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(RESEARCH ARTICLE)

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Polymorphism of Insulin-like Growth Factor-1 Receptor (*IGF1R*/*Taq*I) Gene in Bali Cattle (*Bos javanicus*) and Its Relationship to the Performance Traits

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Abstract

Bali cattle (*Bos javanicus*) are the indigenous beef cattle that able to adapt well in most places in Indonesia. This study was aimed to detect the polymorphism of *Insulin-like growth factor-1 receptor (IGF1R)* gene with PCR-RFLP analysis. Thirty-one (31) of Bali cows from Klungkung Regency of Bali Province were used for the investigation. Research showed that the *IGF1R/Taq*I gene was polymorphic as the existance of SNP g.269333G>T (intron 12). Three genotypes of GG (0.87), GT (0.10) and TT (0.03) were obtained from the PCR-RFLP analysis. While, the frequency of G allele was higher than T (0.92 vs 0.08). However, the genetic diversity in the *IGF1R/Taq*I gene of Bali cattle was low (PIC=0.14) and under the genetic equilibrium ($\chi 2$=3.74). In general, the heterozygous animals had the high performance traits but not differ significantly. In conclusion, The *IGF1R/Taq*I gene has the potency for molecular selection to improve the growth traits of Bali cattle.

Keywords: Bali cattle; *IGF1R*/*Taq*I gene; PCR-RFLP; Performance; Polymorphism

1. Introduction

Bali cattle (*Bos javanicus*) is an indigenous beef cattle of Indonesia that origined from Bali Island. In year 2024, the population of Bali cattle in Bali Island was 396,717 heads and about 30% from the total beef cattle population in Indonesia [1]. Bali bulls with *Gliricidia sepium* base feed able to attain 239.00±21.28 kg of slaughter weight; 121.45±11.10 kg of hot carcass weight; and 51.14±0.51 kg of carcass percentage [2]. In addition, the birth, weaning (205 days of age) and yearling (365 days of age) weights in Bali cattle (male/female) at the breeding station were 17.73±1.72 / 17.55±1.70 kg; 89.50±8.80 / 85.58±9.61 kg; and 142.45±3.25 / 130.25±2.58 kg, respectively [3]. The growth traits in Bali cattle had the moderate heritability (h2) value (0.10 - 0.30) and possible to improve with conventional selection method [4]. Presently, selection of Bali cattle can be performed with molecular selection technique [5]. One of candidate genes in the molecular selection for growth traits in cattle is *Insulin-like growth factor-1 receptor (IGF1R)* gene [6, 7, 8, 9]. In cattle (*Bos taurus*), the IGF1R gene is located at chromosome 21 along 306,876 bp with 24 exons (GenBank: NC_037348.1). The *IGF1R* gene plays a crucial role in the insulin-like growth factor signaling pathway, mediating the action of growth hormone and contributing to skeletal growth and protein synthesis [10]. One mutation site in the bovine *IGF1R* gene was detected in intron 12 (g.269333G>T) and related to the superovulation and pregnancy rate after embryo transfer in cattle [11]. The present study was aimed to detect the mutation site of g.269333G>T in the bovine *IGF1R* gene of Bali cattle with PCR-RFLP analysis. In addition, this study also observed the relationship of the observed mutation site with the performance of Bali cows. The results in this study is important as the early information to assess *IGF1R* gene for the molecular selection in Bali cattle in the future.

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2. Materials and Methods

2.1. Animals and research site

Thirty-one (31) heads of Bali cows were used for the experimental animals. The Bali cows under study were collected from the Satwa Winangun farmers group at Klungkung Regency, Bali Province, Indonesia. This area is located at latitude 8°27'37" - 8°49'00" S and longitude 115°21'28" - 115°37'43" E with the altitude of 100-500 above sea level. In addition, this place has about 24 - 32 ºC of air temperature, 76.80 - 82.20 % of relative humidity, and 1,025 - 3,352 mm/year of rainfall.

2.2. Management of animals

The animals were kept in the individual cage with the artificial insemination (AI) mating system. The feed ration of Elephant grass (*Pennisetum purpureum*), corn leaves (*Zea mays*), field grass (*Cenchrus ciliaris*) and commercial concentrate (3 kg/head) were given in the animals with ad libitum water. The regular health examination and vitamin were managed every 6 months by the veterinarian.

2.3. Animal performance

The body weight and body measurements of Bali cows were recorded for the association analysis. The body weight of animals were measured using digital weight scale (Iconix FX1, New Zealand). Eight body measurements of head length (HL), head width (HW), chest girth (CG), chest width (CW), body length (BL), withers height (WH), rump height (RH) and cannon circumference (CC) were measured referring to Figure 1. The HL was measured from the horn site/ poll to the lower lip. The HW was measured as the widest point of head [12]. The CG was measured as circumference of the chest just behind the foreleg/os costa V. The CW was was measured with caliper as a distance from left to right upper arm or the distance between tuberositas humerisinister and dexter. The BL was measured with stick mesure as the distance between the point of the shoulder (tuber humerus on os humerus) and the pinbone (tuber ischiadicum on os coxa). The WH was measured with a stick-ruler as the distance from the surface of the platform to the dorsal point (os vertebrae thoracalis III) of the withers. The RH was measured with stick measure as the distance from rump (tuber coxae) to the surface of the platform on which the animal stands [13]. The CC was measured as the smallest circumference of the cannon bone of foreleg [14].

2.4. DNA extraction

Approximately 5 mL of blood samples were collected fromjugular veion of animal using venoject needle and EDTA vacutainer tube. The blood samples were stored in the freezer (-20ºC) for the further analysis. In parallel, the DNA templates were extracted from the blood samples using DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's instructions. In this study, the DNA concentration and DNA purify (λ260/λ280) from the DNA extraction process were 20.56±13.71 ng/µL and 1.75±0.31, respectively.

2.5. IGF1R gene amplification

The amplification of *IGF1R* gene was performed in a total volume of 10 µL consisting 3 µL of DNA template; 0.2 µL of each primer; 5 µL of PCR mastermix (MyTaqTM HS Red Mix (Bioline, USA) ; and 1.6 µL of ddH2O. A primer pair from [15] i.e. Forward: 5'- CCC AAT GGA TTG ATC CTC ATG T -3' and Reverse: 5'- GCT GTG TAG TTC CCT GGG TT -3' were used in this study with the amplicon size of 616 bp in the intron 12 region of bovine *IGF1R* gene (Figure 2). Therefore, the PCR analysis was performed in a mastercycler machine (Applied Biosystems, USA) using PCR program: 1 cycle of pre-denaturation at 95ºC for 1 min and followed by 35 cycles of denaturation at 95ºC for 30 s; annealing at 56ºC for 30 s; initial extension at 72ºC for 1 min; and final extension at 72ºC for 5 min.

2.6. Genotyping of IGF1R/TaqI gene and sequencing

Genotyping of *IGF1R/Taq*I gene was performed with RFLP technique in a total reaction of 7 µL comprising 4.2 µL of PCR product; 0.3 µL of *Taq*I restiction enzyme; 0.7 µL of buffer; and 1.8 µL of ddH2O. According to the PCR-RFLP, three patterns of DNA fragment will be observed in *IGF1R/Taq*I gene represent of GG (47 bp & 569 bp), TT (47 bp; 161 bp & 408 bp) and GT (47 bp; 161 bp; 408 bp & 569 bp) genotypes. The forward sequencing analysis was performed by Apical Scientific laboratory (Malaysia) to confirm the mutation site in the differ genotype sample.

2.7. Data analysis

The genetic diversity parameters of genotype frequency, allele frequency, observed heterozygosty (Ho), expected heterozygosity (He), number of effective allele (ne), polymorphic informative content (PIC), and Chi square ($\chi 2$) values

were estimated referring to [16, 17]. Therefore, a General Linear Model (GLM) method were used for association analysis with the mathematical formula:

$Yij = \mu + Gi + Eij$

where, Yij is the observed value; μ is the common mean; Gi is the effect of ith genotype; and Eij is the experimental error. Furthermore, a BioEdit program [18] was used to detect the mutation site in the *IGF1R* gene of Bali cattle

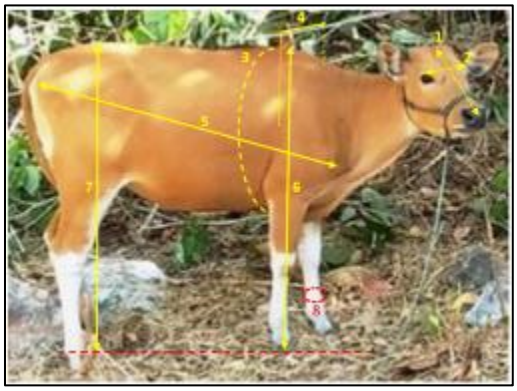

Figure 1 Scheme of body measurements in a Bali cow consisted of head length (1), head width (2), chest girth (3), chest width (4), body length (5), withers height (6), rump height (7) and cannon circumference (8). Source: [19] with modification

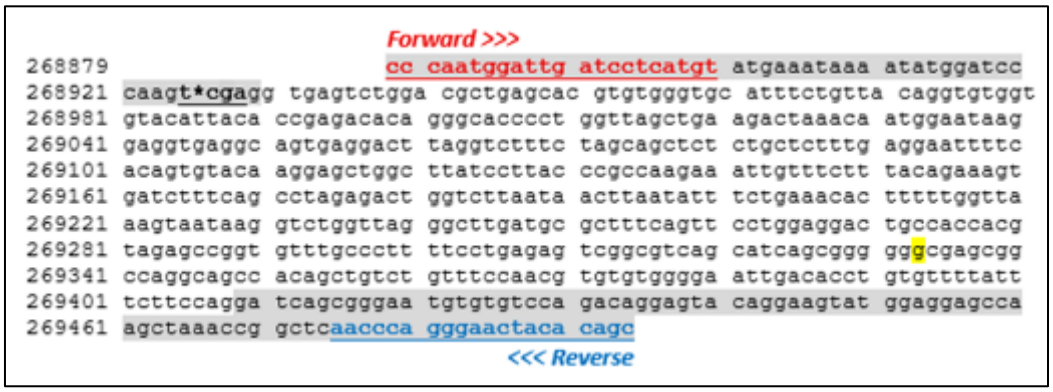

Figure 2 Primer position and the TaqI restriction site (T*CGA) in the target sequence of bovine *IGF1R* gene (GenBank: NC_037348.1) along 616 bp from exon 12 to exon 13 (grey shading). A mutation sites were detected at 269333th (G/T) nucleotides (yellow shading) as reported by [11]

3. Results

Along 616 bp of IGF1R gene was successfully amplified on 1 % agarose gel as shown in Figure 3. In addition, the PCR-RFLP analysis in *IGF1R/Taq*I gene reveals three genotype of GG, TT and GT genotypes in Bali cattle under study (Figure 4). The mutation site of g.269333G>T was detected in the Bali cattle under study with TT genotype (Figure 5). According to Figure 5, no mutation site detected in the *Taq*I restriction site at exon 12 in Bali *IGF1R* gene. However, most of Bali cattle under study had the GG genotype (0.87) and followed by GT (0.10) and TT (0.03) genotypes (Table 1). Commonly, the frequency of G allele was higher than T allele in Bali cattle under study. Hence, the number of effective allele (ne) in g.269333G>T locus was 1.17. The polymorphic informative content (PIC) value in the investigated Bali cattle was low (0.14). In addition, the genetic diversity of *IGF1R/Taq*I was showed under the genetic equilibrium. Moreover, the association analysis revealed that no significant association between *IGF1R/Taq*I gene polymorphism with the body weight and body measurements of Bali cattle under study (Table 2).

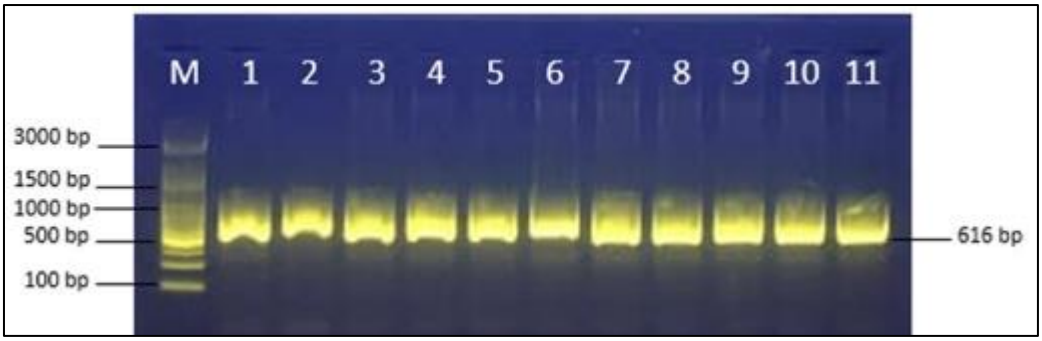

Figure 3 The amplification of *IGF1R* gene in Bali cattle (*Bos javanicus*) along 616 bp on 1% agarose gel. M: DNA ladder 100 bp; Line 1-11: DNA samples

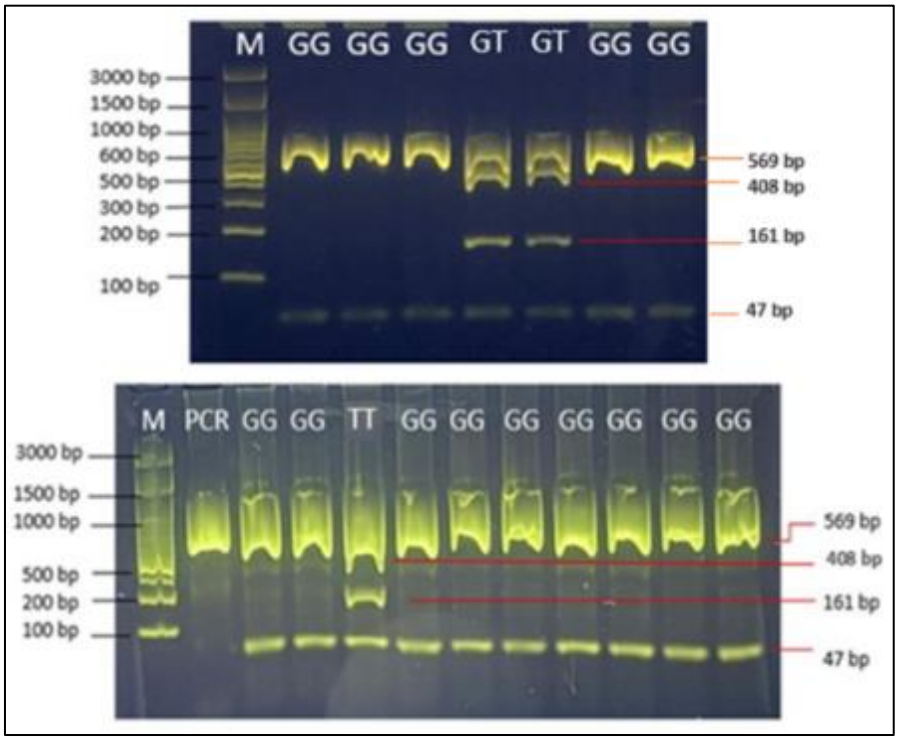

Figure 4 PCR-RFLP of *IGF1R/Taq*I gene in Bali cattle on 2% agarose gel shows three genotypes of GG (47 bp & 569 bp), TT (47 bp; 161 bp & 408 bp) and GT (47 bp; 161 bp; 408 bp & 569 bp). M: DNA ladder 100 bp

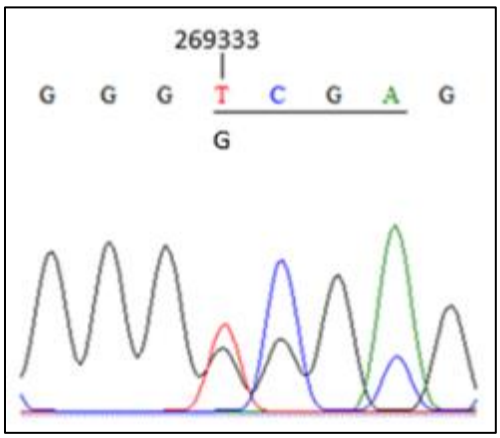

Figure 5 A mutation site (G>T) at 269333th nucleotide in the *IGF1R* gene of Bali cattle (*Bos javanicus*) was formed the *Taq*I restriction site (underline)

Table 1 Genetic diversity in the *IGF1R/Taq*I gene of Bali cattle (*Bos javanicus*)

Genotype frequency (N)			Allele frequency		H_o	H_e	n_e	PIC	χ^2
GG	GT	TT	G	T					
0.87 (27)	0.10 (3)	0.03 (1)	0.92	0.08	0.10	0.15	1.17	0.14	3.74*

N: number of animals; Ho: observed heterozygosity; He: expected heterozygosity; ne: number of effective alleles; PIC: polymorphic informative content; χ2: Chi-square value; *under the genetic equilibrium

Table 2 Association of *IGF1R/Taq*I gene polymorphism with body weight and body measurements of Bali cattle (*Bos javanicus*)

Performance	Genotype		
	GG (N=27)	GT (N=3)	TT (N=1)
Body weight (kg)	215.62±39.88	217.67±18.77	191.00
Head length (cm)	33.85±1.46	35.00±1.00	31.00
Head width (cm)	18.45±1.28	19.67±1.15	18.00
Chest girth (cm)	149.15±7.48	150.33±8.14	135.00
Chest width (cm)	32.25±4.84	30.33±2.52	31.00
Body length (cm)	117.00±9.02	117.67±5.51	106.00
Withers height (cm)	114.60±12.68	115.67±4.51	102.00
Rump height (cm)	114.40±3.59	120.00±4.36	105.00
Cannon circumference (cm)	14.95±0.69	14.33±0.58	15.00

N: number of animals

4. Discussion

The *IGF1R/Taq*I gene in Bali cattle was polymorphic with the low genetic diversity. According to the PIC value, the genetic diversity of genetic marker can be classified as low (<0.20), moderate (0.20-0.30) and high (>0,30) levels [16]. Consequently, the polymorphsm in the intron 12 region of *IGF1R/Taq*I gene can not be used to select Bali cattle in the present study. The superior G allele in the mutation g.269333G>T was also reported in East Anatolian Red (0.87), South Anatolian Red (0.82) and Chinese Holstein (0.59) cattle [6, 11]. Furthermore, [11] reported that polymorphism of g.269333G>T in the *IGF1R* gene had significant association with total number of ova in Chinese Holstein cow with GG as the superior genotype. In this study, a rare TT genotype was found in one sample of Bali cows but its absent in Chinese Holstein cows [11].

In contrast, a mutation site of g.268818A>G in the exon 12 region of bovine *IGF1R* gene had the significant association (P<0.05) with BL at weaning age and WH at yearling age of Madura (*Bos indicus*) cattle [9] due to the weaning weight of Angus cows [7]. In addition, a mutation of g.300532T>C in the 3'UTR region of bovine *IGF1R* gene had significant association (P<0.05) with birth weight, pre-weaned daily gain and weaning weight in Angus cows. [20] obtained seven mutation sites in the *IGF1R* gene that significantly associated with milk quality traits in Chinese Holstein cows i.e. g.261007T>C (intron 8), g.263989G>A (intron 10), g.270011G>A (intron 13), c.278330C>T (exon 16), g. 292249C>T (intron 20), g.296374A>G (intron 20) and g.302167G>C (3'UTR). Despite this, four *IGF1R* gene polymorphisms of in the exon 2 (*IGF1R/MspI* and *IGF1R/Taq*I), exon 10 (*IGF1R/MspI*) and exon 16 *(IGF1R/RsaI*) had the significant association with milk production traits in Simmental cows [8].

In this study, the polymorphism of *IGF1R/Taq*I (intron 2) had the low PIC value and not effective to select the performance of Bali cattle. [16] stated that PIC value had three categories of low (<0.20), moderate (0.20-0.30) and high (>0.30). However, the genetic diversity in the g.269333G>T of Bali cattle was under the genetic diversity. The genetic equilibrium can be caused by selection, migration, crossbreeding, inbreeding and geographical factor [21]. Low PIC value in Bali cattle also reported in the *AKIRIN2/FokI* (0.008) and *TTN/HpyCH4III* (0.008) as reported by [22]. The genetic marker in the intronic region of candidate genes can be influenced the economic traits of livestock. Introns is

important in various cellular processes such as splicing, mRNA transport, nonsense-mediated decay (NMD), expression regulation and transcription initiation [23, 24]. Advances in genomic approaches, such as genome-wide association study (GWAS), have become crucial in livestock genetics research because they enable the identification of single nucleotide polymorphisms (SNPs) and candidate genes involved in controlling complex traits. This approach provides a deeper understanding of the genetic basis of various production traits and supports the development of genome-based breeding programs [25]. In the future, GWAS can be implemented to obtain the genetic markers in livestocks effectively [26]. According to GWAS analysis, many candidate genes of birth weight (*STXBP6* and *TERT*) and adult weight (*SUGT1*) were detected in Bali cattle [27, 28].

5. Conclusion

The *IGF1R/Taq*I gene of Bali cattle was polymorphic with low genetic diversity. Most of the animals population had the GG genotype (0.87) with the dominant G allele (0.92). Therefore, the polymorphism of *IGF1R/Taq*I gene was not significantly associated with the performance of Bali cattle. Hence, the present study confirmed that *IGF1R/Taq*I gene can not be used as the molecular selection in Bali cattle due to the low genetic diversity. In the future, the in-dept study to detect many genetic markers in the different region of *IGF1R* gene is essential to develop a molecular selection in Bali cattle.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval for this study was obtained from Animal Ethics Committees of Faculty of Veterinary Medicine, Udayana University (Approval Number: B/139/UN14.2.9/PT.01.04/2025).

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