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Effect of DGAT1 Gene on Hot and Cold Carcass, Neck and Non-Carcass Traits in Indonesia Sheep

Pengaruh Gen DGAT1 terhadap Karkas Hangat dan Karkas Dingin, Leher dan Non-Karkas pada Domba Indonesia

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ABSTRACT

Quality carcass and non-carcass are two inseparable components that result from slaughtering animals with high economic value. The aims study is effect of polimorfisme DGAT1 gene on hot and cold carcass, neck and non-carcass traits in Indonesia sheep. A total of 50 rams used in this study were collected from 10 Barbados Cross Sheep (BCS), 10 Compass Agrinac Sheep (CAS), 15 Javanese Thin-Tailed Sheep (JTTS), 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 DGAT1 gene polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The effect of the DGAT1 gene and breed with carcass and non-carcass traits were described using a T-test. The result showed that we found two genotypes: CC (466 bp), and CT (76, 390, and 466 bp) in sheep. The DGAT1 gene polymorphisms (g.8539 C>T) were in Hardy-Weinberg Equilibrium (HWE). The DGAT1 gene polymorphisms had a significant (P<0.05) on carcass traits in neck. However, the DGAT1 gene had no significant association (P>0.05) with non-carcass traits. The CT genotype had the highest value of carcass traits compared to CC genotypes. Therefore, the quality of carcass and non-carcass in Indonesian sheep for the DGAT1 gene only affects the neck value.

Keywords: Breed, carcass and non-carcass characteristic, DGAT1 gene, PCR-RFLP, sheep

ABSTRAK

Karkas dan non karkas yang berkualitas merupakan dua komponen yang tidak dapat dipisahkan dari hasil pemotongan hewan yang bernilai ekonomi tinggi. Tujuan Penelitian ini melihat pengaruh polimorfisme DGAT1 terhadap karkas hangan dan karkas dingin serta leher dan non-karkas pada domba Indonesia. Sebanyak 50 ekor domba jantan yang digunakan dalam penelitian ini dikumpulkan dari 10 Domba Barbados Cross (DBS), 10 Domba Compass Agrinac (DCA), 15 Domba Ekor Tipis (DET), dan 15 Domba Jonggol (DJ). Domba dipotong pada umur 10-12 bulan dengan berat badan rata-rata 20.45 kg. Identifikasi polimorfisme gen DGAT1 dilakukan dengan Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Pengaruh gen DGAT1 dengan sifat karkas dan non-karkas dideskripsikan menggunakan uji-T. Hasil penelitian menunjukkan bahwa ditemukan dua genotipe: CC (466 bp), dan CT (76, 390, dan 466 bp) pada domba. Polimorfisme gen DGAT1 (g.8539 C>T) berada dalam Keseimbangan Hardy-Weinberg. Polimorfisme gen DGAT1 berpengaruh (P<0.05) terhadap potongan karkas leher pada domba. Namun, gen DGAT1 tidak memiliki hubungan yang signifikan (P>0.05) dengan sifat non-karkas. Genotipe CT memiliki nilai karakteristik karkas lebih tinggi dibandingkan dengan genotipe CC. Oleh karena itu, kualitas karkas dan non-karkas pada domba Indonesia hanya mempengaruhi potongan karkas leher.

Kata kunci: Breed, karakteristik karkas dan non karkas, gen DGAT1, PCR-RFLP, domba

INTRODUCTION

Carcass and non-carcass are the results of sheep slaughter. Consumers can use the benefits of both in everyday life. High carcass production performance is followed by high slaughter weight (Ashari *et al*. 2018). While non-carcass (offal) can be used as a food ingredient because it has a relatively high nutritional value and the price is relatively low. Baihaqi and Herman (2012) stated that fat tail sheep slaughtered at mature weight had a non-carcass component between 44.8-46.4% of their slaughter weight. Previous research shows that non-carcass has sufficient power for economic value.

Previous studies have reported factors that affect carcass and non-carcass, namely genetic factors (Sedykh *et al.* 2021: Kusuma *et al.* 2020). The polymorphism value between genetics and meat quality, including carcass (carcass percentage), has a value of 26.10-43.10% (Harahap *et al*. 2021), and non-carcass has a value of 25.61% (Junior *et al*. 2016). Research conducted by Sedykh *et al*. 2020 that the value of polymorphism between genetics and meat quality, including carcass (average), is high being 8.49-8.51 points. 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 (Brito *et al*. 2017). These results indicate that selection based on genetics is more effective in increasing genetic superiority for sheep quality, especially carcass and non-carcass characteristics.

Genetically, meat quality, including carcass and non-carcass, is influenced by several potential genes. One of them is diacylglycerol acyltransferase 1 (DGAT1). DGAT1, located on chromosome 9, is included in the metabolic gene stored in adipose tissue (Gunawan *et al.* 2019: Chitraju *et al.* 2019: Fang *et al.* 2012: Patel *et al.* 2012). Relatively higher gene activity of the DGAT group was found in the heart, adipose tissue, and lactating mammary glands (Fang *et al.* 2012). Research conducted by Dai *et al.* (2022) on Southdown Sheep proved a positive correlation between carcasses and DGAT1 polymorphism. Dagong *et al*. (2012) researched carcass and non-carcass effects have been carried out on another gene, using the CAST gene representing three genotypes, namely CAST-11, CAST-12, and CAST-22, proving a negative correlation CAST polymorphisms between non-carcass.

In Indonesian sheep, DGAT1 is associated with fatty acids (Gunawan *et al.* 2019), carcass weight, meat quality, and retail cut (Permana *et al.* 2022). However, the relationship between the DGAT1 gene polymorphism and carcass and non-carcass traits in Indonesian sheep breeds has not been studied. Even though breed diversity also affects carcass and non-carcass characteristics on the quality of sheep. Therefore, this study aimed to analyze the effect of DGAT1 on carcass and non-carcass traits of four breeds of Indonesia sheep, i.e., Barbados Cross Sheep (BCS), Compass Agrinac Sheep (CAS), Javanese Thin-Tailed Sheep (JTTS) and Jonggol sheep (JS).

MATERIALS AND METHODS

Animals

Totals samples used in this study were collected from 50 rams consisted 10 of BCS, 10 of CAS, 15 of JTTS, and 15 of 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 an average body weight of 20.45 kg, aged between 10-12 months (Gunawan *et al*. 2019).

Slaughter Procedure and Sample Collection

Slaughtering was performed according to standard halal methods at a commercial abattoir PT Pramana Pangan Utama (PPU) Bogor, Jawa Barat, Indonesia. 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 hours, 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 extraction. All pieces were put on ice and stored at -20 °C.

DNA extraction and PCR-RFLP Amplification

The longissimus dorsi samples were used for DNA extraction using the Geneaid gSYNC DNA Extraction Kit. The SNP g. 8539 C>T of the DGAT1 gene used in this study refers to Gunawan *et al.* (2019). A pair of primers used to amplify the DGAT1 gene were (F: 5'-CCT CTG CCT TCT TCC ATG AG-'3 and R: 5'-CAG TAC AGC AGC AAG TGG TG-'3) with PCR product of 466 bp (base pair). The PCR amplifications were performed in a 15 µl consisting of 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. PCR amplification using the AB Systems with the initial denaturation at 95 °C for 1 min, then followed by 35 amplification cycles of primer annealing at 60 °C for 15 s, extension at 72 °C for 15 s, and final extension at 72 °C for 1 min. The PCR amplification product was electrophoresis used 1.5% agarose gel.

Statistical Methods

Allele and genotype frequencies

Allele and genotype frequencies were analyzed using genotyping data of four sheep breeds; BCS, CAS, JTTS, and JS. Allele and genotype frequency was calculated with the following formula (Nei and Kumar 2000):

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

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.

Hardy-Weinberg equilibrium values

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

Where $\chi 2$ is the chi-square value, O is the observed values of the i genotype, and E is the expected values of the i genotype (Hartl and Clark 1997).

Association analysis

Association between the DGAT1 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 X_1 and X_2 are the average traits for genotype or breed 1 and 2; n_1 and n_2 are individual numbers of genotype or breed 1 and 2, and δ is the combined standard deviation.

RESULTS AND DISCUSSION

DGAT1 Genotyping

The amplification of the DGAT1 gene fragment was carried out using a PCR Thermal Cycler from Applied Biosystems produced a PCR product length of 466 bp, as shown in Figure 1.The DGAT1 SNP 8.539 C>T in exon 17 was successfully carried out using a primer designed. The results showed 466 bp PCR fragments, previously measured by the ladder on a 1.5 % agarose gel, were sequenced following purification.

PCR-RFLP results showed a polymorphism of the DGAT1 gene indicated by two genotypes consisted CC (466 bp) and CT (466, 390, and 76 bp), whereas TT (390 and 76 bp) was not found in this study (Figure 2). Population's composition of alleles and genotypes is in Hardy Weinberg Equilibrium (Table 1).

Table 1 shows the results of the analysis frequency of genotype CC 96 %, CT 4 %, and yet TT 0 %. However, the CC genotypes still were dominant to CT. The result allele frequency is 98 % for allele C and 2 % for T. According to Gunawan *et al.* (2018), a population is said to be polymorphic if the allele frequency is less than 99%. The study shows that the population in this study is polymorphic in JTTS. Genotype and allele frequencies depend on the breed (Sedykh *et al.* 2021).

The research conducted by Gunawan *et al.* (2018) is in line with this result. The genetic equilibrium of population was evaluated by using $\chi 2$-test (chi-square). The chi-square revealed that the 8.539 C>T of the DGAT1 gene as a whole is not in Hardy-Weinberg Equilibrium, except JTTS. The research conducted by Gauda *et al.* (2022), the Hardy-Weinberg equilibrium uses chi-square < 3.84. Hardy-Weinberg equilibrium can be affected by inbreeding, assortative mating, natural selection, and population subdivision (Novianti 2021).

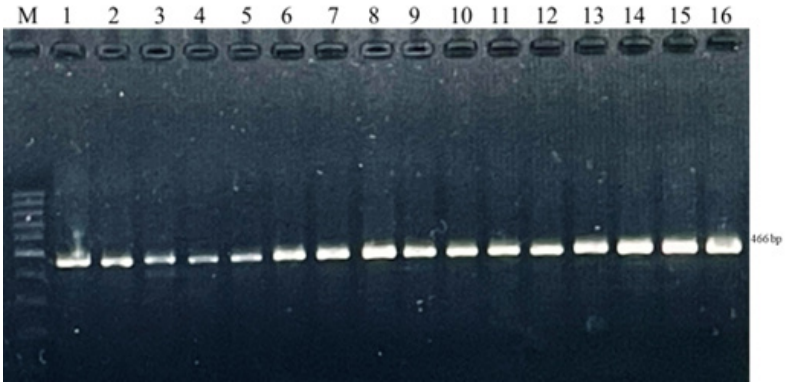

Figure 1. The results of amplifying the DGAT1 gene with a length of 466 bp on 1.5% agarose gel. M= Marker 100 bp; 1-16= sheep samples

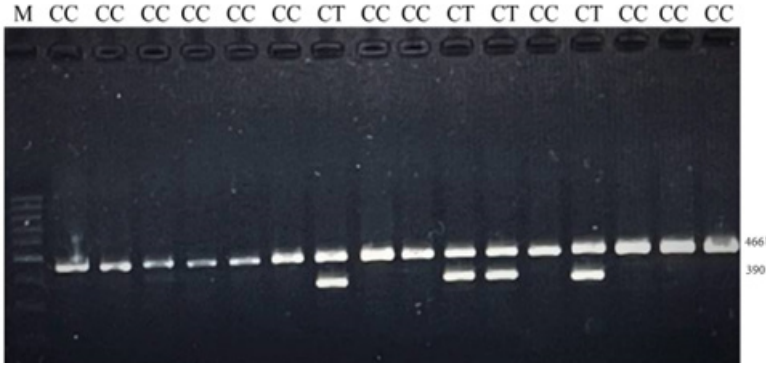

Figure 2. PCR-RFLP results of DGAT1| gene using *AluI* enzyme on agarose gel 2.5%. M= Marker 100 bp

Table 1. Genotype and allele frequencies, and chi-square (χ2) value of DGAT1 gene in Indonesian sheep

Sheep	N	Frequency of genotype			Frequency allele		Chi-square (χ2)
		CC	CT	TT	C	T	
CAS	10	1.00(10)	0.00 (0)	0.00 (0)	1	0	-
BCS	10	1.00 (10)	0.00 (0)	0.00 (0)	1	0	-
JS	15	1.00 (15)	0.00 (0)	0.00 (0)	1	0	-
JTTS	15	0.86 (13)	0.13 (2)	0.00 (0)	0.93	0.06	0.53*
Totals	50	0.96 (48)	0.04 (2)	0.00 (0)	0.98	0.02	54.57

Note: n= samples number, (..) = sample number which CC, CT, TT genotype, χ2 table = 3.84, *= statistically significant, CAS= Compass Agrinac Sheep, BCS= Barbados Cross Sheep, JS= Jonggol sheep, JTTS= Javanese Thin-Tailed Sheep.

Effect of DGAT1 gene on Carcass Traits

The DGAT1 gene polymorphisms had a significant effect (P<0.05) on carcass traits in sheep, i.e., carcass parameters including neck (Table 2). The CT genotype of the DGAT1 gene played a role in increasing carcass characteristics. The CT genotype had a higher carcass characteristic value than the CC genotype. The DGAT1 polymorphism in the neck parameter shows that the CT genotype has a higher value than the CC genotype, which means that the selection of CT genotype sample is selected as a quality sheep with a high neck weight compared to CC. Azizah *et al.* (2020) and Listyarini *et al.* (2022) also reported successively the LEPR gene and OLFML3 gene that carcass traits were affected by gene polymorphism in Indonesian sheep. Sadeghi *et al*. (2020) also reported that carcass traits were affected by DGAT1 polymorphism in Iranian Zel and Lori-Baktiari sheep breeds. Machado *et al*. (2019) reported GH, IGF1, and LEP gene can be candidate genes for related carcasses because they play vital roles in the metabolism process and growth.

The CT genotype has a neck value of 0.64 kg. Research conducted by Junior *et al.* (2016) on Brazil sheep with a a neck value of 0.81 kg. Differences in association values indicate that neck values are also affected by differences between breeds. Dagong *et al.* (2012) reported that commercial cuts of the carcass, which have a high proportion of meat with a low proportion of fat, can produce high economic value.

Low fat is also influenced by the type of muscle that makes up a part of the carcass. The neck are carcass parts composed of skeletal muscles. Anatomically, skeletal muscle tissue is primarily attached to the bones and functions to move the parts of the skeleton so that the fat contained in it is lower than other muscles. Wangko (2014) states that the histological picture of skeletal muscle tissue shows hundreds to thousands of long cylindrical fibers called muscle fibers (fibers). This anatomy and histology overview illustrates that the thick skeletal muscle fibers make the texture rougher so that when held, it becomes tender. The level of tenderness and taste are two important factors influencing consumer preferences in consuming meat (Suwiti *et al*. 2013). Pas *et al*. (2004) stated that the genetic improvement of livestock for meat production with a selection strategy aims to increase the potential for muscle growth, good leanness, and good quality meat when slaughtering. An approach based on molecular selection with the DGAT1 gene can be used as a genetic improvement strategy for carcass quality.

Effect of DGAT1 gene on Non-Carcass Traits

The DGAT1 gene polymorphisms had no significant impact (P>0.05) on non-carcass traits either in sheep (Table 3). All non-carcass parameters associated with DGAT1 were not significant in this study. These findings were consistent with the study by Dagong *et al.* (2012) who reported a non-significant difference among CAST polymorphism with non-carcass traits. Baihaqi and Herman (2012) stated that head and tail were significant in Priangan and JFTS sheep, but in blood and skin were not significant. The difference may be due to the slaughter weight and breeds of sheep. The average weight of non-carcass characteristics in this study was also affected by lower initial body weight. Dagong *et al.* (2012) reported that 21-23 kg body weight produced higher non-carcass blood, head, and tail sections in another gene (CAST-22), respectively 1.038 kg, 2.121 kg, 0.076 kg. Baihaqi and Herman (2012) reported that 32.5-40 kg body weight produced higher non-carcass blood, head, and tail sections, respectively 1.291 kg, 2.182 kg, 0.217 kg. This result shows that body weight affects non-carcass weight in sheep. The highest non-carcass component in the CT genotype was skin, followed by head, limpa, lug, and heart. However, the highest non-carcass component in the CC

Table 2. The effect of the DGAT1 gene on carcass characteristics

Parameters	Genotype			P-Value
	CC	CT	TT	
	(n=48)	(n=2)	(n=0)	
Hot Carcass (kg)	8.02±2.32	10.30±0.01	0.00±0.00	0.175
Cold Carcass (kg)	7.80±2.46	10.26±0.17	0.00±0.00	0.168
Neck (kg)	0.37±0.12b	0.64±0.04a	0.00±0.00	0.003*

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

Table 3. The effect of DGAT1 gene on non-carcass traits

Parameters	Genotype			P-Value
	CC (n=48)	CT (n=2)	TT (n=0)	
Blood	0.70±0.20	0.78±0.06	0.00±0.00	0.621
Head	1.74±0.42	1.96±0.08	0.00±0.00	0.420
Skin	1.82±0.63	2.43±0.25	0.00±0.00	0.188
Foot	0.54±0.10	0.63±0.08	0.00±0.00	0.320
Tail	0.07±0.12	0.08±0.01	0.00±0.00	0.977
Limpa + Lug + Heart	0.67±0.24	0.79±0.02	0.00±0.00	0.501

Note: x = means of non-carcass traits; n= number of samples per genotypes.

genotype was skin, head, and blood. Baihaqi and Herman (2012) reported that the highest non-carcass component was limpa, lug, and heart, followed by skin and head. This study showed that non-carcass weight was also influenced by differences in sex, breed, and body weight.

Physiologically, the function of DGAT1 in sheep metabolism, Chitraju *et al.* (2019) reported that the DGAT1 gene plays an important role in triglycerol metabolism. Triglycerol is a part of fatty acids, in which fatty acids are mainly metabolized in the heart. Azizah *et al*. (2020) reported that the amount of fatty acid composition has an important role in the quality of meat and includes sensory properties such as flavour and juiciness. However, the polymorphism of DGAT1 involvement does not appear to affect non-carcasses, including the heart.

CONCLUSION

The DGAT1 gene was polymorphic in Indonesian sheep. Two genotypes were found in sheep, i.e., CC and CT. The DGAT1 gene polymorphisms significantly affected carcass traits in sheep. The CT genotype had a higher carcass characteristic than other genotypes. Therefore, the quality of carcass and non-carcass in Indonesian sheep for the DGAT1 gene only affects the neck value.

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