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Polymorphism of stearoyl-CoA desaturase (SCD1) gene in Indonesian local cattle

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ABSTRAK

Stearoyl-Coa desaturase (SCD1) merupakan salah satu gen yang terlibat dalam perubahan asam lemak jenuh menjadi tidak jenuh. SNP pada gen SCD1 exon 5, yang mengubah asam amino valin menjadi alanin (Val293Ala) mempengaruhi komposisi asam lemak daging. Penelitian ini bertujuan untuk mengetahui keragaman gen SCD1 pada tiga sapi lokal Indonesia. Keragaman gen SCD1 diidentifikasi menggunakan 98, 20 dan 7 sampel DNA masing-masing sapi lokal Ciamis, Bali/Banteng dan Peranakan Ongole (PO). Keragaman SCD1 dianalisis berdasarkan SNP Val293Ala yang diidentifikasi menggunakan metode PCR_RFLP dengan enzim Aci1. Frekuensi alel dan nilai heterogositas dianalisis menggunakan program POPGENE 32. Hasil penelitian menunjukkan gen SCD1 pada sapi lokal Ciamis dan PO adalah polimorfik. Frekuensi alel T masing-masing sebesar 74,5% dan 71,4% serta alel C masing masing sebesar 25,5% dan 28,6%. Pada sapi lokal Ciamis terdapat tiga genotipe, yaitu TT, CT dan CC dengan frekuensi masing-masing sebesar 52%, 44,9% dan 3,10%, sedangkan pada sapi PO terdapat dua genotipe, yaitu TT dan CT dengan frekuensi masing-masing sebesar 42,9% dan 57,1%. Gen SCD1 pada sapi Bali monomorfik. Keragaman gen SCD1 pada sapi Lokal Ciamis dan PO masing-masing sebesar 0,38 dan 0,44 termasuk dalam kategori sedang.

Kata kunci : sapi lokal Indonesia, gen SCD1, PCR_RFLP

ABSTRACT

Stearoyl-Coa desaturase (SCD1) gene is one of genes that involves in converting saturated fatty acids to unsaturated fatty acids. SNP at exon 5 in SCD1 gene that changes amino acid valine to alanine (V293A) has an influence to meat fatty acid composition. The aim of this research was to analyze SCD1 gene polymorphisms based on SNP V293A at exon 5 of three Indonesian local cattle. The identification of SCD1 gene polymorphisms was done by using 98, 20 and 7 DNA sample from Ciamis, Bali/Banteng, and Ongole Grade (PO) cattle, respectively. PCR_RFLP method with AciI enzim was carried out to identify SNP Val293Ala. Allelic frequencies and heterozygosity value were analyzed by using POPGENE32. The result showed that SCD1 gene at Ciamis local cattle and PO cattle were polymorphic. Their frequencies were 74.5% and 71.4% for T and 25.5% and 28.6% for C, respectively. There were three genotypes on Ciamis local cattle i.e TT, CT and CC with their frequencies were 52%, 44.9% and 3.10%, respectively. There were two genotypes on PO cattle i.e TT and CT with their frequencies were 42.9% and 57.1%, respectively. Meanwhile, SCD1 gene in Bali cattle was monomorphic. Heterozygosity value of SCD1 gene in Ciamis and PO cattle were 0.38 and 0.44, respectively. Their heterozigosities were categorized as medium.

Keywords : Indonesian local cattle,, PCR_RFLP, SCD1 gene

INTRODUCTION

The high consumption of red meat has been supposedly assosiated with diseases in human, such as cardiovacular, obesity, diabetes, neurological, skin damage and cancer (Miyazaki and Ntambi, 2003; Mauvoisin and Mounier, 2011). This is due to red meat contains considerable amount of saturated fatty acids (SFA), that is believed to increase unpleasant cholesterol in blood. Therefore, it is needed to increase unsaturated fatty acids (MUFA/PUFA) to get a healthier meat.

The one of genes which affects fatty acids composition in milk, adipose tissue, and meat is Stearoyl-CoA desaturase-1 (Milannesi *et al*. 2008; Orru *et al*., 2011). The biosynthesis of palmitoleyl-CoA and oleyl-CoA from palmitoyl-CoA and stearoyl-CoA are catalyzing by SCD1 enzyme, these substrates are important for the formation of triacylglycerols, phospholipids, cholesterol, and wax esters (Mauvoisin and Mounier, 2011). The expression of SCD1 gene affects fatty acid composition in phospolipid membrane, trigliseride dan cholesteryl ester, fat metabolism, and obese, it shows substantial role of SCD1 regulation in physiological processes (Scaglia *et al.*, 2009)

Several previous studies indicated that there was high correlation between SCD1 gene variation that was caused by SNP Val293Ala at exon 5, with saturated and unsaturated fatty acid ratio (SFA : PUFA/MUFA) in adipose tissue and meat (Taniguchi *et al*., 2004; Ohsaki *et al*., 2009; Barton *et al.*, 2010; Orru *et al*., 2011). Allelic variation of SCD1 gene in crossbred of Wagyu x Limousin, contributed to fat deposition, marbling, and composition of meat fatty acid (Jiang *et al*. 2008). SNP (Single Nucleotide Polymorphism) at exon 5 (T10329C/ T878C) that converts encoding amino acid Valine to Alanine (Val293Ala) causes difference intramuscular fatty acid composition in Japanese Black (Taniguchi *et al*. 2004; Ohsaki *et al*., 2009). Therefore, this SNP V293A has the potential as a marked assisted selection (MAS) for selection in beef cattle to get better meat quality.

This study was designed to identify SNP (single nucleotide polimorphisms) of SCD1 gene in Indonesian local cattle. Indonesia has many kinds of indigenous cattle such as Bali/Banteng cattle and local cattle likes Ongole Grade (PO), and Ciamis local cattle. There was no information about SCD1 gene in Indonesian cattle.

MATERIALS AND METHODS

Materials

Three breeds of Indonesian local cattle were used to identify polymorphisms of SCD1 genes, there were Ciamis local cattle, Bali/Banteng, and PO cattle. The identification of SCD1 gene polymorphisms were used 98, 20, and 7 DNA samples of Ciamis local cattle, Bali/Banteng, and PO cattle, respectively.

Methods

Single Nucleotide polymorphisms at SCD1 gene was analyzed using PCR_RFLP (Restricted Fragment Length Polymorphism), with Aci1 restricted enzyme that was cut off at GCGG/CGCC site. The primer that was used to amplify SCD1 gene at exon 5 were F:5'TGCCCATATGTATG GATACCG3' and R: 3'CCCAAAGGGGTT CATCATAC5'. These target were amplified with initial denaturation at 95^{o}C for 5 minutes, followed by 33 cycles extended denaturation at 95°C for 30 s, annealing at 56°C for 45 s, extension at 72°C for 1minute, and terminated by cooling down at 72°C for 5 minutes. The excision with restriction enzyme was performed with 5 µl of PCR products mixed with 0.7 µl DW (destilated water), 0.8 µl buffer 0 and 0.5 µl Aci1 enzyme then was incubated at 37°C for 16 h. The PCR_RFLP product was evaluated by 1.5% electrophoresis agarose and visualized by UV transluminator. The product of PCR_RFLP consist of two band patterns on agarose electrophoresis which were 321 bp and 263 bp for T allele and C allele, respectively.

Data Analysis

Allelic, genotype frequencies and heterozygosity were calculated by Nei (1987) formula using POPGENE.32 program.

RESULT AND DISCUSSION

SCD1 Gene Variation

SCD1 gene target was covered at a part of exon 5 and whole of intron 6. It has successfully amplified with forward and reverse primer which was assigned to further PCR-RFLP analysis. PCR product was about 343 bp of length (Figure 1). PCR-RFLP was conducted using Aci1 enzyme, cut off nucleotide sequence at GCGG/CGCC site. PCR_RFLP analysis found two banding patterns on agarose electrophoresis which were 321 bp (T

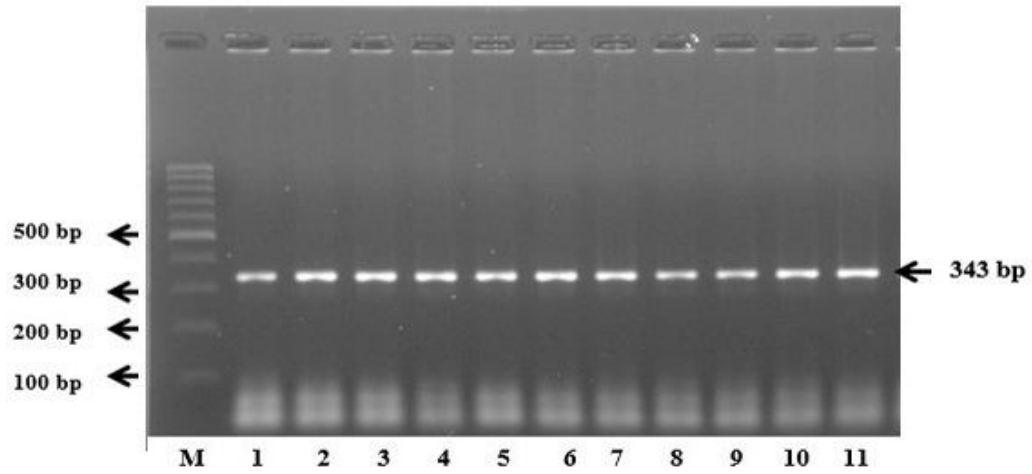

Figure 1. PCR Product of SCD1 Gene 343 bp of Length . M = Marker 100bp, 1- 11 = Sample number

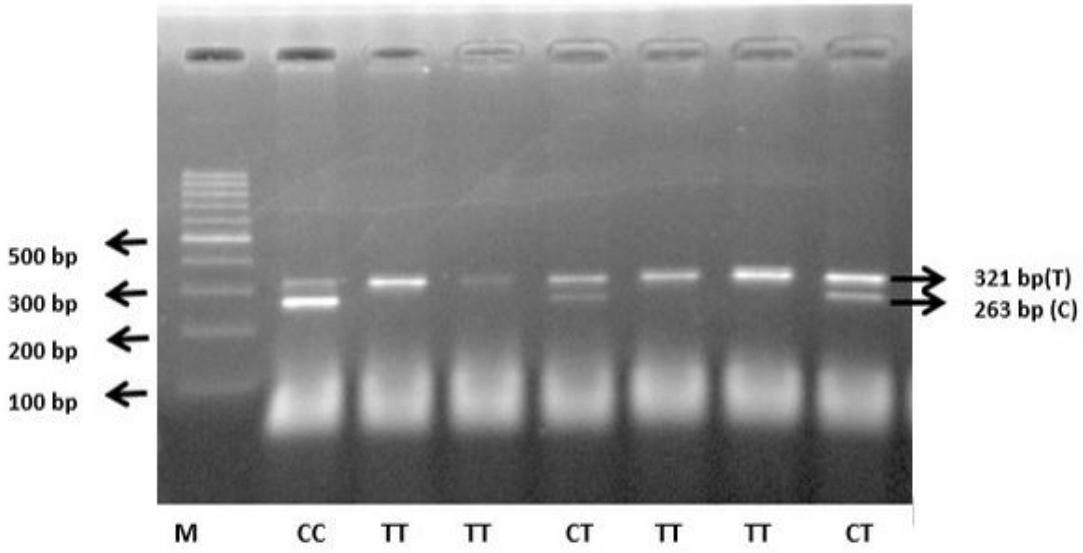

Figure 2. Band and Genotype of PCR- RFLP Product SCD1 Gene (321 bp and 263 bp)

allele) and 263 bp (C allele) as presented in Figure 2. The SNP target was 263 bp of length. Its position at 10329 (T>C) encoding the changes amino acid of Valine to Alanine (Val293Ala) (NCBI no access AY241932.1). Based on this mutation, there were two alleles i.e T and C and three genotypes, namely TT, CT and, CC. The allele and genotype frequencies based on SCD1 gene in each breed were presented in Table 1.

Table 1 showed that higher frequency of T allele as compared to C allele was found in Ciamis local cattle and PO cattle. In the present study, there was no SNP Val293Ala in Bali cattle. The frequency of C allele were low in Ciamis local cattle and PO cattle due to lower frequencies of CC genotype in Ciamis local cattle and that was not found in PO cattle.

These result corresponds with what has been reported by Orru *et al.* (2011) who analyzed 103 Simmental bulls that the frequency of T allele (70%) was higher than C allele (30%). In contrast, higher frequency of C allele than T allele was

Table 1. Allele and Genotype Frequencies of SCD1 Gene in Indonesian Local Cattle

Breed	n	Allele frequency		Genotype frequency		
		T	C	TT	CT	CC
Ciamis cattle	98	0.7449	0.2551	0.5204	0.4490	0.0306
Bali/Banteng	20	1	0	1	0	0
Ongole Grade (PO)	7	0.7143	0.2857	0.4286	0.5714	0

n = the number of samples

Table 2. Observed and Expected Heterozygosity Value of SCD1 Gene in Indonesian Local Cattle

Breed	n	Heterozygosity		
		H_o	H_e	Nei
Ciamis cattle	98	0.4490	0.3820	0.3800
Bali/Banteng	20	0	0	0
Ongole Grade (PO)	7	0.5714	0.4396	0.4082

n = the number of samples; Ho = Observed heterozygosity; He = Expected heterozygosity

reported by several researchers. Barton *et al.* (2010) found 55.5% vs 44.5% on Fleckvieh bulls and Taniguchi *et al.* (2004) who genotyped SCD1 gene by 3 SNPs including SNP at amino acid position V293A in Japanese Black cattle, revealed the average frequency of C allele and T allele were 59% and 41%, respectively, that is due to, there are intensif selection forward to meat quality in this breed, particularly in marbling. The research of Milanesi *et al.* (2008) in Italian cattle revealed that C allele frequency at T878C was spread out 89% in Grey Alpine - 34% in Italian Red Piedmintosa cattle, which showed that C allele in cows was higher that beef cattle. Taniguchi *et al*. (2004), Oka *et al.* (2007) and Ohsaki *et al.* (2009) who analyzed SNP in Japanesse Black cattle which revealed substitution T to C that changed the amino acid coding from Valine (V) to Alanine (A), caused differences in fatty acid composition. The AA (CC) genotype in Japanese Black steers indicate a higher MUFA percentage than VV(TT) and VA(CT), but the SCD1 polymorphism contributed 4% only, to overall MUFA variation (Taniguchi *et al.* 2004). There were differences among genotypes in meat fatty acid composition of Fleckvieh bull, AA (CC) and AV (CT) genotypes cattle have higher MUFA, lower SFA and therefore a higher MUFA / SFA ratio than VV genotype cattle (Barton *et al*. 2010). The SNP V293A in exon 5 was non synonimous mutation, due to change of encoded amino acid Valine to Alanine. Kumar *et al.*, (2009) declare that a non-synonymous single nucleotide polymorphism (nsSNP) is a single amino acid substitution (AAS) in a protein sequence which could change the function of the protein, consequently, it can transform the phenotype.

The SCD1 gene in Bali cattle was monomorphic, there was no mutation in this site, Bali cattle is an Indonesian native cattle, that it is believed domesticated from Banteng. Indonesian goverment has been protect purity of Bali cattle, through cross breed prohibit of Bali cattle with others breed. Many studies showed that there were no variation (monomorphic) on quantitative gene in Bali cattle. Zulkarnain (2010) suggested that the growth hormone receptor gene (GRH/Alul1) in Bali cattle was monomorphic. Furthermore, Ishak *et al.* (2011) declared there was no differencess on nucleotide sequence (monomorphic) of FSH beta-subunit gene in Bali cattle.

SCD1 gene diversity values based on observation and expected heterozygosity of

Ciamis local cattle population and PO cattle, were 0.449, 0.571 and 0.382, 0.440, respectively (Table 2). The heterozygosities value were a medium category.

CONCLUSION

The SCD1 gene in Ciamis local cattle and PO cattle were polymorphic, while it was monomorphic in Bali cattle. The SNP V293A was non synonimous mutation, due to encoding amino acid changed from Valine to Alanine. Frequency of C allele (mutation/ SNPV293A) was lower than T allele in Ciamis local cattle and PO cattle. Heterozygosity of SCD1 gene in Ciamis local cattle and PO cattle were in medium category.

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