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POLYMORPHISM STEAROYL-COA DESATURASE (SCD) GENE AND ASSOCIATON WITH CHARACTERISTICS MEAT IN BALI CATTLE

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ABSTRAK

Stearoyl-CoA desaturase (SCD) adalah enzim yang diproduksi oleh gen SCD, berpengaruh terhadap lemak dalam jaringan adiposa (marbling). Penelitian ini bertujuan untuk mendapatkan keragaman gen SCD pada sapi bali dan asosiasinya terhadap kualitas daging. Jumlah sampel sapi bali adalah 48 ekor terdiri atas jantan 24 ekor dan betina 24 ekor berasal dari BPTU-HMT Sapi Bali di provinsi Bali. Amplifikasi gen SCD menggunakan primer forward 5'-ACCTGGTGTCCTGTTGTTGTGCTTC-3' dan reverse 5'-GATGACCCTACTCTTCTATTTATGC-3'. Keragaman gen SCD diidentifikasi dengan metode direct sequencing. Sifat kualitas daging yaitu tebal longissimus dorsi (TLD), tebal lemak punggung (TLP), tebal lemak rump (TLR), tebal rump (TR), *marbling score* (MS), dan persentase lemak intramuskular (PLIM) di koleksi menggunakan Veterinary *Ultrasound Scanner*. Frekuensi alel dan genotipe dihitung dengan GENEPOP (V3.2) untuk mengetahui apakah polmorfisme gen SCD dalam keseimbangan Hardy-Weinberg. Asosasi *Single Nucleotide Polymorphism* (SNP) gen SCD terhadap kualitas daging dianalisis dengan pendekatan General Linier Model (GLM). Hasil analisis ditemukan 8 SNP yaitu 5 SNP monomorfik (c.10153A>G, c.10318C>A, c.10329C>T, g.10394G>A, g.10486A>C) dan 3 polimorfik (g.10360G>A, g.10428C>T, g.10487G>A) berada dalam keseimbangan H-W. Gen SCD Ditemukan berasosiasi nyata ($P<0.05$) pada SNP g.10428C>T terhadap sifat *marbling score* dan persentase lemak intramuskular. Berdasarkan hasil tersebut SNP g.10428C>T dapat dijadikan sebagai kandidat *marker assisted selection* (MAS).

Kata kunci: Sapi bali, Gen SCD, SNP

ABSTRACT

Stearoyl-CoA desaturase (SCD) is an enzyme produced by SCD gene which is responsible for a conversion of saturated fatty acids (SFA) to mono-unsaturated fatty acid (MUFA) in adipose tissue. This enzyme affects the fats in intramuscular so having influence on marbling. The purpose of this study was to obtain the polymorphisms of the SCD gene and their associations with meat quality traits in Bali cattle. The number of samples used were 48 heads of cattle consisted of 24 bulls and 24 cows from BPTU-HMT Bali cattle in the province of Bali. The SCD gene has been amplified using forward primer 5'-ACC CCT TGG TGT GTG GTT GTT CTT C-3 'and reverses primer 5'-CCT GAC GAT ACT ATG TTT CTA CTT C-3'. The polymorphisms of the SCD gene were identified by direct sequencing method. Meat quality traits such as thick of longissimus dorsi (TLD), thick of back fat (TBF), thick of fat rump (TFR), thick of rump (TR), marbling score (MS), and the percentage of intramuscular fat (PIMF) were analyzed using the Veterinary Ultrasound Scanner. To determine Hardy-Weinberg equilibrium status,

both allele and genotype frequencies were analyzed using GENEPOP program (V3.2). Association of the SCD gene SNP and meat quality traits was analyzed by GLM. This result showed that there were 5 monomorphic SNPs (c.10153A>G, c.10318C>A, c.10329C>T, g.10394G>A, g.10486A>C) and 3 polymorphic SNPs (g.10360G>A, g.10428C>T, g. 10487G>A) were in HW equilibrium. Association analysis showed that g.10428C>T SNP significantly affected marbling score (MS) and percentage of intramuscular fat (PIMF) ($P<0.05$). Based on these results, g.10428C>T SNP of the SCD gene may be used as a candidate marker to select meat quality traits in Bali cattle.

Keywords: Bali cattle, SCD gene, SNP

INTRODUCTION

Bali cattle (*Bos javanicus*) as one of native cattle in Indonesia is a cattle domestication from bull (*Bibos banteng*) (Purwantara *et al.,* 2012). Bali cattle has potential advantages to produce a good meat quality, such as high percentage of meat carcass (48-52%) (Ismail *et al.,*2014), the water holding capacity approximately 66.2% (Dewitri*et al.,* 2015), cooking shrinkage 19-28%, texture score 5-6, and also has a chemical composition of protein content about 17-21% and fat content about 2-7% (Eko and Subandriyo, 2004). According to Pearson (1971) meat quality parameter can be assessed from color, tenderness, texture, flavour and aroma including smell and taste, juiciness, cooking shrinkage, water holding capacity (WHC), pH of the meat, intramuscular fat or marbling. Marbling is the fat composition contained in intramuscular (Soeparno, 2005). Several studies reported that every cattle breed has different variations of marbling score such as Sumba ongole which has marbling score about 2-3 (Priyanto *et al.,* 2015). There are several factors affecting marbling including the type of diet, genetics, condition and location where animals are rare (Pollan, 2006).

The efforts to improve the genetic quality of Bali cattle especially to marbling properties can be applied with selection based on molecular (DNA) or known as marker assisted selection (MAS) (Azrai, 2005). Selection by MAS was conducted for beef cattle (Rezende *et al.,* 2012) and other animals such as chickens (Lahav *et al.,* 2006), goat (White and Donald*,* 2004) and buffalo (Sarika *et al.,*2013).

Meat quality (using marbling score) was controlled by multiple genes such as DGAT1 gene (Karolyid *et al.,* 2012), SREBP gene (Barton *et al.,* 2010) and SCD gene (Ohsaki *et al.,* 2009). Stearoyl-CoA desaturase (SCD) gene is a gene responsible for an enzyme that converts SFA into MUFA on adipose tissue (Corl *et al.,* 2001; Kay *et al.,* 2004). The SCD gene is located on chromosome number 26 which has 6 exons and 5 introns (Ohsaki *et al.,*2009). SCD genes have been studied in Wagyu cattle, Canadian Holstein, Jersey, Fleckvieh cattle and local Korean cattle (Kgwatalala *et al.,* 2007; Milanesi *et al.,* 2008; Barton *et al.,*, 2010; Ohsaki *et al.,*, 2009; Oh *et al.,* 2011). The objective of this study were identification SCD gene exon 5 and intron 5 and their association with meat quality in Bali cattle.

MATERIALS AND METHODS

Cattle

Numbers of Bali cattle used were 48 heads consisted of 24 heads males and 24 females with age 12-15 months from BPTU-HMT Bali cattle in Bali province. Cattle were reared in the same paddock and feed with the same type of forage (*Pennisetum purpureum* and *Phaspalum notatum*) 10% and concentrate at 1% of body weight, respectively. The Meat quality parameters such as thick of longissimus dorsi (TLD), thick of back fat (TBF), thick of fat rump (TFR), thick of rump (TR), marbling score (MS), and percentage of fat intramuscular (PIMF) (Figure 1) was observed using Veterinary Ultrasound Scanner WED-3000V. Data were analyzed using the software Image ultrasound-J NH (ImageJ ®, NIH, USA). The TLD and TBF measurements were scanned on the ribs 12 and 13 (Melendez and Marchello 2014), while the TR and TFR measurementswere conducted between ileum and ischium (Silva *et al.,* 2012). MS measurements were performed by Meat Standards Australia (http://www.wagyu.org.au/marbling/).

Total DNA extraction

Total genome were extraced from blood using DNA Kit Geneaid (modified). The first step was sample preparation of blood samples were taken as many as 300 mL in 1.5 ml tube and was added by a solution of RBC lysis as much as 900 mL then homogenized. After that, those samples were put at room temperature for 10 minutes and

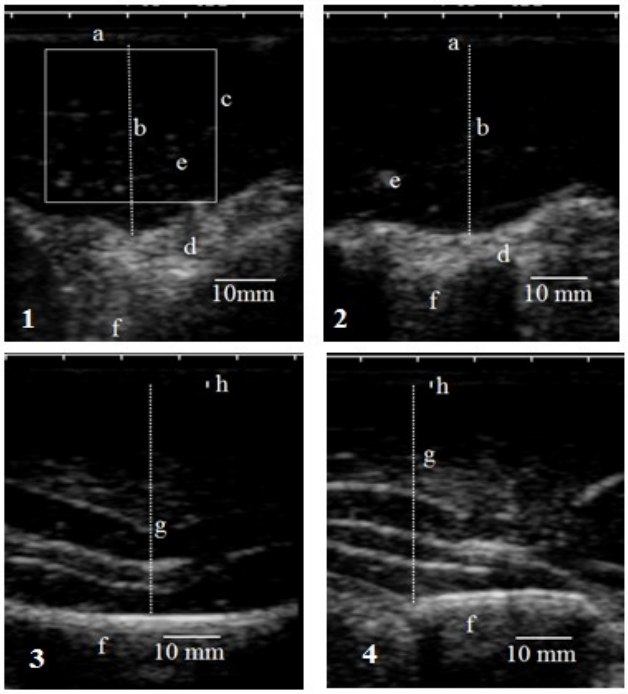

Figure 1. Ultrasonografion the ribs 12 and 13 in bali cattle on vertical (1) and horizontal (2). USG rump thickness on vertikal (3) and horizontal (4). Thickness of backfat (a), thickness oflongissimusdorsi (b), region of persentage IMF 30x30 mm (c), bone (d), intramuscular fat (e), ribs (f), rump thickness (g), and thickness of fat rump (h).

centrifuged at 3000 rpm for 5 minutes then the supernatant was discarded. A total of 100 mL of RBC lysis and 200 mL of GB buffer were added then homogenized using vortex. Samples were incubated at 60° C for 10 minutes and inverted every 3 minutes. Then, RNAse as much as 5 mL was added and incubated at room temperature for 5 minutes. A total of 200 mL of ethanol absolute was added and the sample was moved in GD column then was centrifuged at 14 000 rpm for 5 minutes and 2 ml collection tube was removed. A total of 400 mL of W1 buffer solution was added to the GD column completed by a new collection tube and then centrifuged at 14 000 rpm for 1 minute then supernatant was discarded and centrifugedagain dry GD column. After that, the tube GD column was transferred to 1.5 ml microcentrifuge tube and added by 100 mL pre-heated elution buffer and left for 3 minutes and then centrifuged at 14 000 rpm for 3 minutes. The quality and quantity of DNA was evaluated by spectrophotometer and electrophoresis on 1% agarose gel.

The SCD Gene Amplification

The forward primer 5'-ACC CCT TGG TGT GTG GTT GTT CTT C-3 'and reverse primer 5'-CCT GAC GAT ACT ATG TTT CTA CTT C-3' were designed according to Ohsaki *et al.* (2009). PCR reagents used were1 mL DNA samples and 49 mL solution of premix. Premix compotition were from 0.3 mL of primer, 23.4 mL DW and 25 mL of Promega Green Master Mix. PCR conditions include pre denaturation at 95 °C for 5 minutes, denaturation at 95 °C 10 seconds, annealing at 50 °C 20 sec, elongation at 72 °C for 30 sec and final elongation at 72 °C for 5 minutes and PCR process took a total of 35 cycles. PCR products were identified by electrophoresis with 1.5%agarose gel. PCR products either forward or reverse were sequenced by 1st Base, Selangor Malaysia.

Data analysis

Phenotypic Data. Data of meat quality namely Thickness of longissimus dorsi (TLD), thickness of back fat (TBF), thickness of fat rump (TFR),

thickness of rump (TR), marbling score (MS), and the percentage intramuscular fat (PIMF) were analyzed descriptively.

Sequencing Data. Data of SCD gene sequences were analyzed with Bioedit program (Hall, 1999), and the determination of SNP (single nucleotide polymorphism) was identified using Molecular Evolutionary Genetics Analysis 5 (MEGA5) (Tamura *et al.,* 2011). Allele and genotype frequencies calculated by GENEPOP (V3.2) (Raymond and Rousset, 2001) to determine if polmorfisme SCD gene in Hardy-Weinberg equilibrium.

Association of SCD gene with meat quality: The association between SCD gene with meat quality were analysis using ANCOVA PROC GLM procedure of SAS (Bhuiyan *et al.*, 2009, SAS Institute Inc. 2008). The statistical model used as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_k + \epsilon_{ijk}$$

Where : Y_{ij} = observed values, μ = common average value, α_i = effect of i^{th} genotype, β_j = effect of sex, γ_k = effect of age, ϵi_{jk} = the error

RESULTS AND DISCUSSION

Polymorphism of the SCD gene

The SCD gene was amplified successfully at annealing temperature 50°C for 20 seconds with a length of PCR product 569 bp in Bali cattle (Figure 2). Eight SNPs of the SCD gene were found in Bali cattle (Table 1), they were three SNPs in exon 5(SNPs c.10153A>G, SNP c.10318C>A, SNP c.10329C>T) and five SNPs in intron 5(g.10360G>A,g.10394G>A, g.10428C>T, g.10486A>C and g.10487G>A).

Genotype and allele frequency of SCD gene in Bali cattle are presented in Table 2. The frequency of GA genotype at SNP g.10360G>A was higher than AA and GG genotype. The frequency of TT genotype at SNP g.10428C>T was higher than CT and CC genotypes. Moreover GG genotype was higher than GA and AA genotype at SNPs 10487G>A. SNP g.10360G> A had the highest frequency of allele A (0.52) whereas in SNP g.10428C>T allele T had the highest frequency (0.80). Allele G (0.73) at SNP g.10487G> A had the highest frequency. All three SNPs found (g.10360G> A, g.10428C> T and g.10487G> A) were polymorphic.

The results showed that the SCD gene on locus g.10360G>A, g.10428C>T and g.10487G>A were in HW equillibrium condition. Meanwhile, the SCD gene on locus c.10153A>G, c.10318C>A, c. 10329C>T, c.10394G>A and c.10487G>A could not be analyzed because only one allele was found with an allele frequency was monomorphic. SNPs called balanced/ in equilibrium condition when chi-square (χ^2) value less than 0.05 ($P<0.05$) (Alleandorf *et al.,* 2013). Factors that affect the HW equllibrium in the population were non-random mating, selection, migration, mutation and genetic drift (Noor 2010).

Table 1. SNP SCD Gene Exon 5 on Bali Cattle

Number	SNP	Diversity	Type of Mutation	Change of AA
	Exon 5			
1	c.10153A>G	Monomorphic	Transition	*Synonymus*
2	c.10318C>A	Monomorphic	Transvertion	*Synonymus*
3	c.10329C>T	Monomorphic	Transition	*Non synonymus*
	Intron 5			
4	g.10360G>A	Polymorphic	Transition	-
5	g.10394G>A	Monomorphic	Transition	-
6	g.10428C>T	Polymorphic	Transition	-
7	g.10486A>C	Monomorphic	Transvertion	-
8	g.10487G>A	Polymorphic	Transition	-

Table 2. Genotype and Allele Frequencies of SCD gene on Bali Cattle

Locus	Genotype Frequency			Allele frequency		Chi square (X^2)
c.10153A>G	AA (0.00)	AG (0.00)	GG (1.00)	A (0.00)	G (1.00)	-
c.10318C>A	CC (0.00)	CA (0.00)	AA (1.00)	C (0.00)	A (1.00)	-
c.10329C>T	CC (0.00)	CT (0.00)	TT (1.00)	C (0.00)	T (1.00)	-
g.10360G>A	GG (0.19)	GA (0.58)	AA (0.23)	G (0.48)	A (0.52)	ns
g.10394G>A	GG (0.00)	GA (0.00)	AA (1.00)	G (0.00)	A (1.00)	-
g.10428C>T	CC (0.04)	CT (0.31)	TT (0.65)	C (0.20)	T (0.80)	ns
g.10486A>C	AA (0.00)	AC (0.00)	CC (1.00)	A (0.00)	C (1.00)	-
g.10487G>A	GG (0.52)	GA (0.42)	AA (0.06)	G (0.73)	A (0.27)	ns

Note :, x^2 = Hardy-Weinberg equilibrium, ns : not significant at α 5% ($X^2 \geq 3.84$)

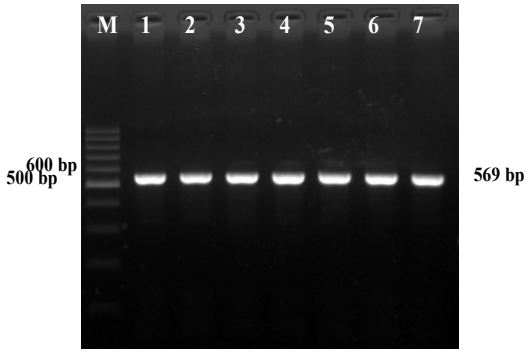

Figure 2. The results of SCD gene exon 5 amplification in Bali cattle. M = marker DNA 100 bp. Line 1-7 is a sample of Bali cattle

Meat Characteristics of Bali Cattle

The following data of meat characteristics of Bali cattle were obtained from ultrasound results as presented in Table 3. Rahma (2010) stated that the area longissimus dorsi on Bali cattle aged 12 months was 16.6-18.0 cm^2. Bali cattle fattened range 2.5-3.5 years had backfat thickness around 8.40 mm (Yosita 2012) which was greater than the results of Bugiwati (2005). Intramuscular of percentage measured by ultrasound had the average 3,509 ± 2,114%. Measurements for meat characteristics also performed on the rump in the intermediate position between the ischium and Illium (between hip and hook), thickness of rump and thickness of fat rump having average about 40.086 ± 4.0895 and 1069 ± 0.345 mm.

Association of the SCD Gene with Meat Characteristics

The association between SCD genes with meat characteristics was presented in Table 4. Significant correlation ($P<0.05$) between SNP g.10428C>T with marbling score (MS) and the percentage of intramuscular fat (PIMF) was found. Bali cattle at SNP g.10428C>T was polymorphic, but *Bos taurus* has genotype CC and it is monomorphic.

CC genotype at the locus g.10428C>T had higher value of marbling and intramuscular fat percentage than CT or TT genotypes. Bali cattle with TT genotype was found to be dominant in comparison with CT and CC. CC genotype had a marbling score of 4.8 which means that it could be classified into the moderate category - slightly abundant. The analysis of the percentage of intramuscular fat showed that the CC genotype had a fairly high percentage when compared with CT or TT genotypes.

Mutation that were found in this study were occured in intronic region 5 of SCD gene, intron

Table 3. Meat Characteristics of Bali Cattle

Characteristics	Phenotype
Thickness of Longissimus dorsi (TLD), (mm)	33.047±5.077
Thickness of Back fat (TBF), (mm)	1.455±0.348
Thickness of Rump (TR), (mm)	40.086±4.0895
Thickness Fat Rump (TFR), (mm)	1.069±0.345
Percentage intramuscular fat (PIMF), (%)	3.509±2.114
Marbling score (MS)	2.333±1.241

Table 4. Association of SNPs in SCD Gene with Meat Characteristics on Bali Cattle

Position of SNP	Genotipe	N	TLD	TLP	RT	RFT	MS
g.10360A>G	AA	9	32.67±1.17	1.51±0.12	38.66±1.16	1.07±0.12	1.83±0.42
	AG	16	33.98±0.83	1.44±0.09	41.39±0.86	1.09±0.09	2.50±0.32
	GG	6	32.26±1.42	1.51±0.15	39.38±1.48	1.06±0.16	2.4±0.54
g.10428C>T	CC*	1	31.49±0.00	1.87±0.00	38.57±0.00	1.17±0.00	4.81±0.00
	CT	7	32.41±1.26	1.55±0.13	40.30±1.39	1.08±0.13	2.80±0.43[a]
	TT	23	33.64±0.69	1.43±0.07	40.29±0.77	1.08±0.08	2.03±0.24[b]
g.10487A>G	AA	3	33.43±1.97	1.33±0.20	39.10±2.03	0.96±0.21	1.51±0.74
	AG	13	33.98±0.95	1.43±0.09	41.74±0.98	1.12±0.10	2.37±0.37
	GG	15	32.69±0.87	1.53±0.09	39.17±0.89	1.07±0.09	2.36±0.33

N : Number of Samples, TLD : Thickness of Longissimus Dorsi, TBF : Thickness of Back Fat, TR : Thickness of Rump, TFR : Thickness of Fat Rump, MS : Marbling Score, PIMF : Percentage intra muskular fat. Means within same row with different superscripts are significant different (P <0.05). Sign* not included in analysis of association

is part of non coding area in DNA. The intron may contain sequences that bind additional transcriptional enhancers or silencers having an affect on transcription. Intron also contain sequences of further regulatory RNAs that may affect the translation and stability of the mRNA and gene. When the mutation occured, RNA can change the gene product. Mutation in these region may influence the gene function. At the level of translation, the introns are involved in regulation of protein production activity. So, mutation in these region having possibility to influence the amount of protein production and function or expression of a gene (Perdew *et al.,* 2006)

Several research of SCD gene have been done in exon 5, Wu *et al.,* (2011) found SNPs at c.10329C>T that was associated with intramuscular fat percentage, the SCD enzyme activity and MUFA concentration in milk in the Italian Holstein (Conte *et al.,* 2006), a melting point in intramuscular fat in Japanese Black cattle (Taniguchi *et al.,* 2004), muscle fat and subcutaneous fat in Fleckvieh cattle (Barton *et al.,* 2009), but did not significantly associated with marbling score in Chinese Simmental cattle (Wu *et al.*, (2011). In addition, the SNP c.10213T> C had association with the high percentage of intramuscular fat in Chinese Simmental cattle (Wu *et al.,*2011). Carcass and meat quality was influenced by several factors including genetic,

species, breed, sex, age, feed including additives (hormones, antibiotics or mineral), and stress (Soeparno 2005).

CONCLUSION

There were 8 SNP consisted of 5 monomorphic SNP and 3 polymorphic SNPs in SCD gene on Bali cattle. SNP g.10428C>T significantly affected to marbling score (MS) and the percentage of intramuscular fat (PIMF), so that SNPs might be used as one of the candidates of MAS in Bali cattle, especially in meat characteristics.

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