

Identification of SNP g.10428C>T of Stearoyl-CoA Desaturase gene related to meat quality in Bali cattle using PCR-RFLP method

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ABSTRAK

Penelitian ini bertujuan untuk menganalisis keragaman Single Nucleotide Polymorphism (SNP) g.10428C>T gen *Stearoyl-CoA Desaturase* (SCD) menggunakan metode PCR-RFLP dan hubungannya dengan karakteristik daging pada sapi Bali. Total 52 sampel darah sapi Bali dari BPTU-HTP Denpasar kemudian dibandingkan dengan sapi Belgian-blue (n=30), Limousine (n=14), Peranakan Ongole (PO) (n=30) serta Wagyu (n=7) digunakan dalam penelitian ini. Keragaman SNP g.10428C>T gen SCD dianalisis dengan teknik PCR-RFLP menggunakan enzim pemotong *HinfI*. Karakteristik kualitas daging pada sapi Bali diduga menggunakan citra ultrasonografi. Frekuensi genotipe dan alel, nilai heterozigositas, dan kesetimbangan Hardy-Weinberg dihitung menggunakan program PopGen 1.32. Asosiasi genotipe dari SNP g.10428C>T gen SCD dengan karakteristik kualitas daging dihitung menggunakan uji-t. Hasil penelitian ditemukan genotipe homozigot (CC dan TT) dan genotipe heterozigot (CT) dari gen SCD|*HinfI* dengan frekuensi alel C (0,26) dan T (0,74) pada sapi Bali. Genotipe CC ditemukan pada sapi Belgian-blue, Limousine, PO, dan Wagyu dengan frekuensi alel C adalah 1,00. Adapun nilai heterozigositas pengamatan ($H_o=0,44$) dan heterozigositas harapan ($H_e=0,39$) yang memperlihatkan keragaman tinggi pada sapi Bali. SNP g.10428C>T gen SCD berasosiasi dengan sifat ketebalan lemak punggung, skor marbling, dan persentase lemak intramuskular. Dengan demikian SNP tersebut memiliki potensi sebagai kandidat marker-assisted selection pada sapi Bali.

Kata Kunci : gen SCD, kualitas daging, PCR-RFLP, sapi Bali, SNP

ABSTRACT

The objectives of this research was to analyze the polymorphisms of SNP g.10428C>T in the SCD gene using PCR-RFLP and its association with meat characteristics in Bali cattle. The number of 52 Bali cattle from BPTU-HTP Denpasar were used in this study and which were compared to Belgian-blue (n=30), Limousine (n=14), Peranakan Ongole (PO) (n=30) and Wagyu (n=7). The polymorphism of the SCD gene was characterized by the use of the PCR-RFLP technique with *HinfI* enzyme restriction. The characteristics of the meat quality in Bali cattle were estimated using ultrasound imaging method. Genotypic and allelic frequencies, heterozygosity values, and the Hardy-Weinberg equilibrium have been calculated using PopGen 1.32. The association between genotypes of SNP g.10428C>T SCD gene and meat quality characteristics was calculated using t-test. The results showed that homozygous genotypes (CC and TT) and heterozygous genotypes (CT) of the SCD|*HinfI* gene with C (0.26) and T (0.74) allele frequencies were and in Bali cattle. The CC genotype was found in Belgian-blue,

Limousine, PO, and Wagyu cattle with the allele C frequency of 1.00. The observed heterozygosity ($H_o=0.44$) higher than expected heterozygosity ($H_e=0.39$) showed high diversity in Bali cattle. Genotypes of SNP g.10428C>T SCD gene were associated with backfat thickness, marbling score, and percentage of intramuscular fat. Thus the SNPs g.10428C>T have potential as marker-assisted selection candidates for Bali cattle.

Keywords: Bali cattle, meat quality, PCR-RFLP, SCD gene, SNP

INTRODUCTION

The potential for both native and local beef cattle and even introduced cattle needs to be optimally utilized. The utilization of the potential of beef cattle that are scattered in Indonesia needs to be done, not only to increase meat production but also the quality of meat. Bali cattle as Indonesian native cattle domesticated from banteng (Martoyo 2012) have the potential to produce high-quality beef (Hafid *et al.* 2019). Suryanto *et al.* (2017) reported that male Bali cattle aged 1.5–2 years had a carcass percentage 54.76%, rib eye area 61.97 cm² and backfat thickness 2.80 mm. According to Jakaria *et al.* (2017) reported that male Bali cattle aged 3 years had, percentage of intramuscular fat 13.48±3.42 mm and marbling score 4.50±1.05. Meat quality is largely determined by several indicators, such as B vitamins, minerals, iron (Martins *et al.* 2018) and includes the characteristics of fatty acids (Nogalski *et al.* 2018).

Meat quality (using marbling score) was controlled by multiple genes such as DGAT1 gene (Li *et al.* 2013), SREBP gene (Barton *et al.* 2010), CAPN1 gene (Li *et al.* 2013) and SCD gene (Ohsaki *et al.* 2009). Stearoyl-CoA desaturase (SCD) is an enzyme produced in the endoplasmic reticulum, which functions to convert saturated SFA into MUFA, especially from palmitic and stearic to palmitoleic and oleic, which are used for triglyceride synthesis (Mauvoisin and Mounier 2011). The SCD gene in cattle have 17088 bp sequence length and consists of 6 exons and 5 introns on chromosome 26 (Ohsaki *et al.* 2009). Several studies have reported that there are an association between stearyl-CoA desaturase (SCD) gene diversity and fatty acid contexture in Wagyu cattle (Taniguchi *et al.* 2004), Chinese Simmental cattle (Wu *et al.* 2012), Holstein cross cattle (Kovalchuk *et al.* 2019), pigs (Estany *et al.* 2014), sheep (Esteves *et al.* 2019) and chickens (Furqon *et al.* 2017).

The relationship between SCD gene diversity and meat quality characteristics is one of the most intensive efforts currently being made to be

used as a candidate for genetic traits or referred to as marker-assisted selection (MAS) (Alwiyah *et al.* 2016). Pereira *et al.* (2008) reported that one method for determining *polymorphism* at the molecular level is Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. The PCR-RFLP method is convenient, time-efficient, especially for specific loci, and does not have to identify unknown mutations or to determine base sequences (Hashim and Al-Shuhaib 2019). Ota *et al.* (2007) The mutation is discriminated by digestion with specific restriction endonucleases and is identified by gel electrophoresis after staining with ethidium bromide (EtBr). SNP g.10428C>T SCD gene was found to be polymorphic in Bali cattle using direct sequencing analysis and significantly associated with the trait of the marbling score and percentage of intramuscular fat (Alwiyah *et al.* 2016). According to Taniguchi *et al.* (2004) the SCD gene is associated with fatty acid composition in meat, IMF deposits (Wu *et al.* 2012) and meat color (Reardon *et al.* 2010). It is important to determine the diversity of SCD genes on SNP g.10428C>T in SCD gene using the PCR-RFLP method in Bali cattle and other beef cattle (Belgian-blue cattle, Limousine cattle, PO cattle, and Wagyu cattle). Based on these reasons, this research was conducted to determine the diversity of SNP g.10428C>T of stearyl-CoA desaturase (SCD) gene in Bali cattle and other beef cattle (Belgian-blue cattle, Limousine cattle, PO cattle, and Wagyu cattle) using the PCR-RFLP method and its relationship with meat quality characteristics.

MATERIALS AND METHODS

Animal and Samples

This study was carried out in the Laboratory of Animal Molecular Genetics, Faculty of Animal Science, IPB University. The samples used consisted of Bali (n=52) from BPTU-HPT Denpasar, Limousine (n=14), and pure breed of

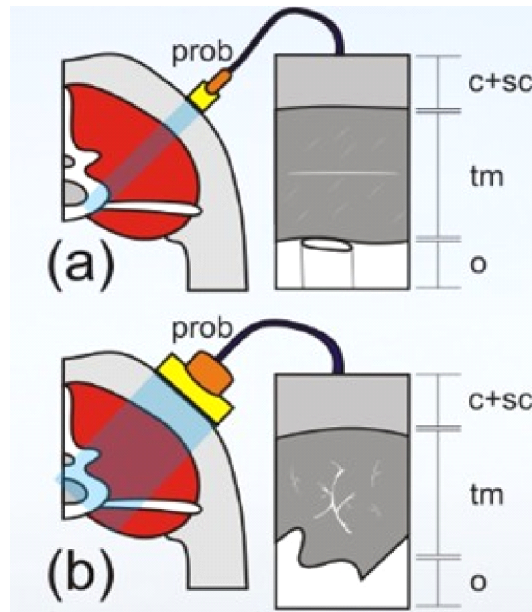


Figure 1. Illustration ultrasound longissimus dorsi in cattle, (a) horizontally (b) vertically, c = cutan, sc = subcutan, tm = thick muscle, o = bone. Source; Ulum *et al.* (2014).

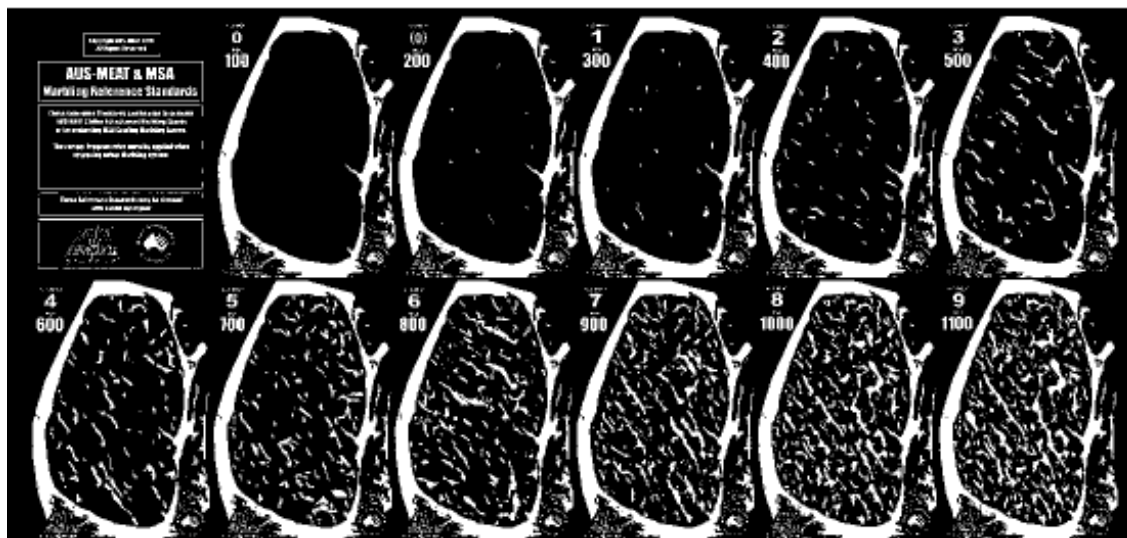


Figure 2. AUS-MEAT and MSA marbling references standards (<http://www.wagyu.org.au/marbling/>).

Belgian-blue (n=30) from BPTU-HPT Padang Mangatas, Peranakan Ongol (n=30) from Kebumen, and pure breed of Wagyu (n=7) from BET Cipelang. The numbers of male Bali cattle used were 52 cattle with an age of 25-35 months.

The meat quality traits analyzed of Bali cattle were carcass and meat characteristics including longissimus dorsi thickness (LDT), backfat thickness (BFT), marbling score (MS)

and percentage of intramuscular fat (PIF) as measured by ultrasonography (Figure 1). Ultrasonographic images were taken at frequencies of 4.5 to 6.5 MHz and depths of 8.8 to 13.0cm. LDT, BFT, MS, and PIF measurements were carried out over ribs 12-13 using Ulum *et al.* (2014) procedures. The ultrasound results were examined using the Image-J NIH software (ImageJ®, NIH, USA). The Marbling Score val-

ue was determined by using the MSA and AUSTRALIAN MEAT (<http://www.wagyu.org.au/marbling/>) (Figure 2).

Genotyping of SCD Gene using PCR RFLP

The SNP g.10428C>T of SCD gene is located on the intron 5 used in this research refers to Alwiyah *et al.* (2016). A pair of primers is 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 according to Ohsaki *et al.* (2009) which partially amplifies exon 5 and intron 5 of SCD gene. The PCR product used consisted of 1 µL DNA sample and 14 µL of premix solution with 0.3 µL of primer, 6.1 µL deionized water, and 7.5 µL of MyTaq HS Red Mix. The PCR amplification using AB System machine with five process consisted of pre denaturation for 1 minutes at 95 °C followed by 35 cycles of denaturation for 15 seconds at 95 °C, annealing for 15 seconds at 51 °C, and extension for 10 seconds at 72 °C, and a final extension for 1 minutes at 72 °C. The PCR products were detected by 1.5% agarose gel electrophoresis by staining with PeqGreen for 35 minutes and visualized using an ultraviolet transilluminator.

The PCR product from the polymorphic region (569 bp) of the SCD gene was excreted with *HinfI* restriction site. The PCR products and *HinfI* restriction enzyme were incubated for 4 hours at 37°C. The products of DNA fragments from PCR-RFLP were interpreted using agarose gel electrophoresis at an intensity of 2% by staining with PeqGreen. Electrophoresis was run for 35 minutes at an average voltage of 100 volts. The fragments were visualized under UV Transilluminator. The PCR-RFLP product consisted CC: 569 bp, CT: 569 bp, 318 bp, and 251 bp, and TT: 318 bp and 251 bp.

Data analysis

Genotype and allele frequencies were analyzed that used genotyping data. Genotype frequency (x_{ii}) and allele frequencies (x_i) was calculated using the formula of Nei and Kumar (2000):

$$x_{ii} = \frac{\sum_{i=1}^n n_{ii}}{N} \quad x_i = \frac{(2n_{ii} + \sum_{i \neq j} n_{ij})}{2N}$$

Where: x_{ii} is the frequency of ii genotype (CC, CT, and TT); x_i is the frequency of i allele (C and T); n_{ij} is the number of the sample of ij geno-

type; n_{ij} is the number of the sample of ij genotype, and N is the population size.

Genetic diversity was calculated using the calculation of the observed heterozygosity value (H_o) and Heterozygosity of expectations (H_e) Nei and Kumar (2000);

$$H_o = \sum_{i \neq j} \frac{N_{1ij}}{N} \quad H_e = 1 - \sum_{i=1}^q x_i^2$$

Where: H_o is heterozygosity of observations; N_{1ij} is number of heterozygous individuals at locus 1, N is total number of samples, H_e is expected heterozygosity, x_i is frequency I, q is total number of samples.

Hardy-Weinberg equilibrium (H-W) (Hartl and Clark 1997):

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

Where: χ^2 is chi-square; O is total of observations genotype to-I; E is total of genotype to expectations to-i.

The association between of the SNP g.10428C>T of SCD gene polymorphism and meat quality traits was performed using t-test procedure to compared between genotypes (Minitab® 18 Software).

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s \sqrt{\left(\frac{1}{n_1}\right) + \left(\frac{1}{n_2}\right)}}$$

$$\text{where } s = \sqrt{\frac{\sum_{i=1}^n (\bar{x}_i - \bar{x}_1)^2 + \sum_{i=1}^n (\bar{x}_i - \bar{x}_2)^2}{n_1 + n_2 - 2}}$$

Where: \bar{x}_1 and \bar{x}_2 is mean of growth traits and meat characteristics of genotypes and 2; n_1 and n_2 is the total of individual genotype 1 and 2, s is combined variance.

Prior to the association analysis, the meat characteristics data were corrected based on age (34 months) and the maintenance system (extensive maintenance) with the attend method (Salamena and Papilaja 2010):

$$X_i \text{ corrected} = \left[\frac{\bar{X}_{\text{standard}}}{\bar{X}_{\text{observations}}} \right] \times X_{\text{observations-i}}$$

Where: X_i corrected = corrected data; $\bar{X}_{\text{standard}}$ = standard group mean; $\bar{X}_{\text{observations}}$ = average of group observations; $X_{\text{observations-i}}$ = value of observations-i.

RESULTS AND DISCUSSION

Diversity of the SCD gene in Beef Cattle

The Stearoyl-CoA desaturase (SCD|*Hinf*I) gene fragment targeting SNP g.10428C>T was successfully amplified at 51°C annealing temperature for 15 seconds with a PCR product length of 569 bp, while the PCR-RFLP genotyping results obtained three types of genotype, i.e. CC, CT and TT genotypes (Figure 4). The results of genotyping from PCR-RFLP analysis of SCD genes in Bali cattle found three genotypes, namely CC genotypes (569 bp), CT genotypes (569 bp, 318 bp, and 251 bp), and TT genotypes (318 bp and 251 bp). Other beef cattle (Belgian-blue cattle, Limousine cattle, PO cattle and Wagyu cattle) only one genotype was found, namely the CC genotype.

Table 1 shows that Bali cattle have the highest TT genotype frequency compared to CT and CC with the allele C and T frequencies 0.26 and 0.74, respectively. Belgian-blue, Limousine, PO and Wagyu cattle breeds only have one genotype and allele, namely genotype CC and allele C, so the frequency of genotype and allele is 1.00. Based on these data, it can be concluded that Bali cattle are polymorphic, while other beef cattle (Belgian-blue cattle, Limousine cattle, PO cattle and Wagyu cattle) are monomorphic. The allele and genotype frequency values equal to or less than 0.99 indicate a diversity of alleles in the population (polymorphic) (Nei and Kumar 2000). This is reinforced by the observed heterogeneity values (H_o) and expectations (H_e), namely 0.44 and 0.39, respectively (Table 2).

The results of the Hardy-Weinberg balance

Table 1. Genotype and Allele Frequencies of SNP g.10428C>T SCD gene

Breed	N	Genotype Frequency			Allele Frequency	
		CC	CT	TT	C	T
Bali	52	0.038 (2)	0.442(23)	0.519(27)	0.260	0.740
Belgian Blue	30	1.000 (30)	0.000(0)	0.000(0)	1.000	0.000
Limousine	14	1.000 (14)	0.000(0)	0.000(0)	1.000	0.000
PO	30	1.000 (30)	0.000(0)	0.000(0)	1.000	0.000
Wagyu	7	1.000 (7)	0.000(0)	0.000(0)	1.000	0.000

The numbers shown in parenthesis are the number of cattle with the genotype specified.

Table 2. Heterozygosity values and the results of the Hardy-Weinberg equilibrium

Breed	n	Heterozigositas		χ^2 test
		H_o	H_e	
Bali	52	0.442	0.388	1.044 ^{ns}
Belgian Blue	30	1.000	0.000	na
Limousine	14	1.000	0.000	na
PO	30	1.000	0.000	na
Wagyu	7	1.000	0.000	na

n = individual number, χ^2 = Chi-square ($\chi^2 < 3.84$, db 1), degree of freedom (df). ns = not significant ($P > 0.05$), na = not applicable

Table 3 Association of SCD gene diversity on SNP g.10428 C>T with meat quality

Characteristics	Genotype gene SCD ($\bar{X} \pm$ Std Dev)			P value		
	CC (n=2)	CT (n=23)	TT (n=27)	CC vs CT	CC vs TT	CT vs TT
LDT (mm)	57.43±6.30	51.40±12.30	51.40±13.60	0.450 ^{ns}	0,449 ^{ns}	0,990 ^{ns}
BFT (mm)	1.48±0.35	1.84±0.39	2.21±0.42	0.407 ^{ns}	0,222 ^{ns}	0,004*
MS	0.84±0.04	1.27±0.42	1.27±0.45	0.001*	0,001*	0,997 ^{ns}
PIF (%)	0.93±0.03	2.02±1.05	1.98±1.09	0.001*	0,001*	0,890 ^{ns}

n = individual number, LDT = Longissimus Dorsi Thickness, BFT = Backfat Thickness, MS = Marbling Score, PIF = Percentage of intramuscular fat, * = significantly ($P < 0.05$), ns = not significant ($P > 0.05$)

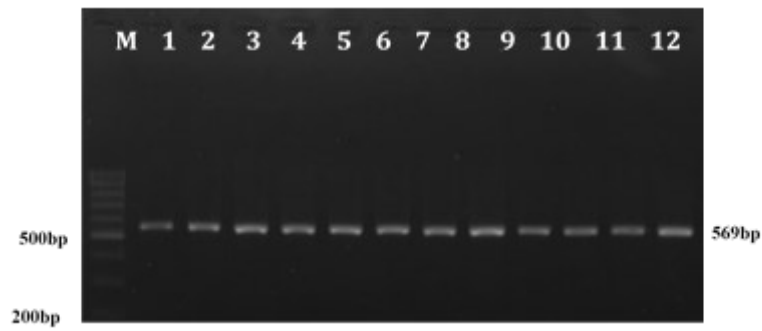


Figure 3. PCR product of SCD gene amplification using 1.5% agarose (M = marker 100 bp; 1-12 = individual samples).

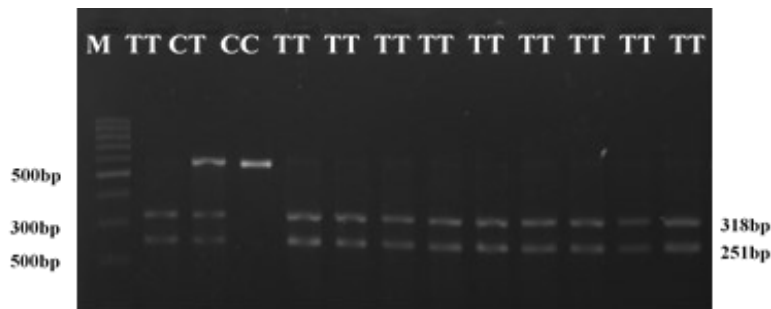


Figure 4. PCR-RFLP technique for genotyping of SCD gene fragments (M = marker 100 bp; CC, CT and TT genotypes).

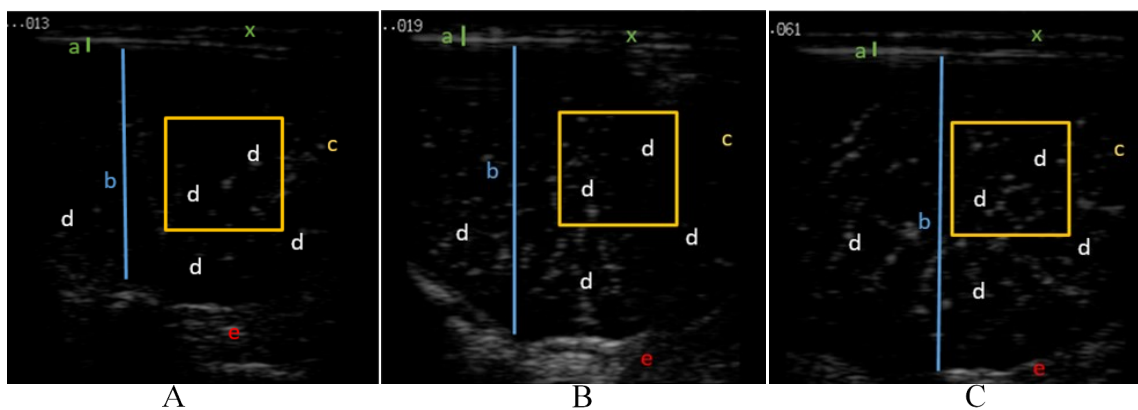


Figure 5. Ultrasound image of 12-13th ribs of Bali cattle in a vertical position. (A). genotype CC (B). genotype CT (C). genotype TT. Subcutaneous fat layer (a). Longissimus dorsi thickness (b). percentage measurement area of 15x15 mm (c) PIF (d). Bone (e). Leather (X).

test on Bali cattle were not significant ($P > 0.05$), indicating that the genotype frequency of the Stearoyl-CoA desaturase (SCD) gene in the Bali cattle population was in balance. Chesnokov and Artemyeva (2015) clarified, a population with H_0

$> H_e$ means that in a population there is random mating (random mating), on the other hand, if $H_0 < H_e$ indicates inbreeding. These results indicate that the Bali cattle population occurs randomly (random mating). Noor (2010) stated, The fac-

tors that influence the balance of allele and genotype frequencies of a population are non-random mating, selection, migration, mutation, and genetic drift. Allendorf *et al.* (2010) reported that the population is in a balanced state because the genotype and allele frequencies are stable from generation to generation.

Association of the SCD Gene with Meat characteristics of Bali Cattle

The catalyst descriptions (LDT, BFT MS, and PIF) of meat were only carried out on Bali cattle, while Belgian-blue cattle, Limousine cattle, PO cattle and Wagyu cattle were not analyzed because they were monomorphic. Association of SCD gene diversity on SNP g.10428 C>T with meat quality in Bali cattle are presented in Table 3. Bali cattle with TT genotypes had higher values for backfat thickness traits, compared to the CC and CT genotypes (Figure 5). The association results showed that the SCD gene at SNP g.10428 C>T had significant impact with backfat thickness, marbling score, and presentation of intramuscular fat (Table 3). In addition, Alwiyah *et al.* (2016) where the SCD gene was associated with marbling score and presentation of intramuscular fat in Bali cattle. According to Li *et al.* (2013), Stearoyl-CoA desaturase (SCD) is one of the genes associated with the fatty acid composition that is solely related to the meat quality. The SCD gene was reported in association with the fatty acid contents of local meat in Ciamis (Hilmia *et al.* 2016), the fatty acid profile of Japanese Black beef (Taniguchi *et al.* 2004), and presentation of intramuscular fat of Simmental Chinese cattle (Wu *et al.* 2012).

The quality of beef in cows is built by several factors, namely gender, age, feed, livestock, housing system, pre-slaughter treatment, and genetics (Raza *et al.* 2019). The genetic influence of carcasses and quality of meat in cattle is relatively large (Ceacero *et al.* 2016). This can be seen from the heritability value of the quality traits of carcass and meat. Heritability value is the inheritance of a trait from parents in offspring that can be used for selection. The parameter of a value that has a high heritability value can be used as the basis for the individual selection method. This will affect the selection accuracy and the selection response which is expected to be greater than the traits with low heritability (Soeroso *et al.* 2009). Information on genetic and molecular parameters based on the SCD gene as candidate markers is expected that

the accuracy of selection according to the desired trait will be better and have a more significant effect.

CONCLUSION

The SCD|*HinfI* gene (SNP g.10428C>T) was monomorphic in Belgian-blue, Limousine, PO, and Wagyu cattle, which was polymorphic in Bali cattle. The SCD|*HinfI* gene (SNP g.10428C>T) contains three genotypes, such as CC, CT, and TT. The SNP g.10428C>T SCD gene was associated with backfat thickness (BFT), marbling score (MS) and percentage of intramuscular fat (PIF). The SNP g.10428C>T SCD gene has the potential as a candidate marker for the selection of Bali cattle with better meat quality.

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