Polymorphism of Insulin-induced Gene 1 (INSIG1) in Bali cattle (Bos javanicus) from small farmer at Badung district, Bali island

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Abstract. Bali cattle is originated from Bali Island, Indonesia and domesticated from Banteng (Bibos banteng). Bali cattle are well known as a beef cattle with good performance, high carcass percentage and good reproduction traits. Carcass and growth traits were influenced by genetic and environment. Genetically, both of them was influenced several gene or polygene. Insulin-induced gene 1 (INSIG1) is one of the gene which effected of growth traits in cattle. The aim of this study was to identify the polymorphism of INSIG1 gene in Bali cattle from small farmer at Badung district, Bali. Fifty three of fresh bloods were taken from Bali cattle and DNA was extracted using high salt method. Detection of SNP A4366G in INSIG1 gene used PCR-RFLP with TaqI restriction enzyme. Data was analyzed by Cervus 3.07 including allele and genotype frequencies, heterozygosity while Hardy Weinberg equilibrium was calculated directly using formula. Results showed that three variants of genotype (AA, AG, and GG) with two alleles, A (0.5%) and G (0.5%) were found in this population and polymorphism was detected. Heterozygosity observed and expected were 0.505 and 0.500, respectively. PIC value reached 0.375. This population was in genetic equilibrium based on HWE. In conclusion, polymorphism of INSIG1 gene in SNP A4366G of Bali cattle from small farmer at Badung district was detected and has genetic diversity. This result could be early genetic information of Bali cattle to identify of their potencies.

1. Introduction
Indonesia is a country with big megafauna and flora biodiversity. Bali cattle is known as Bos javanicus javanicus that originated from Bali Island, Indonesia. It is a domesticated descendant of wild Banteng (Bibos banteng) [1] and one of the important beef cattle breeds contributing supply of protein domestic needs. Comparing with other beef cattle breed in Indonesia i.e., Madura, Ongole Grade, Pesisir, and Donggala, Bali cattle have better adaptation abilities with the marginal environment [2], high fertility with a calving interval of about 11.87 months, and an 88.44% of pregnancy rate [3], and good meat quality [4]. Even more, the percentage carcass of Bali cattle was about 52.72–57.6% [5] higher than
Ongole grade (49.40%) [6], and Madura cattle (47%) [7]. The population reached 4,789,521 heads (32% of the total beef cattle population (14,824,372 heads) that spread out to all Indonesian regions. It showed that Bali cattle is one of the favorable beef cattle in Indonesia. Meanwhile, on Bali island, the population reached 637,473 heads [8].

Cattle performance as growth, carcass trait, reproduction traits were influenced by genetic and environment. Some traits or genetic conditions are controlled by a single gene (monogenic) and it is known as qualitative traits while others traits are controlled by many genes (known polygenic or quantitative traits) [9]. Insulin-induced 1 (INSIG1) is a regulator in lipid metabolism which consisting of two isoforms, INSIG1 and INSIG2 [10]. INSIG1 is an Endoplasmic Reticulum (ER) protein that 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 [11]. Insulin-induced gene 1 mRNA increases dramatically in fat tissue of normal rats at the onset of diet-induced obesity [12].

In previous studies have investigated the association INSIG1 gene with growth traits i.e., Qinchuan cattle [13] and Nanyang cattle [14]. Four SNPs were identified in Qinchuan cattle as 4366 (A>G), 4534 (T>C), 5001 (T>C), and 5235 (G>A). Genotype GG at locus A4366G and CC genotype at locus T4534C and locus at T5001C have better performance in growth and carcass traits (body length, withers height, hip width, slaughter weight, and carcass weight) [13]. Meanwhile in Nanyang cattle, ten SNPs was found as g.A937G (EX1_220A>G), g.C3175A (IVSI+2049C>A), g.C3242T (IVSI+2116C>T), g.G3323A (EX2_72G>A), g.C4623T (EX3_63C>T), g.C4683T (EX3_123C>T), g.C4772G (IVS3+45C>G), g.C5157T (IVS3+430C>T), g.A2518C (IVS3+491A>C), and g.G5235A (IVS3+508G>A), which included four mutation in coding region (AGC/GGC for exon 1, GCC/ACC, TAC/TAT, and CTC/CTT for exon 3) and the others in the introns. Based on the individual, seven common haplotypes were identified based on four mutations in coding region SNPs (AAGGCCTT, AAGGCTCT, AGAGCCTT, AGAGCTCT, GGAACCTT, GGAACCTT, and GGAGCCTT). Based on association analysis, seven haplotypes did not show a significant effect on body weight (birth, 6 months, 12 months, 18 months, and 24 months) [14]. Therefore, the aim of this study was to identify polymorphism of the INSIG1 gene (A4366G) in Bali cattle from small farmer at Badung Bali district for early genetic information.

2. Material and methods

2.1. Animals

Fifty-three of Bali cattle from Badung District of Bali Island, Indonesia were used in this study. Three milliliters of fresh blood were collected from vena jugularis and stored in vacutainer containing K3EDTA (anticoagulant). DNA was extracted using the salting-out method [15]. DNA was stored at -20°C for the next analysis.

2.2. Amplification of INSIG1 gene

The DNA fragment of a specific target (INSIG1 gene) was amplified using Polymerase Chain Reaction (PCR) technique. A pair primer was used in this study, F: 5’-GTGGGACTGTGGATGACT-3’ and R: 5’-GAGGAAGGCGATGTTGAT-3’ [13]. The total volume of PCR mixture is 10 μl containing 5 μl PCR Mastermix (My Taq™ Red Mix, Bioline), 1 μl for each primer (10 pmol/μl), 2 μl water-free nucleic, and 1 μl DNA template. The mixture was performed by PCR Gradient machine (Eppendorf, Germany) with condition program: pre-denaturation 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, extension at 72°C for 45 minutes, and the final extension at 72°C for 10 minutes. Amplicons were checked using 1% agarose gel with electrophoresis program, 1 hour, and 100 Voltage (Bio-Rad, California USA). Then, agarose gel was stained by Ethidium Bromide and visualized under UV light (UV Transilluminator, MajorSciences, California USA).
2.3. Genotyping
The genotyping used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. Single Nucleotide Polymorphism (SNP) A4366G in INSIG1 gene was recognized by TaqI restriction enzyme (Thermo Fisher Scientific, USA). Ten microliters of digest mixture (5 μl amplicon, 1 μl 10x enzyme buffer, 0.3 restriction enzyme (10U/μl), and 3.7 μl water-free nuclease) was incubated in a water bath at 65°C for three hours. Digest product was performed by 2% agarose gel (1 hour and 100 Voltage) and stained using Ethidium Bromide (EtBr). The pattern of the genotype was visualized using UV Transilluminator.

2.4. Data analysis
Allele and genotype frequencies, observed and expected heterozygosity (Ho and He), and Polymorphic Information Content (PIC) were analyzed using software Cervus 3.07 [16], while Hardy-Weinberg Equilibrium was analyzed using direct calculation [17].

3. Results and discussion
A 428 bp specific fragment of the INSIG1 gene was successfully amplified by PCR technique (figure 1). Mutation in 4,366 nt caused changing of nucleotide Adenine to Guanine (A4366G) and recognized by TaqI enzyme (TG^CA). Detection of the mutation used PCR-RFLP method. Three patterns of genotype were found in the Bali cattle population from Badung district i.e. GG, AG, and AA. GG genotype was produced 318 and 110 bp while AA genotype was 428 bp (TaqI enzyme was not recognized of TG^CA restriction site). Then, a heterozygote genotype (AG) was performed 428, 318, and 110 bp (figure 2).

![Figure 1. Visualization of PCR product of INSIG1 gene in Bali cattle (428 bp).](image1)

![Figure 2. Visualization of PCR-RFLP products of INSIG1 gene in Bali cattle. M: Ladder 100 bp; PCR: PCR product (428 bp); lane 1,3,5,8-11,16-18: genotype AG; lane 2,4,14,15: genotype GG; and lane: 6,7: genotype AA.](image2)
Table 1. Allele and genotype frequencies, Heterozygosity (Ho and He), Hardy-Weinberg Equilibrium (HWE), and Polymorphic Information Content (PIC).

| Samples               | N  | Genotype | Allele | Heterozygosity | PIC   | X² Calculated |
|-----------------------|----|----------|--------|----------------|-------|---------------|
| Bali cattle from Badung Bali | 53 | AA (0.245) | AG (0.510) | GG (0.245) | A 0.500 | G 0.500 | Ho 0.505 | He 0.500 | 0.375 | 0.019 |

X² Table =3.841. If X² Table > X² Calculated, was in Hardy Weinberg Equilibrium.

Polymorphic was found in this study which got A and G alleles with high variation (table 1). Frequency of G allele (0.500) equals A allele (0.500) while AG genotype reached 51% in the population. This result almost the same as with the previous study [13], in Qinchuan cattle A allele about 0.4163 and G allele about 0.5837 with dominant AG genotype (48.6%). Genetic polymorphism is defined as the occurrence of multiple alleles at a locus, where at least two alleles occur with a frequency greater than 1% [18]. The same result was obtained by Liu et.al (2012) where the G allele was dominant (0.5837) in Qinchuan cattle from China. The informativeness of genetic marker can be quantitatively measured by a statistic called the Polymorphism Information Content (PIC) [13]. The PIC value of the marker is defined as the expected fraction of informative offspring from pedigree [19]. The PIC value in this study reached 0.375 and included the informative category. There are three category i.e., highly informative (PIC>0.5), informative (0.5>PIC>0.25) and slightly informative (PIC<0.25) [20]. The Bali cattle population was in genetic equilibrium based on Hardy Weinberg Equilibrium (HWE). Theory of HWE can be applied when the population conforms to the several assumptions i.e., 1) population was in natural selection; 2) neither mutation (the origin of new allele) nor migration (the movement of individuals and introducing new alleles into the population); 3) population size; 4) individuals in the population mate randomly [21].

In a previous study, mutation of A4366G in the INSIG1 gene was associated with growth traits. G allele in Qinchuan cattle has a good effect for growth traits and carcass where GG genotype has higher withers height, hip width, slaughter weight, and carcass weight than AG and AA genotypes [13]. Increasing GG genotype in the population has the potential for improvement of genetic quality especially growth and carcass traits. Utilization of a marker genetic (Single Nucleotide Polymorphism or SNP) in breeding selection has accurate and valid results and also decreases of time of the breeding process.

4. Conclusion
Polymorphic of INSIG1 gene was found in Bali cattle population from Badung district, Bali island with the predominant G allele. The population was in Hardy-Weinberg equilibrium. Increasing of G allele in the Bali cattle population maybe can improve genetic quality.

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