Association of DGAT1 Gene Related to Flavor, Odor, Cholesterol, and Mineral in Indonesian Sheep

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(received 14-11-2023; revised 01-02-2023; accepted 16-03-2023)

ABSTRAK

Amri F, Harahap RS, Sumantri C, Inounu I, Depison, Alwi Y, Gunawan A. 2023. Asosiasi gen DGAT1 terkait flavor, odor, kolesterol, dan mineral pada domba Indonesia. JITV 28(2):122-128. DOI: http://dx.doi.org/10.14334/jitv.v28.i2.3152

Diacylglycerol acyltransferase 1 (DGAT1) merupakan salah satu kandidat gen potensial untuk perbaikan kualitas dan gizi daging domba Indonesia. Tujuan penelitian ini untuk mengidentifikasi keragaman gen DGAT1 pada SNP g. 8539 C>T serta kaitannya kandungan flavor dan odor, kolesterol, dan mineral daging domba Indonesia. Total sampel yang diidentifikasi sebanyak 254 ekor domba jantan berumur 10–12 bulan yang terdiri dari 20 ekor domba ekor gemuk (DEG), 107 domba ekor tipis (DET), 10 domba komposit garut (DKG), 10 domba *compass agrinak* (DCA), 10 domba *barbados cross* (DBC), 20 domba garut (DG), 27 domba jonggol (DJ), dan 50 domba lokal jambi (DLJ). Sebanyak 100 ekor domba diantaranya digunakan untuk analisis asosiasi gen DGAT1 dengan kandungan flavor dan odor, kolesterol, dan mineral. Identifikasi keragaman DGAT1|ALuI dianalisis dengan metode PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). Hubungan gen DGAT1 dengan parameter nilai gizi daging dianalisis dengan metode GLM (General Linear Model). Hasil penelitian menunjukkan keragaman gen DGAT1 bersifat polimorfik pada DET, DG dan DEG, sedangkan pada DCA, DBC, DLJ dan DKG bersifat monomorfik. Dua genotipe, CC dan CT ditemukan dalam DET, DG dan DEG. Gen DGAT1 berasosiasi secara signifikan (P<0.05) dengan kandungan flavor dan odor, yaitu senyawa 4-Ethyloctanoic acid (EOA). Genotipe CT memiliki nilai EOA yang lebih tinggi dibandingkan CC. Keragaman gen DGAT1 tidak ditemukan kaitannya dengan kandungan kolesterol dan mineral. Gen DGAT1 dapat dijadikan kandidat marka genetik untuk perbaikan kualitas flavor dan odor domba Indonesia.

Kata Kunci: Kolesterol, DGAT1, Flavor, Odor, Mineral

ABSTRACT

Amri F, Harahap RS, Sumantri C, Inounu I, Depison, Alwi Y, Gunawan A. 2023. Association of DGAT1 Gene related to flavor, odor, cholesterol, and mineral in Indonesian sheep. JITV 28(2):122-128. DOI: http://dx.doi.org/10.14334/jitv.v28.i2.3152

Diacylglycerol acyltransferase 1 (DGAT1) is a potential candidate gene for improving Indonesian lamb's quality and nutrient value. The study aimed to identify the diversity of the DGAT1 gene with SNP g.8539 C>T and its relation to Indonesian lamb's flavor and odor, cholesterol, and mineral. Total of 254 ten to twelve months old sheep consisted of 20 Javanese fat-tail sheep (JFTS), 107 Javanese thin-tail sheep (JTTS), 10 Garut composite sheep (GCS), 10 Compass agrinak sheep (CAS), 10 Barbados cross sheep (BCS), 20 Garut sheep (GS), 27 Jonggol sheep (JS), and 50 Jambi local sheep (JLS). One hundred sheep were used to analyze the association of the DGAT1 gene with flavor and odor content, cholesterol, and mineral. The diversity of DGAT1|*Alu*I was analyzed with the PCR-RFLP method. The association of the DGAT1 gene with the nutritional value of meat was analyzed using the GLM (General Linear Model) method. The results showed that the DGAT gene was polymorphic in JTTS, GS, and JFTS and monomorphic in CAS, BCS, JLS, and GCS. CC and CT genotypes were found in JTTS, GS, and JFTS. SNP g.8539 C>T of DGAT1 gene had a significant association (P<0.05) with flavor and odor, 4-Ethyloctanoic Acid (EOA). However, the DGAT1 gene had no significant association (P>0.05) with cholesterol and mineral. The DGAT1 gene might be marker-assisted selection for improving lamb flavor and odor in Indonesian sheep.

Key Words: Cholesterol, DGAT1, Flavor, Odor, Mineral

INTRODUCTION

Increasing meat consumption per capita is one of the focuses of Indonesia to improve the quality of Indonesian human resources. One potential source to meet this goal is lamb. Sheep are already famous in Indonesia, adapted well to the tropical climate, and are used to being kept by Indonesian people, especially in rural areas. Lamb meat consumers consider flavor and odor the most critical parameters of lamb quality. The leading causes of

sheepmeat odor are the two compounds: branched-chain fatty acid (BCFA), present in all fatty tissue, has been implicated as the cause of such flavors as, has indole, which originated from pastoral diets. Branched-chain fatty acids (BCFAs) consisted of 4-methyl octanoic (MOA), 4- ethyl octanoic (EOA), 4-methyl nonanoic (MNA) acids, 3-methylindole (MI), and 4-methyl phenol (MP) are the chemical compounds that are accepted as the main contributors to flavor and odor (Watkins et al. 2012).

However, it is vital to improve the quality of lamb to increase the acceptance and value of Indonesian lamb. Improvement in the quality of flavor and odor could also stimulate the development of the lamb market (Listyariniet al., 2018). Identifying genes related to lamb quality is the best way to accelerate the improvement of Indonesian lamb through a molecular selection approach. One gene with immense potency as a genetic marker for quality traits of sheep meat is the Diacylglycerol acyltransferase 1 (DGAT1) gene (Gunawan et al. 2019). The DGAT1 gene is located on the reticulum endoplasm membrane and is essential in the intricacy of Glycerol (TG) synthesis. DGAT1, located in adipose tissue, has a role in reserve metabolic energy (Fang et al. 2012; Patel et al. 2012; Chitraju et al. 2019). High activity of DGAT1 has been identified in the liver, adipose tissue, small intestine, and mammary gland (Fang et al. 2012).

Polymorphism of DGAT1 with mutation site in exon 16-17 (DGAT1|AluI) and its association with carcass characteristics in Iranian sheep had been reported by (Mohammadi et al. 2012). Moreover, the DGAT1 gene is also associated with fat composition in goat milk (Li et al. 2013; Zonabend König et al. 2017), saturated fatty acid levels (Hatta et al. 2019), and minerals in cows. In Indonesian sheep, DGAT1 is associated with carcass weight, meat quality and retail cut, fatty acids, and carcass characteristics (Gunawan et al. 2019).

Our previous study has not studied the relationship between DGAT1 gene polymorphisms with flavor and odor, cholesterol, and mineral traits in various breeds of Indonesian sheep. Though the variety of breeds also affects the flavor, odor, cholesterol, and mineral traits in the lamb quality. Therefore, the study has been established to estimate the diversity of the DGAT1 gene and the association of the DGAT1 gene to Indonesian lamb quality traits including, flavor and odor, cholesterol, and mineral.

MATERIALS AND METHODS

Samples

All experiments were approved by the Animal Ethics Commission of the IPB University (approval no.117-2018 IPB). The total of 254 rams used in this study were Javanesse fat-tail sheep (JFTS) (n=20), Javanesse thin-tail sheep (JTTS) (n=107), Garut composite sheep (GCS) (n=10), Compass Agrinak sheep (CAS) (n=10), Barbados cross sheep (BCS) (n=10), Garut sheep (GS) (n=20), Jonggol sheep (JS) (n=27) and Jambi local sheep (JLS) (n=50). Sheep aged 10–12 months with male sex are kept in a caged group. The feed provided during maintenance consisted of elephant grass and concentrate. One hundred sheep were used to analyze the association of the DGAT1 gene with flavor and odor content, cholesterol, and mineral. Data for analysis association study with flavor and odor were obtained from JFTS (n=10), JTTS (n=75), and JS (n=15), while for cholesterol consisted of JFTS (n=10), JTTS (n=45), GCS (n=10), CAS (n=10), and BCS (n=10), JS (n=15). In addition, data analysis for mineral content consisted of JS (n=15) and JTTS (n=85).

DNA extraction and PCR 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 using 1.5% agarose gel.

Genotyping using PCR-RFLP

Genotyping is done by PCR-RFLP technique using AluI cutting enzymes. Five L of amplicons were added to a mix of 0.9 L DW, 0.7 L tango buffer, and 0.4 L AluI enzyme restriction and incubated at 37 °C for 16 hours. The PCR-RFLP product was electrophoresed using 2.5% agarose gel. The DNA fragments that emerged were compared with a 100 bp marker. Genotyping is carried out based on the length of the DNA fragment. The genotype of DGAT1 consisted of CC: 466 bp; TT: 390, 76 bp; and CT: 466, 390, 76 bp.

Flavor and odor analysis

The flavor and odor parameters measured were 4-Methyloctanoic Acid, 4-Ethyloctanoic Acid, 4-Methylnonanoic Acid, Indole, 3-methyl, Phenol, 3-

methyl, Undecanoic Acid, and Phenol, 4-methyl. 500 g of loin samples were carried out for flavor and odor analysis. Flavor and volatile odor compounds were extracted using the Likens-Niceson method, which combines distillation and extraction with a solvent using Gas Chromatography-Mass Spectrophotometry (GC-MS).

Cholesterol content analysis

Cholesterol examination using the method: CHOD-PAP (Cholesterol] Oxidase Peroxidase Aminoantipyrine Phenol) with the principle: measurement of total cholesterol after oxidation and hydrolysis of colorimetric indicator enzymes, namely chinonimine produced and 4-amino antipyrine and phenol with hydrogen peroxide with the help of a peroxide catalyst. Measurements were made at a wavelength of 546 nm. The serum was mixed with cholesterol test reagent, incubated for 10 minutes at 37°C, then read the results. Absorbance readings with blank reagents were carried out for 60 minutes.

Mineral content analysis

The mineral content was analyzed according to AOAC (2015) Official Method 969.08. The longissimus dorsi was used for mineral content analysis. The parameters were analyzed, including iron (Fe), zinc (Zn), kalium (K), and selenium (Se).

Data analysis

Genotype frequency

Genotype frequency is the ratio of genotypes to the total population. Genotype frequency is calculated by comparing the number of genotypes with the population. The genotype frequency was calculated using the following formula:

$$Xi = \frac{\sum_{i=1}^{n} ni}{N}$$

Xii is frequency genotype ii, ni is the number of individual genotypes ii, and N is the number of samples.

Allele frequency

Allele frequency is the ratio of the number of alleles to all alleles in a population. The Allele frequencies were calculated using the following formula:

$$Xi = \frac{(2nii + \sum_{j \neq 1} nij)}{(2N)}$$

Where Xi is allele X-*ith* frequency, nii is the number of individual *ii* genotypes, nij is the number of individuals *ijth* with initial genotype, and N is the number of samples.

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-squared. Hardy-Weinberg equilibrium was calculated using the following formula:

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

Where X^2 is a chi-square value, O is the number of genotypes observed, and E is the number of genotypes expected.

Association analysis

The association between the DGAT1 gene and flavor, odor, cholesterol, and mineral content was analyzed using the General Linear Model (GLM) method with Minitab® 18 Software. The difference was considered statically significant if the p-value <0.05. Pairwise differences between genotype effects were tested by performing the Tukey Model. The formula model was used:

$$Yij = \mu + genotype_i + breed_j + e_{ijk}$$

Where Yij is the performance of the individual lamb for flavor, odor, cholesterol, and mineral content; μ is the average of flavor, odor, cholesterol, and mineral content for each trait; genotype*i* is the fixed effect of *i-th* genotype; breed*j* is the fixed effect of the *j-th* breed; *eijk* is the random error.

RESULTS AND DISCUSSION

DGAT1 gene diversity

The PCR amplification of the DGAT1 gene with mutation C>T in exon 17 was successfully carried out using a primer designed, and the results showed one genotyped CC (466 bp) (Figure 1).

The identification results of DGAT1 using PCR-RFLP with the restriction enzyme AluI showed 2 genotypes: a combination of C and T alleles, namely CC and CT. One band at 466 bp for CC homozygotes, two bands consisting of 390 and 76 indicates TT homozygotes, and three bands at 466 bp, 390 bp, and 76 bp for CT heterozygotes, as shown in Figure 2.

The diversity of the DGAT1 gene was analyzed using the formula for allele frequency, genotype frequency, and Hardy-Weinberg balance. The allele and genotype frequency values and the Hardy-Weinberg balance of the DGAT1 gene are presented in Table 1. The results showed that the C allele was the dominant allele in all sheep populations, whose frequency value ranged from 93-100%, while the T allele frequency was 0-7%.

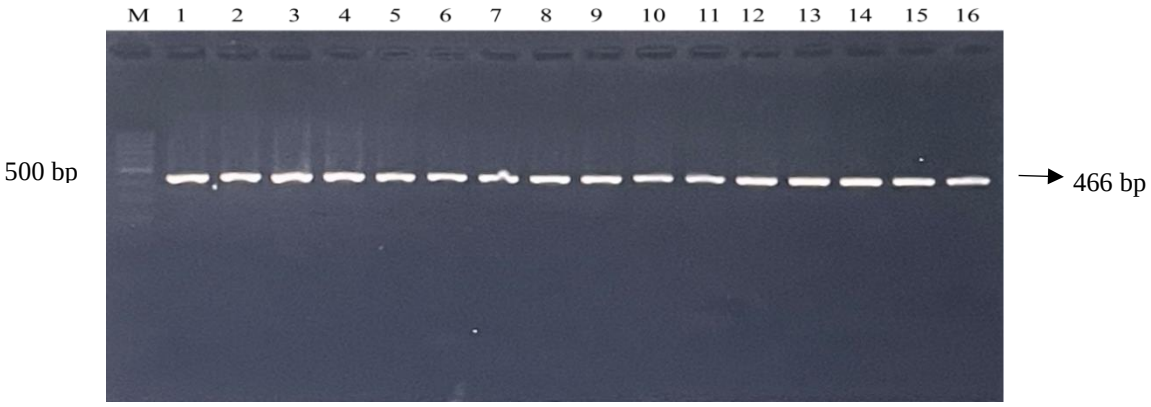

Figure 1. The results of amplifying the DGAT1 gene on 1.5% agarose gel. M= Marker 100 bp; 1-16= sheep samples

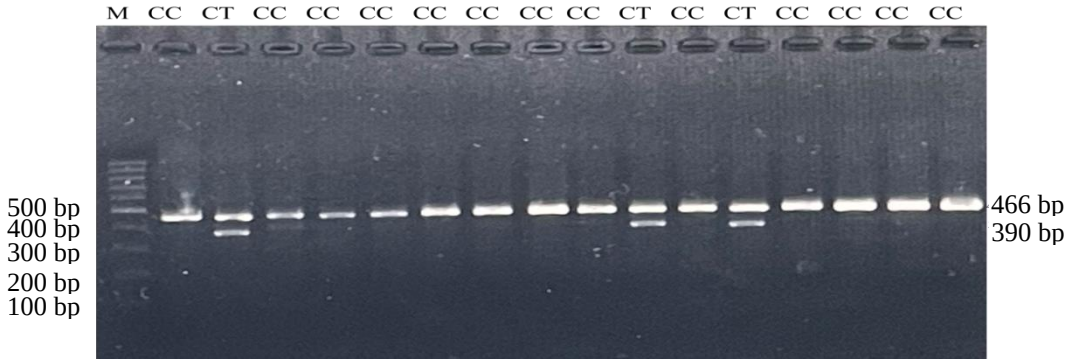

Figure 2. PCR-RFLP results of DGAT1| gene using AluI enzyme on agarose gel 2.5%. M= Marker 100 bp

This indicates that alleles are heterozygous for only 2% of the population. The low frequency of several alleles is most likely the result of random genetic drift (Star & Spencer 2013). The genotype frequencies obtained have different values; the CC genotype was 0.96, while the CT genotype was 0.04. Gene diversity is only found in Javanese fat-tail sheep (JFTS), Javanese thin-tail sheep (JTTS), and Garut sheep (GS). However, the DGAT1 in Compass agrinak sheep (CAS), Barbados cross sheep (BCS), Garut composite sheep (GCS), and Jambi local sheep (JLS) were monomorphic. This can be seen from the allele and genotype frequency values equal to 1.00. In this study, the population in the Hardy-Weinberg equilibrium value was 0.07. One of these factors can occur as a result of the genotype in the population being maintained/constant from generation to generation (Abramovs et al. 2020).

DGAT1 gene association with flavor and odor compounds

Association analysis of the DGAT1 gene with flavor and odor showed a significant association ($P < 0.05$) with 4-Ethyloctanoic Acid, while 4-methyl octanoic, 4-methyl nonanoic, 3-methylindole, 4-methyl phenol, 3-methyl phenol had no significant association with DGAT1. Individuals with the CT genotype had higher EOA values than the CC genotype (Table 2).

This study showed that DGAT1 had the most significant effect on phase I EOA metabolism in the liver. In contrast, the research report by (Listyarini et al. 2018) showed no relationship between EOA and CYP2A6 and KIFI2 genes. (Liu et al. 2012) stated that EOA is found in lamb, which plays a role in flavor compounds that are very important for flavoring in food.

The EOA compound has a relatively high molecular weight (C10H20O2) which has a positive impact because it is not volatile, so it can be used as an agent to repair unpleasant odors from other compounds. Another study related to EOA had a positive impact when the cooking test was carried out. The results did not affect the smell and taste of lamb (Resconi et al. 2013). Cattle reported that the DGAT1 lysine (L)>Alanine (A) polymorphism with an amino Acid substance at 232 K232A was associated with the amount of intramuscular fat that affected flavor and odor.

DGAT1 gene association with cholesterol

The association analysis of the DGAT1 gene with cholesterol showed no significant association ($P > 0.05$). Table 3 shows the results of the analysis. This is in line with the research that has no significant effect on cholesterols FAO products, some genes bound to acetyl-CoA will be incompletely oxidized to ketone bodies and cholesterol (Xue et al. 2018). The DGAT1 gene encodes

Table 1. Genotype and allele frequencies, and chi-square (χ^2) value of DGAT1 gene in Indonesian sheep

Sheep	N	Genotype frequency			Allele frequency		Chi-square (χ^2)
		CC	CT	TT	C	T	
JFTS	20	0.9 (18)	0.1 (2)	0.0	0.95	0.05	0.05^{ns}
JTTS	107	0.97 (104)	0.03 (3)	0.0	0.99	0.01	0.02^{ns}
JS	27	1.0 (27)	0.0 (0)	0.0	1.00	0.00	-
GCS	10	1.0 (10)	0.0 (0)	0.0	1.00	0.00	-
CAS	10	1.0 (10)	0.0 (0)	0.0	1.00	0.00	-
BCS	10	1.0 (10)	0.0 (0)	0.0	1.00	0.00	-
GS	20	0.85 (17)	0.15(3)	0.0	0.93	0.07	0.11^{ns}
JLS	50	1.0 (50)	0.0 (0)	0.0	1.00	0.00	-
Total	254	0.96 (246)	0.04 (8)	0.0	0.98	0.02	0.07^{ns}

N= Total samples; (..)= sample number which CC, CT, TT genotype; χ^2 table= 3.84. ^{ns}= not significant

Table 2. Genotype and association analysis of candidate genes with flavor and odor compounds

Parameters (ug/ul)	Genotype of DGAT1 ($\bar{x}$ ± SE Mean)			P value
	CC (97)	CT (3)	TT (0)	
4-*methyl octanoic* (MOA)	0.28±0.09	0.14±0.11	0.00±0.00	0.648 ^{ns}
4-*ethyl octanoic Acid* (EOA)	0.16±0.02^{b}	0.89±0.63^{a}	0.00±0.00	0.023*
4-*methylnonanoic* (MNA)	1.04±0.27	0.12±0.06	0.00±0.00	0.457 ^{ns}
3-*methylindole* (MI /Skatole)	0.03±0.01	0.02±0.01	0.00±0.00	0.838 ^{ns}
3-*methyl phenol* (MP)	3.22±0.69	1.11±1.11	0.00±0.00	0.767 ^{ns}
4-*methyl phenol (*MP)	0.001±0.0004	0.001±0.001	0.00±0.00	0.840 ^{ns}

$\bar{x}$= means of carcass traits; SE= standard error; (..)= the number of samples per genotypes; superscript a, b showed a significant difference at 5%; *=Significant at P<0.05; ^{ns}= Not significant. Different superscripts on the same row are different (P<0.05)

Table 3. Genotype and association analysis of candidate genes with cholesterol

Parameters (%)	Genotype of DGAT1 ($\bar{x}$ ± SE Mean)			P value
	CC (97)	CT(3)	TT(0)	
Cholesterol	7.17±2.58	6.59±2.41	0.00±0.00	0.686^{ns}

$\bar{x}$= means of carcass traits, SE= standard error, (..)= number of sample per genotypes, ^{ns}= not significant

Table 4. Genotype and association analysis of candidate genes with mineral

Parameters (mg/100 g)	Genotype of DGAT1 ($\bar{x}$ ± SE Mean)			P value
	CC (97)	CT (3)	TT (0)	
Fe	1.87±0.79	1.68±0.54	0.00±0.00	0.679^{ns}
Zn	2.62±0.99	2.47±0.51	0.00±0.00	0.807^{ns}
K	274.53±85.99	258.3±83.1	0.00±0.00	0.748^{ns}
Se	0.61±0.31	0.63±0.14	0.00±0.00	0.901^{ns}

$\bar{x}$= means of carcass traits, SE= standard error, (..)= number of sample per genotypes, ^{ns}= not significant

an enzyme containing 489 amino acids that catalyze the synthesis of TG by covalently linking TG to the substrate acetyl CoA. The study showed that acetyl-CoA incompletely binds to the DGAT1 gene during the formation of TG, which then leads to cholesterol oxidation. The relationship of cholesterol (VLDL and LDL) with triglycerides seen from forming very low-density lipoprotein (VLDL) will depend on the fatty acids available from adipose TG. VLDL is the primary lipoprotein in TG transport. TG synthesized by the liver is packaged into VLDL for distribution to peripheral tissues such as adipose tissue, heart, and skeletal muscle. The remaining VLDL is converted to intermediate-density lipoprotein (IDL) and then to (low-density lipoprotein) LDL by hepatic plasma triglyceride lipase (HTGL). Other genes associated with cholesterol control were reported by (Liang et al. 2020) in Chinese thin-tailed and fat-tailed sheep that the SREBF1 and SREBF2 genes affect cholesterol metabolism in the liver.

DGAT1 gene association with mineral

The association analysis of the DGAT1 gene polymorphism showed that the DGAT1 gene had no significant effect (P>0.05) on the mineral components, consisting of (Fe, Zn, K, and Se). Table 4 shows the results of the analysis.

However, the mean Fe obtained in our study ranged from 1.68-1.87 mg/ 100 g, Zn ranged from 2.47-2.62 mg/ 100g, K ranged from 258.3-274.53 mg/ 100g, and Se ranged from 0.61-0.63 mg/100 g. The data shows that the potassium content has the highest value of other mineral components. This happens because K is included in the macro-minerals. Humans' average macro-mineral requirement is more significant than 100 mg/day (Prashanth et al. 2015). Meanwhile, Fe, Zn, and Se are micro-minerals with the need for Fe and Zn (15 mg) and Se (55-70 mg) (Sigdel & Janaswamy 2020).

Minerals as essential macro and micronutrients function to maintain body resistance. Fe functions in oxygen transport in hemoglobin and is a component of several enzymes, including cytochromes, required for energy generation (Piskin et al. 2022). Deficiency of Fe and Se causes short bowel disease, which causes epithelial and mesenchymal dysfunction that affects the function and immune system of the gastrointestinal tract (Riaz & Mehmood 2012). Meat is a significant source of total iron (Fe) and heme iron, primarily of myoglobin and hemoglobin (Cabrera & Saadoun 2014). Potassium plays a role in maintaining cellular osmolarity and acid-base balance, as well as transmission of nerve stimulation and regulating heart and muscle function (Yamada & Inaba 2021). Zinc is an essential enzyme component, such as carbonic-anhydrase in red blood cells and dehydrogenase in the liver, and is a cofactor that increases enzyme activity. Zn deficiency causes dermatitis, slow growth, sexual maturity, infertility, and immunodeficiency (Khanam 2018).

CONCLUSION

The DGAT1 gene was polymorphic in JFTS, JTTS, and GS, while in CAS, BCS, JLS, JS, and GCS were monomorphic. The CC genotype is still dominant in Indonesian sheep. The DGAT1 gene was significantly associated with flavor odor, namely 4-Ethyloctanoic Acid (EOA). Sheep inheriting the CT genotype had higher EOA compared to the CC genotype. However, there is no significant association between DGAT1 diversity with cholesterol and mineral content. The diversity of the DGAT1 gene might contribute to the flavor odor compound in Indonesian sheep.

ACKNOWLEDGEMENT

This research funding by Dana Masyarakat IPB University under the project of Riset Kolaborasi Universitas Jambi – IPB University with the following number: 4008/IT3.L1/PT.01.03/M/T/2022 date 1 June 2022.

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