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# Association of Stearoyl-CoA Desaturase Gene Polymorphisms on Milk Fatty Acid Composition of Holstein Friesian Cows in Indonesia

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## ABSTRACT

**Milk fatty acid composition determines milk quality and the health of consumers. Stearoyl-CoA desaturase (SCD) gene controls lipid metabolism in the mammary gland, which plays an essential role in catalyzing saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA). This study aimed to analyze the genetic diversity of the SCD gene and its effect on the milk fatty acid quality of Holstein Friesian (HF). Genetic variants were identified on 226 HF cattle from various dairy breeding Stations in West Java, Central Java, and East Java in Indonesia. Blood samples were extracted and amplified using Real-Time Polymerase Chain Reaction (RT-PCR) technique based on TaqMan Probe to determine the genotype of the SCD gene at g.10153A>G locus. Milk samples were analyzed by Gas Chromatography and Mass Spectrometry (GCMS) on 48 lactating cows. The SCD g.10153A>G produced three genotypes, namely AA (0.112), AG (0.640), and GG (0.271); and two alleles, namely A (0.409) and G (0.591). Genotype polymorphism of the SCD g.10153A>G had a significant effect ($p<0.05$) on SFA, including propionic acid (C3:0), butyric acid (C4:0), caprylic acid (C8:0), and pentacosylic acid (C25:0); and MUFA including hexadecenoic acid (C16:1) and total fatty acids. The SCD g.10153A>G SNP (AG genotype) could be one of the candidates as a genetic marker to assist selection in reducing SFA and increasing MUFA in the milk content of HF cows in Indonesia.**

*Keywords: fatty acid; genotype; Holstein Friesian; RT-PCR; SCD gene*

## INTRODUCTION

Milk is a highly nutritious animal food product that is beneficial for humans due to the content of immunoglobulins (Conneely *et al.*, 2013), a source of Conjugated Linoleic Acid (CLA) (Bittante & Cecchinato, 2013; Ferlay & Chilliard, 2020; Nishimori *et al.*, 2017; Stanton *et al.*, 2020; Yilmaz-Ersan *et al.*, 2018). However, currently, some people avoid food products containing SFA (including dairy products) due to generative and neo-generative diseases (Livingstone *et al.*, 2012; Markiewicz-Kęszycka *et al.*, 2013).

Animal feed technology innovations have been developed to reduce SFA content in dairy, meat, and egg products. Food products containing low SFA have begun to be produced to offer an alternative to healthier livestock products (Nguyen *et al.*, 2019; Średnicka-Tober *et al.*, 2016). Milk fatty acid composition allows being modified through the application of selection in breeding programs (Valenti *et al.*, 2019). Molecular technology development by identifying single nucleotide polymorphism (SNP) of major genes provides room for improvement in milk production and quality. Exploration of various functional SNPs of potential lactation genes, therefore, needs to be done. Changes in DNA structure will affect the expression, transcriptional regulation, and amino acid sequence of the genes (Criado-Mesas *et al.*, 2020). Gene polymorphisms related to the fatty acid quality of livestock products have been extensively studied, such as in dairy cattle (Alim *et al.*, 2012; Asmarasari *et al.*, 2020), goats (Avilés *et al.*, 2016), pigs (Fernández-Barroso *et al.*, 2020), and chicken (Furqon *et al.*, 2017).

The biosynthesis of milk fatty acids is regulated in a series of enzymatic processes that accumulate the biosynthesized fatty acids in triacyl glycerides (TAGs) in milk. Important enzymes that regulate a range of milk biosynthesis include such as stearoyl-CoA desaturase (SCD), acetyl CoA carboxylase (ACCA), and diacylglycerol acyltransferase (DGAT) (Ntambi & Miyazaki, 2004; Azis *et al.*, 2020). The Stearoyl-CoA desaturase enzyme encoded by the bovine SCD gene is located on chromosome 26 (bovine chromosome 26/BTA-26) which is expressed in adipose tissue and mammary glands (Criado-Mesas *et al.*, 2020). Stearoyl-CoA desaturase (SCD) is a key enzyme in milk fatty acid desaturase that encodes the delta-9 desaturase enzyme in catalyzing fatty acids from SFA to MUFA and PUFA (Livingstone *et*

*al.,* 2012). The biosynthesis of palmitoyl-CoA and oleyl-CoA from palmitoyl-CoA and stearoyl-CoA, catalyzed by the enzyme SCD, is a substrate for the formation of triacylglycerols, phospholipids, cholesterol, and wax esters (Mauvoisin & Mounier, 2011; Uemoto *et al.,* 2012).

The SCD gene has a base length of 17 kbp, consisting of 6 exons and 5 introns. It encodes a protein of 359 amino acids. Northern-Blot analysis showed a single transcript of approximately 5 kbp is present in the mammary gland and adipose tissue, which was characterized by an unusually long 3'UTR region of 3.8 kbp (Bernard *et al.,* 2013). The SCD gene has a strong relationship with milk production, milk fat, and milk protein (Alim *et al.,* 2012), as well as fat and milk fatty acid composition (Li *et al.,* 2016; Maharani *et al.,* 2013; Oh *et al.,* 2013). Several genes involved in metabolic pathways or catabolism in the BTA-26 region provide SCD, LIPF (gastric lipase), and mitochondrial GPAM (glycerol-3-phosphate acyltransferase) (Roy *et al.,* 2006; Wang *et al.,* 2021).

Based on the previous study information on the function of the SCD (Alim *et al.,* 2012; Li *et al.,* 2016; Maharani *et al.,* 2013; Oh *et al.,* 2013), there was a strong indication that the SCD gene could be used as a molecular marker to improve milk fatty acids quality in dairy cattle. The SCD gene polymorphism allows it to be used as a marker in explaining the variability of milk fatty acid composition in HF cattle in Indonesia. This study aimed to analyze the genetic variant of the SCD gene, especially at g.10153A>G SNP using Real-time Polymerase Chain Reaction (RT-PCR) and its association with milk fatty acid composition of HF cattle in Indonesia.

## MATERIALS AND METHODS

### Blood Samples

Blood samples in this study were obtained from several dairy cattle breeding centers (West Java, Central Java, and East Java) in Indonesia. The total samples (262 heads) were obtained from the Indonesian Research Institute for Animal Production (IRIAP) West Java (99 heads), Animal Husbandry Training Center of Cikole (AHTCC) West Java (41 heads), Animal Breeding Center and Forage Feed of Baturraden (ABCFFB) Central Java (76 heads), Lembang Artificial Insemination (Lembang AI) (17 heads), and Singosari Artificial Insemination Station (Singosari AI) East Java (29 heads). This research has received Animal Care and Use Committee (No. 030-KEP-UB-2022) from Universitas Brawijaya.

### Milk Samples

Milk samples came from lactating HF cows (48 heads.) within lactation (2-8 months) and 1-4 lactation periods from Animal Breeding Center and Forage Feed of Baturraden (ABCFFB). Milk samples were analyzed using Gas Chromatography and Mass Spectrometry (GC-MS), conducted at the Technical Implementation Unit of the Integrated Laboratory of Diponegoro University, Semarang. An esterified milk fat sample (0.5 L) was injected into the GC column by automatic sampling. Separation was carried out on the RTx1-MS Resttech column 30 m x 0.5 mm ID 0.25 m, with Polydimethyl Ciloxan stationary phase, injector temperature 280 °C, column temperature 70 °C increased to 300 °C with an increase of 10 °C/minute, The eluent used was helium gas with a flow rate of 1.15 mL/minute. MS detector used is Electron Multifer Detector (EMD) 70 MeV. Results of the analysis in the form of a mass spectrum were displayed on the GC-MS software after being run and compared with the wiley9.lib library.

### DNA Extraction

The total blood samples were extracted using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific). Genomic DNA was extracted by 200 µL following the recommended protocol with some modifications. A series of extraction procedures were carried out in the Molecular Genetics Laboratory, Faculty of Animal Science, IPB University. The results of DNA extraction were examined using a spectrophotometer to determine the quality and concentration of the extracted DNA. All extracted DNA samples fulfilling the eligibility criteria were ready to be used for the PCR analysis procedure.

### Primer-Probe Design of the SCD Gene

Primers and probes of the SCD gene were designed using primer express software version 3.0.1 (Perkin-Elmer, Applied Biosystems) at the g.10153A>G mutation site. The DNA sequences of the SCD gene were obtained from GenBank with No. Access: AY241932. Forward primary sequence: 5'-TCC TGT TGT TGT GCT TCA TCC T -3'; primary reverse: 5'-ACG GAA TAA GGT GGC AAA AAA C -3'. The forward and reverse primer sequences of the SCD gene are enclosed by two probe labels. The forward primer was flanked by probe1: 5'-(FAM) CAC ACT CGT GCC GTG-3' with fluorescence FAM™ dye TaqMan®MGB probe to identify specific G alleles, while the reverse primer was flanked by probe 2 (VIC) 5'-CAC ACT CGT GCC ATG -3' with fluorescence VIC™ dye TaqMan®MGB probe to identify specific A allele (Figure 1).

### Genotyping SCD Gene

Amplification of the SCD gene used RT-PCR to identify g.10153A>G SNP of this gene. Conditions for the thermal cycler from the RT-PCR were annealing temperature at 60 °C for 1 minute, denaturation and extension at 95 °C for 20 seconds, and 3 seconds for 40 cycles, respectively. DNA samples were distributed into MicroAmp Optical 96-Well reaction plates. While the composition of the reagent mix consisted of 5 uL TaqMan®GTXpressTM Master Mix containing buffer, Uracil-N-glycosylase, deoxyribonucleotides, uridine, passive reference dye (ROX), and TaGold DNA polymerase (Applied Biosystems, Foster City, CA USA), 0.5 uL primer and Taqman-MGB probe (forward and reverse) and the rest of RT-PCR grade water 3.5 L. The mixture was then distributed by 10 µL into MicroAmp

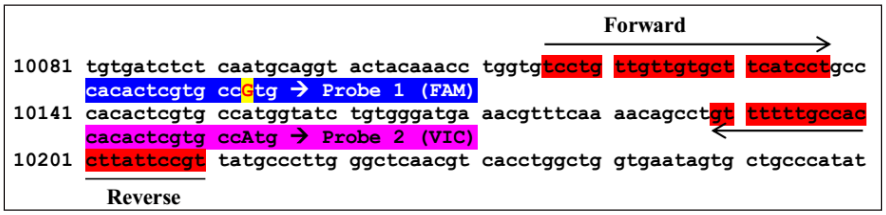


Figure 1. Primer (forward-reverse) and probes 1 and 2 of the SCD gene of HF cattle

Optical 96-Well reaction plates with a ratio of DNA and mix 1:9 (vol/vol), and non-template DNA was used as a control. It was then covered with sealing foil, centrifuged at a speed of 2500 rpm for 3 minutes, and put into an RT-PCR machine (Applied Biosystems 7500 Fast Real-Time PCR System). Each sample was run in RT-PCR. A specific amplification process was observed through the movement of the fluorescence curve from the baseline to the exponential phase that passed the cycle threshold. Determination of the genotype of the SCD gene at locus g.10153A>G was based on the fluorescence received by the sensor on the RT-PCR. The AA genotype was indicated by FAM fluorescence in blue color. The AG genotype was indicated by VIC fluorescence, and FAM was indicated by blue/pink and/or green fluorescence. The GG genotype was indicated by fluorescent VIC in pink/red color. The data results were received by 7500 software v2.0.6 (Applied Biosystems, Foster City, CA, USA) in Microsoft excel format.

### Data Analysis

Genotype data from the SCD gene at g.10153A>G locus of dairy cattle were analyzed using genotype frequency and allele frequency by popgen32 software version 1.31. Genotypic association of genotypes the SCD g.10153A>G SNP on individual milk fatty acids was studied by the general linear model (GLM) by SAS ver. 9.2 packet. The following presented mathematical model:

$$Y_{ijklmn} = \mu + G_i + L_j + P_k + S_l + T_m + \varepsilon_n$$

where, $Y_{ijklmn}$ was the individual component of milk fatty acids, $\mu$ was the overall mean for each trait (24 fatty acids), $G_i$ was the effect of genotype i (AA, AG, and GG), $L_j$ was the effect of month of lactation (2-4, 5-8), $P_k$ was the effect of lactation period (1, 2, 3-4), $S_L$ was the effect of calving season (1-6, 7-12), $T_m$ was the effect of calving year, $\varepsilon_{ijklmn}$ was the effect of random residual.

## RESULTS

### Amplification Results of the SCD Gene

The SCD gene amplifications of all observed HF cattle were successfully done. The SCD gene amplification used the TagMan-MGB Probe on the RT-PCR machine. The thermal cycle conditions consisted of a denaturation temperature of 95 °C, an annealing temperature of 60 °C for 1 minute, and an extension temperature of 95 °C for 3 minutes. The DNA fragment amplification of the SCD at g.10153A>G locus resulted in three genotypes, namely AA, AG, and GG (Figures 2, 3, and 4). The AA genotype was presented by a blue fluorescent curve, the AG genotype with blue and pink fluorescence curves, and the GG genotype with a pink fluorescence curve. The genotype results were more clearly visible in each plot with different fluorescence (Figure 5). The blue plot showed the AA genotype, the green color was for the AG genotype, and the pink color was for the GG genotype.

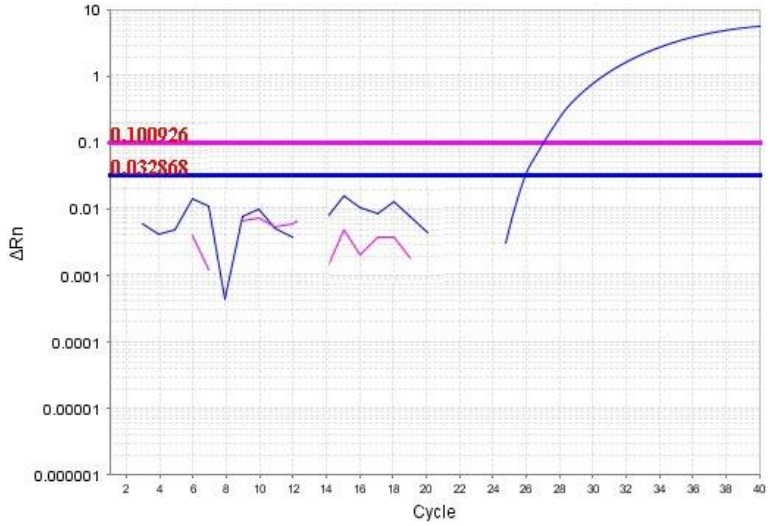


Figure 2. Amplification curve of AA genotype of the SCD gene at g.10153A>G locus in HF cattle. = AHGJ8BB-A; = AHGJ8BB-G.

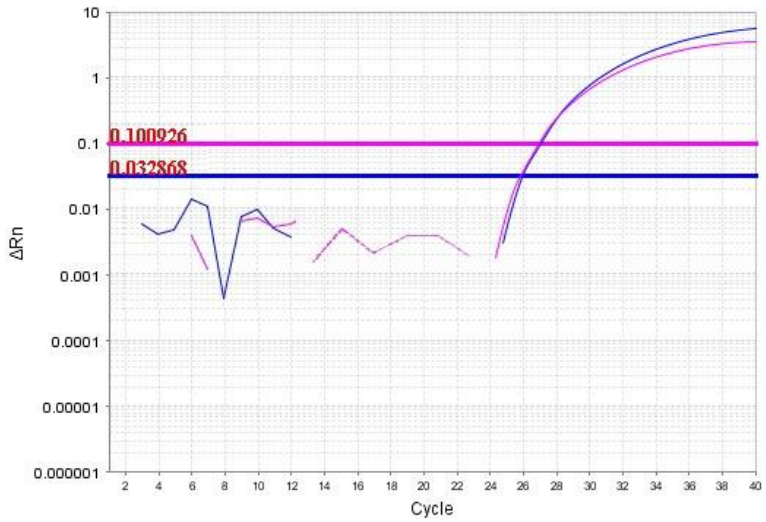


Figure 3. Amplification curve of AG genotype of the SCD gene at g.10153A>G locus in HF cattle. = AHGJ8BB-A; = AHGJ8BB-G.

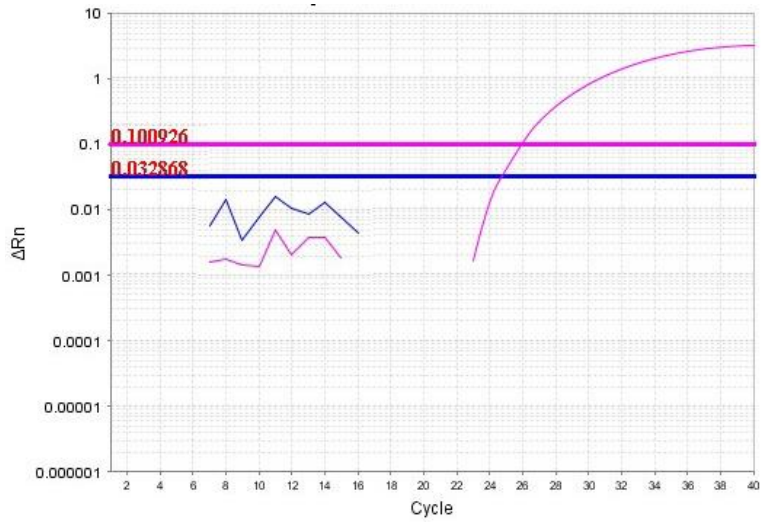


Figure 4. Amplification curve of GG genotype of the SCD gene at g.10153A>G locus in HF cattle. = AHGJ8BB-A; = AHGJ8BB-G.

### Genetic Polymorphism of the SCD Gene

Genetic polymorphisms of the SCD gene at g.10153A>G locus were presented in terms of genotype frequency and allele frequency (Table 1). The results showed the highest genotype frequency was for the AG genotype (0.640), followed by the GG genotype (0.271), and the lowest was for the AA genotype (0.112). There were two dairy cattle stations without the AA genotype, namely for HF females in AHTCC and HF males in Singosari AIS. Based on the genotype frequency results, the AA genotype was at the lowest frequency resulting in a very low frequency of the A allele. The G allele frequency (0.591) was generally higher than the A allele frequency (0.409).

### Association of the SCD Gene Polymorphism with Milk Fatty Acids

Associations between genotypes of the SCD gene at g.10153A>G locus with individual milk fatty acids were presented in Table 2. There were 24 types of milk fatty acids successfully analyzed, ranging from fatty acids with short to long carbon chains. Based on the lengths of the carbon chain, milk fatty acids were divided into three types of fatty acids, namely saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). Association study of the genotypes of the SCD gene with total milk fatty acids and SFA were significantly different ($p<0.05$), while MUFA and PUFA were not significantly different ($p>0.05$). The AG genotype cows produced the highest total fatty (85.95±5.30%), followed by the GG genotype (85.37±6.54%), and the lowest was by the AA genotype (83.55±9.74%).

## DISCUSSION

The results of allele frequency and genotype frequency of the SCD gene at g.10153A>G locus (Table 1) totally from the highest frequencies of the genotype of AG (0.640), GG (0.271), and AA (0.112). The AA geno-

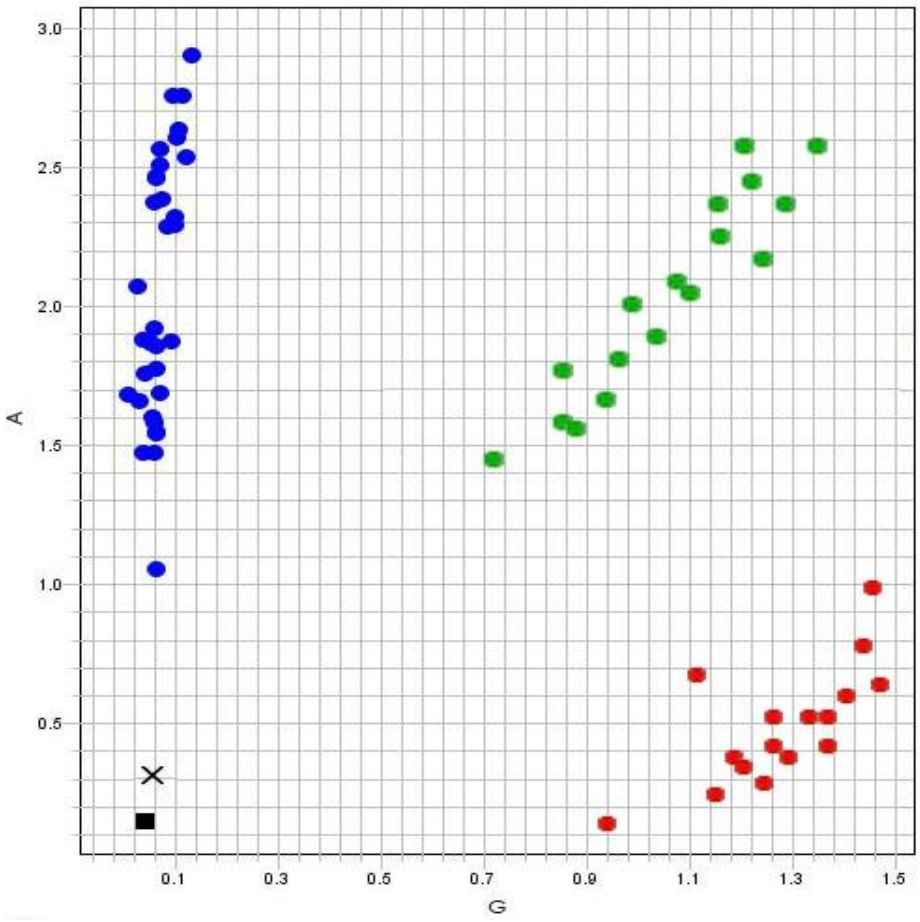


Figure 5. Allelic discrimination plots of AA, AG, and GG genotypes the SCD gene at g.10153A>G locus of HF cattle. ●= A/A, ●= A/G, ●= G/G, and X= undetermined.

Table 1. Genotype frequency and allele frequency of the SCD1 gene polymorphism in HF cattle by location

| Location | n | Genotype frequency | | | Allele frequency | |
|---|---|---|---|---|---|---|
| | | AA | AG | GG | A | G |
| IRIAP | 99 | 0.152 | 0.333 | 0.515 | 0.318 | 0.682 |
| ABCFFB | 76 | 0.118 | 0.526 | 0.355 | 0.382 | 0.618 |
| AHTCC | 41 | 0.000 | 0.805 | 0.195 | 0.402 | 0.598 |
| Singosari AIS | 29 | 0.000 | 0.828 | 0.172 | 0.414 | 0.586 |
| Lembang AIS | 17 | 0.176 | 0.706 | 0.118 | 0.529 | 0.471 |
| Total | 262 | 0.112 | 0.640 | 0.271 | 0.409 | 0.591 |

Note: n was the number of DNA samples of observable HF cattle. IRIAP= Indonesian Research Institute for Animal Production, Bogor, West Java; ABCFFB= Animal Breeding Center and Forage Feed of Baturraden, Banyumas, Central Java; AHTCC= Animal Husbandry Training Center of Cikole, Bandung, West Java; Singosari AIS= Singosari Artificial Insemination Station, Malang, East Java; Lembang AIS= Lembang Artificial Insemination Station, Bandung, West Java, Indonesia.

type was not found in HF cattle from two locations of AHTCC and Singosari AIS. Almost all HF cows were inseminated using frozen semen from HF bulls from the two AI stations of Singosari AIS and Lembang AIS. No AA genotype was found in HF cows in AHTCC, indicating that frozen semen in AI mating of HF cows in this location probably came from Singosari AIS. HF bulls in Lembang AIS also had a low frequency of the AA genotype (0.176) and were dominated by the AG genotype. This condition would come into the assumption that no AA genotype was found in this location.

In general, the frequency of the GG genotype was higher than that of the AA genotype (Table 1). This condition can be caused by both HF females and males of the DNA sources not having the SNP mutation of the SCD gene at g.10153A>G locus. Another possibility could be caused by the A allele as the wild-type allele and the G allele as the mutant allele one. Mutations could be due to substitution, insertion, and deletion at the DNA level (Gunawan *et al.*, 2018; Mia *et al.*, 2013). The SCD gene at g.10153A>G locus for all locations could be said to be polymorphic as it had no genotype frequency of less than 0.01. Previous studies of the SCD

Table 2. Milk fatty acid composition of Holstein Friesian cow with different stearoyl-CoA desaturase (SCD) genotype

| Milk fatty acid (%) | Genotype | | |
|---|---|---|---|
| | AA (n=6) | AG (n=25) | GG (n=16) |
| Saturated fatty acid (SFA) | | | |
| a. Short chain (C3-C9) | | | |
| Propionic (C3:0) | 0.29±1.09$^{ab}$ | 0.78±0.59$^{b}$ | 0.09±0.68$^{a}$ |
| Butyric (C4:0) | 0.03±0.24$^{a}$ | 0.08±0.13$^{a}$ | 0.43±0.15$^{b}$ |
| Caproic (C6:0) | 0.42±0.33 | 0.86±0.18 | 0.69±0.20 |
| Caprylic (C8:0) | 0.49±0.75$^{a}$ | 1.09±0.41$^{b}$ | 0.69±0.46$^{ab}$ |
| Nonanoic (C9:0) | 0.56±0.09 | 0.57±0.05 | 0.65±0.54 |
| Sub total | 1.79 | 3.38 | 2.55 |
| b. Medium chain (C10-C14) | | | |
| Capric (C10:0) | 2.07±0.17 | 1.65±0.09 | 1.77±0.11 |
| Lauric (C12:0) | 12.15±0.45 | 12.77±0.24 | 12.79±0.28 |
| Tridecanoic (C13:0) | 0.59±0.16 | 0.88±0.09 | 0.72±0.10 |
| Myristic (C14:0) | 18.67±1.10 | 18.09±0.60 | 18.66±0.68 |
| Sub total | 33.48 | 33.39 | 33.94 |
| c. Long chain (C14-C22) | | | |
| Pentadecanoic (C15:0) | 5.61±0.31 | 5.15±0.17 | 5.06±0.19 |
| Palmitic (C16:0) | 21.44±1.22 | 21.78±0.67 | 21.53±0.76 |
| Stearic (C18:0) | 1.27±0.23 | 1.48±0.13 | 1.46±0.14 |
| Arachidic (C20:0) | 0.99±0.07 | 1.13±0.04 | 1.15±0.05 |
| Behenic (C22:0) | 0.76±0.19 | 0.97±0.10 | 1.11±0.12 |
| Pentacosylic (C25:0) | 1.87±0.35$^{a}$ | 2.68±0.19$^{b}$ | 2.84±0.22$^{b}$ |
| Sub total | 31.94 | 33.19 | 33.15 |
| Unsaturated fatty acid (UFA) | | | |
| a. MUFA (C14:1) – (C22:1) | | | |
| Caproleic (C10:1) | 0.49±0.75 | 1.09±0.41 | 0.69±0.46 |
| Myristoleic (C14:1) | 2.78±0.20 | 2.69±0.10 | 2.54±0.12 |
| Hexadecenoic (C16:1) | 6.99±1.09$^{b}$ | 5.92±0.60$^{a}$ | 6.05±0.68$^{a}$ |
| Heptadecanoic (C17:1) | 0.97±0.28 | 1.20±0.15 | 1.18±0.17 |
| Eicosanoic (C20:1) | 1.62±0.22 | 1.59±0.12 | 1.68±0.13 |
| Dodecanoic (C12:1 n-3) | 0.75±0.03 | 0.74±0.01 | 0.75±0.02 |
| Erucic (C22:1) | 1.02±0.16 | 0.98±0.09 | 0.94±0.11 |
| Sub total | 14.62 | 14.21 | 13.83 |
| b. PUFA (C4:2) – (C22:4) | | | |
| Linoleic (C18:2 n-6) | 0.73±0.19 | 0.65±0.10 | 0.75±0.12 |
| Arachidonic (C20:4 n-3) | 0.99±0.07 | 1.13±0.04 | 1.15±0.05 |
| Sub total | 1.72 | 1.78 | 1.9 |
| Total fatty acid | 83.55±9.74$^{a}$ | 85.95±5.30$^{b}$ | 85.37±6.54$^{b}$ |
| SFA | 67.21±6.75$^{a}$ | 69.96±3.68$^{b}$ | 69.64±4.68$^{b}$ |
| MUFA | 14.62±2.73 | 14.21±1.48 | 13.83±1.69 |
| PUFA | 1.72±0.26 | 1.78±0.14 | 1.90±0.17 |

Note: $^{a, ab, b}$ means in the same row with different superscripts differ significantly ($p<0.05$).

gene polymorphism reported that the C allele of this gene had a strong effect on the total desaturase index. This gene could be one of the candidate genes to improve milk production (Pauciullo *et al.*, 2010).

The association of variant genotypes of the SCD gene at g.10153A>G locus on fatty acid composition in this study gave significant effects ($p<0.05$) on several saturated fatty acids, namely propionic acid (C3:0), butyric acid (C4:0), caprylic acid (C8:0), and pentacosylic acid (C25:0). In MUFA, there was only one fatty acid to be significantly different, namely palmitoleic acid (C16:1), on the contrary, in PUFA there was no significantly different ($p>0.05$). The AG genotype produced the highest percentage of total fatty acids, followed by the GG genotype, while the lowest was for the AA genotype. Similarly, the results for the saturated fatty acid (SFA) showed that the AG genotype was the highest, followed by the GG genotype, and the lowest was the AA genotype. In contrast to total fatty acids and saturated fatty acids, the AA genotype had the highest proportion of MUFA compared to the AG and GG genotypes. Based on these results, it could be possible that different selections occurred in HF among locations so that the SCD gene polymorphism at g.10153A>G SNP had different effects on fatty acid compositions.

The occurrence of mutations at the amino acid level could affect the regulation and function of enzymes (Rincon *et al.,* 2012; Uemoto *et al.,* 2012; Valenti *et al.,* 2019). A specific effector system controlled the SCD adipose thermogenesis through lipid partitioning between lipogenesis and oxidation (Ntambi & Miyazaki, 2004). Lacking SCD did not significantly affect the quality of milk fatty acids, even though the feeding requirement was sufficient (Tudisco *et al.,* 2012). SCD deficiency could also attenuate lipid accumulation due to a lack of peroxisome proliferative receptor-a (PPAR-a). The role of PPAR-a was one of the key transcription factors in inducing transcription of fatty acid-oxidation (Bernard *et al.,* 2013; Choi *et al.,* 2015).

Previous studies reported the existence of eight SNPs in exon 5 of the SCD gene that gave significant associations to intramuscular fat in Japanese Black steers (Yokota *et al.,* 2012), fatty acid composition (Moioli *et al.,* 2007), beef marbling scores, and the amount of MUFA and CLA (Pewan *et al.,* 2020; Yokota *et al.,* 2012; Ropka-Molik *et al.,* 2017). Moioili *et al*. (2007) added that gene polymorphisms provided significant differences between several cattle breeds (Jersey, Piedmontese, and Valdostana). The results of this study indicated that the presence of variant genotypes in the SCD gene at g.10153A>G locus can be one of the causes of differences in the milk fatty acid composition of HF cattle. Thus, the SCD gene g.10153A>G SNP could be considered as a candidate for one of the genetic markers in improving the milk fatty acid composition in dairy cattle.

The SCD gene amplification used RT-PCR technology by utilizing the TaqMan MGB Probe. The TaqMan MGB Probe was designed to attach and bind to very specific target sequences (Azis *et al.,* 2020). The benefits of the TaqMan MGB Probe have colored fluorescence so that in the amplification process, each cycle can be observed in real-time and without an electrophoresis process like other PCR methods (RFLP and RAPD). The TaqMan MGB Probe as a detection system has been widely used in gene quantification (Wang *et al.,* 2017). The TaqMan MGB probe has a reporter at the 5′ end and a quencher at 3′. The function of the reporter at 5′ as a donor fluorophore and the quencher as a fluorophore receiver hybridizes in the annealing phase (Babafemi *et al.,* 2017). The fluorescence signal is generated from the hybridization process of the two that match the target, namely wild-type hybridization on a wild-type target and hybridization type mutant on a mutant target. The amplification results of the SCD gene are curves and genotype clusters. The fluorescence curves produced in the amplification process of the SCD polymorphism were the result of hybridization between the TaqMan Probe (Figures 2, 3, and 4). The results of the amplification of the SCD gene at g.10153A>G locus was in the form of fluorescence curves showing the AA genotype in a blue curve, the AG genotype in blue and pink, and the GG genotype in pink. Meanwhile, genotype clusters also produce three criteria (Figure 5).

The SCD gene or Stearoyl-CoA Desaturase gene is important in influencing the de novo synthesis of milk fatty acids. SCD enzyme in biological systems has a key role in cellular biosynthesis to produce MUFA from SFA (Alsharari *et al.,* 2020). The SCD enzyme is located in the endoplasmic reticulum, which acts as a catalyst for the double bond between the 9 and 10 carbon atoms of saturated fatty acids (Li *et al.,* 2016; Mauvoisin & Mounier, 2011). The cellular products included stearic acid (C18:0) and palmitic acid (C16:0), which are derived from the carbon atom double bonds at positions 9 and 10 of SFA (Livingstone *et al.,* 2012). The SCD enzyme is known as a candidate gene to change the proportion of SFA and UFA (unsaturated fatty acids), especially MUFA. Based on previous studies have reported that genetic polymorphisms of the SCD gene were associated with fatty acid quality, provided in dairy cattle (Alim *et al.,* 2012; Asmarasari *et al.,* 2020), goats (Avilés *et al.,* 2016), pigs (Fernández-Barroso *et al.,* 2020), and chicken (Furqon *et al.,* 2017). Differences in fatty acid composition can be caused by differences in feed consumption (Alsharari *et al.,* 2020), breed, and genetic factors (Anggraeni *et al.,* 2020). The SCD enzyme activity was significantly related to unsaturated fatty acids (UFA), especially in bovine fat tissue (Li *et al.,* 2016).

The effect of the SCD gene polymorphism is an association between the diversity of the SCD gene and milk fatty acid composition. This diversity is associated with SFA, MUFA, and PUFA in milk. In this study, cows with AA genotypes had lower SFA values than cows with AG and GG genotypes. Based on the study, this gene is important in reducing SFA in milk. Likewise, cows with the AA genotype had higher MUFA values than the other genotypes (AG and GG), where these fatty acids positively affected health. Therefore, the results of this study can be applied practically (although further research is needed) in its application, namely, the selection of cows with the AA genotype can reduce SFA content and increase MUFA content in milk.

## CONCLUSION

Genotyping SCD gene at g.10153A>G locus in HF cattle resulted in AA, AG, and GG genotypes. The SCD genotype polymorphisms were significantly associated with SFA providing propionic acid (C3:0), butyric acid (C4:0), Caprylic acid (C8:0), and Pentacosylic acid (C25:0); as well as MUFA (Hexadecenoic acid (C16:1) and total fatty acid. The SCD gene g.10153A>G SNP (AA genotype) was potential as the candidate molecular marker to assist selection in improving milk fatty acids in HF cattle.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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