is less than 99%. The sheep population used in this study is in Hardy-Weinberg equilibrium because χ^2 count < χ^2 table. The Hardy-Weinberg equilibrium law holds true if there is no migration, selection, and mutation. Mutation is a chemical change in a gene that causes a change in the function of the gene (Noor, 2010). Disequilibrium in Bali cattle

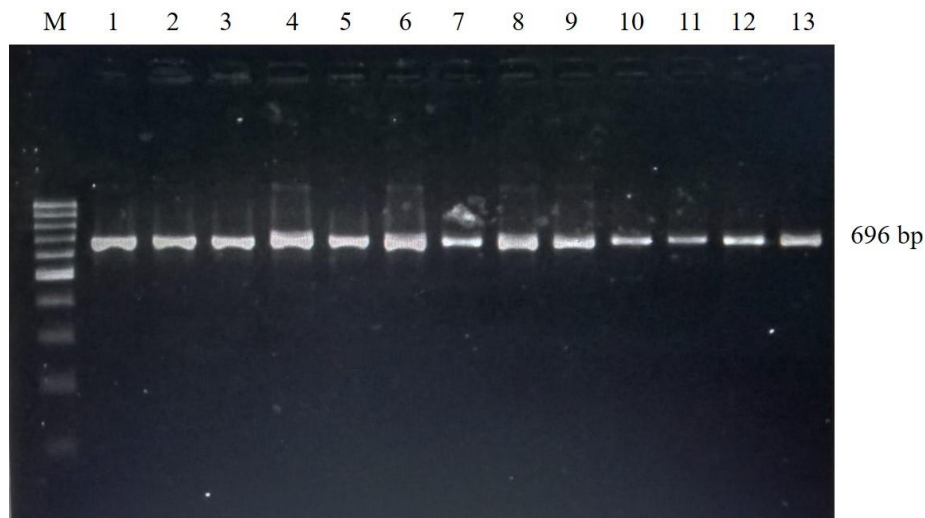


Figure 1. Results of KIF12 gene amplification on 1.5% agarose gel. M = marker 100 bp ladder size standard; Line 1-13 = individual sample of sheep.

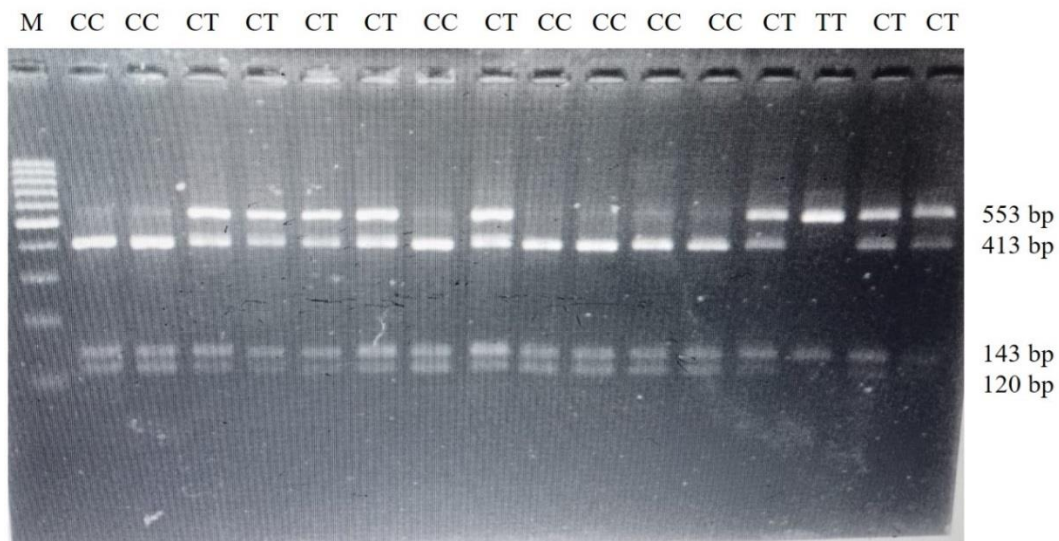


Figure 2. PCR-RFLP results for the KIF12|Bfal on 2% agarose gel. M= 100 bp markers; 1, 2, 7, 9, 10, 11, 12 = CC genotype; 3, 4, 5, 6, 8, 13, 15, and 16 = CT genotype; 14 = TT genotype.

genetic diversity might be caused by intensive selection, non-random mating, and mutation (Khasanah *et al.*, 2016).

Association of KIF12 gene polymorphisms on sheep carcass characteristics

The association of the KIF12 gene with carcass characteristics showed a significant relationship ($P < 0.05$) between the KIF12 gene and the carcass length of sheep. The CC, CT, and TT genotypes in sheep had carcass length values of 78.74 cm, 69.30 cm, and 66.17 cm, respectively. The results obtained from the association of the KIF12 gene on sheep carcass characteristics are presented in Table 2. Sheep with the CC genotype had a higher carcass length than the CT and TT genotypes. In contrast to the research by Gunawan *et al.* (2019) that sheep with the CT genotype in the DGAT1 gene had a higher carcass length than the CC genotype. This indicates that carcass length is influenced by many genes, one of which is the KIF12. Carcass length in sheep is related to slaughter weight, the higher the carcass length, the higher the slaughter weight (Lapian *et al.*, 2013). Bone which is the main component of carcass has a relatively slow growth rate compared to muscle and fat growth

(Suryadi, 2006). Other factors that affect the body size of livestock are breed, sex, feed, temperature, and climate (Sugeng, 2003).

Association of KIF12 gene polymorphisms with lamb quality

The results of the analysis showed that the KIF12 gene at the g.9617965 C>T mutation point had a significant association ($P < 0.05$) with pH and water holding capacity (WHC) in lamb. The association of the KIF12 gene on lamb meat quality is presented in Table 3. The KIF12 gene has also been described as a primary ciliary-associated protein, which is thought to be associated with meat quality (Stalke *et al.*, 2021). The KIF12 gene is a hepatocyte nuclear factor 1-beta (HNF1b) target gene in mice that is expressed in the liver and other tissues such as thyroid, stomach, small intestine, large intestine, prostate, breast, pancreas, and kidney (Gong *et al.*, 2009). In addition, KIF12 has been shown to be required for normal mitosis, localization of myosin II in the cleavage furrow, and cytokinesis in the dictyostelium which is involved in delivering the plasma membrane to the cell division pathway (Lakshmikanth *et al.*, 2004; Agop *et al.*, 2009).

Table 1. Frequency of genotype and allele of KIF12 gene and chi square test

Sheep	N	Genotype frequency			Alel frequency		Hardy-Weinberg equilibrium	
		CC	CT	TT	C	T	X^2_{count}	X^2_{table}
BCS	10	0.50 (5)	0.50 (5)	0.00 (0)	0.75	0.25	1.11	3.84
JTT	52	0.23 (12)	0.50 (26)	0.27 (14)	0.48	0.52	0.0001	3.84
JS	15	0.20 (3)	0.60 (9)	0.20 (3)	0.50	0.50	0.60	3.84
GS	15	0.47 (7)	0.47 (7)	0.06 (1)	0.70	0.30	0.19	3.84
CAS	10	0.40 (4)	0.40 (4)	0.20 (2)	0.60	0.40	0.28	3.84
TOTAL	102	0.30 (31)	0.50 (51)	0.20 (20)	0.55	0.45	0.01	3.84

BCS= Barbados cross sheep; JTT= Javanese thin tail; JS= Jonggol sheep; GS= Garut sheep; CAS= Compass agrinak sheep; N= total of samples.

Table 2. Association of KIF12 gene on carcass characteristics

Carcass characteristics	Genotype			P value
	CC (n=31)	CT (n=51)	TT (n=20)	
Live weight (kg)	23.59±4.98	22.14±3.54	22.63±3.16	0.28
Warm carcass (kg)	9.34±2.83	8.40±0.71	9.19±1.67	0.11
Carcass percentage (%)	40.35±6.20	39.81±4.39	41.71±4.02	0.34
Carcass length (cm)	78.74±19.34 ^a	69.30±15.22 ^b	66.17±15.31 ^b	0.01*
Cold carcass (kg)	9.05±2.95	8.23±1.79	9.19±1.79	0.13

n= number of samples; * significantly different (P<0.05) at level $\alpha=0.05$.

Table 3. Association of KIF12 gene on meat quality

Meat quality	Genotype			P value
	CC (n=31)	CT (n=51)	TT (n=20)	
pH	6.25±0.64 ^a	5.96±0.55 ^{ab}	5.77±0.42 ^b	0.007
Tenderness (kg/cm ²)	3.75±0.87	3.69±0.88	3.87±0.69	0.74
Cooking loss (%)	45.84±7.92	47.42±7.69	43.19±8.32	0.13
WHC (%)	27.39±3.07 ^b	27.23±2.55 ^b	30.02±34.47 ^a	0.004*

n= number of samples, * significantly different (P<0.05) at level $\alpha=0.05$.

The CC, CT, and TT genotypes had pH values of 6.25, 5.96, and 5.77, respectively. The TT genotype had the most normal pH value of 5.77. According to Lawrie (2003) reported that the normal pH value in fresh meat generally ranges from 5.4 to 5.8. The highest pH value in the KIF12 gene was found in the CC genotype of sheep. The high pH value is caused by a decrease in glycogen which can cause lactic acid clots to produce a high pH value (Dewi, 2012). The high pH value causes the meat to have a closed structure, dark in color with a dry meat surface because the liquid meat is tightly bound to protein (Lawrie, 2003). The protein in the KIF12 gene is involved in the post-mortem process.

Sheep with genotypes CC, CT, and TT had water holding capacity (WHC) of 27.39%, 27.23%, 30.02%, respectively. The TT genotype lamb produced the highest water holding capacity (WHC) in meat. According to Ockerman (1983) the higher the meat that has WHC, the higher the protein and carbohydrate content will be. The increase in WHC in TT genotype sheep may be due to changes in fat and protein in lamb caused by proteolytic enzymes (Suantika *et al.*, 2017). Proteolytic enzymes damage muscle membranes, resulting in the diffusion of ions into meat proteins (Rosita *et al.*, 2019). The KIF12 gene has an association with the physical properties of meat with parameters of fatness, cooking loss, and water holding capacity (WHC) of lamb. The KIF12 gene has great potential to be used as a candidate marker in sheep selection programs to produce better meat quality.

Conclusions

SNP g. 9617965 C>T KIF12 gene in BCS, JTT, JS, GS, CAS is polymorphic. The allele frequency in all types of sheep was in Hardy-Weinberg equilibrium. The KIF12 gene was significantly associated with lamb meat quality, namely pH and water holding capacity (WHC), as well as carcass characteristics, namely lamb carcass length. The results of this study indicate that the KIF12 gene has the potential to be used

as a candidate marker in sheep selection programs to improve meat quality and carcass characteristics.

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