Genetic Polymorphism of SCD1 Gene of Holstein-Friesian Cows in Indonesia

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ABSTRAK

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Stearoyl-Coenzyme A Desaturase 1 (SCD1) tergolong sebagai famili dari asam lemak jenuh. Pada ternak ruminansia yang menghasilkan susu, protein SCD1 sering kali diekspresikan pada kelenjar menyusui dan relevan berpengaruh pada komposisi susu dan produk susu lainnya. Polimorfisme gen SCD1 pada sapi Friesian Holstein (FH) dapat digunakan sebagai dasar seleksi ternak secara molekuler untuk meningkatkan produktivitas. Tujuan dari studi ini adalah untuk mengetahui polimorfisme gen SCD1 pada sapi Friesian Holstein yang ada di Indonesia. Sebanyak 162 ekor sapi FH yang berasal dari 4 lokasi (Bogor, Sukabumi, Tasikmalaya dan Enrekang) digunakan dalam penelitian ini. Genotyping gen SCD1 menggunakan metode PCR-RFLP dengan enzim restriksi NcoI. Hasil menunjukkan bahwa tiga genotip (AA, AV dan VV) dan dua alel (A dan V) telah berhasil ditemukan dan bersifat polimorfik. Alel A ditemukan dominan (0.63) pada seluruh sampel dan dalam keseimbangan genetik. Frekuensi alel A tertinggi ditemukan di lokasi Sukabumi (0.78) sedangkan yang terendah di Bogor (0.55). Nilai heterosigotitas teramati (Ho) dan harapan (He) sebesar 0,471 dan 0,470. Kesimpulan, polimorfisme ditemukan pada semua lokasi dengan alel A sebagai alel dominan. Hasil ini dapat menjadi informasi genetik awal sapi FH di Indonesia dan untuk membentuk strategi *breeding* sapi perah agar dapat meningkatkan produktivitas khususnya peningkatan lemak susu sehat.

Kata Kunci: Sapi Perah Friesian Holstein, SCD1, Polimorfisme

ABSTRACT

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Stearoyl-Coenzyme A desaturase 1 (SCD1) belongs to the fatty acid family of desaturases. In lactating ruminants, the SCD1 protein is highly expressed in the mammary gland and is relevant for the fatty acid composition of milk and dairy products. Polymorphism of SCD1 gene in Holstein-Friesian (HF) cows could be used as a basis of molecular selection of cattle in order to increase their productivity. The aim of this study was to investigate the polymorphism of SCD1 gene of Holstein-Friesian cows in Indonesia. A total of 162 blood samples of HF cows were collected from four different locations i.e. Bogor, Sukabumi, Tasikmalaya and Enrekang districts. Genotyping of SCD1 gene used PCR-RFLP method with NcoI restriction enzyme. The result showed that three genotypes (AA, AV and VV) and two alleles (A and V) have successfully found and polymorphic. A allele was dominant in all populations (0.63) and in Hardy Weinberg Equilibrium. The highest A allele was found in Sukabumi (0.78) and the lowest was in Bogor (0.55). Heterozigosity observed and expected reached 0.471 and 0.470, respectively. In conclusion, genetic polymorphism was found in all population with dominant of A allele. This finding can be used as a early genetic information of Holstein-Friesian cattle in Indonesia and to build breeding strategy for improving of productivity especially improving of healthy fat milk.

Key Words: HF Dairy Cow, SCD1, Polymorphisms

INTRODUCTION

The gene encoding Steroyl-Coenzyme A Desaturase 1 (SCD) was mapped to bovine chromosome 26 (Campbell et al. 2001), where some QTLs for fat yield and other milk traits have been also identified. Based on Genome Wide Association Study in Dutch Dairy cattle, fat content in milk was influenced from several genes and one of them was SCD1 in BTA26 (Bouwman et al. 2011).

Considerable amount of studies have shown significant role of genotypes SCD1A293V in exon 5 toward the configuration of fatty acids in milk and milk production traits (Taniguchi et al. 2004; Moioli et al. 2007; Kgwatalala et al. 2009; Clark et al. 2010; Kulig et al. 2016). Hence, traits selection through different type of genotype can be beneficial to change the composition of milk fat. A high amount of saturated fatty acids and a low amount of (poly) unsaturated fatty acids contribute to milk traits. So, essential to human health, the upsurge

in unsaturated fatty acid amount in milk has become important objective in some studies (Taniguchi et al. 2004; Schennink et al. 2008; Kgwatalala et al. 2009; Mashhadi et al. 2012).

SCD is a key enzyme responsible in desaturation of fatty acid in mammary gland and others tissues. Stearoyl-CoA desaturase (SCD) plays an important role in bovine mammary lipid metabolism as it introduces a cis-double bond in the D9 position of a wide range of fatty acids (FA). It has a substrate preference of C18:0 and to a lesser extent C16:0, which are converted into C18:1 cis-9 and C16:1 cis-9, respectively (Ntambi 2004). C18:1 cis-9 has a far lower melting point than C18:0. Therefore, the SCD plays a vital role in keeping the fluidity of cell membrane and milk fat. The fatty-acid (FA) fluidity was determined by the length and quantity of FA chains included in the orientation of its paired-chains. Since the FA profile influence milk fluidity, it is used in triglyceride synthesis of milk fat. Milk fluidity can be reduced by decreasing the number of both FA's short and medium-chains. This occurs because of inhibition of the synthesis of FA mammary de novo (Maxin et al. 2011; Jacobs et al. 2013). SCD is also responsible for the conversion of C18:1 trans-11 to C18:2 cis-9, trans-11, which has been perceived related to several medical benefits towards human, one of them is anti-carsinogenic and anti-atherogenic effect (Bhattacharya A, Banu J, Rahman M 2006; Reynolds CM and Roche HM 2010).

The objective of this study is to identify the possibility of polymorphism in SCD gene using PCR-RFLP technique. The study also investigates the correlation of SCD gene polymorphism to milk fat production. The finding of this study is substantially useful in a trait selection design of dairy cattles which will improve of milk fatty-acid productivity. In practice, the selected dairy cattles from this design can be delivered either to small farmers or industries and trigger improvement in milk production.

MATERIALS AND METHODS

DNA Samples

A total of 162 fresh blood samples of Holstein-Friesian dairy cows were used as DNA resources for genotyping of SCD1 gene. Three milliliters of blood of each individual cow were collected into vacutainer tube containing K_3EDTA via jugular vein. All of samples were taken from four districts i.e. Enrekang, Tasikmalaya, Sukabumi and Bogor (Table 1.) Genomic DNA isolation was performed using Genomic DNA mini kit (Geneaid Biotech Ltd., Taiwan) by following to the the manufacturer's instructions. The collected DNA was stored at -20°C for further use.

Table 1. Details of the number samples of Holstein-Friesian dairy cows in Indonesia used for genotyping of SCD1 genes

Provinces	Districts	Number of animals
South Sulawesi	Enrekang	45
West Java	Tasikmalaya	19
West Java	Sukabumi	23
West Java	Bogor	75
Total		162

Genotyping

Genotyping of SCD1 gene was conducted by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. A 400 bp of specific fragment was amplified using a pair primer of forward: 5-CCCATTCGCTCTTGTTCTGT-3 and reverse 5-CGTGGTCTTGCTGTGGACT-3 (Kgwatalala et al. 2009). The PCR was performed in a 10 µl reaction mixture containing 5 µl PCR Master Mix (My Taq HS Red mix Bioline, USA), 1 µl each of primer (10pmol/µl), 2 µl water free nucleases and 1 µl DNA template. The PCR mixture was run in a thermal cycler machine (Eppendorft, Germany) following program pre-denaturation 94^{o}C for 5 minutes, 35 cycles of denaturation at 94^{o}C for 45 seconds, annealing 56.7^{o}C for 30 seconds, extension 72^{o}C for 45 seconds and final extension 72^{o}C for 5 minutes. PCR products were checked using 1% agarose gel (100 V, 60 minutes) and visualized under UV light by UV Transilluminator (Major Science, USA).

RFLP method was performed using *Nco*I enzyme (Kgwatalala et al. 2009). The cutting site of *Nco*I enzyme is 5'-C*CATGG-3'. The PCR product were digested in a total volume 10 µl containing 5 µl PCR product, 0.3 µl NCoI restriction enzyme (10U/µl), 1 µl reaction 10x buffer and 3.7 µl ddH2O. The mixture was then incubated at 37^{o}C for 3 hours and inactivated at temperature of 65^{o}C for 20 minutes. The digested PCR products were electrophoresed using 2.5% agarose gel for an hour of 100 Voltage. The fragments were stained using GelRed for an hour and visualized under UV light by UV Transiluminator (Major Science, USA).

Data analysis

Genotypes and allele frequencies, heterozygosity observed (Ho), heterozygosity expected (He) and Hardy Weinberg Equilibrium (HWE) was direct calculated by Nei dan Kumar (Nei 2000).

RESULTS AND DISCUSSION

A 400 bp targeted-fragment of the SCD1 gene were successfully amplified using Polymerase Chains Reaction (PCR) technique in all samples (Figure 1). Mutation at position 10329 caused changing of Cytosine to Thymine that influenced of amino acid Alanine (A) to Valin (V). The mutation was known as SNP A293V and recognized by *Nco*I restriction enzyme (5'-C*CATGG-3') (Taniguchi et al., 2004). SCD1|*Nco*I restriction analysis revealed that there were three genotypic patterns and two alleles (A and V) in all of Holstein-Friesian dairy cow population. This finding is similar as described by (Taniguchi et al. 2004; Mele et al. 2007; Milannesi et al. 2008). Those three patterns were single fragment of 200 bp (referred to AA genotype), single fragment of 400 bp (VV genotype) and two fragments of 200 bp and 400 bp were a heterozygous of AV genotype (Figure 2).

The genotype and allele frequencies of SCD1 gene for HF dairy cow in all population were presented in Table 3. The frequency of AA genotype of FH dairy cow in Sukabumi (0.61) was higher than those in Tasikmalaya (0.42), Enrekang (0.36) and Bogor (0.23). Frequency of the AV genotype in Bogor population (0.65) was higher than those in Enrekang (0.58), Tasikmalaya (0.42) and Sukabumi (0.35). The results showed that those FH dairy cows with the VV genotype were low in all population (Enrekang = 0.06; Tasikmalaya = 0.16; Sukabumi = 0.04; Bogor = 0.12). The allele frequencies for HF dairy cows in all population were under HWE condition, indicating random mating with respect to this locus. Gene equilibrium in population is achieved when selection, mutation, migration and genetic drift are absent (Falconer and T.F.C. Makay 1996). Average value of heterozigosity observed (Ho) reached 0.471 while heterozigosity expected was 0.470. Those values refected of genetic diversity of SCDI gene in four populations.

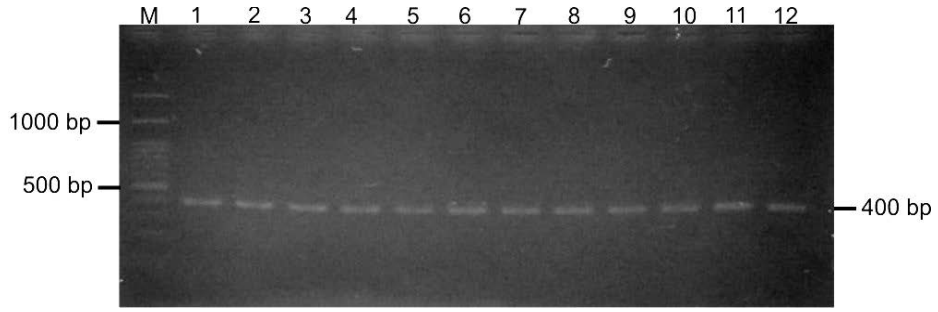

Figure 1. Visualization of PCR product of SCD1 gene from HF dairy cow samples (1-12); M = 100 bp DNA ladder

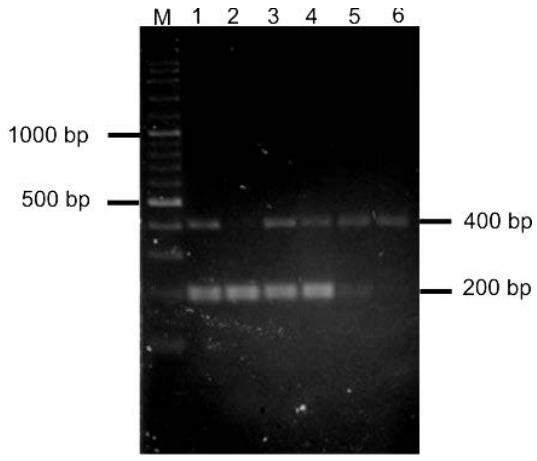

Figure 2. Visualization of PCR-RFLP products of SCD1 gene.
M = 100 bp DNA ladder
Line 1,3-5 = AV genotypes
Line 2 = AA genotype
Line 6 = VV genotype

Table 2. Allelic and Genotypic Frequencies, heterozigosity and HWE value of SCD1 Gene in Friesian Holstein

Population	N	Allelic Frequency		Genotypic Allelic			Ho	He	HWE	
		A	V	AA	AV	VV			(χ2value)	(χ2tab)
Enrekang	45	0.64	0.36	0.36	0.58	0.06	0.463	0.458	3.060	3.841
Tasikmalaya	19	0.63	0.37	0.42	0.42	0.16	0.478	0.465	0.172	3.841
Sukabumi	23	0.78	0.22	0.61	0.35	0.04	0.348	0.340	0.011	3.841
Bogor	75	0.55	0.45	0.23	0.65	0.12	0.498	0.494	7.762	3.841
Total	162	0.62	0.38	0.34	0.56	0.10	0.472	0.471	6.008	3.841

N = Number of samples
Ho = Heterozygosity observed
He = Heterozygosity expected
HWE = Hardy-Weinberg Equilibrium (χ2value< χ2tab {0,05} means that the frequency of the genotypic population is under HWE condition)

Table 3. Allelic and Genotypic Frequencies of SCD1 Gene in Numerous Dairy Cow Breeds

Breed	N	Allelic Frequencies		Genotypic Frequencies			References
		A	V	AA	AV	VV	
Isfahan Holstein	408	0.58	0.48	0.26	0.65	0.09	Nanaei et al. 2013
Iranian Holstein	394	0.76	0.24	0.60	0.32	0.08	Mashhadi et al. 2012
Canadian Jersey	525	0.81	0.19	0.69	0.24	0.07	Kgwatalala et al. 2009
Italian Brown	351	0.18	0.82	0.04	0.29	0.67	Conte et al. 2010
Italian Holstein	297	0.57	0.43	0.27	0.60	0.13	Mele et al. 2007
Holstein	143	0.71	0.29	0.50	0.42	0.08	Clark et al. 2010
Polish Holstein-Friesian	100	0.77	0.23	0.58	0.36	0.06	Kesek et al. 2017

The distribution of the SCD1|*Nco*I alleles is characterized by a higher frequency of the A allele compared to the V allele in most of HF dairy cow population studied (Table 2). Similar results with previous studies, Isfahan Holstein (0.58 vs 0.48) (Nanaei et al. 2013), Iranian Holstein (0.76 vs 0.24) (Mashhadi et al. 2012), Polish Holstein (0.77 vs 0.23) (Kesek et al. 2017). In contrastly, Italian Brown and Piedmontese breed from Italy have V allele higher than A allele (0.82 vs 0.18 (Conte et al. 2010) and 0.58 vs 0.42 (Moioli et al. 2007), respectively). Italian Brown cattle in Conte et al. (2010) study has positive of F_{IS} value or inbreeding coefficient (0.05) comparing Italian Holstein (-0.23) that might caused genetic selection or the other cases. Based on F_{ST} coefficient value for evaluating whether the effect of genetic selection or genetic drift, moderate F_{ST} value was in between Italian brown and Italian Holstein (0.16) and high value was in between Italian brown cattle and Jersey (0.40). High F_{ST} value indicated an elevated distance between Italian brown cattle and the other dairy breed. Macciotta et al (2008) reported that V allele higher than A allele also (0.566 and 0.434) with big population 538 Italian dairy cattle lactation.

Based on previous studies were known that SCD1|*Nco*I gene associated with fat milk content. Mele et al. (2007) studied milk fatty acid in Canadian Holstein and reported that AA genotype has higher in three fatty acid content (9.3% of *cis*-9 C14:1; 37.9% of *cis*-9 C18:1; and 11% of MUFA) than VV genotype. In CLA (*cis*-9, *trans*-11) concentration, AA genotype has higher than AV and VV genotypes (0.37; 0.36 and 0.33 g/100 g of total lipid, respectively). Herck (2009) investigated in Holstein that A allele was associated with higher proportion of C10:1, C12:1, C14:1, C18:0, and C18:1 trans 11 than V allele.

Different result has reported by Komisarek and Dorynek (Komisarek, J. and Dorynek 2009) which showed a positive effect of VV genotype on fat percentage. Futhermore, Macciotta et al. (2008) found that VV genotype has the greatest fat yield (1.22 kg/d) whereas AV (1.193 kg/d) and AA (1.186 kg/d) in Italian Holstein. Added by Conte et al. (2010) in Italian Brown cattle, VV allele has higher C14:1 cis 9 and DI 14 fatty acid (18.3% and 20.6%, respectively) than AV and AA genotypes. Higher proportion of C10:0, C12:0, C14:0, C16:1 and CLA was found in V allele of Holstein also (Herck, 2009).

Polymorphism of SCD1|*Nco*I gene in all of locations of HF dairy cows could be as early genetic information to improve of genetic quality through molecular breeding strategy. High of fat milk especially CLA and unsaturated fatty acid can be focus to get healthy milk.

CONCLUSION

This study, demonstrated that there was a genetic polymorphism in samples collected from four locations of Friesian Holstein dairy cow population in Indonesia with dominance A allele. This finding may lead to further investigation on association of SCD1 gene polymorphism on milk fat yield in Friesian Holstein in Indonesia with higher sample of cows.

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