Polymorphism of *Insulin-induced gene 1 (INSIG1)* of three local beef cattle in Indonesia

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**Abstract.** Insulin-induced gene 1 (INSIG1) encoded protein that blocks proteolysis activity from protein sterol regulatory element binding proteins (SREBP). The role gene plays of cholesterol, fatty acid, and glucose metabolism. Indonesia has many several beef cattle which has good quality of growth trait. The aim of this study was to identify polymorphism of INSIG1 gene (SNP 4366 (A>G) and 4534 (T>C)) of three local beef cattle in Indonesia (Bali, Pasundan and Ongole Decendent). One hundred and twenty seven samples were used in this study consisting of Bali cattle (46) from Pulukan Bali Island, Pasundan (36) from UPTD BPPT Beef cattle Ciamis West Java and Ongole Grade (OD) (45). DNA was extracted from whole blood using High Salt method then genotyping used PCR-RFLP method with Rsal and TaqI enzymes. Parameters in this study were genotype and allele frequencies, heterozigosity observed (Ho), expected (He), Hardy-Weinberg Equilibrium (HWE) and Polymorphism Information Content (PIC). Result showed that a 428 bp of DNA fragment was successfully amplified and digested. Three variant of genotypes with two alleles were identified. For SNP 4366 (A>G) were AA, AG and GG with dominant of G allele while SNP 4534 (T>C) were CC, CT and TT with dominant C allele. Both of SNPs in each of breed populations was in Hardy-Weinberg Equilibrium and polymorphic. Additionally, PIC value reached moderate. In conclusion, polymorphism was found in beef cattle and it could be early study for genetic diversity.

**Keywords:** INSIG1 gene, genetic polymorphism, Bali cattle, Pasundan cattle, Ongole Descendent cattle, growth trait

1. **Introduction**

In 2045, Indonesia’s population is predicted to reach 320 millions people [1]. This is in line with the increasing need of meat as a protein source. In 2019, national meat consumption reached 2.56 kg/capita/year with the national meat production was 504.080.000 tons (from cattle) and supplied by national livestock (17.467.000 heads). To fulfill the national meat consumption, Indonesia have imported of cattle and beef meat frozen from several countries such as Australia, New Zealand, India and Brazil for supply of protein consumption [2]. Indonesia is a big mega biodiversity for flora and fauna. Indonesia has many breed of beef cattle which adapted with tropical environment i.e. Bali cattle, Madura cattle, Pasundan cattle, Ongole Decendent, Jabres cattle, Kantingan cattle, Sumba Ongole cattle, Pesisir cattle and Aceh cattle. Therefore, their performances genetically are good especially growth trait, reproduction and heat tolerance [3]. Growth performance in cattle was influenced by genetic and environment. Genetically, growth was role played by polygene.

Insulin-induced 1 (INSIG1) is a regulator in lipid metabolism which consisting of two isoforms, INSIG1 and INSIG2 [4]. INSIG1 as an Endoplasmic Reticulum (ER) protein binds the sterol-sensing domain of SREBP cleavage-activating protein (SCAP) and facilitates retention of SCAP/SREBP...
complex in ER. INSIG1 plays a central role in cholesterol homeostasis [5]. INSIG1, PPARG and PPARGC1A genes were associated with transcription regulation during lactation in dairy cattle [6]. Recent 12 genes had high expression in mammary gland association with milk cholesterol content. Those were TMEM120B, INSIG1, FLNB, RPN2, RASAL1, ARF4, MYL9, GRN, ORAI1, PLBD2, AQP1, and RSRC2 [7]. In the case of beef cattle, the genetic information about INSIG1 gene is limited. Polymorphism of INSIG1 gene was found in four SNPs, 4366 (A>G), 4534 (T>C) from intron 2 while 5001 (T>C) and 5235 (G>A) from intron 3 [8]. Those SNPs were associated with growth and carcass traits. Then, ten haplotypes of INSIG1 gene in Nanyang cattle i.e g.A937G; g.C3175A; g.C3242T; g.G3323A; g.C4623T; g.C4683T; g.C4772G; g.C5157T; g.A5218C; and g.G5235A. Polymorphism was found but association of those haplotype combination SNPs with growth traits were not significant [9]. Genetic information about growth traits is needed and important to build of breeding strategy of Indonesian beef cattle. Perhaps, it can improve their genetic quality and performances. So, the aim of this study was to identify of genetic polymorphism of Insulin-induced gene (INSIG1) especially SNP 4366 (A>G) and 4534 (T>C) in three beef cattle in Indonesia.

2. Materials and methods

2.1. Animals and DNA extraction

One hundred and twenty seven samples of Indonesian beef cattle were used in this study that consisted of Bali cattle (46) from Pulukan Bali island, Pasundan (36) from UPTD BPPT Beef cattle Ciamis West Java and Ongole Grade (OD) (45) from Sembawa (36), and Grobogan East Java (9). Three milliliter of whole blood samples were taken from vena caudalis and collected at vacuntainer tube with anticoagulant (K3EDTA). DNA was extracted using high salt method [10]. DNA products were stored at -20°C.

2.2. Genotyping

Genotyping of INSIG1 gene used Restriction Fragment Length Polymorphism (PCR-RFLP) method. A pair primer of INSIG1 gene was applied in this study F: 5’-GTGGGACTGTGGATGACT-3’ and R: 5’-GAGGAAGGCCATGGTGAT-3’ [8], which covered two SNPs (A4366G and T4534C). Total volume of PCR amplification was performed in total 10 μl consisting of 5 μl PCR Mastermix (Bioline), 1 μl of each primer (10pmol/μl), 2 μl water free nuclease and 1 μl DNA template. PCR condition was pre-denaturation at 94°C for 5 minutes, followed by 35 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 64°C, 45 seconds extension at 72°C and then a final extension at 72°C for 10 minutes. The amplified products were electrophoresed by 1% agarose gel in 1xTBE buffer with 100 Voltage for 1 hour. The gel was stained using Ethidium bromide. Visualization of gel was under UV light (UV Transilluminator MajorScience, USA).

Detection of genotype pattern in each of samples used RFLP method with enzyme restriction TaqI (TG^CA) for SNP 4366 (A>G) and Rsal (GT^AC) for SNP 4534 (T>C). PCR product were digested in a total volume 15 μl containing 2 μl PCR product, 1.5 μl buffer enzyme, 0.3 μl Rsal and TaqI enzyme restrictions, and 11.2 μl water free nuclease. The mixture was incubated at 65°C for 3 hours. Digest product were checked using 2% agarose gel in 1xTBE buffer with 100 Voltage for 1 hour. The gel was stained using Ethidium bromide. Visualization of gel was under UV light (UV Transilluminator MajorScience, USA).

2.3. Data analysis

Genotype data of each samples and breed were calculated for allele frequency, genotype frequency, heterozygote observed, heterozygote expected and Polymorphic Information Content (PIC) using Cervus 3.0.7 software [11]. While Hardy Weinberg Equilibrium (HWE) value calculated directly [12; 13]:

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

where:
χ² : Chi-Square test value
O : the number of observed genotypes
E : the number of expected genotypes

3. Results and discussion

The bovine INSIG1 gene maps to chromosome 4 which consist of five exons and six introns [9]. A 428 bp DNA fragment of INSIG1 gene was successfully amplified using a pair specific primer [8] (Figure 1). For genotyping (PCR-RFLP method), in this study used two SNPs of INSIG1 gene in intron 2, 4366 (A>G) and 4534 (T>C) (GenBank accession No. NC_007302.4). Mutation in 4366 nt caused changing of nucleotide Adenine to Guanine and recognized by TaqI enzyme (TG^CA) while mutation in 4534 nt changed Thymine to Cytosine and detected by RsaI enzyme (GT^AC). For SNP 4366 (A>G), GG genotype was produced 318 and 110 bp while AA genotype was 428 bp. It caused TaqI enzyme was not recognized of TG^CA restriction site. Then, heterozygote genotype (AG) was performed 428, 318, and 110 bp. While, for SNP 4534 (T>C), will produced three genotypes CC (428 bp), CT (428, 278, 150 bp) and TT (278 and 150 bp) (Figure 2).

Figure 1. Visualization of PCR product of INSIG1 gene (428 bp).

Figure 2. Visualization of PCR-RFLP product of INSIG1 gene at SNP 4366 (A>G). M: Ladder 100 bp; Genotype AA: 428bp; AG: 428,318,110 bp; GG: 318,110 bp.
According to Table 1, G and C allele in both of SNPs were dominant allele, 0.665 and 0.720 respectively. Interestingly, frequency of genotype of local beef cattle in two SNPs was unique. Especially, Bali cattle has dominant allele A (at SNP 4366 (A>G) 0.51 and allele C (at SNP 4534 T>C) 0.989. Genotype TT was not found in Bali cattle population. It may be caused by different sub species. Bali cattle is known as *Bos sondaicus* or *Bos javanicus*. Bali cattle is a domesticated descendant of the wild Banteng [14] have similar phenotype with Banteng (*Bos banteng*) [15]. That statement was supported based on molecular analysis mitochondrial DNA cytochrome b [16], Y chromosome and microsatellite [17]. So, this study explored Bali cattle from Pulukan-Bali Island where of origin island of Bali cattle was domesticated. Whereas, Pasundan and Ongole Decendent cattle included *Bos indicus* cluster [18; 19].

Study about *INSIG1* gene in cattle is limited. In previous study, four novel SNPs (4366 (A>G), 4534 (T>C), 5001 (T>C) an 5235 (G>A)) in bovine (Qinchuan cattle from China) have published and given information that the SNPs were associated with phenotype traits (body measurements, slaughter and carcass weight) [8]. At SNP 4366 (A>G), the genotype GG was known significantly affecting in hip width, carcass and slaughter weight of China cattle (*Bos taurus*). For SNP 4534 (T>C) was significant in body length and withers height (genotype CC). Additionally, SNP 5001 T>C was identified that genotype CC has highest of withers height (152.667±1.055 cm). While, SNP 5235 (G>A) was not

Table 1. Genotype and allele frequencies, heterozigosity, PIC value and HWE of the *INSIG1* gene (two SNPs) in beef cattle.

| Mutations (SNPs) | Breed          | N  | Genotypes frequencies | Alleles | Heterozygosity | PIC  | HWE (X²) |
|------------------|----------------|----|-----------------------|---------|----------------|------|----------|
|                  |                |    | AA        | AG        | GG        | A   | G        | Observed (Ho) | Expected (He) |              |          |
|                  | Pasundan cattle| 36 | 1        | 10       | 25       | 0.167 | 0.833 | 0.282       | 0.278         | 0.239       | 0.000     |
|                  | Bali cattle    | 46 | (2.77)   | (27.77)  | (69.44)  | 9    | 5.11   | 0.489       | 0.505         | 0.500       | 0.375     | 1.400     |
|                  | Ongole Grade cattle| 45 | 4       | 18       | 23       | 0.289 | 0.711 | 0.415       | 0.411         | 0.326       | 0.031     |
|                  | Total          | 127| 15       | 55       | 57       | 0.335 | 0.665 | 0.447       | 0.445         | 0.346       | 0.096     |

|                  |                |    | CC        | CT        | TT        | C   | T       | Ho  | He    | PIC  | HWE (X²) |
|                  | Pasundan cattle| 36 | 16       | 18       | 2        | 0.694 | 0.306 | 0.430       | 0.424         | 0.334       | 1.143     |
|                  | Bali cattle    | 46 | (44.40)  | (50.00)  | (5.60)   | 9    | 0.989 | 0.011       | 0.022         | 0.022       | 0.021     | 0.006     |
|                  | Ongole Grade cattle| 45 | 11       | 20       | 14       | 0.467 | 0.533 | 0.503       | 0.489         | 0.374       | 0.517     |
|                  | Total          | 127| 72       | 39       | 16       | 0.72  | 0.28  | 0.404       | 0.403         | 0.322       | 7.169     |

X²: 3.841; PIC: Polymorphic Information Content; HWE: Hardy-Weinberg Equilibrium

Figure 3. Visualization of PCR-RFLP product of *INSIG1* gene at SNP 4534 (T>C). M: Ladder 100 bp; PCR: PCR product (undigest); CC: 428 bp; CT: 428, 278, 150 bp; TT: 278, 150 bp.
significant in all of phenotype traits. The other study, seven common allele haplotypes were identified based on four coding region in Nanyang cattle. The haplotype GACT (frequency 75.4%) was the most prevalent haplotype [9]. In other study, INSIG1 appears to play an important role in cattle muscle adipogenesis [20]. PPARG and SREBF1 activate transcription of INSIG1 and regulate its expression during adipogenesis [21]. The INSIG1 gene was one of candidate genes that related to obesity trait in human. This gene is key element for cholesterol processing and binding to the SREBP Cleavage-Activating-Protein (SCAP) [22]. Substitution at position -169 (C>T) in promoter was discovered polymorphism and associated with plasma glucose concentration in two cohort of healthy. The SNP related significant with lower post-load plasma glucose concentration. The INSIG1 gene plays a role in glucose homeostasis [23].

All of samples both of SNPs and breeds were in Hardy-Weinberg Equilibrium (HWE). It showed that all of population was in random mating, no selection, migration and genetic drift [24]. Polymorphic Information Content (PIC) value is often used to measure the informativeness of genetic marker for linkage studies [25]. The PIC was identified in moderate value (0.239-0.326), moderate value of PIC or informative was 0.5>PIC>0.25 [26]. Genetic information in this study is important to know of genetic diversity of local beef cattle in Indonesia and explored about their potential genetic marker. It could be used as early genetic information to construct of breeding system based on molecular level (DNA) and improved of their genetic quality.

4. Conclusion
Polymorphic INSIG1 gene was found in both of SNPs (4366 (A>G) and 4534 (T>C) and all of beef local cattle breeds. Three variants genotype and two alleles were identified (GG, GC, CC for SNP 4366 and CC, CT, TT for SNP 4534). G and C allele were dominant allele. Hardy-Weinberg Equilibrium was found in all samples and SNPs with PIC value reached moderate. This genetic information could be used to know genetic variation INSIG1 gene and to get potential genetic in local beef cattle (Bos indicus and Bos sondaicus) in Indonesia.

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