



## Genome-wide Association Study of Birth Weight in Bali Cattle (*Bos javanicus*)

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### ABSTRACT

The genome-wide association study is popularly used to identify markers associated with high economic traits in cattle breeds worldwide. However, this method has never been applied to Bali cattle (*Bos javanicus*), which are indigenous to Indonesia. Therefore, this study aimed to determine whether the GWAS can also be employed to figure out genomic regions potentially affecting Bali cattle's birth weight trait. In this study, phenotypic and genotypic data of 48 Bali cattle were used to figure out genomic regions which are potentially affecting the birth weight trait of Bali cattle. Those samples were genotyped using an Illumina bead chip array with 53,218 single nucleotide polymorphisms (SNPs). The estimation of the variance proportion explained by each SNP was conducted by a restricted maximum likelihood (REML) approach. The result showed that the birth weight trait in Bali cattle was affected by multiple regions dispersed over the genome, especially chromosomes 20 and 21. This study demonstrated that GWAS can be used effectively and discovered two intriguing regions related to quantitative trait loci and genes, namely *STXBP6* and *TERT*, which previously had been linked to cattle birth weight and growth. These findings are important for developing and refining genomic-based selection programs for Bali cattle in Indonesia.

**Keywords:** Bali cattle; birth weight; GWAS; *STXBP6*; *TERT*

### INTRODUCTION

Growth traits are usually considered in cattle breeding programs due to their relationships with beef production and other economically important traits (Smith *et al.*, 2019). Body weight is an easily measured growth-related trait that breeders place a high value on (Utsunomiya *et al.*, 2013). To adhere to their breeding schemes, the cattle are commonly weighed at predetermined age stages, including birth, weaning, and yearling weights. In general, birth weight influences average daily gain, mature weight, and carcass weight (Flores *et al.*, 2022), as well as reproduction traits such as age at first calving and gestation length (Lopez *et al.*, 2020). Consequently, birth weight is one of the criteria that must be considered in the preliminary beef cattle selection (Assan, 2012). The relationship of birth weight with those traits can be explored more deeply using molecular genetic studies, especially genetic architecture.

Many genes have an impact on growth characteristics (Bullock, 2021). Casas & Kehrli (2016) explained that several genes had been linked to this trait, including Myostatin (*MSTN*) in *Bos taurus* Autosome (BTA)

2, Calpastatin (*CAST*) in BTA 7, Leptin (*LEP*) in BTA 4, and  $\mu$ -Calpain (*CAPN*) in BTA 29. Single Nucleotide Polymorphism (SNP) in a gene is a variation of a single nucleotide among individuals (Eggen, 2012). SNP is often used in selection activities because it is powerful. For example, g.25940T>C SNP of the *MOGAT1* is significantly associated with body weight and chest depth in Chinese cattle (Lyu *et al.*, 2021). Polymorphisms in the *PLAG1* are known to be associated with growth traits in Chinese cattle (Zhong *et al.*, 2019). An SNP in the *PLAG1* is also found to be associated with the body length of Bali cattle (Cahyadi *et al.*, 2022). Currently, the availability of high-density SNP genotyping technology makes it possible to explore loci responsible for quantitative traits at the genomic level. The loci information on the genomic regions affecting respective traits could be useful to predict the potential of production at an early age, possibly completing genomic breeding values (Eggen, 2012).

Bali cattle are the only Indonesian native cattle with a unique history and genetic background hypothesized to be domesticated from wild *Banteng* (Martoyo, 2012; Suwiti *et al.*, 2017). Domestication of Bali cattle in

Southeast Asia was estimated to be around 5,000 Before the Present (Mohamad *et al.*, 2019) or more than ~3,400 years ago (Sudrajad *et al.*, 2020). Bali cattle possess unique mechanisms of adaptation to tropical environments. They show a good tolerance even when faced with poor conditions such as low-quality feed (Martoyo, 2012). Therefore, this cattle breed was reported to have spread almost throughout Indonesia, southern Asia, Southeast Asia, and Australia. However, the growth performance of Bali cattle is thought to be low, with an average daily gain of 0.30 kg/day, which could be attributed to management and genetic factors (Suwiti *et al.*, 2017).

The development of molecular technology provides many opportunities for further genetic exploration. The genome-wide association study (GWAS), a very powerful approach, is popularly used to identify genomic regions and genomic variations related to phenotypic traits (Uffelmann *et al.*, 2021). This approach can predict significant quantitative trait loci and could be implemented to predict genetics in many livestock species (Sharma *et al.*, 2015). To date, the majority of the GWAS studies were carried out in the hump-less cattle subspecies, which is known as taurine cattle (*Bos primigenius taurus*) and also in the humped bovine subspecies such as indicine or zebu cattle (*Bos primigenius indicus*), but no such study has been reported for *Bos javanicus* (Bali cattle). In a previous study, Utsunomiya *et al.* (2013) discovered that the most significant marker was closely linked with birth weight in Brazilian Nelore cows (Zebu or *Bos indicus*) located at BTA 14, the same region previously discovered on taurine. The area (about 1 Mb) contains several genes, namely *CHCHD7*, *LYN*, *MOS*, *PENK*, *PLAG1*, *RPS20*, *RDHE2*, and *SDR16C5*. Moreover, other studies found that calf birth weight was also associated with markers in the other autosomes, i.e., BTA 9 (Buzanskas *et al.*, 2014) and BTA 22 (Zepeda-Batista *et al.*, 2021). In this study, the first examination regarding a genome-wide scan for nucleotide markers associated with body weight traits in the Bali cattle population is reported. Therefore, this study explored putative SNPs and genomic regions close to them to reveal prospective functional correlations with calf birth weight.

## MATERIALS AND METHODS

### Ethical Clearance

All animal procedures related to sample collections of Bali cattle were approved by the Commission of Ethical Clearance of National Research and Innovation Agency No. 82/Klirens/X/2021.

### Animals and Phenotypes

The studied Bali cattle population was raised in a Breeding Center of Bali cattle - Pulukan, Bali Island, Indonesia. This institution aims to breed superior cattle through quantitative and qualitative selection programs. Birth and weaning weights became a major concern used as growth parameters in the selection program

(Supriyantono *et al.*, 2011). Body weight traits (i.e., birth, weaning, and yearling weights) of 48 heads Bali cattle consisting of 24 males and 24 females were obtained from their historical databases, which well-trained staffs have collected. Cattle weighing is a routine activity implemented in the breeding center and follows the standard operational procedure. Phenotype data were tabulated and descriptively analyzed.

### Genotypes and Quality Control

Blood of 48 observed samples (3 mL per animal) was collected from the jugular vein by the veterinary staff. DNA was extracted from those whole blood samples and conducted by following the protocol of the Wizard® Genomic DNA Purification Kit (Promega Corporation, USA). DNA concentration was quantified using Picogreen (Thermo Fisher Scientific Inc., USA). Moreover, DNA purity was evaluated using a NanoDrop (Thermo Fisher Scientific Inc., USA). The quality of DNA used in this study was set to at least 20 ng/μL, and the purity of extracted DNA was more than 1.8. Genomic data were generated using the Beadchip of Illumina Bovine SNP50 v3 containing 53,218 SNPs scattered uniformly throughout the bovine genome (Illumina Inc., Macrogen, Korea).

Before analyzing the genome-wide association study, genomic data was filtered using PLINK v1.9 to remove low-quality data (Chang *et al.*, 2015). Criteria of genotype data filtering were as follows: (1) the Hardy-Weinberg Equilibrium value was not less than  $1 \times 10^{-5}$ ; (2) the SNP call rate was more than 90%, (3) the minor allele frequency was less than 1%, and (4) the proportion of empty genotypes for each variant and empty SNP variants for each individual must not exceed than 10%. Moreover, the dataset included bovine chromosomes 1 to 29.

### Genome-Wide Association Study

Estimation of the association of any individual SNP with quantitative traits was carried out using a mixed linear model by the following formula:

$$y = Xb + Zu + e$$

where  $y$  is the observation vector for birth weight,  $b$  is the fixed effect vector,  $u$  is the random additive genetic effect vector,  $X$  and  $Z$  are incidence matrices,  $e$  is random errors. In addition, a Restricted Maximum Likelihood (REML) analysis was used for genomic analysis in GCTA (Yang *et al.*, 2013). This method uses the genomic relationship matrix (GRM) to estimate the variance explained by all SNPs in phenotypes using REML analysis and the GRM. The GCTA software was also utilized to predict genomic heritability based on the genotype and phenotype variations.

A *Bos\_taurus\_UMD\_3.1.1* assembly was used as a reference genome assembly. The gene ontology was carried out using the Genome Browser of Ensembl (<http://www.ensembl.org/index.html>). Moreover, functional analysis was completed in BovineMine v.1.6 (Elsik *et al.*, 2016).

## RESULTS

The descriptive statistics of the body weight of Bali cattle are presented in Table 1. The average weights of 48 Bali cattle at birth, weaning, and yearling was 19.63±2.23 kg, 76.39±26.23 kg, and 120.69±46.04 kg, respectively, with phenotypic variances of 4.96, 688.14, and 2,119.88. The highest birth weight of Bali cattle was 25 kg, while the lowest birth weight was only 15 kg. At weaning and yearling, the highest and lowest weights were 31 kg and 135 kg, 44 kg and 223 kg, respectively. The estimated genomic heritability value for birth weight in the Bali cattle population was 0.02±1.79.

To ensure the samples variability, the genomic structure of the Bali cattle population in the breeding center have been previously explained by Sudrajad *et al.* (2022), i.e., observed heterozygosity ( $H_o$ ) 0.30, expected heterozygosity ( $H_e$ ) 0.26, and negative inbreeding coefficient (-0.19). The quality control process yielded 49,439 cleaned variants, accounting for 93% of the total SNPs.

Table 1. Descriptive statistics of body weight in the Bali cattle population

Trait	Mean	SD	Min.	Max.	$\sigma^2P$
BW (kg)	19.63	2.23	15	25	4.96
WW (kg)	76.39	26.23	31	135	688.14
YW (kg)	120.69	46.04	44	223	2,119.88

Note:  $\sigma^2P$ : phenotypic variance, SD: standard deviation, BW: birth weight, WW: weaning weight, YW: yearling weight.

Table 2. The details of significant SNPs, candidate genes, and their effects on traits of interest in cattle

No.	BTA	SNP name	Candidate gene	Expression	References
1	21	rs109322139	<i>STXBP6</i>	Reproduction efficiency in beef cattle Birth weight in Brahman cattle	McDanel <i>et al.</i> (2014) Martinez <i>et al.</i> (2017)
2	20	rs110404528	<i>TERT</i>	Cell development Bovine embryo development Birth weight in cattle	Ren <i>et al.</i> (2022); Durán Aguilar <i>et al.</i> (2017); Lai <i>et al.</i> (2020) Iqbal <i>et al.</i> (2011); Gilchrist <i>et al.</i> (2015) Crispim <i>et al.</i> (2015); Weng <i>et al.</i> (2015)

Note: BTA= *Bos taurus* Autosomes.

Those SNPs were then included in the GWAS analysis. The GWAS result for birth weight is shown in Figure 1. Based on the Manhattan GWAS plot, there were two regions which are significantly affecting the birth weight in the Bali cattle population. This study found two quantitative trait nucleotides that can potentially be used as genetic markers for the birth weight in Bali cattle, i.e., rs110404528 and rs109322139 (Table 2). The rs110404528 was a part of the *TERT* located in BTA 20, and rs110404528 was located in the BTA 21 nearby *STXBP6*.

## DISCUSSION

Birth weight is considered as an economically important trait in the beef cattle industry and is also commonly the first quantitative trait to be measured in calves (Utsunomiya *et al.*, 2013). In the Bali cattle population, Kaswati *et al.* (2013) reported that Bali cattle birth, weaning, and yearling weights in 2006-2009 at Bali Cattle Breeding Center in Puluhan (Bali Island) reached 17.8±1.08 kg, 88.59±16.15 kg, and 131.12±25.50 kg, respectively. Maintained in the same place, the birth weight of Bali cattle in this study was slightly higher, reaching 19.63±2.23 kg (Table 1). Moreover, the average weaning and yearling weights identified in our study were slightly lower, i.e., 76.39±26.23 kg and 120.69±46.04 kg, respectively. Bali cattle are said to have lower body weights than the other cattle breeds (Suwiti *et al.*, 2017), but this can be improved through intensive rearing and the availability of nutritional feed. Riwu & Kihe (2015)

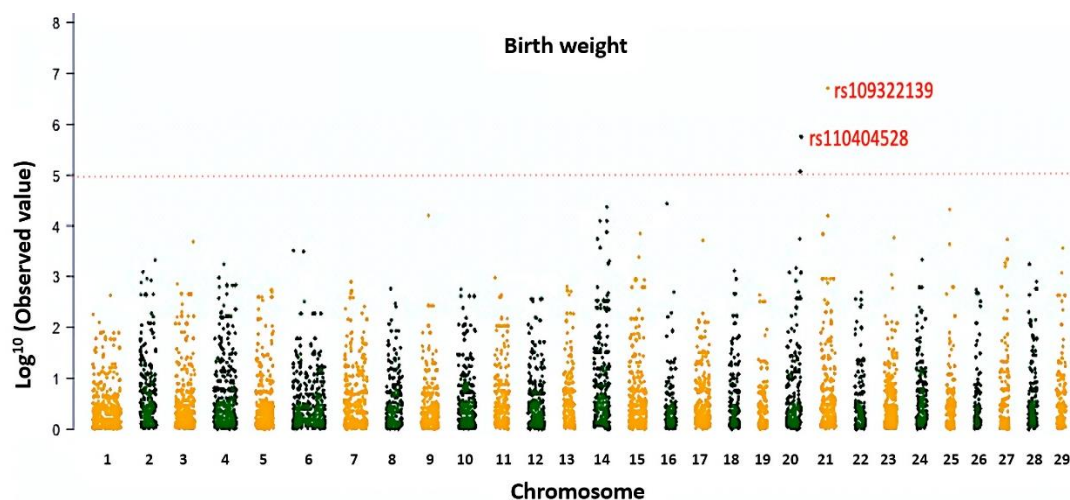


Figure 1. Manhattan plot of GWAS for birth weight in Bali cattle

explained that after feeding correction in pregnant cows, the birth weights of Bali cattle increase, whereas Volkandari *et al.* (2020) reported that Bali bulls in intensive rearing outperform semi-intensive rearing.

The heritability of birth weight in Bali cattle has been estimated to be low, i.e., 0.06 to 0.08 (Riwu & Kihe, 2015) and  $0.09 \pm 0.07$  (Gunawan & Jakaria, 2011), whereas the value of genomic heritability in our study was  $0.02 \pm 1.79$ . The lower heritability value, in this case, could be attributed to the small number of samples used, but it could also be due to the presence of a significant environmental influence on the observed trait, as evidenced by the higher standard deviation value. However, the maternal genetics effects were not considered in our analysis due to the insufficiency of pedigree records and the limitation of GCTA software to include the environmental effects (Kumar *et al.*, 2015). Furthermore, heterozygosity is a useful metric that must be checked prior to GWAS (Gondro *et al.*, 2013). Despite our small sample size, the heterozygosity value of the Bali cattle population in the breeding center is higher than that of the Bali cattle population in the farmer with low inbreeding coefficients (Sudrajad *et al.*, 2022) so that we can be certain of the sample's variability. Moreover, the value of  $H_o$  was not too high when compared to  $H_e$ , indicating that there was no DNA contamination or samples deviated from the population (Gondro *et al.*, 2013).

More in-depth information could be explored with the latest technological approach, namely GWAS. Based on our findings, we found two significant SNPs associated with birth weight in Bali cattle, rs110404528, located at BTA 20, and rs109322139 at BTA 21 (Figure 1). The rs110404528 is located at the intron region of the *TERT*, while rs109322139 is an intergenic variant nearby *STXBP6* (Table 2). In biological processes, the *STXBP6* gene is linked to a protein containing complex subunit organization. This is important for mammals in the process of cell division during growth (Nazar *et al.*, 2021). Therefore, *STXBP6* gene is very likely to have a relationship with body weight. It is possible to explain why several genes on chromosomes 21 and 20 are associated with the Bali cattle birth weight. Previous studies have established a link between the *STXBP6* on BTA 21 and reproductive efficiency in beef cattle (McDanel *et al.*, 2014). Another study found that BTA 21 was related to birth weight in Brahman cattle (Martínez *et al.*, 2017).

Furthermore, numerous studies have found a link between chromosome 20 and cattle growth parameters ranging from birth weight to weaning weight to adult weight (Crispim *et al.*, 2015; Weng *et al.*, 2015; and Martínez *et al.*, 2017). Ren *et al.* (2022) went into greater detail about how the *TERT* gene on BTA 20 is linked to cell development in calves. The *TERT* gene product is a component of the telomerase enzyme. *TERT* expression has demonstrated intriguing cellular properties that may be appealing for tissue engineering, such as increased *in vitro* cell proliferation and differentiation (Hartwig *et al.*, 2012). Other studies have discovered that the *TERT* gene influences telomerase activity during the development of bovine embryos (Gilchrist *et al.*, 2015; Iqbal *et al.*, 2011). Moreover, in a GWAS study on

Holstein cattle, this gene was also associated with other cell development functions, i.e., milk somatic cell score (Durán *et al.*, 2017), immune system, skin hyperplasia, and wound healing (Lai *et al.*, 2020). Based on these studies, the current study's findings that several variants tend to be associated with the Bali cattle's birth weight can be used as a reference. More research and validation efforts with a larger population of Bali cattle are required to increase the significance of the findings of this study.

## CONCLUSION

In this study, a genome wide association study was suitable for identifying genomic regions significantly affecting the birth weight of Bali cattle. Those regions were considered to be the candidate genes (i.e., *STXBP6* and *TERT*) that were previously identified as affecting the birth weight and calf growth traits in beef cattle. These results should be considered a basis genomic selection program for refining and developing Bali cattle in Indonesia.

## CONFLICT OF INTEREST

The authors have declared no competing interests.

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