The 84-bp Indel Polymorphism of The Sterol Regulatory Element-Binding Protein 1 (SREBP1) Gene in Several Cattle Breeds in Indonesia

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ABSTRAK

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Gen *Sterol regulatory element-binding protein 1* (*SREBP1*) merupakan gen pengkode SREBP1, salah satu famili faktor transkripsi yang memiliki peran kunci dalam homeostasis lipid serta metabolisme asam lemak. Penelitian ini bertujuan untuk mendeteksi polimorfisme indel 84-bp di daerah intron 5 gen SREBP1 (84-bp indel) pada beberapa bangsa sapi di Indonesia. Hasil penelitian ini berguna dalam seleksi genetik berbasis molekuler untuk produksi daging sehat pada sapi. Penelitian ini menggunakan total 740 ekor dari enam bangsa sapi (Limousin, Simmental, Friesian Holstein, Bali, Sumbawa dan Pasundan) yang berasal dari dua Balai Inseminasi Buatan Nasional dan peternak rakyat. Polimorfisme 84-bp indel dideteksi menggunakan metode polymerase chain reaction (PCR) dan divisualisasikan menggunakan sistem elektroforesis gel agarosa. Hasil penelitian menunjukkan alel L (tipe insersi) merupakan alel yang umum dan ditemukan terfiksasi pada sapi Holstein-Friesian, Bali dan Sumbawa (1,00). Sedangkan alel S (tipe delesi) ditemukan pada sapi Limousin, Simmental dan Pasundan dengan frekuensi berturut-turut 0,24; 0,16 dan 0,01. Keberadaan alel S pada sapi Pasundan hanya ditemukan pada genotipe heterozigot LS (0,03). Kesimpulannya, gen *SREBP1* (84-bp indel) ditemukan dalam kondisi polimorfik pada sapi Limousin, Simmental dan Pasundan, tetapi monomorfik pada sapi Friesian-Holstein, Bali dan Sumbawa.

Kata Kunci: Asam Lemak, Indel, Sapi Lokal Indonesia, Gen *SREBP1*

ABSTRACT

Anwar S, Putra WPB, Khaerunnisa I, Wulandari AS, Prihatin KW, Sutikno. 2023. The 84-bp Indel Polymorphism of *Sterol Regulatory Element-Binding Protein 1* (*SREBP1*) Gene in Several Cattle Breeds in Indonesia. JITV 28(2):102-111. DOI:http://dx.doi.org/10.14334/jitv.v28.i2.3-46.

Sterol regulatory element-binding protein 1 (*SREBP1*) gene is a gene that encodes SREBP1, a family of transcription factors that have a key role in lipid homeostasis as well as fatty acid metabolisms. The present study aimed to detect the 84-bp indel polymorphism in the intron 5 of the SREBP1 gene (84-bp indel) in several cattle breeds in Indonesia. A total of 740 cattle of six breeds (Limousin, Simmental, Holstein-Friesian, Bali, Sumbawa, and Pasundan) from two National Artificial Insemination Centers (NAICs) and smallholder farmers were used in this study. The detection of 84-bp indel polymorphism was performed using the polymerase chain reaction (PCR) method and visualized through a gel agarose electrophoresis system. The study showed that the L allele (insertion-type) was common and is fixed in Holstein-Friesian, Bali, and Sumbawa cattle (1.00). Meanwhile, the S allele (deletion-type) was found in Limousin, Simmental, and Pasundan cattle with a frequency of 0.24, 0.16, and 0.01, respectively. The presence of the S allele in Pasundan cattle was only found in the form of the heterozygous LS genotype (0.03). In conclusion, the 84-bp indel of the SREBP1 gene was found to be polymorphic in Limousin, Simmental, and Pasundan cattle, but monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle.

Key Words: Fatty Acid, Indel, Indonesian Local Cattle, *SREBP1* Gene

INTRODUCTION

The *sterol regulatory element-binding proteins* (SREBPs) are transcription factors that play a key role in lipid homeostasis (Eberlé et al. 2004). These transcription factors are recognized as master regulators of cholesterogenesis and lipogenesis in mammals since their expression was found to influence transcriptional activation in more than 30 genes encoding enzymes for cholesterol, fatty acid (FA), triglyceride (TG), and phospholipid synthesis (Eberlé et al. 2004). The SREBPs bind to sterol regulatory elements (SREs) in the promoter regions of target genes to stimulate transcription (Shimano & Sato 2017).

There are three SREBP isoforms, including SREBP1a, SREBP1c, and SREBP2 (Bionaz et al. 2020). SREBP1a and 1c regulate genes involved in fatty acid and TG synthesis, while SREBP2 primarily activates genes involved in cholesterol biosynthesis (Deng et al. 2014). Furthermore, both SREBP1a and 1c are encoded by the same gene (*SREBP1*) but their transcripts are produced from alternative splicing that differs in the first exon (exon 1a and exon 1c) (Eberlé et al. 2004). However, SREBP1c is the predominant SREBP subtype expressed in most animal tissues (Deng et al. 2014).

The *sterol regulatory element-binding transcription protein 1* (*SREBP1*) gene has several synonyms: *ADD1*, *SREBF1*, and *SREBP-1* gene (NCBI, 2021). The polymorphism of the *SREBP1* gene in cattle was first reported by Hoashi et al. (2007) in Japanese Black and Holstein-Friesian cattle. They found no polymorphisms in the full-length coding sequence (CDS) region. However, they found the 84-bp indel polymorphism in intron 5 of the SREBP1 gene (84-indel) in Japanese Black cattle with two allele types. The 84-bp insertion is denoted as a long-type (or L allele), while the 84-bp deletion as a short-type (or S allele). These studies were then expanded to other cattle breeds.

SREBP1 or also known as sterol regulatory element binding transcription factor 1 (SREBF1) is also essential in the fat metabolism of ruminants because it also influences the fat characteristics of both meat and dairy products. This has been demonstrated in cattle (Harvatine & Bauman 2006; Proskura et al. 2017; Gamarra et al. 2021; Kawaguchi et al. 2021), buffalo (Deng et al. 2017; Xu et al. 2019), goat (Xu et al. 2016), and sheep (Tsiplakou et al. 2015; Liang et al. 2020; Carcangiu et al. 2021). In bovine, the *SREBP1* gene is located on chromosome 19 with 21 exons and encodes 1183 amino acids (Hoashi et al. 2007). The sequence of bovine SREBP1 mRNA has a high similarity (82%) to the SREBP1c isoform of hominids (Human and chimpanzee) (Gamarra et al. 2021). In addition, SREBP1 expression was positively correlated with FA in several cattle breeds (Gamarra et al. 2018). The differential mRNA expression of *SREBP1* has been reported in different tissues (Bhuiyan et al. 2009; Li et al. 2018) and different cattle breeds (Gamarra et al. 2018). The increased expression of *SREBP1* indicates an elevated capacity of *de novo* synthesis of FA, which leads to an enhanced lipid accumulation in bovine muscle (Li et al. 2018). *In vitro* studies in bovine hepatocytes and mammary epithelial cells showed that silencing or knocking down the SREBP1c gene decreased lipogenic gene expression and caused a decrease in lipid or FA synthesis (Ma & Corl 2012; Deng et al. 2014). In addition, lipopolysaccharides (LPS) from bacterial endotoxin can decrease milk fat synthesis via decreasing expression of SREBP1 in dairy cows (Wang et al. 2018). Therefore, the *SREBP1* gene is one of the promising candidate genes that determine FA composition and fat-related traits both in the meat of beef cattle (Han et al. 2013; Xu et al. 2013) and milk in dairy cattle (Harvatine & Bauman 2006; Nafikov et al. 2013; Li et al. 2014). Furthermore, SREBP1 together with other key FA metabolism-associated genes can be used for the genetic selection of healthy meat in beef cattle (Zhou et al. 2022).

Several studies have found several variants of the *SREBP1* gene that are spread in both coding and non-coding regions (Hoashi et al. 2007; Huang et al. 2011; Rincon et al. 2012; Lee et al. 2013). An 84-bp indel polymorphism (84-bp indel) in intron 5 of the *bovine SREBP1* gene was first reported by Hoashi et al. (2007). This polymorphism is popular and frequently found to affect the FA composition in several cattle breeds: Japanese Black cattle (Hoashi et al. 2007; Ohsaki et al. 2009), Hanwoo (Bhuiyan et al. 2009), Simmental (Xu et al. 2013), and Angus × Charolais crossbred (Han et al. 2013). The S allele (84-bp deletion) of the *SREBP1* gene has been found to contribute to elevated levels of good fatty acid than the L allele, and vice versa. Despite this, the polymorphism has also been found to affect carcass weight (Ohsaki et al. 2009) and growth traits in beef cattle (Huang et al. 2011)

Determinations of the genetic diversity of the 84-bp indel of the *SREBP1* gene among various cattle breeds in Indonesia is important to obtain the genetic marker for meat quality, especially for FA composition. However, information regarding the genetic diversity of the *SREBP1* gene in Indonesian cattle is limited. Therefore, the purpose of this study was to detect the genetic polymorphism of the 84-bp indel of the *SREBP1* gene in several cattle breeds in Indonesia i.e., Limousin, Simmental, Holstein-Friesian, Bali, Sumbawa, and Pasundan cattle breeds.

MATERIALS AND METHODS

DNA samples

This study involved a total of 740 animals, where 202 out of the samples of them were in the form of frozen semen collected from bulls at the two National Artificial Insemination Centers (NAICs) of Indonesia, i.e. Lembang and Singosari (consisting of 100 Limousin, 62 Simmental, and 40 Holstein-Friesian). An amount of 538 samples were collected from the blood of heifers and bulls kept by small-holder farmers (consisting of 100 Holstein-Friesian, 100 Sumbawa, 180 Bali, and 158 Pasundan). The sampling locations are presented in Table 1. Genomic DNA was extracted from both frozen semen and whole-blood samples by using a gSYNC DNA extraction kit (Genaid Biotech

Table 1. Sampling location for blood samples of cattle kept by small-holder farmers

Breed	Sampling Location (Districts)	n
Holstein-Friesian	Bogor, Enrekang, Sukabumi, Tasikmalaya	100
Sumbawa	Sumbawa	100
Bali	Barru, Enrekang, Klungkung	180
Pasundan	Ciamis, Majalengka, Pangandaran, Tasikmalaya	158

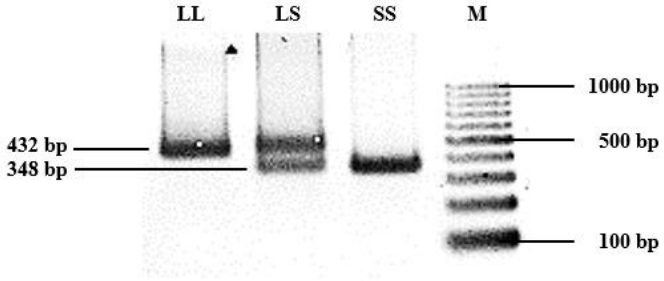

Figure 1. Visualization of the 84-bp indel polymorphism amplification products of the *SREBP1* gene in a 2% agarose gel (w/v). M: 100-bp DNA ladder. It showed genotypes of LL (432 bp), SS (348 bp), and LS (432 bp and 348 bp)

Ltd., Taiwan) and a blood/cell DNA mini kit (Genaid Biotech Ltd., Taiwan), respectively. All collected genomic DNA products were stored at -20 °C until further analysis was performed.

Genotyping

Genotyping of the 84-bp indel polymorphism in intron 5 of the bovine *SREBP1* gene (84-bp indel) was performed using the PCR amplification method. The primer pairs are according to Hoashi et al. (2007) with modification as follows: SREBP1-F: 5'-CCA CAA CGC CAT CGA GAA ACG CTA C -3' and SREBP1-R: 5'-GGC CTT CCC TGA CCA CCC AAC TTA G -3'. The PCR reactions were carried out in a total volume of 10 µL containing 10-12 ng/µL of DNA template, 4 µL of Go Taq Green Mastermix (Promega, USA), 0.2 µM of each primer (forward and reverse), and nuclease-free water up to a final volume of 10 µL. The PCR reaction was accomplished in a gradient thermal cycler (Eppendorf, Germany) under the following cycling conditions: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 95°C for 25 s, primer annealing at 59°C for 25 s, extension at 72°C for 25 s and final extension at 72°C for 5 min. The PCR products were electrophoresed in 2% of agarose gel at 100 V for 30 min and stained with GelRed®10,000X solution (Biotium, USA). The amplicons were visualized with a G-BOX Gel Documentation System (Syngene, UK).

The genotype of each individual was determined based on the pattern and size of the fragments observed in the gel. The 100-bp DNA ladder will be used as the standard for determining fragment size estimations. The LL genotype was indicated by the presence of one 432 bp fragment. The SS genotype is indicated by the presence of one 348 bp fragment due to an 84 bp deletion. Meanwhile, the LS genotype was shown with two fragments of 432 and 348 bp respectively. The representation of the fragment patterns of each genotype is shown in Figure 1.

Sequencing

The presence of 84-bp indel polymorphism was confirmed by forward and reverse sequencing reactions from one representative sample of different genotypes (LL, LS, and SS). The amplified products were sent to 1st BASE Sanger Sequencing services (Apical Scientific Sdn Bhd., Malaysia) for sequencing using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and capillary electrophoresis in ABI PRISM®310 Genetic Analyzer (Applied Biosystems, USA). The sequence data of the *SREBP1* gene in this study were then aligned with NCBI genome reference of NC_037346.1 and Genbank Accession number of AB355704.1 (as a representation of 84-bp insertion type) and AB355705.1 (as a representation of 84-bp deletion type) using the BioEdit software version 7.2 (Hall 1999).

Data analysis

Genetic diversity parameters were genotype frequencies (LL, LS, and SS), allele frequencies (L and

S), Hardy-Weinberg Equilibrium test, observed heterozygosity (Ho), and expected heterozygosity (He). All parameters were performed using GenAlEx v6.5 (Peakall & Smouse 2012).

RESULTS AND DISCUSSION

In the present study, specific DNA fragments of the bovine *SREBP1* gene were successfully amplified by producing three genotypes: LL (432 bp), LS (432 and 348 bp), and SS (348 bp) (Figure 1). An 84-bp indel polymorphism was also confirmed in this study by DNA sequence analysis (Figure 2). This polymorphism could be traced with rsID number rs133958066 at dbSNP database, which is relatively located between 34643011 to 34643094 (g.34643012_34643095del or c.1065+85_1065+168del) based on the bovine genome assembly NC_037346.1 (transcript ID: NM_0011113302.1) from the NCBI database or ENSBTAG00000007884 (transcript ID: ENSBTAT000) from the Ensembl database. This is equivalent to a base position between 84 to 167 under Genbank Accession No. AB355704.1 (AB355704.1:g.85_167del).

The genotype and allele frequencies of the 84-bp indel polymorphism are displayed in Table 2. In this study, the L allele was found to be dominant in all cattle breeds and was fixed in Holstein-Friesian (H-F), Bali, and Sumbawa cattle. Conversely, the S allele, as a minor allele, was detected in Limousin, Simmental, and Pasundan cattle with frequencies of 0.24, 0.16, and 0.01, respectively. As stated by Allendorf and Luikart (2007), a locus is considered polymorphic if the most common allele is at a frequency less than 99% (or 0.99). This suggests that the 84-bp indel locus was found to be polymorphic in Limousin, Simmental, and Pasundan cattle, while it was found to be monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle. Additionally, while the frequency of homozygous SS genotypes was low in all breeds studied, Limousin cattle had the highest frequency of SS genotypes (0.07).

The previous studies on the genotype and allele frequencies of the 84-bp indel polymorphism in various cattle breeds are presented in Table 3. Our study supports previous findings, which showed the fixation of the L allele in H-F cattle (0.00) (Hoashi et al. 2007; Kaneda et al. 2011; Proskura 2013) indicating that the S allele was not present in this breed. On the other hand, Japanese Black cattle showed the highest frequency of the S allele among the breeds studied. The high frequency of the S allele (0.21) in Snow Dragon beef cattle is believed to originate mainly from Wagyu cattle, as Snow Dragon beef cattle are the result of crossbreeding between Wagyu (as a terminal-paternal breed) and F1-crossbred cows (Limousin x Fuzhou yellow cattle) (Liu et al. 2012). The frequencies of the S allele in Limousin (0.24) and Simmental (0.16) cattle in our study are consistent with previous studies on Limousin (0.22) (Bhuiyan et al. 2009) and Simmental (0.17) (Xu et al. 2013) cattle, respectively. However, Bhuiyan et al. (2009), did not detect the S allele in Simmental cattle (0.00) which might be due to the smaller sample size (n=49) compared to the study conducted by Xu et al. (2013) (n=314). As stated by Hartl (1988), the allele frequency within a given sample is an estimate of the true population allele frequency, and therefore, the larger the sample size, the more accurate the estimate. Based on this, Hartl (1988) recommended using a sample of 100 individuals or more whenever possible. Meanwhile, the S allele was at a very low frequency in Pasundan cattle (0.01). This has also been observed in Brahman (0.05) and Red Chittagong (0.01) (Bhuiyan et al. 2009), as well as Canadian crossbreeds (0.01) (Han et al. 2013).

Table 2. Genotype and allele frequencies of the 84-bp indel polymorphism in the *SREBP1* gene in various cattle breeds in Indonesia

Breed	N	Genotype frequency			Allele Frequency		$\chi 2$	Prob	Sig	Ho	He
		LL (n)	LS (n)	SS (n)	L	S					
Limousin	100	0.58 (58)	0.35 (35)	0.07 (7)	0.76	0.24	0.291	0.590*	ns	0.350	0.370
Simmental	62	0.69 (43)	0.29 (18)	0.02 (1)	0.84	0.16	0.331	0.565*	ns	0.290	0.271
Holstein-Friesian	140	1.00 (140)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	-	-	-
Bali	180	1.00 (100)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	-	-	-
Sumbawa	100	1.00 (100)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	-	-	-
Pasundan	158	0.97 (154)	0.03 (4)	0.00 (0)	0.99	0.01	0.026	0.872*	ns	0.025	0.025

N= number of individual samples, χ^2= Chi-squared value, prob= probability, ns = not significant. *The probability of the observed chi-squared value being greater than 0.05 (P>0.05) means that the population is in Hardy-Weinberg Equilibrium, H = Observed heterozygosity, He= Expected heterozygosity.

Table **3**. Genotype and allele frequencies of the 84-bp indel polymorphism in the *SREBP1* gene in various cattle breeds reported in previous studies

Breed	N	Genotype frequency			Allele Frequency		Ho*	He*	Ref
		LL (n)	LS (n)	SS (n)	L	S			
Japanese Black[1]	72	0.39 (28)	0.49 (35)	0.12 (9)	0.63	0.37	0.49	0.46	Hoashi et al. (2007)
Japanese Black[2]	606	0.16 (98)	0.72 (437)	0.12 (71)	0.52	0.48	0.72	0.50	Hoashi et al. (2007)
Japanese Black[3]	417	0.46 (190)	0.47 (196)	0.07 (31)	0.69	0.31	0.47	0.43	Ohsaki et al. (2009)
Japanese Black[4]	233	0.45 (106)	0.43 (101)	0.11 (26)	0.67	0.33	0.43	0.44	Ohsaki et al. (2009)
Japanese Black[5]	480	0.24 (115)	0.49 (235)	0.27 (130)	0.49	0.51	0.49	0.50	Matsuhashi et al. (2011)
Japanese Black[6]	539	0.47 (256)	0.41 (219)	0.12 (64)	0.68	0.32	0.44	0.44	Sasazaki (2021)
Holstein-Friesian	96	1.00 (96)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Hoashi et al. (2007)
Holstein-Friesian	30	1.00 (30)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Kaneda et al. (2011)
Holstein-Friesian	175	1.00 (175)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Chinese Holstein	94	1.00 (94)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Huang et al. (2011)
Bos indicus[7]	30	1.00 (30)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Kaneda et al. (2011)
Jersey	50	1.00 (50)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Charolais	43	1.00 (43)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Angus	49	1.00 (49)	0.00 (0)	0.00 (0)	1.00	0.00	-	-	Proskura (2013)
Montbéliarde	187	0.55 (103)	0.41 (77)	0.04 (7)	0.76	0.24	0.41	0.37	Proskura (2013)
Nanyang	265	0.85 (226)	0.15 (39)	0.00 (0)	0.93	0.07	0.15	0.14	Huang et al. (2011)
Qinchuan	235	0.90 (211)	0.10 (24)	0.00 (0)	0.95	0.05	0.10	0.10	Huang et al. (2011)
Jiaxian Red	441	0.73 (322)	0.27 (119)	0.00 (0)	0.87	0.13	0.27	0.23	Huang et al. (2011)
Hanwoo	62	0.52 (46)	0.40 (35)	0.08 (7)	0.72	0.28	0.40	0.40	Bhuiyan et al. (2009)
Hanwoo	348	0.59 (206)	0.30 (104)	0.11 (38)	0.74	0.26	0.30	0.38	Lee et al. (2013)
Canadian crossbred[8]	225	0.98 (220)	0.02 (5)	0.00 (0)	0.99	0.01	0.02	0.02	Han et al. (2013)
Simmental (bulls)	314	0.67 (212)	0.32 (99)	0.01 (3)	0.83	0.17	0.31	0.28	Xu et al. (2013)
Snow Dragon Black	128	0.62 (79)	0.34 (44)	0.04 (5)	0.79	0.21	0.34	0.33	Xu et al. (2013)

N= number of individual samples; [1]Collected from all over regions in Japan; [2]Collected from progeny testing station at Hokkaido and Hiroshima; Japan, [3]Collected from field populations in Miyazaki, Japan; [4]Collected from all over Japan for field progeny testing carried out by the Wagyu Registry Association; [5]Collected from commercial populations in Gifu, Japan; [6]Collected from Hyogo Prefecture, Japan; [7]Consisted of 10 Cambodian, 10 Myanmar, and 10 Laotian native cattle; [8]Crossbred (Angus x Charolais) commercial steers; *Calculated from genotypic and allelic data in cited references

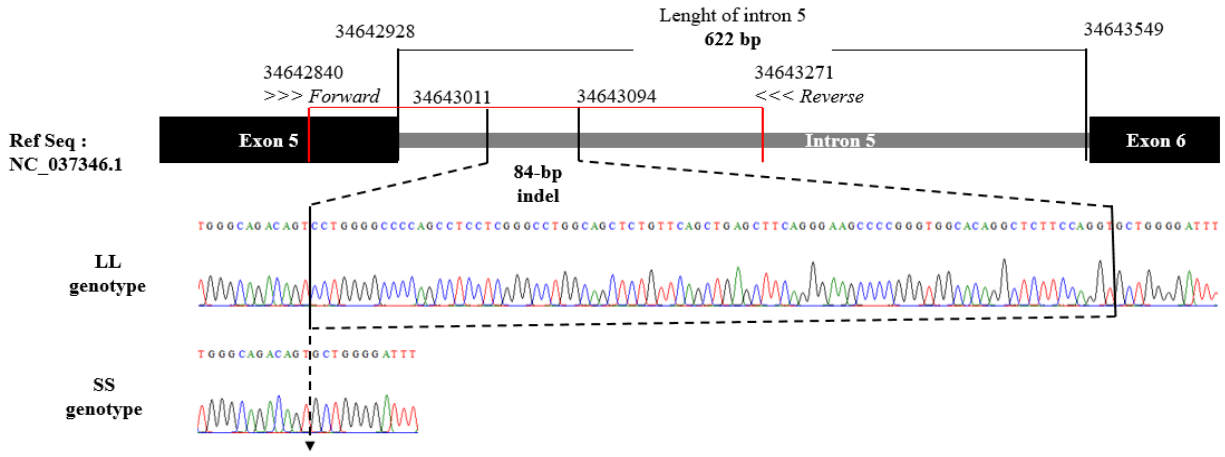

Figure 2. Chromatogram sequence comparison between long-type (LL genotype) and short-type (SS genotype) sequences of 84-bp indel polymorphism in intron 5 of the bovine *SREBP1* gene. Redline is the position of flanking primers (forward and reverse) used in the present study. The chromatogram showed the absence of an 84-bp sequence in the SS genotype.

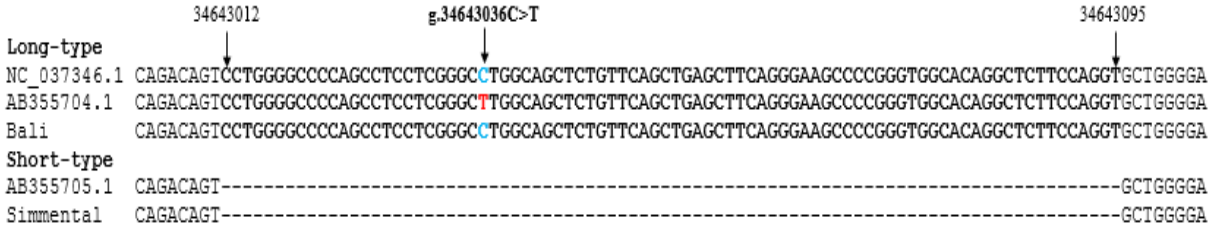

Figure 3. Sequence alignment of long-type and short-type of 84-bp indel polymorphism of the bovine *SREBP1* gene between Genbank (NC_037346.1, AB355704.1, and AB355705.1) and representative sample sequences in the present study (Bali and Simmental). The 84-bp insertion sequences are indicated in black bold letters. Novel SNP is indicated in blue and red letters.

The observed heterozygosity (Ho) values for Limousin, Simmental, and Pasundan cattle were 0.35, 0.29, and 0.02, respectively. This suggests that the level of heterozygosity was low in Pasundan cattle, while Limousin and Simmental cattle had a higher Ho value compared to Nanyang (0.15), Qincuan (0.10), and Canadian crossbred cattle (0.02). On the other hand, Japanese Black cattle had the highest Ho value ranging from 0.43 to 0.72 among all the cattle breeds studied which ranged from 0.02 to 0.41. Therefore, the 84-bp indel marker has a high potential to be used in marker-assisted selection in Japanese Black cattle.

The frequency of the L allele was observed to be higher than that of the S allele in all cattle breeds studied. Gamarra et al. (2021), speculate that the presence of the S allele in some *Bos taurus* breeds could have appeared after *Bos indicus* and *Bos taurus* species differentiation around 1.7 to 2 million years ago. The S allele is thought to be specific and may not be segregating widely among different cattle breed populations as has been declared in several previous reports (Hoashi et al. 2007; Bhuiyan et al. 2009). To enrich the information on S and L allele frequencies in various breeds, we also analyzed the genotypes of other bull breeds in NAICs, including Angus (n=9), Brangus (n=9), Ongole (n=22), Brahman (n=6), Aceh (n=4), and Madura (n=5). The results indicated that the S allele was not found in all of the bulls (unpublished data). Therefore the S allele tends to be absent in *Bos indicus* breeds as observed in Cambodian, Myanmar, and Laotian native cattle (Kaneda et al. 2011) as well as in Sumbawa cattle. In addition, our study provides new information that the S allele was also absent in the *Bos javanicus* species (Bali cattle). Meanwhile, the presence of a small number of individuals carrying the S allele in Pasundan cattle (*Bos indicus* x *Bos javanicus*) could not be explained with certainty whether the S allele was segregated in the population or due to crossbreeding with *Bos taurus* cattle as presumed in *F94L-MSTN* mutation (Anwar et al. 2020).

The SREBP family of transcription factors plays a crucial role in regulating the transcriptional activation of genes involved in the synthesis of fatty acids, particularly those that contain the sterol regulatory element (SRE). SREBPs bind to SREs located in the promoter regions of target genes, thus affecting gene expression. Target genes of SREBP1c isoform as in bovine, including low-density lipoprotein (LDL) receptor, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), insulin-induced gene-1 (INSIG1), S14, glucokinase (GK), phosphoenolpyruvate carboxykinase (PEPCK),

and sodium/iodide symporter (NIS) which are important in milk fat synthesis, glucose synthesis and lactation in cattle (Rincon et al. 2012; Li et al. 2014; Wen et al. 2016). Target gene activation by SREBP has been demonstrated in several studies. The study conducted by Wen et al. (2016) proved that there was a decrease and increase in mRNA expression and protein concentration of NIS through inhibition, silencing, and overexpression of SREBP and by reporter gene and DNA-binding assay experiments. A study by Gamarra et al. (2018) showed a significant positive correlation between SREBP1 and SCD1 gene expression levels in several cattle breeds. The important role of SREBP was also shown in the study of Han et al. (2018) where the promoter activity of FASN2 and FASN3 was due to overexpression of SREBP1. However, this effect can be enhanced by the co-overexpression of SCAP + SREBP1, where SCAP is an essential protein for SREBP activation. However, the 84-bp indel polymorphism of SREBP1 itself was found to not affect SCD1 gene expression. Furthermore, Hoashi et al. (2007) found no interaction between one SNP in the SCD1 gene and the 84-bp indel polymorphism of the SREBP1 gene in influencing the percentage of monounsaturated fatty acids (MUFA) and melting points in intramuscular fat. This suggests that the 84-bp indel might influence the transcription efficiency of SREBP1 itself and thus have an indirect effect on the quality of fat in cattle.

Although it is unclear the effect of the presence of the 84-bp indel in intron 5 of the SREBP1 gene on gene regulation, the association between 84-bp indel polymorphism and FA composition in several beef cattle breeds has been reported in several previous studies. In general, the S allele was favored as it is significantly associated with increased levels of healthy FAs (Hoashi et al. 2007; Bhuiyan et al. 2009; Gamarra et al. 2021). Even though some studies found no significant effect in commercial Japanese Black, and commercial Korean cattle (Matsuhashi et al. 2011; Lee et al. 2013) and inconsistent results between progeny testing and field populations in Japanese black cattle (Ohsaki et al. 2009). This could be due to the relatively small effect of the *SREBP1* gene on the field population (Ohsaki et al. 2009) or may have a different linkage phase between the DNA marker and the causative mutations in different beef cattle populations (Han et al. 2013).

In addition to FA compositions, the 84-bp indel was also associated with growth and carcass traits and body size in some cattle breeds. Ohsaki et al. (2009) found that the SS genotype has a higher carcass weight ($P = 0.0451$) than LS and LL genotypes in Japanese Black cattle. Huang et al. (2011), reported that the LS genotype had significantly higher birth weight, body weight, and average daily gain compared to the LL genotype at birth, 6-, 18-, and 24-month old ($P < 0.05$ or $P<0.01$) in Nanyang cattle. A recent study showed that *SREBP1* gene 84-bp indel was significantly associated with back fat thickness, ultrasound *longissimus muscle* depth, and body size traits in Chinese Qincuan cattle (Gao et al. 2022). In contrast, in the case of Xinjiang brown dairy cattle, Peng et al. (2020) found that 84-bp indel was significantly associated with 305-day milk production and milk protein yield in the second and third parity, but the SS genotype tended to be lower than the LS and LL genotypes.

Introns are non-coding sequences and therefore variations in the intronic regions are considered to have less functional significance compared to variations in the exonic regions in determining specific traits. Despite this, some studies have demonstrated an association between variants in the intronic regions of certain genes and the fatty acid composition of both types of meat (Srikanth et al. 2015; Otto et al. 2022) and milk (Jiang et al. 2016; Li et al. 2016) in cattle. According to Hoashi et al. (2007), the 84-bp indel polymorphism in the intronic region of the SREBP1 gene can directly affect the translation efficiency of the gene, and the presence of insertion of 84 nucleotides is thought to have a regulatory-like function on micro RNA (miRNA) in controlling the expression of genes related to fat quality in cattle. However, this argument needs to be further investigated.

Micro RNA (miRNA) is a small non-coding RNA (ncRNA) that regulates gene expression (Catalanotto et al. 2016). It works by binding to specific regions within its target mRNA, such as the promoter, 5'UTR, 3'UTR, or coding sequences, to either inhibit, silence or induce the expression of target genes (O'Brien et al. 2018). Although the mechanism of the 84-bp indel in the intron 5 of the *SREBP1* gene in influencing fat quality has not been reported, several studies have shown the effect of indel polymorphisms in the intronic region on the expression of genes and/or phenotype in various animals such as chicken (Zhang et al. 2014), pig (Cui et al. 2019), goat (Gao et al. 2020; Wang et al. 2020), and cattle (Zhao et al. 2018; Jiang et al. 2019). Cui et al. (2019) reported that three intronic indels (11-bp and 16-bp insertions and 17-deletion) are associated with the mRNA expression of the KDM6A gene and testicular measurement traits in male piglets. In addition, the *in silico* analysis showed that the presence of the indel in the KDM6A gene affects the binding ability of several transcription factors in transcription binding sites.

Interestingly, according to the results of the sequence alignment analysis in our study, there is evidence of an SNP within an 84-bp insertion-type sequence at the position 34643036 according to RefSeq NC_037346.1 (g.34643036C>T). The sequence in RefSeq NC_037346.1 and Bali cattle was found to be Cytosine (C), while it was Thymine (T) in Japanese

Black cattle (Genbank: AB355704.1), as shown in Figure 3. This SNP may be a breed-specific variant exclusive to Japanese cattle populations. However, further confirmation is necessary through studies on larger cattle breed populations.

CONCLUSION

This study concluded that the L allele (insertion-type) was found to be a common allele across all breeds studied. Furthermore, the 84-bp indel of the *SREBP1* gene was found to be polymorphic only in Limousin, Simmental, and Pasundan cattle, but monomorphic in Holstein-Friesian, Bali, and Sumbawa cattle.

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