Hasil-hasil Penelitian

Sapi Bali

Kerjasama:
BPTU-HPT Denpasar - Kementerian Pertanian,
Fakultas Pertanian Universitas Sebelas Maret (UNS),
Badan Riset dan Inovasi Nasional

HASIL-HASIL PENELITIAN SAPI BALI

TIM PENELITI

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KATA PENGANTAR

Sapi Bali yang juga dikenal dengan nama *Bos javanicus* merupakan salah satu plasma nutfah yang dimiliki oleh Indonesia. Keberadaannya memiliki peran yang potensial, mengingat karakteristiknya yakni performan yang bagus dalam hal daya pertumbuhannya di daerah tropis serta prosentase karkasnya. Berdasarkan data statistik, populasi sapi Bali secara nasional menduduki peringkat pertama, hal ini mendorong para peneliti khususnya di bidang peternakan untuk terus menggali lebih jauh potensi yang dimiliki dan dapat dikembangkan pada sapi Bali, salah satunya dengan teknologi molekuler. Dengan mengikuti perkembangan terkini pada riset molekuler, teknologi genotyping keseluruhan genom dengan menggunakan Bovine 50K SNP beadchip dihadirkan dalam penelitian kami.

Dokumen ini merupakan kumpulan dari hasil-hasil penelitian yang telah kami laksanakan terkait Sapi Bali. Namun demikian, belum keseluruhan hasil penelitian kami sampaikan dalam bendel dokumen ini dikarenakan masih terdapat naskah yang masih dalam proses publikasi, terutama yang terkait dengan analisis *Genome Wide Association Study* (GWAS) terkait sifat fenotip pada Sapi Bali dan studi validasinya. Naskah yang terpublikasi di masa berikutnya akan kami laporkan secara terpisah kepada BPTU-HPT Denpasar.

Harapan kami, dokumen ini dapat memberikan gambaran mengenai penerapan teknologi SNP dalam eksplorasi struktur dan penanda genetik pada genom sapi Bali. Disadari bahwa apa yang telah kami laksanakan ini masih jauh dari sempurna, oleh karena itu kami sangat mengharapkan adanya saran dan masukan guna perbaikan ke depan. Ucapan terima kasih dan penghargaan kami haturkan kepada semua pihak yang telah membantu terlaksananya kegiatan serta berpartisipasi, khususnya kepada Kepala dan seluruh staf di BPTU-HPT Denpasar. Kami berharap semoga informasi yang disampaikan dapat bermanfaat, terutama dalam upaya pelestarian dan pengembangan ternak lokal Sapi Bali.

Bali, 19 Oktober 2022

Tim Peneliti

DAFTAR ISI

Tema 1.

Profil Genom dan Struktur Populasi Sapi Bali

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RESEARCH ARTICLE
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Genomic structure of Bali cattle based on linkage disequilibrium and effective population size analyses using 50K single nucleotide polymorphisms data

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Abstract

Background and aim: Bali Cattle (*Bos j. javanicus*) is a local breed originating in Indonesia, accounting for 32.3% of the total cattle population. To date, no studies of the genetic structure and demographic status of Bali cattle have been conducted, even though the breeding of Bali cattle has a long and unique history that is likely to have impacted its genetic diversity. Therefore, a study that used molecular breeding technologies to characterize the demography of Bali cattle would be timely. This study aimed to examine genome diversity in Bali cattle and estimate the linkage disequilibrium (LD) and effective population size (N_e) values in the cattle population.

Materials and Methods: In this study, we explored the population structure and genetic diversity of Bali cattle using genomic-level analyses. Our study primarily studied cattle that had been bred in livestock breeding centers since these breeds had subsequently spread throughout Indonesia. We focused on characterizing the genetic structure, determining the level of LD present, and estimating the N_e of the Bali cattle population. The genomic data used for this study were obtained from DNA samples of 48 Bali cattle collected at the Breeding Center of Bali Cattle as well as 54 genomic samples from Bali cattle collected elsewhere in Indonesia that had been used in recent publications. This genomic dataset included exclusively 50K single nucleotide polymorphisms (SNP) array (Illumina Bovine 50SNP bead chip, Illumina, USA) data.

Results: We found that the LD values of Bali cattle from the breeding center and those raised elsewhere were 0.48±0.43 and 0.39±0.40, respectively. Subsequently, the N_e value of Bali cattle from the breeding center and farmers was 151 and 96, respectively.

Conclusion: Our results suggest that the selection program of the breeding center is beneficial for maintaining the genetic diversity of Bali cattle.

Keywords: Bali cattle, effective population size, genomic data, genetic structure, linkage disequilibrium.

Introduction

Bali cattle (*Bos j. javanicus*) have a unique history and genetic background, as they are the only native Indonesian cattle that were domesticated by the Banteng [1,2]. Bali cattle are the most suitable for several regions in Indonesia because they possess unique adaptations to the tropical environment, including a high growth rate even when given low-quality feed [2]. These characteristics make Bali cattle attractive for farming in Indonesia; at the time of the last census, Bali cattle were the predominant breed in Indonesia, representing 32.3% of all cattle in the country [3]. The breeding of Bali cattle in Indonesia has a long history and began with the isolation of its ancestors on the Island of Bali. More recently, a breeding station was established, which facilitated the spreading of Bali cattle throughout Indonesia [4]. The phases of history involved likely have affected the genomes of present-day Bali cattle. Nevertheless, to date, no studies that examined the genetic structure and demographic status of Bali cattle using genomic technologies have been conducted. Decker *et al*. [5] used genome data from 20 Bali cattle in a study of the genetic composition of wild cattle from around the world, but detailed descriptions of some cattle types, including Bali cattle, remain to be explored. The use of molecular technologies in animal husbandry has become more ubiquitous, chiefly because these technologies facilitate more efficient breeding and greater genetic improvement within livestock breeding programs. At present, the emergence of new products and new findings related to genetic markers associated with

desirable livestock traits present great opportunities for further improving the livestock breeding process. In Indonesia, genomic-level analyses have become an important part of research programs designed to support the improvement of livestock genetic quality. For example, such analyses have been utilized to evaluate Indonesian beef and dairy cattle genomes [4,6], as well as the relationship between *PLAG1* (a gene on chromosome 14 of the cattle genome) with Ongole cattle birth weight [7].

Single nucleotide polymorphisms (SNPs) are a type of variation in nucleotide sequence found in DNA. SNPs are often stable and may also be directly related to protein function; therefore, they are one of the best genetic markers used by breeding programs [8]. A project to explore sequence variation in the cattle genome began in 2003 and was published in 2004, revealing that several SNPs were found [9]. Currently, automated SNP detection devices exist that can search for SNP variation in the cattle genome; these include the Bovine SNP50 Beadchip v3, which can detect up to 53,218 SNPs in the cattle genome (Illumina Inc., USA).

Linkage disequilibrium (LD) is one of the important parameters in population genetics; it reflects the degree of correlation between allelic variants found at different loci in a given population. Therefore, LD values reflect the distance between genetic markers and the level of inbreeding (genetic proximity) of individuals in a population. LD values are smaller if the genetic distance is greater; this may reflect historical migration, mutation, recombination, or selection in a population [10,11]. LD values can also be used to estimate population genetic parameters such as the effective population size (N_e) [4,12]. Understanding the N_e is critically important for trying to maintain genetic diversity in a livestock population, since a high N_e reflects a great deal of genetic variation within this population. In contrast, a low N_e indicates that less genetic variation is present.

This study aimed to examine genome diversity in Bali cattle and estimate the LD and N_e values in the cattle population. Specifically, we seek to examine the genomic effects of the selection and breeding programs of the Breeding Center of Bali Cattle Breeding; to do so, we will compare LD and N_e values of cattle bred at the breeding center with other Bali cattle populations bred by private farmers. Estimates will be obtained using the Bovine 50SNP Beadchip (Illumina Inc.). The results of this study may be useful as an assessment of breed development in Indonesian Bali cattle and may also be used as a basis for future policies regarding Bali cattle.

Materials and Methods

Ethical approval

All animal procedures related to sample collection were approved by the Ethical Clearance Commission at National Research and Innovation Agency (Badan Riset dan Inovasi Nasional) No. 82/Klirens/X/2021.

Study period and location

The study was carried out from March to November 2021. A total of 48 Bali cattle blood samples were obtained from the Breeding Center of Bali Cattle (Denpasar, Indonesia). The DNA isolation and related works were conducted at the Division of Biology, Integrated Laboratory of Universitas Sebelas Maret. In addition, genomic analysis was conducted at Macrogen (Korea).

Genomic data sources for Bali cattle

We obtained genomic data from 102 samples of Bali cattle. Forty-eight of these samples came from DNA isolated from the blood of 48 Bali cattle bred at the Breeding Center of Bali Cattle (Denpasar, Indonesia). The other 54 samples included 20 samples uploaded by Decker *et al*. [5] to the DRYAD database (http://datadryad.org/), 18 samples from the appendix of a publication by Hartati *et al*. [7], and 16 samples sourced from data of Sudrajad [13], which we used to represent Bali cattle bred by farmers. The Bali cattle at the breeding center were produced by a series of quantitative and qualitative selection processes designed to breed superior cattle [14]. By contrast, Bali cattle produced by farmers are simply those that had been bred according to the habits of the farmers that owned them.

DNA isolation was carried out as per the method of Sambrook *et al*. [15] at the Integrated Laboratory of Universitas Sebelas Maret (Surakarta, Indonesia). DNA concentration quantification was performed using Picogreen (Thermo Fisher Scientific Inc., USA), and evaluations of DNA purity were performed using a NanoDrop (Thermo Fisher Scientific Inc., USA) device. DNA samples were deemed to be of sufficient quality if the concentration was at least 20 ng/μL and the absorbance ratio at 260 and 280 nm was more than 1.8. DNA samples were then screened using the Illumina Bovine SNP50 v3 Beadchip (Illumina Inc.) at Macrogen (Korea) to obtain genomic data. This chip contains a genotyping array comprising 53,218 SNPs spread uniformly throughout the bovine genome.

Genome data quality control

Before analyzing the genomic structure and genetic diversity of Bali cattle, we filtered our genomic data to remove low-quality data using PLINK v1.07 (Purcell Lab, Harvard Medical School, Boston, USA) [16]. Quality control filtering for genotype data was performed using the following criteria: SNP variants would be maintained if the Hardy–Weinberg Equilibrium value was not $<1\times10^{-4}$, the SNP call rate was >90%, the minor allele frequency was <1%, and the proportion of empty SNP variants for each individual and the proportion of empty genotypes for each variant must not exceed 10% [13]. In addition, our dataset included all autosomes, which in cattle includes chromosomes 1-29.

Analysis of the genetic structure

Analysis of the genetic structure was performed in both Bali cattle populations. First, we calculated the expected heterozygosity (H_e) and observed heterozygosity (H_o) values and determined the value of the inbreeding coefficient (F_{IS}) as well as the average genomic relationship matrix (GRM). The H_o and H_e values were calculated using the *hierfstat* package as implemented in R v.3.2.2 (The R Foundation for Statistical Computing, Vienna, Austria) [17]. These calculations are based on the formula compiled by Nei [18], as follows:

$$H_o = \sum_{i \neq j} \frac{N_{1ij}}{N}$$

$$H_e = 1 - \frac{1}{m} \sum_{i=1}^{m} \sum_{a=1}^{k} p_a^2$$

N: The number of samples tested
N_{1ij}: Heterozygosity at each locus
m: The number of loci tested
p_a^2 : Allele frequency – a from all k allele

The F_{IS} value was also calculated using the same R package (The R Foundation for Statistical Computing) through the formula described by Weir and Cockerham [19], as follows:

$$F_{IS} = 1 - \frac{H_I}{H_S}$$

H_I: The average H_o of all samples in a population
H_S: The average H_e of all samples in a population

The GRM value was calculated using Genome-wide Complex Trait Analysis v1.25.2 (University of Queensland, Australia) [20]. This program generates two outputs: The first corresponds to SNP relationships within an individual (diagonal), and the second corresponds to SNP relationships between individuals in the same population (off-diagonal). Sample variability within each population was determined using a negative value on the off-diagonal variance.

LD

Various statistics have been introduced to measure LD. In our study, we calculated the LD using the r^2 formula [21], as follows:

$$r^2 = \frac{\left(f(AB) * f(ab) - f(Ab) * f(aB)\right)^2}{f(A) * f(a) * f(B) * f(b)}$$

f(x) designates the frequency of the *x* allele. LD value estimation was performed using PLINK v1.07 (Purcell Lab) [16] and was visualized using the size of the allele distance (kb) through R v.3.2.2 (The R Foundation for Statistical Computing) [17]. PLINK was used as a measurement tool, and the *--r2* command was used to obtain the LD value of SNP pairs. Next, the *--ld-window-r2* command was used to report all SNP pairs [16]. LD values range from 0 to 1; a value of 1 indicates a strong correlation between variants.

Effective population size (N_e)

The effective population size (N_e) was estimated using the LD value and Sved's formula, as presented by Xu *et al*. [12], as follows:

$$N_e = \frac{1}{4c} * \left(\frac{1}{r^2} - 1 \right)$$

c designates the recombination distance in Morgan's units. N_e was estimated using R v.3.2.2 (The R Foundation for Statistical Computing) [17]. Next, N_e was plotted following estimated times in horizontal ordinate, which was obtained by $(2c)^{-1}$. We graphed the resulting relationship using the *ggplot2* package in R to obtain usable plots.

Results and Discussion

Bali cattle genome data conditions

The SNP microarray we utilized in this study screened for 53,218 SNPs (Illumina Inc.). Our genotyping dataset showed that the average percentage of SNPs detected (i.e., the call rate) in the Bali cattle genome was 97.8%. In addition, the average genotype score for each SNP was 0.7. These figures suggest that the level of reliability of the SNP data used in this study is high. Supplementary Figure-1 presents a further analysis of the reliability of our genotyping dataset.

The number of SNPs from Bali cattle genome data from the breeding center obtained after the genome data quality control process was 49,439 variants (93%). Conversely, the number of clean SNPs in farmers' Bali cattle genome data was 52,886 variants [13]. The reduced number of variants is mainly because some genotypes cannot be well identified during the genotyping process [16].

Structure of the Bali cattle genome

Table-1 [5,7,13] summarizes the results of our comparative genome structure analysis of the two types of Bali cattle. Table-1 shows that the Bali cattle samples from the breeding center showed higher heterozygosity than the samples obtained from the farmers' Bali cattle. In addition, the inbreeding coefficient and the measure of relationships between individuals from the breeding center population samples were also lower than the corresponding measurements of the samples of the farmers' cattle. At the same time, the effective population size value was much higher. Taken together, these figures indicate that Bali cattle from the breeding center are more diverse and have lower levels of inbreeding than the farmers' cattle.

Furthermore, the diagonal GRM value (which represents the average relationship between variants in an individual within a population) of the Bali cattle from the breeding center was higher than that of Bali cattle reared by local farmers (Table-1). This indicates that the variant in the Bali cattle from the breeding center is uniformly present in that population. This is likely a result of the cattle breeding program applied by the breeding center to select desirable characteristics. When

Table-1: Summary statistics of observed Bali cattle populations.

Bali cattle population	No. of samples	Observed SNPs in BTA	H_o[1]	H_e[2]	F_{IS}[3]	GRM[4]		LD[5] (SD)	Recent N_e[6]
						Diagonal	Off-diagonal		
Breeding center	48	49,439	0.30	0.26	−0.19	0.70	−0.021	0.48 (0.43)	151
Farmers[7]	54	52,886	0.12	0.08	−0.16	0.57	−0.011	0.39 (0.40)	96

[1]Observed heterozygosity. [2]Expected heterozygosity. [3]Inbreeding coefficient. [4]Average of the genomic relationship matrix referring to inbreeding (diagonal) and outbreeding (off-diagonal). [5]Linkage disequilibrium as estimated using the r^2 method. [6]Effective population size. [7]Data sources: Decker *et al*. [5], Hartati *et al*. [7], and Sudrajad [13]

considered together, our results suggest that the selection program of the Bali cattle breeding center is successful. Moreover, our findings agree with other genomic structure analyses of livestock populations [22].

LD

The uniformity of variants present in the genomes of Bali cattle from the breeding center can be verified by checking the average LD value. As per our knowledge, this the first study of LD in Bali cattle; our results show that the calculated LD value of the Bali cattle population from the breeding center (0.48±0.43) was higher than the LD value of Bali cattle bred by farmers (0.39±0.40) (Table-1). This indicates that LD patterns will be population-specific, since LD depends on the genetic events experienced by individuals within a population [23]. We hypothesize that the higher LD of Bali cattle from the breeding center might be caused by the selective breeding conducted on the population, especially since other farmer-bred Bali cattle did not experience a similar selection. This is significant because selection can affect the level of variance uniformity [24]. Moreover, since high LD values result from recent selection, low levels of LD may, therefore, reflect a low selection intensity in the farmer-bred population [25,26].

Figure-1 shows a graph of changes in the estimated LD values in interallelic distances up to 500 kb for Bali cattle from the breeding center. Here, we see that average LD values were high over a short allele distance and continually decreased as the allele distance increased. This trend has also been found in other cattle populations [11,12,23,26]. Moreover, when compared with LD values of cattle from other countries as calculated by Perez O'Brien *et al*. [26], the LD values of the Bali cattle population bred at the breeding center were higher than the corresponding values for *Bos indicus* cattle (0.39) but lower than for *Bos taurus* cattle (0.59). By contrast, Bali cattle bred by farmers showed average LD values that were equivalent to those of the *B. indicus* cattle. In general, LD values can differ between populations even within a single cattle breed, since large LD values are dependent on changes in genetic composition experienced by individuals in the population [11,23]. LD values will tend to be low if the selection intensity in the population is low, and there is the possibility of crossing with native animals, as is often the case with *B. indicus* cattle in developing countries [26].

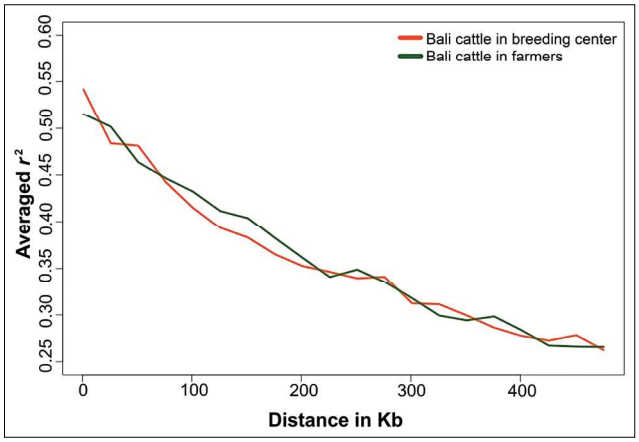

Figure-1: Graph of LD value of Bali cattle populations.

Effective population size (N_e)

In this study, we found strong trends for N_e values in the Bali cattle population. Relative to N_e values from several generations ago, the effective population size of Bali cattle has experienced a sharp decline. However, in the more distant past (i.e., from 40 generations ago until the present) N_e values were increased (Figure-2; Supplementary Table-1) [5,7,13]. This is in contrast to the most common pattern, in which the N_e of a population was much higher in the past and has decreased continuously until the current generation [4,12,23,25].

The effective population size illustrates a pattern of genetic variation that can be used to explain population diversity from the past to the present [23]. Thus, we constructed a graph of the N_e values of the Bali cattle populations in this study. This graph illustrates that there have been efforts to increase the genetic variation within the Bali cattle population since approximately 40 generations ago. Taken together, our analysis of effective population size shows that in Indonesia, the establishment of a Bali cattle breeding unit in 1976 and the subsequent distribution of Bali cattle to other regions, as well as efforts to artificially breed Bali cattle [2,4,14,22], have helped to maintain genetic diversity in the studied populations of Bali cattle.

The N_e values of the two Bali cattle populations studied here are both far greater than the minimum threshold of the Food and Agricultural Organization for determining whether a cattle population is far from extinction (i.e., an effective population size of 50 for each generation) [27]. Future efforts should then

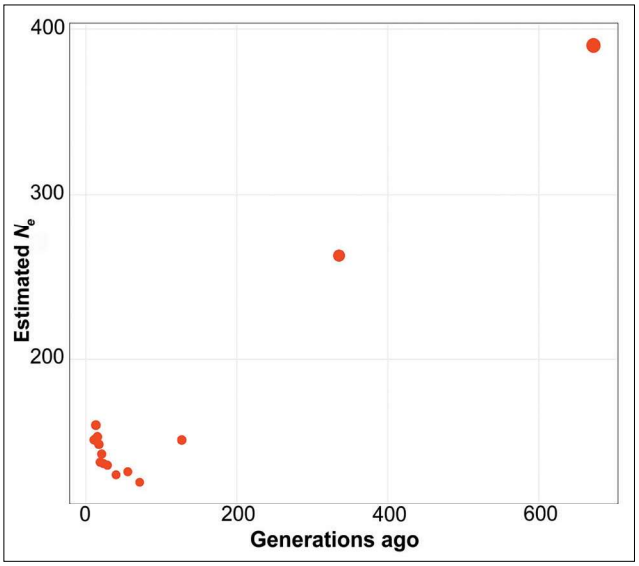

Figure-2: Graph of the effective population size of Bali cattle.

help to maintain the genetic diversity of Bali cattle populations.

Conclusion

Based on observed heterozygosity levels, inbreeding coefficients, and GRM values, we conclude that the Bali cattle population bred at the breeding center has a higher level of genetic diversity than the Bali cattle bred by farmers. Moreover, the LD value of Bali cattle falls just above *B. indicus* and just below *B. taurus*, and the effective population size remains high. The livestock selection program implemented by the Breeding Center of Bali Cattle may, therefore, be influential in helping to maintain the genetic diversity of Bali cattle. The results of this study can be used as a basis for developing and improving Bali cattle breeding policies in the future.

Authors' Contributions

PS and MC: Conceptualization, investigation and data curation, software, validation, writing original draft and review and editing. RYK: Investigation and data curation and Methodology. SDV: Investigation and data curation, methodology, validation, and review and editing. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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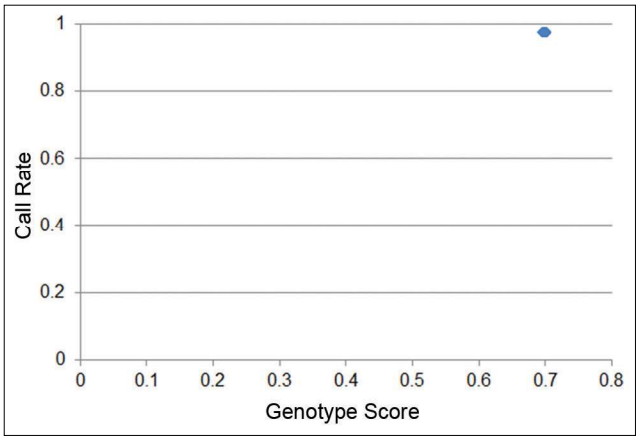
Supplementary materials

Supplementary Table-1: The historical effective population size (N_e).

Bali cattle in the breeding center																								
N_e	151	160	153	148	137	142	136	135	130	132	125	151	262	390	766	1980	-	-	-	-	-	-	-	-
Generation	11	13	15	17	19	21	23	28	40	55	71	127	335	672	1482	5104	-	-	-	-	-	-	-	-
Bali cattle in the locals[1]																								
N_e	96	112	118	119	133	128	136	143	155	170	173	193	191	230	269	305	324	377	528	515	613	737	773	769
Generation	12	14	16	19	22	26	31	37	44	53	65	79	97	120	149	186	233	292	367	452	553	658	756	848

[1]Data Sources: Decker *et al*. [5], Hartati *et al*. [7], Sudrajad [13]



Supplementary Figure-1: The level of reliability of SNPs in the Bali cattle genome.

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Estimasi Ukuran Populasi Efektif Sapi Bali Berdasarkan Data Genom

(Estimation of Effective Population Size for Bali Cattle Based on Genomic Data)

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ABSTRACT

Bali cattle (*Bos javanicus*) is a local cattle and native to Indonesia, which currently has a population of 32.3% nationally. Efforts to develop Bali cattle in Indonesia have gone through a long history that allows an influence on their genetic status. This study aimed to identify the status of Bali cattle genetic diversity in terms of effective population size (N_e) by utilizing genomic technology. The genome data used amounted to 48 samples from the DNA of 48 Bali cattle in BPTU – Denpasar, which were processed using Illumina Bovine SNP50 v3 beadchip. Data quality control with PLINK software and N_e estimation was calculated using R software. From the analysis it was found that from 53,218 SNPs, the average callrate was 97.8%. The N_e value of the Bali cattle population has experienced a very sharp decline and has risen again since 40 generations ago. At present, the N_e value of Bali cattle was 151, greater than the minimum value required by FAO so that a population was protected from extinction. Efforts and support from various parties are needed to maintain the diversity status of Bali cattle.

Key words: Effective population size, Bali cattle, genome

ABSTRAK

Sapi Bali (*Bos javanicus*) merupakan sapi lokal yang asli Indonesia, yang saat ini populasinya mencapai 32,3% secara nasional. Upaya pengembangan sapi Bali di Indonesia telah melewati sejarah panjang yang memungkinkan terjadinya pengaruh terhadap status genetiknya. Penelitian ini bertujuan untuk mengidentifikasi status keragaman genetik sapi Bali dari sisi ukuran populasi efektif (N_e) dengan memanfaatkan teknologi genom. Data genom yang digunakan berjumlah 48 sampel yang berasal dari DNA 48 ekor sapi Bali di BPTU sapi Bali Denpasar yang diproses dengan menggunakan *Illumina Bovine SNP50 v3 beadchip*. Kontrol kualitas data dengan *software* PLINK dan estimasi N_e dikalkulasikan dengan menggunakan *software* R. Dari analisis diketahui bahwa dari 53.218 SNP pada beadchip, rata-rata *callrate* sebesar 97,8%. Nilai N_e populasi sapi Bali pernah mengalami penurunan yang sangat tajam dan naik kembali sejak 40 generasi yang lalu. Pada masa sekarang ini nilai N_e sapi Bali sebesar 151, lebih besar dari nilai minimum yang dipersyaratkan FAO agar suatu populasi terhindar dari kepunahan. Perlu upaya dan dukungan dari semua pihak guna mempertahankan status keragaman sapi Bali.

Kata kunci: Ukuran populasi efektif, sapi Bali, genom

PENDAHULUAN

Sapi Bali (*Bos javanicus*) apabila ditinjau dari kajian sejarah dan genetik, merupakan sapi lokal yang asli Indonesia (Mohamad et al. 2009; Sutarno & Setyawan 2016). Sapi ini merupakan keturunan Banteng yang diperkirakan proses domestikasinya berlangsung lebih dari 2.000 tahun yang lalu. Sapi Bali disebut sebagai sapi yang paling cocok untuk dibudidayakan di berbagai daerah di Indonesia, sebab dapat beradaptasi di lingkungan tropis dengan pertumbuhan dan proporsi karkas yang baik walaupun dengan keterbatasan sumber daya pakan. Karakter/sifat inilah yang menjadikan sapi Bali dapat diunggulkan dan digemari oleh peternak. Sehingga berdasarkan hasil sensus disebutkan bahwa sapi Bali merupakan bangsa sapi dengan populasi terbesar di Indonesia, yakni mencapai 32,3% (Kementan & BPS 2011).

Upaya pengembangan sapi Bali di Indonesia melewati sejarah yang cukup panjang, mulai dari isolasi sapi Bali di pulau Bali, dibentuknya balai pembibitan, hingga proses penyebarannya ke berbagai wilayah di Indonesia. Tentu saja fase-fase tersebut akan berpengaruh terhadap status genetik sapi Bali saat ini. Selama ini, kajian mengenai struktur genetik dan status demografi sapi Bali, khususnya mengenai ukuran populasi efektifnya belum pernah dilakukan. Memahami mengenai ukuran populasi efektif (N_e) sapi Bali sangat penting sebab upaya mempertahankan status keragaman genetik sapi Bali merupakan salah satu target yang ingin dicapai oleh pemerintah, dalam hal ini Balai Pembibitan Ternak Unggul (BPTU) sapi Bali. Nilai N_e yang tinggi mengindikasikan bahwa terdapat variasi genetik yang besar pada populasi ternak tersebut, demikian juga sebaliknya (Sudrajad 2016).

Seiring pesatnya perkembangan teknologi, riset terkait pemanfaatan teknologi molekuler untuk pemuliaan telah masuk ke ranah genom. Genom pada ternak merupakan informasi keseluruhan DNA yang berada dalam sel di tubuh ternak tersebut. Oleh karena itu teknologi genom dipercaya dapat memetakan gen pada tubuh ternak secara lebih tepat (Hocquette et al. 2007). Berbagai metode analisis untuk mengeksplorasi informasi genom saat ini telah berkembang dan dimanfaatkan oleh banyak kalangan, termasuk para peneliti di Indonesia. Informasi genom tersebut juga dapat dimanfaatkan untuk mengestimasikan nilai N_e dari suatu populasi.

Penelitian ini merupakan studi pendahuluan guna mengidentifikasi nilai ukuran populasi efektif sapi Bali dengan memanfaatkan data genom. Hasil penelitian ini diharapkan dapat digunakan sebagai dasar penelitian lanjutan mengenai keragaman genetik sapi Bali dan bermanfaat untuk digunakan sebagai bahan evaluasi pengembangan sapi Bali, khususnya di BPTU sapi Bali.

MATERI DAN METODE

Data genom

Data genom sapi Bali yang digunakan dalam penelitian ini berjumlah 48 sampel, berasal dari DNA hasil isolasi dari darah 48 ekor sapi Bali yang dibudidayakan di BPTU Denpasar. Isolasi DNA dilaksanakan dengan mengikuti metode Sambrook et al. (1989). Pengecekan konsentrasi DNA dilaksanakan dengan Picogreen (Thermo Fisher Scientific Inc., USA) dan pengecekan kemurnian DNA dilakukan dengan menggunakan NanoDrop (Thermo Fisher Scientific Inc., USA). Sampel DNA dikatakan memenuhi standar kualitas apabila konsentrasi minimal 20 ng/µl dan kemurniannya >1,5. Selanjutnya sampel DNA

diproses dengan menggunakan *Illumina Bovine SNP50 v3 Beadchip* untuk mendapatkan data genom yang terdiri dari 53.218 SNP.

Kontrol kualitas data genom

Data genotip harus terlebih dahulu dibersihkan dari data yang memiliki kualitas rendah menggunakan program PLINK v1.07 (Purcell et al. 2007). Kontrol kualitas untuk data genotip menggunakan kriteria sebagai berikut: variasi SNP akan dipertahankan apabila nilai *Hardy-Weinberg Equilibrium* (HWE) tidak kurang dari 1 x 10^{-4}, nilai persentase SNP yang ditemui (*call rate*) lebih besar dari 90%, frekuensi allel minor kurang dari 1%, varian SNP kosong dalam setiap individu dan genotip kosong dalam setiap varian tidak boleh lebih dari 10% (Sudrajad et al. 2016). Selain itu, kromosom yang digunakan adalah kromosom tubuh (*autosome*) yakni kromosom 1-29.

Estimasi ukuran populasi efektif

Nilai N_e diestimasikan dengan berdasarkan rumus yang telah disampaikan oleh Hayes et al (2003) sebagai berikut:

$$N_e = \frac{1}{4c} * \left(\frac{1}{r^2} - 1\right)$$

Dalam rumus tersebut, simbol c merupakan jarak rekombinasi dalam satuan Morgans. Sedangkan r^2 merupakan asosiasi non-acak alel-alel pada lokus yang berbeda di dalam suatu populasi, yang dihitung menggunakan rumus yang dijelaskan oleh Sved (1971). Estimasi nilai N_e dikalkulasikan dengan menggunakan *software* R v.3.2.2 (R Core Team 2015).

HASIL DAN PEMBAHASAN

Kondisi data genom sapi Bali

Jumlah SNP yang digunakan dalam penelitian ini sebanyak 53.218 varian (Illumina Inc., USA). Berdasarkan data hasil *genotyping*, rata-rata persentase SNP yang ditemui (*call rate*) di genom sapi Bali sebesar 97,8%. Selain itu skor genotip yang ditemui dalam setiap SNP rata-rata sebesar 0,7. Hal ini berarti tingkat realibilitas data SNP dalam penelitian ini tinggi. Secara total, jumlah SNP dari data genom sapi Bali di BPTU Denpasar yang didapatkan setelah proses kontrol kualitas data genom sebanyak 49.439 varian (93%). Berkurangnya jumlah varian tersebut terutama disebabkan karena beberapa genotip tidak dapat teridentifikasi dengan baik pada saat proses *genotyping* (Purcell et al. 2007).

Ukuran populasi efektif sapi Bali

Dalam penelitian ini, terdapat pola yang unik untuk nilai N_e pada populasi sapi Bali. Apabila ditinjau dari perubahan nilai ukuran populasi efektif (N_e) dari beberapa generasi yang lalu, dapat diketahui bahwa ukuran populasi efektif sapi Bali pernah mengalami penurunan yang tajam. Namun sejak 40 generasi yang lalu hingga sekarang nilai N_e cenderung mengalami kenaikan kembali (Tabel 1). Biasanya, nilai N_e suatu populasi cenderung tinggi pada generasi yang lalu dan akan turun secara kontinyu hingga generasi sekarang (Flury et al. 2010; Sudrajad et al. 2016).

Nilai N_e merupakan gambaran dari sebuah pola variasi genetik, sehingga dapat digunakan untuk menjelaskan status keragaman pada suatu populasi saat ini dan di masa lampau (Flury et al. 2010). Oleh karena itu, grafik nilai N_e populasi sapi Bali dalam penelitian ini menggambarkan bahwa terdapat upaya untuk meningkatkan variasi genetik sapi Bali sejak 40 generasi yang lalu. Upaya tersebut secara nyata dapat dibuktikan bahwa di Indonesia, pembentukan unit pembibitan sapi Bali (BPTU Sapi Bali) sejak tahun 1976, pelaksanaan penyebaran sapi Bali ke beberapa daerah lain serta upaya seleksi sapi Bali unggul yang pernah digalakkan telah memberikan hasil yang baik guna menjaga keragaman genetik sapi Bali.

Tabel 1. Ukuran populasi efektif sapi Bali dari beberapa generasi

Generasi yang lalu	N_e	Generasi yang lalu	N_e	Generasi yang lalu	N_e
1.482	766	55	132	19	137
672	390	40	130	17	148
335	262	28	135	15	153
127	151	23	136	13	160
71	125	21	142	11	151

Nilai N_e populasi sapi Bali saat ini masih jauh lebih besar apabila dibandingkan batas minimum yang dipersyaratkan oleh *Food and Agricultural Organization* (FAO) agar suatu populasi sapi dapat dikatakan jauh dari ancaman kepunahan, yaitu 50 pada setiap generasi (Lu et al. 2012). Upaya yang diperlukan selanjutnya adalah bagaimana mempertahankan status keragaman populasi sapi Bali yang baik tersebut.

KESIMPULAN

Ukuran populasi efektif sapi Bali sejak 40 generasi yang lalu mengalami kenaikan dan saat ini mencapai nilai 151. Nilai ini lebih besar dari syarat minimal yang ditetapkan FAO. Perlu upaya dan dukungan dari berbagai pihak guna mempertahankan status keragaman genetik sapi Bali.

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ANALISIS KERAGAMAN GENETIK SAPI BALI MENGGUNAKAN TEKNOLOGI GENOM

SKRIPSI
untuk memenuhi sebagian persyaratan
guna memperoleh derajat Sarjana Peternakan
di Fakultas Pertanian
Universitas Sebelas Maret

Program Studi Peternakan

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FAKULTAS PERTANIAN
UNIVERSITAS SEBELAS MARET
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2022

ANALISIS KERAGAMAN GENETIK SAPI BALI MENGGUNAKAN TEKNOLOGI GENOM

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ABSTRAK

Sapi Bali merupakan salah satu ternak asli Indonesia yang perlu ditingkatkan nilai ekonomisnya melalui seleksi ternak berdasarkan genetik dengan memanfaatkan teknologi genom. Penelitian ini bertujuan untuk mengetahui tingkat keragaman genetik pada sapi Bali menggunakan *SNP Bovine 50K Beadchip*. Penelitian dilakukan terhadap 54 data genotip sapi Bali dari peternakan rakyat dan 48 data genotip sapi Bali dari BPTU-HPT Denpasar. Penelitian dilaksanakan pada bulan April hingga Oktober 2021. Penelitian dilakukan mulai dari koleksi DNA melalui darah, ekstrasi DNA, *genotyping*, kemudian analisis data. Analisis data dilakukan dengan menggunakan tiga perangkat lunak yaitu PLINK v1.9., R v4.0.4., dan GCTA v1.93.2. Hasil penelitian ini menunjukkan heterozigositas pada sapi Bali BPTU-HPT lebih rendah daripada sapi Bali peternakan rakyat namun keduanya menunjukkan adanya keragaman genetik pada masing-masing populasi. Nilai FIS menunjukkan nilai negatif pada sapi Bali BPTU-HPT lebih kecil daripada sapi Bali peternakan rakyat yang menandakan tingkat perkawinan sedarah pada sapi Bali BPTU-HPT lebih rendah, namun nilai FST menunjukkan keduanya memiliki kedekatan genetik antar populasi. Nilai *off-diagonal* GRM yang negatif menunjukkan bahwa pada masing-masing individu dalam populasi tidak berhubungan erat secara genetik. Analisis MDS menunjukkan bahwa sapi Bali BPTU-HPT memiliki kelompok yang lebih menyebar daripada sapi Bali peternakan rakyat, namun kedua populasi masih berada dalam kelompok yang sama. Kesimpulan dari penelitian ini adalah tingkat keragaman genetik pada sapi Bali BPTU-HPT lebih terjaga daripada sapi Bali peternakan rakyat, namun demikian kedua populasi masih memiliki tingkat kedekatan genetik yang tinggi.

Kata Kunci: Sapi Bali, Keragaman genetik, Teknologi genom, SNP.

Tema 2.

Asosiasi Penanda Genetik Terhadap Sifat Pertumbuhan Sapi Bali

Identification of 19-bp indel of the Pleomorphic Adenoma Gene 1 in Bali cattle population

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Abstract. Pleomorphic adenoma gene 1 (PLAG1) is a zinc finger transcription factor gene located on bovine chromosome 14 (BTA14) affecting body size and reproduction traits in cattle. The objective of this study was to identify 19-bp indel of the PLAG1 gene in Bali cattle population. A total of 96 blood samples of Bali Cattle were collected from Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BPTU-HPT) Denpasar. Genomic DNA was extracted from blood samples and used to detect 19-bp indel of the PLAG1 gene using following primer pair 5'-TCCGAACAACAGGTGAGGGAGAAAT-3' and 5'-CCACTTCAGG-GGTGCTCTAGGTTTG-3'. The polymerase chain reaction (PCR) products using DNA pool samples were sequenced to validate the PCR product and to find out novel polymorphism in Bali cattle population. The result showed that there was no variation found in Bali cattle population based on 19-bp indel of the PLAG1 gene, which is indicated by 123 bp DNA band. However, sequence analysis of the PLAG1 gene resulted in a novel single nucleotide polymorphism (SNP) at nucleotide number 32235 of the PLAG1 gene that changed guanine (G) to adenine (A). This novel SNP could be furthermore genotyped and it might be a potential candidate marker for body size and reproduction traits in Bali cattle.

1 Introduction

Cattle is one of livestock commodity producing meat who are considerable contributing to national beef supply. In 2019, beef production reaches 504,802.29 tons which contributes around 10.32% of total meat production in Indonesia. Beef is the second highest meat commodity after broiler in fulfilling meat national needs [1]. Cattle plays an important role in national meat supply. Therefore, it is very important to improve productivity and population of Indonesian local cattle, especially whose genetically superior such as Bali cattle *(Bos javanicus*).

Bali cattle is Indonesian native cattle breed which is hypothesized to be originated from domestication of wild Banteng (*Bibos banteng syn Bos sondaicus*) long time ago. Some

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advantages of Bali cattle are more adaptable with Indonesian tropical environment, high efficiency of production and reproduction such as high fertility (80-85%), calving interval around 12 to 14 months, and high carcass percentage. Moreover, sexual maturity of Bali cattle is started at the age of 18 months, the estrus cycle in heifer ranges from 16 to 23 days, its estrus time is 36 to 48 hours with 18 to 27 hours of fertile period, and Bali cattle's fertility is much higher than *Bos taurus* cattle [2]. In addition, body weight and measurement traits are the easiest growth traits to be observed consisting of birth weight, actual body weight, withers height, body length, chest girth, waist height, and chest depth [3]. Those traits are well-known as quantitative traits which are economically and biologically important traits and able to be used as indicators in selecting superior Bali cattle [4]. The development of recent molecular technique allows human being to improve native cattle production genetically through marker assisted selection.

A candidate gene well-known involving in cattle growth is Pleomorphic adenoma gene 1 abbreviated PLAG1. It is also known as a gene controlling growth and body size of cattle due to directly affecting *Insulin-like growth factor* 1 (IGF1) serum [5-6]. Previous study reported that a 19-bp indel namely rs523753416 located in intron 1 of the PLAG1 gene is associated with growth and body measurement traits of Chinese cattle population [7]. It is also associated with birth weight of Indonesian Peranakan Ongole cattle [8]. In addition, PLAG1 gene affects carcass traits of Nellore cattle [9]. The PLAG1 gene is not well-studied in Indonesian cattle. Therefore, the objective of this study was to identify 19-bp indel of the PLAG1 gene in Bali cattle population.

2 Materials and methods

2.1 Bali cattle population and DNA extraction

A total of 96 Bali cattle were used in this study. They were 2 to 3 years old which are originated from BPTU-HPT Denpasar, the Province of Bali. The blood samples were collected from *vena jugularis* by using 18G vacutainer needle and 3 ml tube containing EDTA. Those blood samples were then used to extract genomic DNA based on high concentrated salt method described by Montgomery and Sise [10]. Moreover, extracted genomic DNA was quantified to evaluate DNA concentration and purity using NanoPhotometer (P-Class®, Implen, Munchen, Jerman). The genomic DNA was stored at -20ºC until used.

2.2 Amplification of the PLAG1 gene and sequencing analysis

Amplification of the PLAG1 gene was conducted using primer pairs previously reported by Xu et al. [7] as follows: 5'-TCCGAACAACAGGTGAGGGAGAAAT-3' as forward primer and 5'- CCACTTCAGGGGTGCTCTAGGTTTG-3' as reverse primer. A polymerase chain reaction (PCR) was carried out in total volume of 25 µL containing 12.5µL Go Taq® Green Master Mix (Promega, Madison, USA), 9.5 µL nuclease free water, 1 µL each primer, and 1 µL genomic DNA template. The PCR was initiated by pre-denaturation at 95°C for 5 minutes and followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Final extension was applied at 72°C for 10 minutes to complete the reaction. Furthermore, the PCR product was checked on 2% agarose gels stained by ethidium bromide using submarine electrophoresis system (Mufid ex, Advance, Japan) at 110 volt for 35 minutes. Then, it was visualized on the Glite UV Gel Doc System® (Pacific Image Electronic Co., Ltd., New Taipei City, Taiwan). In addition, three

DNA pool samples containing three different individual genomic DNA was sequenced using 1st BASE sequencing service (APICAL SCIENTIFIC Laboratory, Selangor, Malaysia).

2.3 Data analysis

The PLAG1 gene sequence obtained in this study was analyzed using BioEdit Sequence Aligment Editor Version 7.2.5 to extract and to get clear the PLAG1 gene sequences. To identify 19-bp indel and other polymorphism within the gene, all sequences were aligned using Clustal Omega software which is accessible online (https://www.ebi.ac.uk/Tools/-msa/clustalo/).

3 Results and discussion

3.1 Amplification of 19-bp indel of the PLAG1 gene

Amplification of the PLAG1 gene fragment in Bali cattle population resulted 123 bp PCR product (Figure 1). This result was in line with those predicted for detection 19-bp indel of the PLAG1 gene. It showed that the DNA isolated from Bali cattle blood was adequate to be used for PCR. The success of DNA amplification depends on the accuracy of the primers used to anneal with the right site of the DNA template [11]. In the PCR, the function of oligonucleotide primer is to hybridize with the DNA template, to define the specific fragment of DNA to be amplified, and also at the same time to provide a hydroxy group (-OH) at the 3' end which is needed for the DNA extension [12].

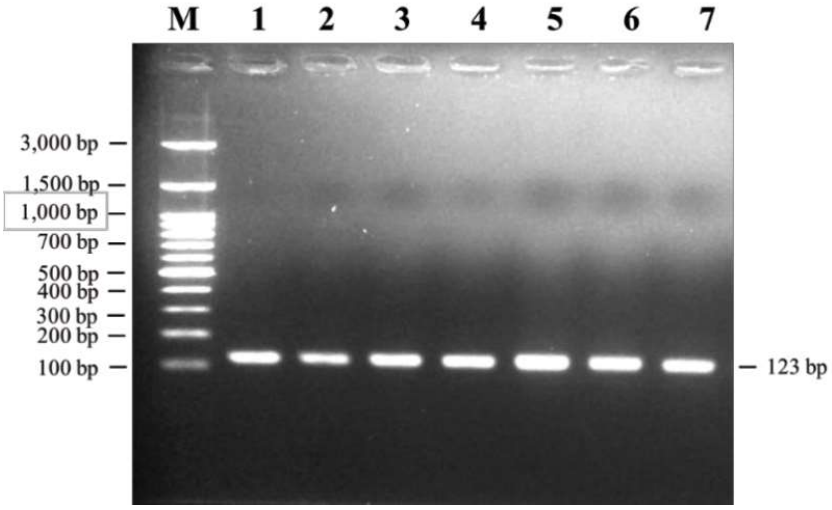

Fig. 1. The PCR product of 19-bp indel of the PLAG1 gene. M is 100 bp marker ladder; 1-7 are individual Bali cattle sample with DD genotype.

The PLAG 1 gene is located on chromosome 14 in cattle and has 4 exons [13]. Identification of 19-bp indel of the PLAG 1 gene in Bali cattle found only one genotype (DD) indicated by 123 bp DNA band (Figure 1). In identification of 19-bp indel in PLAG1 gene, the expected PCR products are 142 bp DNA band for homozygous wild type (WW), 142 and 123 bp for heterozygous (WD), and 123 bp for homozygous deletion (DD) [7]. Bali cattle genotype which tends to be less varied (monomorphic) is due to the phenomenon of phenotypic plasticity as a result of natural selection [14]. In addition, the ability of an individual to display more than one morphology is a response to environmental changes which is known as phenotypic plasticity [15].

3.2 Analysis of the PLAG1 gene sequence

Sequence alignment analysis of the PLAG1 gene fragment identified an intronic single nucleotide polymorphism (SNP) located at nucleotide number 32235 of the PLAG1 gene. This SNP was a kind of substitution mutation that changed Guanine (G) to Adenine (A) (Figure 2A and 2B). The 19-bp indel did not vary since only DD variant found which were proved by identical PCR products size for all samples used in this study (Figure 1) and identical nucleotide sequences based on alignment analysis (Figure 2C). Previous study reported that there are three variants for 19-bp indel location [7]. Moreover, polymorphisms in the PLAG1 gene are associated with body size and growth traits of beef cattle [7,16-18]. Therefore, the novel SNP located at nucleotide number 32235 of the PLAG1 gene could be promising marker to be genotyped in Bali cattle since PLAG1 gene is well-studied gene affecting cattle growth. Unfortunately, there was no restriction enzyme site for this novel SNP, direct sequencing could be the right approach to genotype this SNP.

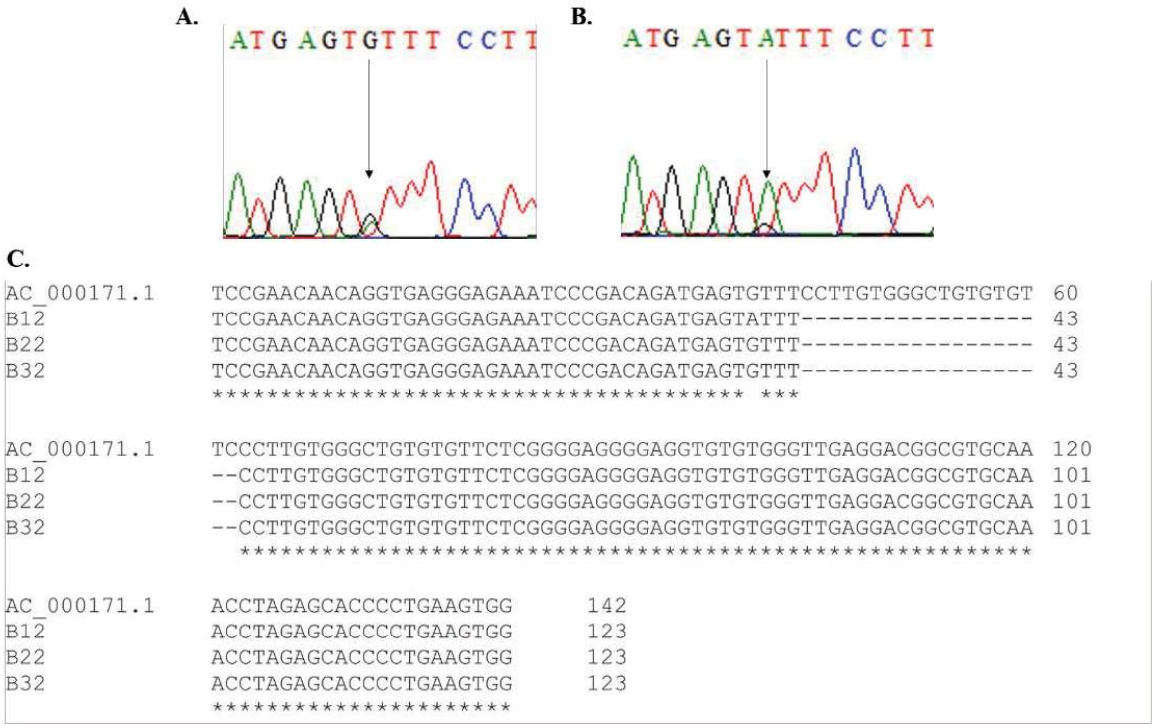

Fig. 2. A novel SNP of the PLAG1 gene fragment identified in this study. A. G variant at nucleotide number 32235 of the PLAG1 gene, B. A variant at nucleotide number 32235 of the PLAG1 gene, C. Alignment of PLAG1 sequences.

4 Conclusion

The 19-bp indel of the PLAG1 gene did not vary where only DD genotype identified in Bali cattle population. However, a novel SNP located at nucleotide number 32235 of the PLAG1 could be promising genetic marker to be genotyped for association study.

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RESEARCH ARTICLE
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Association of pleomorphic adenoma gene 1 with body weight and measurement of Bali cattle (*Bos javanicus*)

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Abstract

Background and Aim: Pleomorphic adenoma gene 1 (*PLAG1*) encodes a multifunctional transcription factor that controls many genes and pathways and is associated with cattle body weight and measurements. This study aimed to evaluate the association between *PLAG1* polymorphisms with body weight and measurements in Bali cattle.

Materials and Methods: A total of 87 Bali cattle, consisting of 48 bulls and 39 heifers at the Breeding Center for Bali Cattle, were used as the population in this study. Cattle were 2 years old and kept semi-intensively in the pasture. Phenotype data consisting of body weight, withers height, body length, chest girth, waist height, and chest depth were measured. Birth weight data were obtained from birth records, and weight gain, adjusted weaning weight, and yearling weight were calculated using formulas. Blood samples were taken from the jugular vein as much as 5 mL, and genomic DNA was isolated using the salting-out method. Polymerase chain reaction (PCR) was performed to amplify three target polymorphisms, namely, g.48308 C>T, g.32212 (19 bp indel), and g.45233 T>C. The presence of a 19 bp indel was determined by direct observation of the PCR product on a 2% agarose gel. Two other polymorphisms were detected by PCR-restriction fragment length polymorphism using the restriction endonuclease enzymes *Sac*II and *Bcl*I. *PLAG1* genotype and phenotype associations were analyzed using a general linear model.

Results: The results showed that two of the target polymorphisms in *PLAG1* did not vary. The DD genotype indicated by 123 bp of PCR product was the only genotype identified for g.32212 19 bp indel, and TT genotype was the only genotype found for g.45233 T>C single-nucleotide polymorphism (SNP). Conversely, g.48308 C>T SNP was found to be polymorphic. In addition, the g.48308 C>T polymorphism of *PLAG1* was significantly associated with body length of Bali cattle. Cattle with the CC genotype had a greater body length than the other two genotypes.

Conclusion: The g.48308 C>T SNP in *PLAG1* was associated with Bali cattle body length characteristics. This finding could be used as a basis for selecting Bali cattle based on body length characteristics.

Keywords: association study, Bali cattle, body length, growth trait, Pleomorphic adenoma gene 1.

Introduction

Bali cattle (*Bos javanicus*) are indigenous Indonesian cattle, which are hypothesized to have originated from the domestication of wild *Banteng* long ago. Bali cattle breed well on Bali Island because the Balinese culture venerates cattle [1]. In 1986, the Government of Indonesia established the Pulukan Breeding Center which is now known as the Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BPTU-HPT) Denpasar. It was founded 10 years after the Bali cattle breeding and development project started. The project focused on the improvement of Bali cattle population and its genetic quality by conventional breeding program [1]. Bali cattle have several advantageous characteristics, including good adaptability to tropical climates, high fertility, resistance to parasites, and low meat fat content [2,3]. Therefore, Bali cattle are one of the national cattle genetic resources that need to be maintained and used sustainably to optimize these advantages. Bali cattle are also listed as a cattle breed by the Food and Agriculture Organization [4].

Bali cattle are a cattle breed with the largest population in Indonesia, reaching 32.3% [5]. Therefore, Bali cattle play an important role in satisfying national meat consumption of 2.31 kg per capita, equating to the need for 624,162 tons of red meat [6]. However, this high demand is not supported by

the amount of meat produced, which only reached 515,600 tons in 2020 [7]. The low production of meat could be overcome by utilizing the potential of Bali cattle as a native Indonesian genetic resource. Improving the genetic quality of Bali cattle must be conducted continuously and in a planned manner with measurable milestones. One way to increase the productivity of Bali cattle is through marker-assisted selection (MAS). The development of molecular genetics has enabled the identification of multiple genes and genetic markers associated with genes responsible for desired phenotypic traits, including quantitative trait loci (QTL) or genomic regions affecting quantitative traits and genes for a particular trait [8,9]. A widely reported gene affecting livestock productivity is the pleomorphic adenoma gene 1 (*PLAG1*) gene [10].

PLAG1 is a member of the pleomorphic adenoma gene family along with *PLAGL1* and *PLAGL2*, which express a class of zinc-finger proteins [11]. The *PLAG1* encodes a multifunctional transcription factor that controls many genes and pathways, such as the Insulin-like growth factor (IGF)-II, IGF-1R, and WNT pathways [12]. Previous studies have reported that *PLAG1* affects the stature and body weight of dairy and beef cattle [13,14]. Hartati *et al*. [10] found that a single-nucleotide polymorphism (SNP) in *PLAG1* was positively associated with Indonesian Peranakan Ongole cattle body measurements. *PLAG1* plays a role in controlling the increase in body measurements and height in Japanese Black cattle [15]. In addition to body measurements, polymorphisms in *PLAG1* also affect cattle body weight and reproductive characteristics [16]. Xu *et al.* [17] reported that a 19 bp indel in *PLAG1* was associated with growth traits and body measurements in Pinan, Xianan, and Jiaxian cattle in China. Zhong *et al*. [18] reported that the g.48308C>T polymorphism of *PLAG1* significantly affected height and chest girth in five Chinese cattle breeds, and individuals with the CC genotype were preferred for these traits.

Few studies have evaluated *PLAG1* in *B. javanicus* because of the limited characteristics of uniform cattle in a large population [19,20]. This study aimed to evaluate the association between *PLAG1* polymorphisms with body weight and measurements in Bali cattle.

Materials and Methods

Ethical approval

All animal procedures related to samples of Bali cattle were approved by the Ethical Clearance Commission, National Research and Innovation Agency (BRIN) No. 81/Klirens/X/2021.

Study period and location

The study was conducted from March to November 2021. Blood samples of Bali cattle were collected at the Breeding Center of Bali Cattle (Denpasar, Indonesia). The laboratory works were carried out at the Division of Biology, Integrated Laboratory of Universitas Sebelas Maret.

Bali cattle population and measurement of phenotype data

Bali cattle used in this study were raised in the Bali cattle breeding center located at Pangyangan, Pekutatan, Jembrana Regency, Bali. The breeding center is located at an altitude of 125 m above sea level. It has an average rainfall of 485 mm/month with a temperature ranging from 22 to 30°C and average relative humidity of 70%.

A total of 87 Bali cattle, consisting of 48 bulls and 39 heifers, were obtained from two male paddocks and two female paddocks. The average age of the cattle was 2 years, and they were maintained semi-intensively with complete records. Up to 4 kg/animal of additional feed was given in the form of concentrate in the morning. Although cattle easily received forage from the paddock, additional king grass was given twice in the morning and afternoon, at a rate of 15 kg/animal. Drinking water was provided *ad libitum*. This management was the same for all cattle kept in the paddock. All cattle in this study had been vaccinated for *Septicemia epizootica* and Jembrana diseases.

Body weight data were measured using a Tru-Test EziWeigh7i digital scale (Datamars, Auckland, New Zealand) and expressed in kilograms. Body measurement data consisting of withers height (a), body length (b), chest girth (c), chest depth (d), and waist height (e) were measured using a ruler and measuring tape following SNI [21] and were calculated in centimeter (Figure-1). Birth weight data were obtained from records at BPTU-HPT Bali Cattle. Furthermore, data on weight gain, weaning weight, and yearling weight were calculated using the formulas reported by Chenette and Frahm [22] as follows:

$$WG = \left(\frac{BWi - BW}{AA} \right)$$

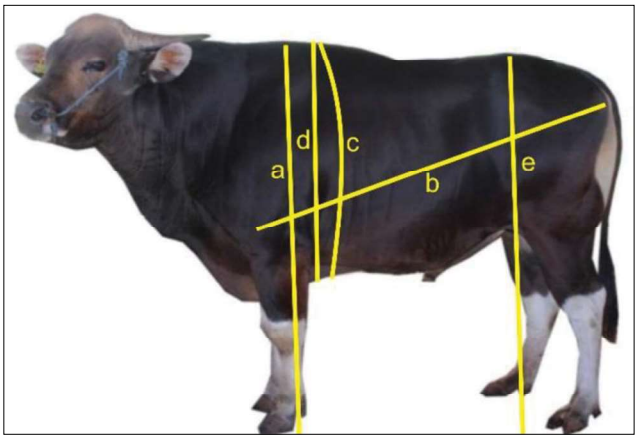

Figure-1: Body measurement of Bali cattle according to SNI 7651-4: 2017. Line (a) represents withers height; (b) body length; (c) chest girth; (d) chest depth; and (e) waist height.

$$WW = \left(\frac{AW - BW}{AA} \right) \times 205 + BW$$

$$YW = \left(\frac{AW - WW}{AA - WA} \right) \times 160 + WW$$

Where, WG is weight gain, BWi is actual body weight, BW is birth weight, WW is adjusted 205 d weaning weight, AW is actual body weight, YW is adjusted 365 days yearling weight, AA is actual age (d), and WA is weaning age. Body weight was expressed in kilograms, and age was expressed in days.

Blood sample collection and DNA extraction

Blood samples were collected from the jugular vein of the cattle as much as 5 mL using an 18G vacuum needle in a 10 mL Vacutainer tube containing EDTA. The collected blood samples were stored in the cooling box at 0°C during transportation and kept in the refrigerator at 4°C until further analysis. DNA extraction from blood samples was conducted following the high salt method protocol by Montgomery and Sise [23]. The genomic DNA obtained was quantified using a NanoPhotometer (P-Class®, Implen, Munich, Germany); DNA concentration (ng/μL) and DNA purity were obtained by comparing the optical density at 260 and 280 nm. The concentration of genomic DNA in this study was set at a minimum of 20 ng/μL and purity greater than 1.8.

Amplification and genotyping of *PLAG1*

The amplification of *PLAG1* targeted three polymorphisms, namely, g.48308 C>T, g.32212 (19 bp indel), and g.45233 T>C. Polymerase chain reaction (PCR) was conducted using a MiniAmp® Thermal cycler machine (Thermo Fisher Scientific, Singapore). The PCR reaction consisted of 12.5 μL GoTaq(R) Green Master Mix (Promega, Madison, USA), 9.5 μL nuclease-free water (1st BASE, Singapore), 1 μL of each primer (Table-1) [17,18], and 1 μL of template DNA. All materials were mixed in a PCR tube with a total volume of 25 μL. The amplification of *PLAG1* was initiated by pre-denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing, and extension at 72°C for 30 s, and the reaction was completed after a final extension at 72°C for 10 min. The annealing temperature and time are listed in Table-1. The PCR products were run on a 2% agarose gel stained with ethidium bromide (Promega) using the Submarine Electrophoresis System Mufid ex (Advance, Tokyo, Japan) for 30 min at 110 V. The 100 bp marker ladder (Geneaid, Taiwan) was used as the standard for the DNA band size. The agarose gel was then visualized using a Gel Documentation System (Glite UV, Pacific Image, Taiwan).

Genotyping was conducted by observing DNA bands of PCR products to detect the 19 bp indel, and digestion of PCR products was conducted using the PCR-restriction fragment length polymorphism (PCR-RFLP) technique to detect other polymorphisms. PCR-RFLP was performed according to the FastDigest *Sac*II protocol for the g.48308 C>T SNP and Fast Digest *Bcl*I for the g.45233 T>C SNP (Thermo Fisher Scientific Inc., Vilnius, Lithuania). The individual Bali cattle genotype was determined by 2% agarose gel electrophoresis of the digested PCR product. The gel was then visualized under UV light using a Gel Documentation System. PCR products of three DNA pools were sequenced to confirm amplicon size (Apical Scientific, Malaysia).

Statistical analysis

Genotype and allele frequencies were calculated, and Pearson's Chi-square test was conducted to verify the Hardy-Weinberg equilibrium (HWE) status. A general linear model was applied to evaluate the effects of *PLAG1* polymorphisms on body weight and measurements using MINITAB version 14.0 software (Minitab Inc., USA). Association analysis was performed using the following model:

$$Y_{ijk} = \mu + G_i + S_j + \varepsilon_{ijk}$$

Where, Y_{ijk} is the phenotype of the k^{th} animal, μ is the population mean, Gi is the fixed effect of genotype, Sj is the fixed effect of sex, and ε_{ijk} is the residual error associated with the k^{th} animal. Tukey's test was performed to determine pairwise differences among the genotypes.

Results

Amplification and genotyping of *PLAG1*

Three DNA fragments specific to *PLAG1*, 628 bp, 123 bp, and 767 bp, were successfully amplified using PCR (Figure-2). The 628 bp DNA fragment represented the g.48308 C>T SNP located in the 3'UTR region and was recognized by *Sac*II. There were three genotypes, namely, the CC (502 bp and 126 bp), TC (628 bp, 502 bp, and 126 bp), and TT genotypes (628 bp, the fragment could not be digested by the

Table-1: Primer pairs of pleomorphic adenoma gene 1 used in this study.

Polymorphism	Primer (5' to 3')	Annealing (°C/s)	Amplicon (bp)	RE	Reference
g. 48308 C>T	F: gcgcgtatcagtcaggacat R: cctttgcctgttgctttccc	58/45	628	*Sac*II	[18]
g. 32212 19 bp indel	F: tccgaacaacaggtgagggagaaat R: ccacttcaggggtgctctaggtttg	60/30	142/123	-	[17]
g. 45233 T>C	F: gcgtgaaggagaagaagcac R: gatcgggttatagggagggc	56/45	767	*Bcl*I	This study

RE = Restriction enzyme

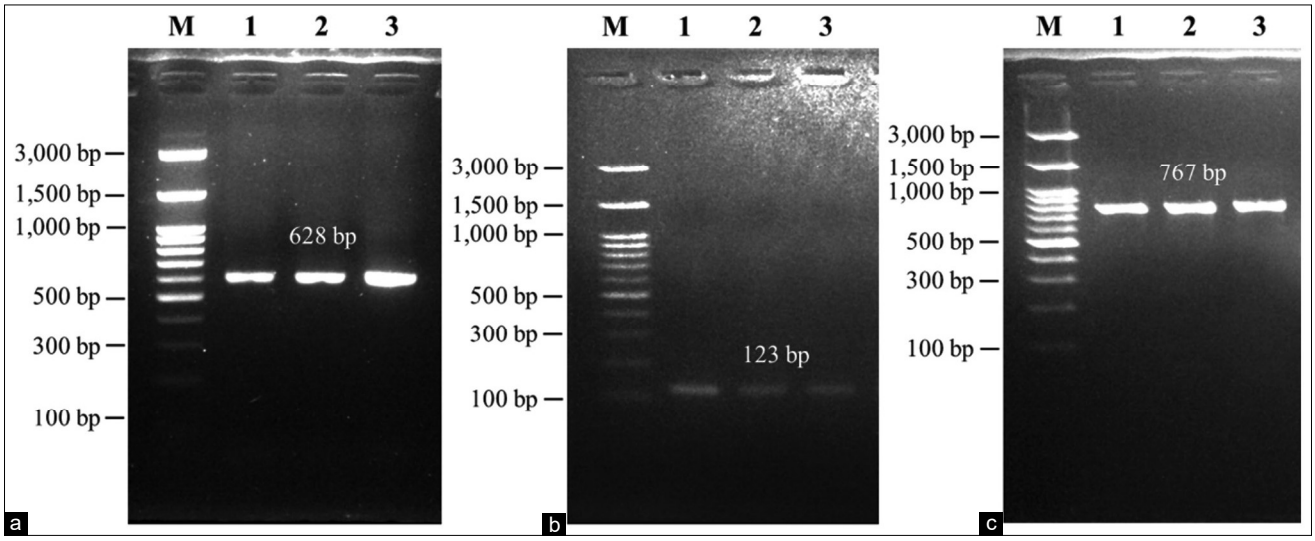

Figure-2: Amplification of pleomorphic adenoma gene 1 polymorphisms. (a) Is an amplicon for detecting the g.48308 C>T single-nucleotide polymorphism (SNP), (b) for detecting the 19 bp indel, and (c) for detecting the g.45233 T>C SNP.

restriction enzyme). Thus, this study identified three genotypes in the Bali cattle population (Figure-3).

A 19 bp indel is located in intron 3, specifically at nucleotide number 32212, of *PLAG1*. This polymorphism was detected by the presence of a 19 bp indel, giving rise to three types of genotypes, namely, II, ID, and DD. Genotype II was characterized by the appearance of one band at 142 bp and the DD genotype at 123 bp, whereas the heterozygous ID produced two bands, 142 bp and 123 bp. The genotype detected in the Bali cattle population was the monomorphic DD genotype (100%). Another SNP identified in *PLAG1*, namely, the g.45233 T>C SNP, located in exon 4, is a missense variant that has been successfully amplified in a 767 bp DNA fragment. However, this SNP did not vary in the Bali cattle population.

Allele and genotype frequencies of *PLAG1* in Bali cattle

The analysis showed that the CC genotype was dominant (88.5%), with an allele frequency of 0.925 (Table-2). Furthermore, based on the HWE analysis, the Bali cattle population experienced an imbalance, as indicated by the HWE value of 15.21, which was greater than the X^2 value with $p<0.05$. Furthermore, the 19 bp indel and g.45233 T>C SNP were monomorphic. All individual Bali cattle genotypes were homozygous (DD and TT) for both polymorphisms. Thus, they could not be used for the association analyses.

Association of *PLAG1* polymorphisms with body weight and traits

Analysis of the associations between the g.48308 C>T SNP and body size and weight in Bali cattle are shown in Table-3. Statistically, the SNP g.48308 C>T was significantly associated with body length in the Bali cattle population. Individuals with the CC genotype had a greater body length than those with the other genotypes (p=0.004). In addition, these SNPs tended to be significantly associated with waist height and chest depth in Bali cattle (p=0.066 and p=0.067, respectively).

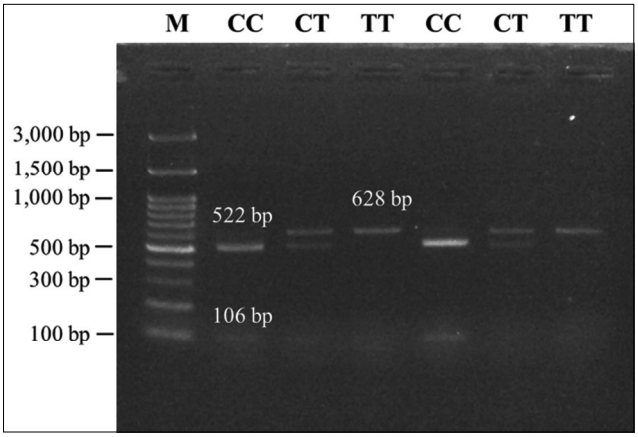

Figure-3: Genotyping of the pleomorphic adenoma gene 1 based on the g.48308 C>T single-nucleotide polymorphism. M is the 100 bp marker ladder, CC represents Bali cattle with the CC genotype, CT represents Bali cattle with the CT genotype, and TT represents Bali cattle with the TT genotype.

Table-2: Allele and genotype frequencies of the g.48308 C>T single-nucleotide polymorphism of pleomorphic adenoma gene 1.

Genotype frequency			Allele frequency		χ^2	p-value
CC	CT	TT	C	T		
0.8850	0.0805	0.0345	0.925	0.075	15.21	9.6429E-05

χ^2=Chi-square

Discussion

PLAG1 is a proto-oncogene that encodes a zinc-finger containing transcription factor and is involved in many pathways. It is located on bovine chromosome 14 (BTA14). Based on the ensemble database, *PLAG1* spans nucleotides 23,330,541-23,375,751 bp on BTA14 and has five exons, four introns, and five transcripts [24]. This study is the first to determine an association between the g.48308 C>T SNP of *PLAG1* and body length in a Bali cattle

Table-3: Association between the g.48308 C>T single-nucleotide polymorphism of pleomorphic adenoma gene 1 and body weight and traits in Bali cattle.

Trait	Mean±SE			p-value
	CC (n=77)	CT (n=7)	TT (n=3)	
Birth weight (kg)	18.94±0.28	18.86±0.71	19.00±1.00	0.794
Weaning weight (kg)	72.03±2.66	67.66±4.30	74.76±1.06	0.103
Yearling weight (kg)	113.47±4.64	105.75±7.45	118.27±2.27	0.101
Body weight (kg)	214.34±8.22	209.00±13.80	187.00±18.60	0.069†
Weight gain (kg/day)	0.26±0.01	0.24±0.02	0.27±0.01	0.101
Withers height (cm)	112.19±0.69	110.14±2.37	109.67±3.33	0.142
Body length (cm)	107.52[a]±1.17	101.86[b]±2.65	103.67[b]±1.86	0.004**
Chest girth (cm)	147.04±1.72	147.71±3.33	140.00±4.04	0.109
Waist height (cm)	111.86±0.67	107.86±2.65	109.50±1.50	0.066†
Chest depth (cm)	57.468±0.71	55.57±1.73	55.50±2.50	0.067†

SE is standard error; n is number of samples; ** indicates highly significant effect; † indicates a suggestive-significance effect.

population. Individuals with the CC genotype possess a greater body length than cattle with other genotypes. This polymorphism has previously been reported to be associated with growth traits in five breeds of cattle in China, revealing that cattle with the CC genotype had greater height and chest girth than cattle with the TT genotype [18]. However, the analysis showed that the Bali cattle population in this study was not in HWE. This can be caused by migration, mutation, recombination, or selection efforts in the population [25]. The Bali cattle population used in this study was obtained from BPTU-HPT Bali cattle, which are allotted the task of providing superior Bali cattle. A selection process may have been employed by the agency considering that its task is to maintain and provide superior Bali cattle breeds for the community. In addition, Bali cattle breed well on Bali Island because Balinese culture respects cattle and this culture is not found in other parts of Indonesia [1,3,4]. This study also identified two other polymorphisms, namely, 19 bp indel and SNP g.45233 T>C, but these two polymorphisms were monomorphic and could not be associated with body weight and measurement traits. Previous research conducted by Xu *et al*. [17] who showed a positive association between the 19 bp indel in *PLAG1* and growth traits in Pinan, Xianan, and Jiaxian cattle in China. Different results were reported by Peng *et al*. [26] who found that the 19 bp indel in *PLAG1* was not associated with phenotypic traits of Xianjian brown, red steppe, and Yunling cattle. *PLAG1* has been widely reported to be associated with growth traits, body size or stature, and reproductive traits in various breeds of cattle [10,13-18]. It affects hip height, growth rate, carcass weight, body condition score, birth weight, and weight at different stages of age [27-30]. It is also responsible for growth physiology [31] in milk production, body size, coat color, and muscle formation in cattle [32]. The association between birth weight and *PLAG1* genotype has been verified in a Friesian Holstein dairy cattle population. The results showed that *PLAG1* is related to body size regulation [33]. This statement is supported by Abi Habib *et al*. [31] who reported that *PLAG1* plays a role in growth physiology. Functional mutations in the bovine *PLAG1* have also been reported to be associated with stature in beef cattle [12]. In addition, an epistatic interaction of the *PLAG1* polymorphism with other genes, such as *IGF2* and insulin, has been reported in cattle [34]. QTL regions that significantly affect livestock height have been mapped to a region on chromosome 14. The mapping of quantitative trait nucleotides (QTNs) to the *PLAG1-CHCHD7* intergenic region shows a positive association with cattle body size [13]. Moreover, a pleiotropic QTN named bovine HD1400007259 in the *PLAG1-CHCHD7* gene region of BTA14 was shown to be significantly associated with bicep and calf muscle size [35].

Selection based on genetic markers of growth traits is very effective in improving cattle performance. Bali cattle, indigenous Indonesian beef cattle, must be given more attention, and productivity must be increased through MAS [36]. Genetic marker-based livestock selection programs have been very effective because they can be conducted as early as needed; thus, they are more efficient than conventional livestock selection [37]. In addition, growth traits have a heritability of up to 0.43, which means that 43% of growth traits are affected by genetics [38]. The nature of growth is represented by body weight and measurements of livestock. This study showed that *PLAG1* is one of the candidate genes responsible for growth traits of Bali cattle since g.48308 C>T SNP was significantly affected body length of Bali cattle. Therefore, the results of this study could be used as a basis for developing policies to improve the genetic quality of Bali cattle by the government through the BPTU-HPT Bali Cattle. Consequently, the function of the Bali Cattle Breeding Center as a provider of superior Bali cattle breeds could be optimized.

Conclusion

The g.48308 C>T SNP of *PLAG1* was associated with the body length trait of Bali cattle. This finding could be used as a foundation for selecting Bali cattle based on body measurement characteristics using

MAS, which is much more effective and efficient than conventional phenotypic selection. However, validation in different populations with a larger number of Bali cattle should be taken into account to achieve more reliable results.

Authors' Contributions

MC: Conceptualization, methodology, formal analysis, funding acquisition, supervision, and writing – review and editing. SS: Validation, investigation, data curation, writing – original draft preparation. MID: Validation, writing – original draft, and project administration. TAB: Validation and writing – original draft. YY and JR: Resources and writing – review and editing. SDV and PS: Investigation and writing – original draft. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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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 T*aq*I 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, GGAACTCT, 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 K_3EDTA (anticoagulant). DNA was extracted using the salting-out method [15]. DNA was stored at -20^{o}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'-GAGGAAGGCGATGGTGAT-3' [13]. The total volume of PCR mixture is 10 µl containing 5 µl PCR Mastermix (My Taq^{TM} Red Mix, Bioline), 1 µl for each primer (10 pmol/µl), 2 µl water-free nuclease, and 1 µl DNA template. The mixture was performed by PCR Gradient machine (Eppendorft, Germany) with condition program: pre-denaturation 94^{o}C for 5 minutes, followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 64^{o}C for 30 seconds, extension at 72^{o}C for 45 minutes, and the final extension at 72^{o}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 T*aq*I 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 *Taq*I 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 (T*aq*I enzyme was not recognized of TG^CA restriction site). Then, a heterozygote genotype (AG) was performed 428, 318, and 110 bp (figure 2).

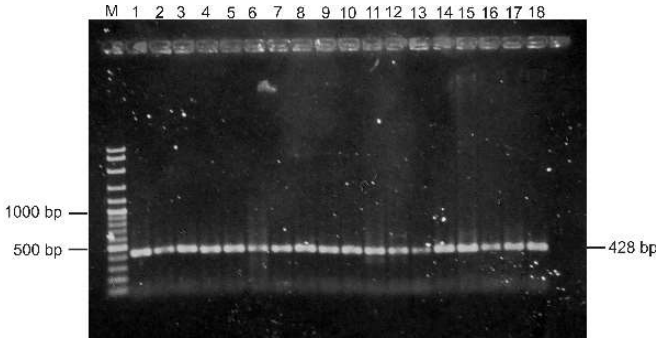

Figure 1. Visualization of PCR product of *INSIG1* gene in Bali cattle (428 bp).

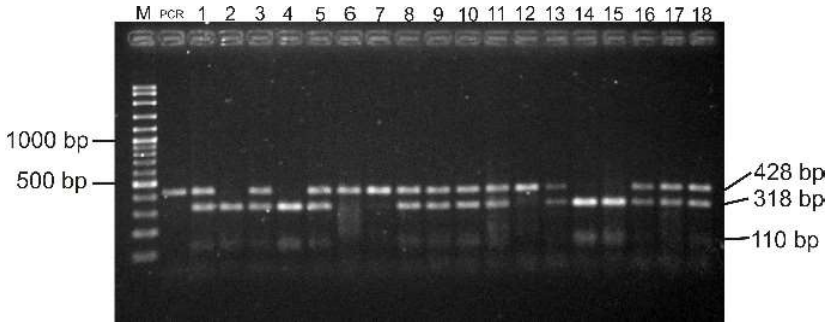

Figure 2. Visualization of PCR-RFLP products of *INSIG1* gene in Bali cattle. M: Ladder 100 bp; PCR: PCR product (428 bp); lane 1,3,5,8-11,16-18: genotype AG; lane 2,4,14,15: genotype GG; and lane: 6,7 : genotype AA.

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	X^2 Calculated
		AA	AG	GG	A	G	Ho	He		
Bali cattle from Badung Bali	53	13 (0.245)	27 (0.510)	13 (0.245)	0.500	0.500	0.505	0.500	0.375	0.019

X^2 Table =3.841. If X^2 Table > X^2 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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Tema 3.

Kajian Manfaat Pola Pemeliharaan Intensif Pada Sapi Bali

DAMPAK SISTEM PEMELIHARAAN INTENSIF DAN SEMI INTENSIF TERHADAP UKURAN TUBUH SAPI BALI JANTAN DI BALAI PEMBIBITAN TERNAK UNGGUL (BPTU) SAPI BALI

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ABSTRACT

Bali cattle is originated from Bali Island, Indonesia and it is spread out to Malaysia. Bali cattle are well known as a beef cattle with high carcass percentage and good reproduction trait. There are several different maintenance systems in Bali cattle i.e extensive, intensive and semi intensive. The aim of this study was to evaluate body sizes of Bali cattle reared under intensive and semi intensive systems at Breeding Center of Bali Cattle (Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BPTU-HPT) Pulukan). Twenty Bali bulls from BPTU Pulukan were used. Body measurement including body length, high of hip, high of shoulder, chest circumference, chest depth, and body weight of Bali bull under two rearing models have been measured. Independent sample T-test has been performed to differentiate body sizes of Bali bull by using SPSS 17.0 software. Result showed that all body sizes of Bali cattle reared under intensive system was significantly better than semi intensive system ($P<0.05$). Body weight of Bali cattle in intensive systems reached 373.20±36.09 kg, on the other hand, Bali bull reared under semi intensive was only 210.75±30.14 kg. In addition, body measurements of Bali bull raised intensively was significantly higher than Bali bulls raised under semi intensive system ($P<0.05$). It might be caused by Bali bulls in intensive rearing system got richer and more complete nutritional values, therefore their genetic growth performances can be fully expressed. In conclusion, intensive rearing system could be applied to reach better growth performance of Bali bull and it could be utilized as a strategy of breeding program to fulfill national beef needs.

Keywords: *body sizes, Bali bull, rearing system, intensive, semi intensive, Breeding Center of Bali cattle*

ABSTRAK

Sapi Bali berasal dari pulau Bali Indonesia dan tersebar sampai wilayah Malaysia. Sapi Bali dikenal sebagai sapi potong dengan persentase karkas tinggi dan performans reproduksi yang baik. Terdapat tiga sistem pemeliharaan ternak sapi Bali yaitu ekstensif, intensif dan semi intensif. Tujuan dari penelitian ini adalah untuk mengevaluasi ukuran tubuh sapi Bali jantan yang dipelihara pada sistem pemeliharaan intensif dan semi intensif di BPTU Sapi Bali Pulukan Bali. Sebanyak 20 sapi Bali jantan yang dipelihara dengan sistem pemeliharaan berbeda diukur Panjang Badan, Tinggi Pinggul, Tinggi Gumba, Lingkar Dada, Dalam Dada, dan Berat Badannya. Analisis data menggunakan independent sample t-test untuk membedakan rata-rata ukuran tubuh sapi Bali dengan software SPSS. 17.0. Hasil menunjukkan bahwa ukuran tubuh sapi Bali jantan pada pemeliharaan intensif di BPTU Sapi Bali lebih baik dibanding dengan pemeliharaan semi intensif ($P<0,05$). Berat badan sapi Bali pada sistem pemeliharaan intensif mencapai 373,20 ± 36,09 kg sedangkan pada semi intensif hanya 210,75±30,14 kg. Ukuran tubuh sapi Bali pada sistem pemeliharaan intensif lebih tinggi dibandingkan semi intensif ($P<0,05$). Hal ini disebabkan sapi Bali jantan yang dipelihara pada sistem intensif memperoleh nutrisi pakan yang lebih baik dan komplit sehingga potensi genetik pertumbuhan terekspresi penuh. Dengan demikian, sistem pemeliharaan intensif pada sapi Bali jantan dapat diaplikasikan untuk memperoleh performans pertumbuhan yang lebih baik dan dapat digunakan untuk program pemuliaan atau seleksi dalam pemenuhan daging nasional kedepannya.

Kata kunci: *ukuran tubuh, sistem pemeliharaan, intensif, semi intensif, BPTU Sapi Bali*

PENDAHULUAN

Kebutuhan nasional akan protein hewani khususnya daging sapi semakin meningkat dengan seiringnya pertambahan penduduk Indonesia. Berdasarkan Buku Proyeksi Penduduk Indonesia 2010-2035, populasi penduduk mencapai 305 juta jiwa pada tahun 2035. Keadaan tersebut dapat menimbulkan permasalahan dalam hal penyediaan protein hewani daging kedepanya. Saat ini tahun 2017, Indonesia melakukan impor sapi bakalan sebesar 168.588.530 kg dan daging beku sebanyak 118.646.837 kg untuk memenuhi kebutuhan daging nasional. Populasi sapi nasional baru mencapai 16.429.000 ekor (Dirjen PKH: Buku Statistik Peternakan dan Kesehatan Hewan, 2018).

Indonesia mempunyai banyak rumpun sapi yang telah adaptif dengan lingkungan tropis salah satunya sapi Bali. Menurut Hardjosubroto (1994); Martodjo (2012), sapi Bali berasal dari domestikasi Banteng (*Bos banteng*). Populasi sapi Bali mencapai 4.789. 521 ekor (PSPK, 2011) yang telah tersebar hampir diseluruh wilayah Indonesia. Sapi Bali mempunyai keunggulan dibanding dengan rumpun bangsa sapi lainnya yaitu fertilitas atau produktivitas reproduksi yang baik, persentase karkas yang tinggi, dan mudah beradaptasi pada lingkungan baru (Mondang dan Talib, 2015). Persentase kebuntingan sapi Bali mencapai 86,56%, *calf crop* mencapai 83,4% (Astiti, 2018) dan umur beranak pertama berkisar 32-44 bulan tergantung dengan pakan yang dikonsumsi disetiap daerah (Gunawan *et al*., 2011). Sedangkan persentase karkas dapat mencapai 56 % (Astiti, 2018).

Terdapat tiga sistem pemeliharaan ternak yaitu ekstensif, intensif dan semi intensif. Pemeliharaan sistem intensif sering digunakan pada sapi potong di Indonesia karena lebih efisien dalam hal pemberian pakan, pembersihan kandang, penanganan penyakit dan memandikan ternak (Sugeng, 2000). Sistem pemeliharaan ekstensif dan semi intensif sering digunakan apabila pemeliharaan sapi berbasis integrasi dengan tanaman seperti kelapa sawit. Mondang dan Talib (2015), ketiga model pemeliharaan sapi Bali di perkebunan kelapa sawit memberikan dampak positif terhadap pengembangan sapi dan usaha perkebunan kelapa sawit.

Balai Pembibitan Ternak Unggul-Hijauan Pakan Ternak (BPTU-HPT) Denpasar merupakan *breeding center* atau pusat pembibitan khusus untuk sapi Bali yang terletak di Pulukan Bali dengan populasi mencapai 959 ekor pada tahun 2018 dengan jumlah penjantan sebanyak 63 ekor pejantan muda umur 1-2 tahun, 64 ekor pejantan muda umur 2-3 tahun dan 83 pejantan (Andreas, 2019). Informasi sistem pemeliharaan pada sapi Bali jantan penting untuk dilakukan agar diperoleh strategi pemeliharaan yang cocok baik untuk tujuan pembibitan (peningkatan mutu genetik) dan penggemukan. Sehingga tujuan dari penelitian ini adalah untuk mengetahui ukuran tubuh (sifat pertumbuhan) sapi Bali jantan yang diperlihara dalam dua sistem yang berbeda yaitu intensif dan semi intensif di Balai Pembibitan Ternak Unggul (BPTU) Sapi Bali.

METODE PENELITIAN

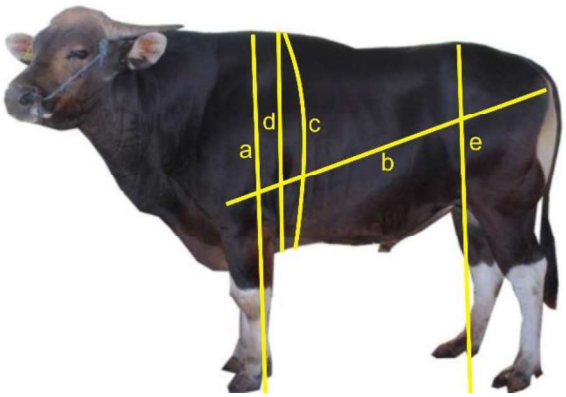
Ternak

Sebanyak 20 sapi Bali jantan dewasa berasal dari *breeding center* sapi Bali milik BPTU-HPT Denpasar yang terletak di Pulukan Bali digunakan dalam penelitian ini dan dipelihara dalam dua sistem manajemen pemeliharaan yang berbeda (Intensif dan Semi Intensif). Penentuan umur sapi berdasarkan SNI 7651-4: 2017, pergantian satu pasang gigi ditaksir pada umur 18 hingga 24 bulan sedangkan dua pasang gigi yaitu lebih dari 24 hingga 36 bulan.

Parameter

Parameter yang diamati dan diukur pada sapi Bali jantan dewasa yaitu berat badan dan ukuran tubuh yang meliputi:

a) Tinggi Pundak diukur dari permukaan lantai yang rata sampai bagian tertingi pundak melewati bagian skapulla secara tegak lurus dengan menggunakan tingkat ukur (SNI 7651-4: 2017).
b) Panjang Badan diukur dari bongkol bahu (*tuberositas humeri*) sampai ujung tulang duduk (*tuber ischii*) menggunakan tongkat ukur (SNI 7651-4: 2017).
c) Lingkar dada, diukur dengan melingkarkan pita ukur pada bagia dada dibelakang bahu (*Os scapula*) (SNI 7651-4: 2017).
d) Dalam Dada diukur dari bagian dada bagian bawah hingga dada bagian atas menggunakan tongkat ukur
e) Tinggi Pinggul diukur dari permukaan lantai yang rata sampai baguan tertinggi pinggul menggunakan tongkat ukur



Gambar 1. Pengukuran tubuh sapi. Ket: a) Tinggi Pundak; b) Panjang Badan; c) Lingkar Dada; (SNI 7651-4: 2017); d) Dalam dada; e) Tinggi Pinggul

Analisis Data

Data berat badan dan ukuran tubuh sapi Bali pada kedua sistem pemeliharan tersebut dianalisis dengan uji *independent sample t-test* menggunakan *software* SPSS 17.0.

HASIL DAN PEMBAHASAN

Secara garis besar pola pemeliharaan sapi terdiri dari ektensif, intensif dan kombinasi keduanya. Menurut Williamson and Payne (1993) pada sistem pemeliharaan ekstensif, ternak dipelihara secara bebas dan merumput tumbuhan yang ada dialam. Pada sistem ini ternak dilepas dengan komposisi jantan dan beberapa betina dalam satu populasi. Untuk sistem intensif yaitu ternak dipelihara dengan dalam kandang yang dibuat khusus. Parakkasi (1999) menambahkan bahwa pada pemeliharaan intensif, pemberian pakan hijauan secara *cut and carry*. Sedangkan sistem pemeliharaan semi intensif merupakan gabungan cara pemeliharaan ekstensif dan intensif dan masih memerlukan campur tangan manusia.

Sistem pemeliharaan sapi Bali jantan dewasa di BPTU HPT Denpasar menerapkan dua sistem, yaitu semi intensif dan intensif. Pada sistem intensif, sapi Bali jantan dikandangkan selama 24 jam (sistem *tail to tail*) sedangkan pada semi intensif sebaliknya dimana sapi dipelihara dalam *paddock* dengan induk dan calon induk terpisah dengan pejantan. Sistem perkawinan yang diterapkan yaitu inseminasi buatan dan kawin alam.

Berat dan ukuran tubuh merupakan salah satu indikator dalam performans pertumbuhan sapi. Hasil pengukuran menunjukkan bahwa sapi Bali jantan dewasa pada sistem pemeliharaan intensif mempunyai performans pertumbuhan lebih baik dibanding dengan sistem semi intensif ($P<0,05$) (Tabel 1). Hasil tersebut sesuai dengan penelitian Mullik dan Gusti (2009) dan Arisasmita (2018).

Tabel 1.

Ukuran tubuh sapi Bali jantan pada dua sistem pemeliharaan yang berbeda

Parameter	Model Pemeliharaan	
	Intensif	Semi intensif
Berat badan (kg)	373.20±36.09[a]	210.75±30.14[b]
Panjang Badan (cm)	127.20±3.73[a]	104.50±7.15[b]
Lingkar Dada (cm)	177.80±6.12[a]	147.20±8.81[b]
Dalam Dada (cm)	69.70±2.21[a]	56.30±4.67[b]
Tinggi Pinggul (cm)	121.50±2.99[a]	109.60±6.57[b]
Tinggi Pundak (cm)	121.90±3.73[a]	111.30±5.49[b]

[a,b]superskrip pada baris yang berbeda menunjukkan hasil yang signifikan (P<0.05)

Pertumbuhan dipengaruhi oleh banyak faktor seperti genetik dan lingkungan, salah satunya adalah pola pemeliharaan. Pada sistem semi intensif, ternak mempunyai kesempatan untuk bergerak dari satu tempat ketempat lainnya lebih tinggi dibanding dengan sistem intensif sehingga energi yang dikeluarkan menjadi lebih tinggi. Menurut Williamson and Payne (1993), ternak yang dipelihara pada sistem ekstensif dapat mencapai bobot potong 3 hingga 6 tahun lebih lama dibanding sistem pemeliharaan lainnya.

Selain pola pemeliharaaan, faktor pakan merupakan faktor lain yang berpengaruh terhadap pertumbuhan sapi Bali jantan (berat dan ukuran badan). Pakan yang diterapkan pada sapi Bali di BPTU HPT Denpasar berupa hijauan dan konsentrat (*complete feed*) sebagai pakan tambahan dengan Protein Kasar (PK) 14%. Pada sistem intensif, hijauan diberikan secara berkala sebanyak 20 kg per hari dan konsentrat 6 kg dengan pemberian minum secara ad libitum. Sedangkan pada sistem semi intensif, hijauan diperoleh dari rumput yang tumbuh dipadang penggembalaan. Jenis rumput yang tumbuh berupa *Penniisetum purpureum* dan *Paspalum natatum cv*. *Competidor*). Jumlah dan kadar PK konsentrat yang diberikan sama dengan sistem intensif. Air minum berupa ad libitum. Pertambahan berat badan tergantung dari suplai asam amino dan energi yang ditransfer ke jaringan tubuh (Poppi and McLennan, 1995).

Kompetisi perolehan pakan antar ternak pada sistem semi intensif dimungkinkan juga menjadi faktor lain yang berpengaruh terhadap rendahnya performans pertumbuhan dibanding pola intensif. Sistem pemeliharaan sapi Bali jantan secara intensif dapat menjadi alternatif untuk memperoleh performans pertumbuhan sapi Bali yang lebih optimal terutama dalam tujuan usaha penggemukan sapi.

KESIMPULAN

Sapi Bali jantan yang dipelihara pada sistem intensif mempunyai berat badan dan ukuran tubuh lebih baik dibanding dengan sapi yang dipelihara dengan sistem semi intensif. Dengan demikian, sistem pemeliharaan intensif pada sapi Bali jantan dapat diaplikasikan untuk memperoleh performans pertumbuhan yang lebih baik dan dapat digunakan untuk program pemuliaan atau seleksi untuk pemenuhan daging nasional kedepannya.

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Naskah Dalam Proses Publikasi Selanjutnya

Genome-wide Association Study Reveal Variants Related to Birth Weight in Bali Cattle (*Bos javanicus*)

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ABSTRACT

Phenotypic and genotypic data of 48 Bali cattle were used to figure out genomic regions which are potentially affecting the body weight traits of Bali cattle (Bos javanicus). Those samples were genotyped using Illumina bead chip array with 53,218 single nucleotide polymorphisms (SNPs). Estimation of variance proportion explained by each SNP was conducted by a restricted maximum likelihood (REML) approach. The result showed that body weight traits at different stages of age which are also dependent on the trait component were affected by multiple regions dispersed over the genome. This study found two interesting regions that related to quantitative trait loci (QTLs) and genes which are previously reported, such as STXBP6 and TERT affecting birth weight and calf growth in beef cattle. These identified regions may contribute for genomic-based selection programs development and refinement of Bali cattle in Indonesia.

Keywords: GWAS, Bali cattle, birth weight, STXBP6, TERT

Identifikasi Mutasi Gen *STXBP6* Pada Sapi Bali (*Bos javanicus*) Melalui Metode *Sequencing*

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Abstrak

Syntaxin Binding Protein 6 (*STXBP6*) merupakan gen yang terkait dengan protein yang mengandung organisasi subunit kompleks dalam proses biologis. Gen *STXBP6* memiliki peran dalam pembelahan sel selama pertumbuhan dan perbaikan jaringan yang rusak. Penelitian ini bertujuan untuk mengidentifikasi mutasi gen *STXBP6* pada sapi Bali. Sebanyak 9 sapi Bali yang berasal dari Balai Pembibitan Ternak Unggul Sapi Bali di Denpasar, Bali digunakan dalam penelitian ini. Sampel darah diambil sebanyak 5 ml melalui vena jugularis yang kemudian diekstraksi menggunakan metode "*High Salt Methode*". Amplifikasi frgamen gen *STXBP6* dilakukan dengan menggunakan tiga pasang primers pada sampel DNA sapi Bali yang telah dibagi menjadi 3 pool. Hasil amplifikasi fragmen gen *STXBP6* selanjutnya dilakukan sekuensing menggunakan jasa dari PT. Genetica Science. *Sequencing* fragmen gen *STXBP6* dilakukan secara dua arah, yakni menggunakan *forward primer* dan *reverse primer*. Hasil *sequencing* susunan basa nukleotida dari fragmen gen *STXBP6* disejajarkan sekuens refrensi (*GenBank Accession* No. NC_037347.1) untuk mengetahui letak mutasi yang ada. Persejajaran sekuens dilakukan dengan menggunakan bantuan *software Microsoft Office Word* dan *BioEdit Sequence Alignment Version 7.2.5* serta menggunakan situs pendukung *Clustal Omega*. Hasil sekuens aligment ditemukan sebanyak 4 mutasi pada fragmen gen STXBP6. Mutasi yang berhasil diidentifikasi adalah g.616 T>C, g.613 -/C, g.58180 C>T, dan g.58158 A>G. Mutasi pada gen *STXBP6* sangat berotensi dijadikan sebagai marker genetic sebagai penciri dari sapi Bali. Mutasi yang ditemukan tidak berpotensi dijadikan marker genetik untuk sifat produksi sapi Bali dikarenakan mutasi tersebut bersifat *monomorphic.*

Kata kunci: Mutasi, Kandidat gen, *STXBP6*, Sapi Bali.