

DGAT1 gene polymorphism and their association with fat deposition and carcass quality in Pasundan cattle of Indonesia

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Abstract. Dudi D, Hilmia N, Khaerunnisa I, Mushawwir A. 2023. *DGAT1 gene polymorphism and their association with fat deposition and carcass quality in Pasundan cattle of Indonesia. Biodiversitas 24: 4202-4208.* The bovine Acyl-CoA: Diacylglycerol O-acyltransferase 1 (DGAT1) gene is crucial to milk and meat quality in cattle. The K232A DGAT1 mutation was broadly used as a milk and meat quality genetic marker. Pasundan cattle are Indonesian local cattle from West Java. These local cattle have adapted to the tropical environment, environmental factors are not obstructing their development. The DGAT1 gene polymorphism information and their contribution to fat deposition and carcass quality in Pasundan cattle is very limited. The objective of this study was to examine the genetic polymorphism of the K232A DGAT1 gene and its association with fat deposition and carcass quality in Pasundan cattle population. The gene polymorphisms were identified using PCR, and direct sequencing to discover a single nucleotide polymorphism (SNP). All sequencing results (ABI trace files) were analyzed in FinchTV, BioEdit, and Molecular Evolutionary Genetic Analysis (MEGA) 6.0. Genotyping was performed on 80 Pasundan cattle. In comparison, Ongole-Grade (n=5), Bali (n=2), Simmental (n=2), Limousin (n=2), Madura (n=2), and Pesisir (n=2) were also used in this study. Their association with fat deposition and carcass quality was evaluated on seven heads of AK genotype and 23 heads of KK genotype. According to the sequencing result, two SNPs were found at g.201G>A and g.202C>A, respectively. The K232A DGAT1 locus was polymorphic in Pasundan cattle population with the K and A allele frequencies of 0.956 and 0.044, respectively. In addition, DGAT1 gene polymorphisms are not associated with back fat thickness, longissimus dorsi area, rump area, and intramuscular fat.

Keywords: Carcass quality, DGAT1 gene, fat deposition, Pasundan cattle

INTRODUCTION

In most species, the Diacylglycerol Acyltransferase 1 (DGAT1) gene encodes a protein of about 500 amino acids with a molecular mass of around 55 kDa (Khan et al. 2021; Pathak et al. 2022). DGAT1 is one of the key genes that play an important role in fat metabolism. This gene is located on the endoplasmic reticulum membrane which is the only specific protein for triacylglycerol synthesis. Most studies in K232A DGAT1 polymorphisms have been conducted in various tropic and sub-tropic cattle breeds, including Bali cattle, White Fulani and Bourgo cattle (Alwiyah et al. 2018; Houaga et al. 2018). As well as the association of this gene with lipid metabolism (Mushawwir et al. 2021a,b). Previous research reports have confirmed that this gene is important in regulating fat deposition in tissues. This gene's existence is of significant economic value as one of the selection considerations for developing animals. However, the DGAT1 gene polymorphism information in Indonesian cattle is very limited.

A non-synonymous mutation was reported in various cattle breeds, buffalo and associated with milk fat composition and biochemical indicators in cow blood serum, including triacylglycerides, non-esterified fatty acids, lactate dehydrogenase, total bilirubin, and total immunoglobulins in Slovak Spotted cattle breed (Lešková

et al. 2013) and dairy cattle (Mushawwir et al. 2020, 2021b; Kharazi et al. 2022; Tanuwiria et al. 2022b). This mutation has occurred in the exon 8 DGAT1 gene, replacing AA nucleotides with GC. This substitution replaced the amino acid lysine (K) with alanine (A), encoded with K232A (Hilmia et al. 2013). The SNP on DGAT1 gene, c.572A>G and c.1416T>G significantly affected back fat thickness, longissimus muscle area, marbling score, fat color and Warner-Bratzler shear force on Chinese commercial cattle. These base-pair differences also show consequences for different patterns of lipid metabolism, lipid transport and gene expression related to lipid modulation in several local Indonesian cattle breeds (Mushawwir et al. 2020, 2021b). These SNP markers may be effective for the marker-assisted selection of meat and carcass quality traits and added new evidence that DGAT1 gene is an important candidate gene for improving meat and carcass quality in the beef cattle industry. Moreover, the effect of this K232A mutation was reported in meat quality, especially related to marbling traits. It is reported that diversity in the DGAT1 gene significantly affected Warner Bratzler Share Force of the Longissimus Dorsi muscle and had an important effect on Gannan Yark meat tenderness (Gao et al. 2020).

Pasundan cattle breed is classified as an Indonesian local cattle breed originating from West Java, according to

the ministerial decree of the Ministry of Agriculture, Republic of Indonesia (No. 1051/Kpts/SR.120/10/2014). The Population of Pasundan cattle in West Java reached 152,832 with an average calving interval of 13 months. Based on 3 microsatellite markers, Pasundan cattle were close to Ongole grade and Bali cattle (Hilmia et al. 2013). Moreover, based on 12 microsatellite markers, Pasundan cattle are identified as *Bos indicus* (Agung et al. 2019). Therefore, escalating the potential of Pasundan cattle as one of the endemic livestock in West Java is very beneficial. Based on genetic and physiological aspects, this local cow has adapted well to the geography and climatology of Indonesia (Mushawwir et al. 2020). Previous studies have shown that an increase in environmental temperature does not affect the thermoregulation pattern, as well as the low quality and availability of feed, which does not show a decrease in immunity. These scientific facts provide a strong basis for the development of Pasundan cattle. Targeted selection of certain traits of economic value, especially related to the regulation of fat deposition dictated by specific genes, is an interesting study and has not been reported by previous researchers. However, the association of the *DGAT1* gene with tissue fat deposition has been reported in other cattle.

Information on *DGAT1* gene diversity in local Indonesian cattle linked with fat deposition and carcass quality is limited. Based on this gap, this study aims to determine the diversity of the *DGAT1* K232A gene and its relationship to fat deposition and the quality of carcass components in Pasundan cattle as candidates for carcass quality marker genes.

MATERIALS AND METHODS

Animals, blood, and DNA samples

Therefore, 80 Pasundan cattle (n=80) were used to study polymorphisms in the *DGAT* gene. These cattle were collected from Beef Cattle Breeding and Artificial Insemination Development Center, Ciamis, West Java. Ongole-Grade (n=5), Bali (n=2), Simmental (n=2), Limousin (n=2), Madura (n=2), and Pesisir (n=2) cattle

were also used in this study as the comparison. All samples belong to the Laboratory of Animal Molecular Genetics, Faculty of Animal Science, IPB University, Bogor, Indonesia. Blood sample from each individual was collected from the jugular vein with a syringe and tube containing EDTA. The blood DNA extraction protocol was run according to (Russell and Sambrook 2021) with minor modification by Norgen Blood Genomic DNA Isolation Mini Kit Dx. The relationship evaluation between *DGAT1* gene diversity with fat deposition and carcass quality involved 30 cows over 2 years old. These cattle with genetic diversity were identified based on SNP K232, consisting of 23 cows of KK, and 7 AK genotypes.

Amplification and genotyping

Specific fragment amplification of the exon 8 *DGAT1* gene (Figure 1) was done using Polymerase Chain Reaction (PCR) method with thermocycler machine (GeneAmp® PCR System 9700, Applied Bio Systems™, Foster City, USA). Amplification was performed in 50µL total volume containing DNA samples (50 ng/µL), primer (0.5 pmol), GoTaq Green Master Mix (1 unit, Promega, Madison, USA), and water. Primers used in this study were according to (Winter et al. 2020), consisting of F primer (5'-CACCATCCTCTTCTCAAG-3') and R primer (5'-GGAAGCGCTTTCGGATG-3'). The amplification process was run in 30 cycles consisting of denaturation (95°C, 10 sec), annealing (50°C, 20 sec), and extension (72°C, 30 sec).

The PCR product was visualized on agarose gel electrophoresis with peqGREEN DNA/RNADye (VWR, Leicestershire, UK) above UV Transilluminator (Alpha Imager, Alpha Innotech, Santa Clara, USA); the agarose concentration for PCR products was 1.5%. Direct sequencing was performed on an ABI-PRISM3730 sequencer (1st Base, Singapore) to determine mutations.

The relationship of genetic diversity of the *DGAT1* gene with fat deposition and carcass quality was evaluated in two different genotypes of AK and KK. The parameters of Subcutaneous thickness (SC), Intramuscular Fat (IMF), Rump Area (RA), and Longissimus Dorsi (LD) areas were measured using Ultra Sonography (USG).

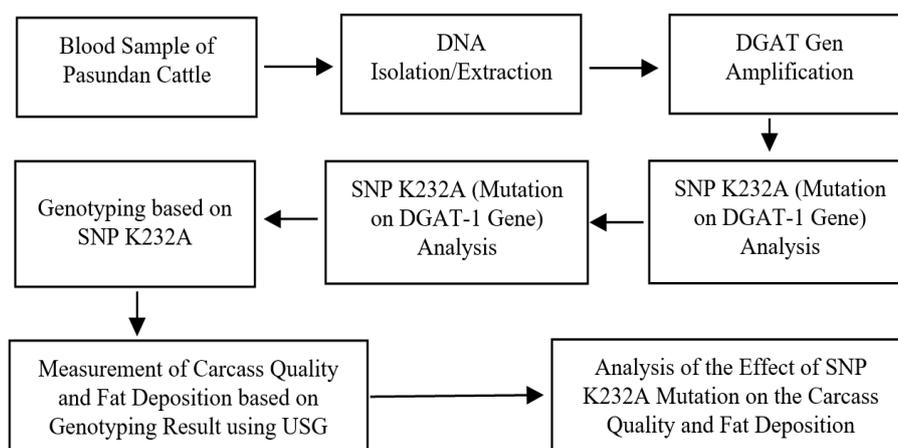


Figure 1. Flow chart of the research methods

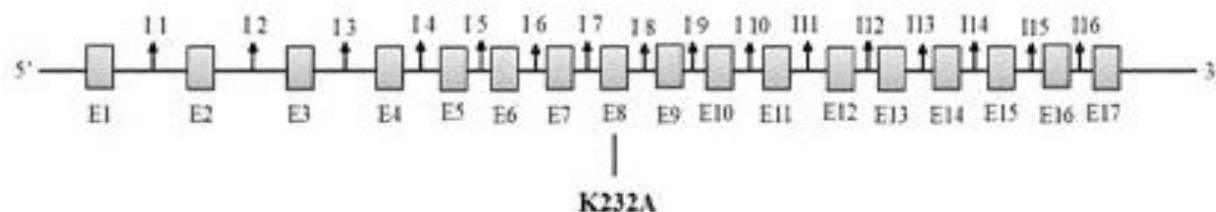


Figure 2. Bovine *DGAT1* gene map, the K232A mutation is located in exon 8. E1-E17: Exon 1-exon 17, I1-I16: Intron 1-intron 16

Relationship between *DGAT1* gene diversity with Subcutaneous Thickness (SC), Longissimus Dorsi (LD) Area, Rump Area (RA) and Intramuscular Fat Percentage (IMF)

The results of observations of the influence of *DGAT1* gene diversity in Pasundan cattle shown by different genotypes against Back Fat thickness (BF), Longissimus Dorsi (LD) Area, Rump Area (LR) and Intramuscular Fat percentage (IMF) using ultrasonography, are presented in Table 2.

Diacylglycerol Acyltransferase 1 (*DGAT1*) is a key gene that has an important role in fat metabolism. This gene is located on the endoplasmic reticulum membrane, the only specific protein for triacylglycerol synthesis. *DGAT1* gene encodes the catalyzing enzyme of the reaction between diacylglycerol and acyl-CoA. This reaction is the final process in the synthesis of triglycerides (Alwiyah et al. 2018; Khan et al. 2021). The results showed that *DGAT1* gene diversity had no significant effect ($P > 0.05$), on Back Fat thickness (BF), Longissimus Dorsi (LD), Rump Area (RA) and Intramuscular Fat (IMF). The thickness of back fat in Pasundan cattle results of this study is much lower than the research of a previous study (Kęsek-Woźniak et al. 2020). The thickness of beef cattle ranges from 6.3 to 13.1 mm, also lower than the results of studies on Bali cows and PO each by 8.4 mm and 6.03 mm (Yosita et al. 2017). The low thickness of back fat in Pasundan cows is suspected because the feed provided is only sufficient for maintenance. The main feed is grass, concentrate is only given as much as 1% dry matter of body weight so that fat deposition is less, while fat is formed because of the excess energy consumed.

Furthermore, the results showed differences in backfat thickness in the *DGAT1* AK and KK genotypes were not significantly different ($P > 0.05$). The results of this study follow the previous studies (Anggraeni 2019; Dudášová et al. 2021; Samuel et al. 2022) which reported no difference in subcutaneous thickness on Hanwoo Korean cattle using SNP K232A. Furthermore, a previous study (Fortes et al. 2019) suggested no difference in back fat thickness in cattle with AA and AK genotypes, respectively, at 3.19 ± 1.76 cm and 3.43 ± 1.46 cm. In addition, high-temperature environments lead to higher evaporation activity and thermoregulation rates. This state requires more energy; therefore, fat is widely used as an energy precursor rather than for deposition.

However, several studies show different results which reported that the *DGAT1* gene affects the thickness of back

fat in cattle; cows with the KK genotype have better back fat thickness than cows with AK/AA genotypes, respectively $5, 3 \pm 0.12$ cm and 4.9 ± 0.06 cm (Nikolina et al. 2013). Back fat thickness is a quantitative trait influenced by many gene pairs. Some of the genes involved in fat metabolism, including back fat, are the Leptin gene, Growth Hormone, Thyroglobulin, and *DGAT2* (Fortes et al. 2019; Kharazi et al. 2022; Tanuwiria et al. 2022a).

The results of the study LD area in Pasundan cattle vary greatly from 24.32 to 38.82 cm², with a coefficient of variation of 34% (Hilmia et al. 2015). The LD area of Pasundan cattle is smaller than the results of the previous study, which suggests that LD in local Ciamis cattle is 48.84 – 49.85 cm² (Hilmia et al. 2015). The area of Longissimus dorsi is affected by the growth process of the body's cells, which in its development requires adequate nutrition. Pasundan cattle feed research is dominated by forages of limited quality, namely elephant grass, corn straw, and sometimes rice straw in the dry season; the concentrate is only given as much as 1% of dry matter of body weight. Furthermore, it was revealed that the area of longissimus dorsi cattle fed 75% concentrates was significantly higher ($P < 0.05$) than cattle with 50% concentrate feed (Ningrat 2010). Based on that previous study, the low breadth of longissimus dorsi is obtained because the feed provided is insufficient for muscle and fat growth.

The results showed that the mutations did not affect the LD area, for KK and AK were 13.05 ± 7.04 and 16.50 ± 7.38 , respectively. The results of this study are in line with previous studies suggesting that there is no significant relationship of *DGAT* gene diversity with LD area, KK / TC genotype of 77.39 ± 2.02 , KK / TT 78.67 ± 2.10 , KA / TC and KA / TT respectively - each by 75.04 ± 2.01 and 77.37 ± 1.74 [23]. Furthermore, another study showed the diversity of the *DGAT* gene did not affect the area of longissimus dorsi in Nelore cattle (Fortes et al. 2019).

Table 2. Carcass component on different genotypes based on SNP K232A on *DGAT1* gene

Carcass component	Genotype	
	KK (n=7)	AK (n=23)
Backfat thickness (cm)	0.24 ± 0.089	0.27 ± 0.081
Longissimus Dorsi Area (cm ²)	13.05 ± 7.04	16.50 ± 7.38
Rump Area (cm ²)	18.42 ± 9.02	19.89 ± 3.43
Intramuscular Fat (%)	3.93 ± 2.64	4.31 ± 2.85

n: Number of samples

Table 1. Genotype and allele frequency of K232A DGAT1 loci in various cattle populations

Breed	n	Genotype frequency			Allele frequency		χ^2
		KK	AK	AA	K	A	
<i>Pasundan</i>	80	0.91	0.09	0.00	0.96	0.04	0.167 ^{ns}
<i>Ongole</i> Grade	5	1.00	0.00	0.00	1.00	0.00	-
<i>Bali</i>	2	1.00	0.00	0.00	1.00	0.00	-
Simmental	2	0.00	1.00	0.00	0.50	0.50	2.000 ^{ns}
Limousine	2	0.00	0.00	1.00	0.00	1.00	-
<i>Madura</i>	2	1.00	0.00	0.00	1.00	0.00	-
<i>Pesisir</i>	2	1.00	0.00	0.00	1.00	0.00	-
Overall	95	0.90	0.10	0.02	0.95	0.05	

Note: n: number of sample, ns: not significant, *: significantly different ($\chi^2_{0.05} = 3.84$)

The quantitative phenotype is affected by multiple genes/polygene pairs, so in addition to DGAT, other genes are involved in the growth of LD sections. It was believed that the carcass composition, namely the longissimus dorsi region, is affected by the myostatin gene, the callipygous gene and the IGF 2 gene (Insulin-like Growth Factor 2) (Devine and Dikeman 2014). In addition, another study suggested that several candidate genes contributed to economic profitability as growth and fat deposition, such as polymorphisms in DGAT1, GHRHR, CAST, SERPINA3 and HSPB1 genes with several live weights, SCD5 and DGAT1 with rib-eye area, and PPARGC1A with GR, hot carcass weight and valuable cuts weight (Amstrong et al. 2018).

The IMF fat in Pasundan cattle is higher than the results (3.93±2.64 to 4.31±2.85) of previous study in Holstein German cattle ranging from 6% to 7.9% and in Charolais cattle from 3.79 to 4.15% (Babii et al. 2018; Bhattarai et al. 2019). It was suggested that DGAT1 acts as the final catalytic enzyme of triglyceride synthesis (Mushawwir et al. 2011; Bovenhuis et al. 2016; Park et al. 2018; Adriani et al. 2021). Triglyceride synthesis is the key to intramuscular fat deposition, balanced by triglyceride absorption and degradation. The study's results on the effect of DGAT gene diversity on intramuscular fat deposition revealed that they were not significantly different ($P > 0.05$). These results are consistent with the previous study which showed that Holstein German cattle with KK, KA and AA genotypes did not differ in the IMF meat content, respectively, at 7.70±0.78, 5.96±0.48 and 6.00±0.56, then at Charolais cattle also did not show a significant difference, KA and AA values were 3.69±0.79 and 4.15±0.49, respectively (Babii et al. 2018). Different research results were shown by another study which reported that mutations in the DGAT gene in K232A and T11993C influenced the marbling genotype score of KK/TC, KA/TT, KK/TT, and KA/TC, respectively, in the sequence of 2, 51±0.27, 2.26±0.21, 1.83±0.26 and 1.48±0.28 (Bovenhuis et al. 2016). Furthermore, the results of another study in Angus, Charolais, Hereford, Limousin, and Simmental cattle, suggest that DGAT 1 also influences marbling deposition (Ekerljung 2017). The difference in temperature between tropical and sub-tropical countries is an important factor in the expression of DGAT-1 in regulating fat deposition in muscle tissue. On the contrary, the rate of fat deposition is

not so important for muscle tissue, to prevent inhibition of heat evaporation of Pasundan cattle.

This intramuscular fat deposition is influenced by several genes, such as diacylglycerol O-acyltransferase 1 (DGAT 1), thyroglobulin (TG), growth hormone (GH), and stearoyl-coenzyme A desaturase (SCD) (Mauri  et al. 2017; Houaga et al. 2018). At the same time, another study found that TG polymorphisms impacted increasing intramuscular fat in 50 male Holstein beef samples (Ardicli et al. 2018). Several studies have shown that genes involved in intramuscular fat deposition in ruminants are FABP3 (H-FABP), PPARG, DGAT1, LPL, ACACA, FASN (FAS), FABP4, CPT1B and SCD (Guo et al. 2014).

Diacylglycerol acyltransferases (DGATs), including DGAT1 and DGAT2, are important enzymes for catalyzing the terminal step in the formation of TGs through the acylation of diacylglycerol (DAG) with a fatty acyl-CoA and thus regulating lipid digestion, absorption, and glycerol lipid metabolism pathways (Bhatt-Wessel et al. 2018), indicating their major functions in both the remodeling and *de novo* production pathways of TAG synthesis. Both abundantly expressed in the small intestine are DGAT1 and DGAT2, indicating a major function in dietary absorption. Mice with targeted deletion of DGAT1 show a pause in circulating postprandial hypertriglyceridemia and a decline in chylomicron development to help this notion (Cruz et al. 2019; Khan et al. 2021; Li et al. 2021). The DGAT1 role in dietary fat absorption was further confirmed by the production of an ADGAT1-specific enzyme inhibitor, XP620, which greatly reduced apolipoprotein B (apoB) secretion in Caco-2 cells, TAG and DAG synthesis in primary enterocytes, and dietary fat absorption in rats (Angiolillo et al. 2016; Hill et al. 2016; Li et al. 2019; Rahmanian et al. 2022).

Specifically in obesity, DGAT1 polymorphisms have been related to a certain form of human obesity (Ludwig et al. 2022). In plants, the single amino acid polymorphism of DGAT1gen is related to the oil content of maize. Similar to a single nucleotide polymorphism of DGAT1 that is consistent with milk fat content in cattle (Scotti et al. 2020). Following the differentiation of 3T3-L1 into mature adipocytes, the DGAT1 protein amounts increased dramatically. Overexpression of DGAT1 and DGAT2 substantially increases TAG production in mature adipocytes (Carvajal et al. 2016; Li et al. 2019; Elzaki et al. 2022). Conversely, mice with DGAT1 deficiency also

decreased adiposity and tolerance to high-fat diet-induced obesity, followed by enhanced energy consumption and hyperlocomotive operation (Agrawal et al. 2018; Tanuwirya et al. 2022b). Improvements in adipocyte lipid metabolism likely trigger this metabolic phenotype due to DGAT1 deficiency. Wild-type mice with adipose transplanted from DGAT1-deficient mice exhibited physiological traits identical to DGAT1-knockout mice, including tolerance to diet-induced obesity and increased insulin sensitivity (Mohammed et al. 2015; Mushawwir et al. 2023). In humans, the full-length wild-type DGAT1 or DGAT2 expression, restored normal lipid metabolism in the intestinal epithelium. Mutations in DGAT1 reduced the expression of its product; dermal fibroblasts and intestinal organoids derived from these patients had altered lipid metabolism and were susceptible to lipid-induced cell death. These findings indicate the importance of DGAT1 in fat metabolism and lipotoxicity in the intestinal epithelium (van Rijn et al. 2018).

Environmental factors seem to impair the DGAT1 gene mutation expression in Pasundan cattle. In this situation, the efficiency of Pasundan cattle must be assisted by implementing acceptable management criteria for the genetic ability to be adequately articulated. Effective feed management, where livestock must get sufficient feed intake in terms of quality and quantity, is a factor that will promote the expression of genotypes.

In conclusion, the K232A DGAT1 locus was polymorphic in the Pasundan cattle population, with the K and A allele frequencies of 0.96 and 0.04, respectively. The diversity of DGAT1 does not affect the thickness of back fat, area of Longissimus Dorsi, area of Rump and percentage of intramuscular fat.

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