World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Detection of *Myogenic Factor 5* (*MYF5*/*Taq*I) gene monomorphism in Bali Cattle *(Bos javanicus)*

Tiya Imran Wahyuni [1,*], Dewi Ayu Warmadewi [2], Widya Pintaka Bayu Putra [3], Hartati Hartati [3] and I Gusti Agung Arta Putra [2]

[1] *Master's Program, Faculty of Animal Husbandry, Udayana University, Denpasar, Bali 80361, Indonesia.*
[2] *Faculty of Animal Husbandry, Udayana University, Denpasar, Bali 80361, Indonesia.*
[3] *Research Center for Applied Zoology, National Research and Innovation Agency, Bogor, West java 16911, Indonesia.*

World Journal of Biology Pharmacy and Health Sciences, 2026, 26(03), 001-007

Publication history: Received on 21 April 2026; revised on 29 May 2026; accepted on 01 June 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.26.3.0324

Abstract

Bali cattle (*Bos javanicus*) are the indigenous cattle of Indonesia that kept for meat production purpose. This study was aimed to observe the genetic diversity in the *Myogenic Factor 5 (MYF5)* gene with PCR-RFLP analysis. Thirty-one (31) heads of Bali cows from Klungkung Regency, Bali Province were used for the investigation. Notably, PCR-RFLP analysis was performed to detect the mutation site g.1948A>G in the intron 2 of bovine *MYF5*/*Taq*I gene. Nonetheless, no polymorphism in the *MYF5*/*Taq*I gene of Bali cattle. Hence, all animals under study were identified as GG genotype. Despite this, two mutation sites of g.1707T>C and g.1795C>T in the *MYF5* gene were occurred in Bali cattle and not observed in other *bovidae* species. Furthermore, the intron 2 sequence of *MYF5* gene in Bali cattle is closed to *Bos frontalis* and *Bos grunniens*. In conclusion, the *MYF5*/*Taq*I gene in Bali cattle was monomorphic. In the future, in-dept study to investigate the other regions of *MYF5* gene is important to obtain the genetic markers of growth traits in Bali cattle in the future.

Keywords: Bali cattle; Monomorphism; *MYF5*/*Taq*I gene; PCR-RFLP

1. Introduction

Bali cattle (*Bos javanicus*) is an indigenous breed of cattle from Bali Island, Indonesia. Presently, this cattle able to adapt well in the most of places in Indonesia with various environmental conditions. as a beef cattle, the Bali bulls able to attain the slaughter weight and carcass weight of 209.25±18.48 kg and 115.50±11.12 kg, respectively [1]. Actually, the genetic potency of Bali cattle can be improved by molecular selection. *Myogenic factor 5 (MYF5)* gene is one of candidate genes affecting the growth traits of cattl [2]. The *MYF5* gene has an important role in themechanism of skeletal muscle formation and theformation of lateral sclerotome derivatives [3], myocyte determination and differentiation [4] and also controls myogenesis and myoblast growth duringprenatal embryo development [5]. Despite this, *MYF5* gene also controls the expression of some myogenic regulatory factors i.e. MYOD and *MYF5* [6]. In cattle, the *MYF5* is located at chromosome 5 with 3 exons [2].

Many studies reported that a single nucleotide polymorphism (SNP) of g.1948A>G was occurred in the intron 2 gene of *bovine MYF5* gene and had significant association with the growth traits of Korean Hanwoo [7], Chinese Qinchuan [2,8] and Friesian Holstein [9] cattle breeds. Unfortunately, no study reports the existance of SNP g.1948A>G in the *MYF5* gene of Bali cattle. Hence, the present study was aimed to detect SNP g.1948A>G that important for the genetic marker of growth traits in Bali cattle. The results of this study is important as the early information to evaluate *MYF5* gene of Bali cattle for the molecular selection program in the future.

* Corresponding author: Tiya Imran Wahyuni

2. Materials and Methods

2.1. Animals and research site

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

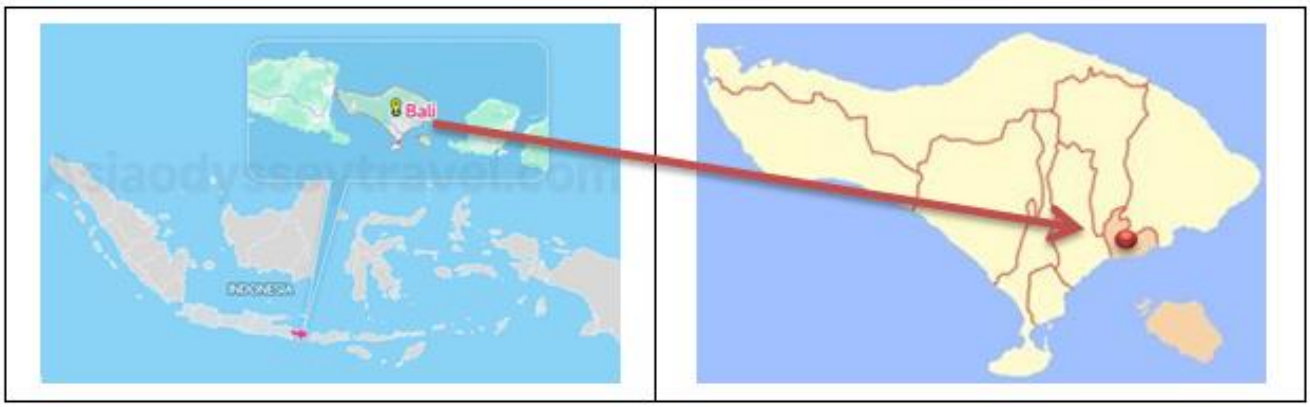

Figure 1 Location of the research side (red circle) at Kelungkung Regency (orange area), Bali Province, Indonesia

2.2. Management of animals

The Bali cattle were kept intensively in the individual cage. An artificial mating and natural mating systems were managed in the animals. The forage feeds of Elephant grass (*Pennisetum purpureum*), corn leaves, banana stem, and rice straw were given to the animals with composition of 15-18 kg head^{-1}. The concentrate was given two times a day at 07.00-08.00 am to 02.00-04.00 pm with the composition of 3 kg head^{-1} day^{-1} for male and 2 kg head^{-1} day^{-1} for female with ad libitum water. The vaccinations, vitamins and anthelmintic medication treatments were performed every six months.

2.3. Ethical Statement

Ethical approval for this study was obtained from Animal Ethics Committees of Faculty of Veterinary Medicine, Udayana University, Indonesia (Approval Certificate No: B/139/UN14.2.9/PT.01.04/2025)

2.4. DNA extraction

Approximately 3 ml of blood samples were taken from *jugular vein* using venoject and vacutainer tube containing EDTA. The collected blood samples were stored at freezer -20 ºC until the further analysis. Therefore, the DNA samples were extracted from the blood samples using DNA extraction Kit (Geneaid, Taiwan) following the manufacturer's instructions. In this study about 20.56±13.71 ng µL^{-1} of DNA concentration and 1.75±0.31of DNA purity ($\lambda 260\ \lambda 280^{-1}$) were obtained based on thats procedure.

2.5. Amplification of *bovine MYF5* gene

The amplification of *bovine MYF5* gene was performed in 10 µL of total reaction consisting 3 µL of DNA template; 0.20 µL of each primer; 5 µL of Dream*Taq* PCR Master Mix (Thermo Scientific, USA) and 1.60 µL of ddH_2O. The primer pair in the present study referring to Çinar et al. (2018) as follows: *MYF5*-F: 5'- AGA GCA GCA GTT TTG ACA GC -3' and *MYF5*-R: 5'- GCA ATC CAA GCT GGA TAA GG -3' with the amplicon size of 490 bp (GenBank: M95684.1). The PCR analysis was operated in the Mastercycler machine (Applied Biosystems, USA) with 1 cycle of pre-denaturation at 95 ºC for 1 min and followed by 35 cycles of denaturtion at 95 ºC for 15 s; annealing at 55 ºC for 15 s; initial extension at 72 ºC for 10 s; and final extension at 4 min.

2.6. Genotyping

Genotyping in the locus SNP g.1948A>G was performed with PCR-RFLP analysis. A PCR-RFLP analysis was performed in 7 µL of total reaction consisting 4.2 µL of PCR product; 0.3 µL of *Taq*I restriction enzyme (T*CGA); 0.7 µL of buffer; and 1.8 µL of ddH_2O. The digestion of *MYF5*/*Taq*I gene was performed at 65 ºC for 2 h. According to the PCR-RFLP analysis, AA genotype is signed by 1 DNA fragment (490 bp); GG genotype with 2 DNA fragments (123 bp & 367 bp); and AG genotype with 3 DNA fragments (123 bp; 367 bp & 490 bp).

2.7. Electrophoresis

Electrophoresis was performed using 1× TBE solution at 100 V for 30 m with 1-2 % agarosegel containing 2 µl of FloroSafe DNA Stain (First Base, Malaysia). The DNA vizualisation was managed using UV transillumitaor light (cleaver scientific) and captured with a digital camera.

2.8. Data analysis and sequencing

The genetic diversity of genotype frequency, allele frequency, observed heterozygosity (H_o), expected heterozygosity (H_e), number of effective allele (n_e), polymorphic informative content (PIC) and Chi-square (χ^2) values were estimated referring to [10]. Sequencing analysis was performed by Apical Scientific (Malaysia). The sequence alignment was performed with BioEdit package [11]. While, the MEGA package [12] was used to construct the dendogram of animals based on partial *MYF5* gene sequence. In this study, four *Bovidae* species of *Bos taurus* (GenBank: M95684.1), *Bos indicus* (GenBank: EF197851), *Bos frontalis* (GenBank: EF197848), *Bos grunniens* (GenBank: EF197849) were involved to construct the dendogram with the *Bubalus bubalis* (GenBank: EF197850) as the outgroup.

3. Results

Along 490 bp of the *MYF5* gene was amplified on 1% agarose gel as shown in Figure 2. The PCR-RFLP revealed that all animal samples were characterized as GG genotype (Figure 3). Hence, the *MYF5*/*Taq*I gene of Bali cattle was monomorphic with the G allele detected in thats locus (Table 1). In addition, the sequence alignment of partial *MYF5* gene among *Bovidae* species revealed that two transition mutations at 77th (T>C) and 165th (C>T) positions were occurred in Bali cattle (*Bos javanicus* species) and represent of the genetic marker of this species (Figure 4). In addition, two transversion mutations at 346th (C>A) and 348th (A>C) were observed in *Bos taurus* and also the genetic marker for this species. In addition, the partial *MYF5* gene sequence of Bali cattle was closed to *Bos frontalis* and *Bos grunniens.* While, the partial *MYF5* gene sequence in *Bos taurus* and *Bos indicus* were closed and classified in the separated cluster. The partial sequence of the intron 2 region of *MYF5* gene in Bali cattle has been deposited in the GenBank database under the accession number PZ229733.

Table 1 Genetic diversity in the *MYF5*/*Taq*I gene of Bali cattle

Genotype frequency (N)			**Allele frequency**		**H_o**	**H_e**	**n_e**	**PIC**	**χ^2**
AA	GG	AG	A	G					
0.00 (0)	1.00 (31)	0.00 (0)	0.00	1.00	0.00	0.00	1.00	0.00	-

N: number of animals; H_o: observed heterozygosity; H_e: expected heterozygosity; n_e:number of effective allele; PIC: polymorphic informative content; χ^2: Chi-square value

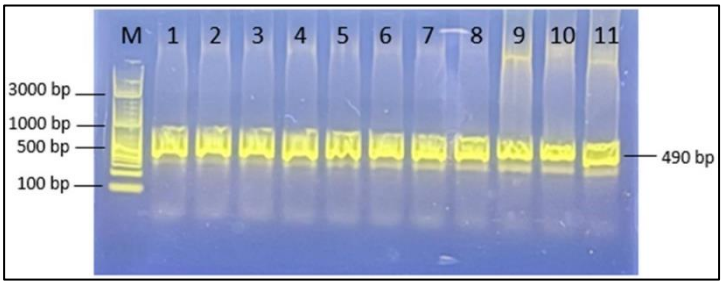

Figure 2 Amplification of Bali cattle *MYF5* gene (490 bp) on 1% agarose gel. M: DNA ladder 100 bp; Line 1-11: DNA samples

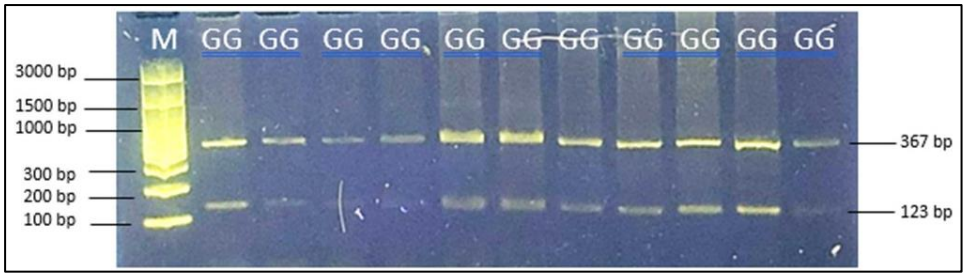

Figure 3 Genotyping of *MYF5*/*Taq*I gene in Bali cattle on 2% agarose gel with monomorphic of GG genotype. M: DNA ladder 100 bp

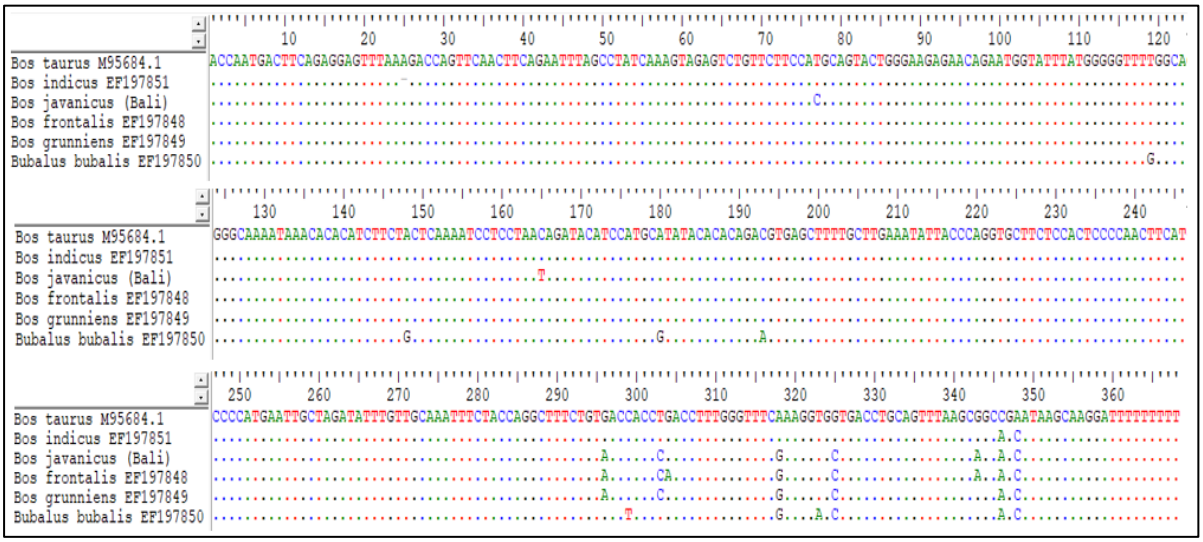

Figure 4 Alignment in the intron 2 region of *MYF5* gene among *Bovidae* species. The mutation of g.1948A>G (arrow) is located at 318th nucleotide**.**

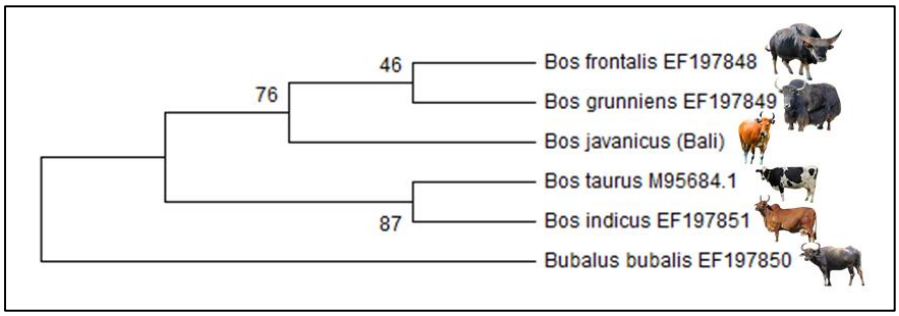

Figure 5 Clustering of the partial *MYF5* gene sequence (intron 2) among *Bovidae* species

4. Discussion

The *MYF5*/*Taq*I gene of Bali cattle was monomorphic with GG as the fixed genotype. In contrast, many study reported the polymorphism of *MYF5*/*Taq*I gene in cattle with the G allele frequency of 0.827 (Nanyang); 0.752 (Jiaxian); 0.758 (Qinchuan); 0.390 (Friesian Holstein) and 0.410 for Brown Swiss [8, 9, 13]. The monomorphism of *MYF5*/*Taq*I in Bali cattle can be caused by a natural selection. Many studies reported the monomorphism in the candidate genes of Bali cattle such as *Stearoyl-CoA Desaturase 1 (SCD1), Thyroglobulin (TG5/BstYI), Endothelial Differentiation Sphingolipid G-Protein-coupled Receptor 1 (EDG1/MscI), Adiponectin (ADIPOQ)*, 3'UTR region of *Pleomorphic Adenoma Gene 1 (PLAG1), Pituitary-Specific Transcription Factor-1 (Pit-1/Hinf*I), *Sterol regulatory element-binding protein 1 (SREBP1),* and *Mitochondrial transcription factor A (TFAM)* genes [14-21]. However, Ujan et al. [22] obtained the mutation site of g.526T>A at the exon 1 of *bovine MYF5* gene with significance association with back fat thickness and meat tenderness in Chinese *Bos taurus*. Interestingly, Saputra et al. [23] reported a mutation of g.643G>A in the exon 1 of *bovine MYF5*/*Msp*I gene with low genetic

diversity. In general, four mutation sites were detected in the *bovine MYF5* gene (GenBank: M95684.1) at g.1948A>G (intron 2); g.1870A>G (intron 2); g.1918C>T; (intron 2) and g.2667G>A (exon 3) as reported by Zhao et al. [2].

However, polymorphism in the *Myostatin (MSTN), Calpain (CAPN1)* and *FRAS1-Related Extracellular Matrix Protein 2 (FREM2)* genes had the significance association with meat quality of Bali cattle [24, 25, 26]. In addition, the *Leptin (LEP/BsAI), Growth Hormone (GH/MspI)* and *Growth Hormone Receptor (GHR/CviAII)* genes in Bali cattle were polymorphic and potential for improving the growth traits with molecular selection [27, 28, 29]. The sequence alignment revealed that the intron 2 sequence of *MYF5* gene in Bali cattle, *Bos frontalis* and *Bos grunniens* had the similarity and informed that these cattle had the different genetic structure compared with *Bos taurus* and *Bos indicus.* Consequently, Bali cattle may be had the specific mutation type in their *MYF5* gene. According to mitochondrial DNA sequence of 16S rRNA region, Bali cattle also classified in the same cluster with *Bos frontalis* and *Bos grunniens* [30]. Furthermore, a genome-wide association study (GWAS) can be performed in Bali cattle to obtain the genetic markers for the growth traits accurately. Fortunately, many candidate genes were identified as the genetic marker for with the birth weight *(STXBP6, TERT)* and adult weight *(SF3A3)* of Bali cattle based on GWAS [31, 32].

5. Conclusion

The *MYF5*/*Taq*I gene of Bali cattle was monomorphic with the GG as the fixed genotype. The intron 2 sequence of *MYF5* gene in Bali cattle (*Bos javanicus*), *Bos frontalis* and *Bos grunniens* had the similarity and its explains the mutation type in *Bos taurus* and *Bos indicus* may be not occurred in Bali cattle. In the future, detecting the genetic diversity in the other region of *MYF5* gene is important to obtain the genetic marker for the growth traits in Bali cattle.

Compliance with ethical standards

Acknowledgments

Author's thank to the farmers at Satwa Winangun farmers group for their permission to collect the blood samples of Bali cattle.

Disclosure of conflict of interest

The authors confirm there are no conflicts of interest associated with this study

References

[1] Bulkaini. Production performance and carcass quality of male Bali cattle by feeding fermented pineapple peel. Asian Journal of Applied Research for Community Development and Empowerment. 2022; 6. doi: 10.29165/ajarcde.v6i2.96

[2] Zhao C, Raza SHA, Khan R, Sabek A, Khan S, Ullah I, Memon S, El-Aziz AHA, Shah MA, Shijun L, Wang L, Liu X, Zhang Y, Gui L, Zan L. Genetic variants in MYF5 affected growth traits and beef quality traits in Chinese Qinchuan cattle. Genomics. 2020; 112: 2804-2812. doi: 10.1016/j.ygeno.2020.03.018

[3] Hernandez-Hernandez JM, Garcia-Gonzalez EG, Caroline E, Brun CE, Rudnicki MA. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Seminars in Cell & Developmental Biology. 2017; 72:10-18. doi: 10.1016/j.semcdb.2017.11.010

[4] Ciecierska A, Motyl T, Sadkowski T. Transcriptomic profile of primary culture of skeletal muscle cells isolated from semitendinosus muscle of beef and dairy bulls. International Journal of Molecular Sciences. 2020; 21(13): 47-94

[5] Zhou D, Xu H, Chen W, Wang Y, Zhang M, Yang T. Study on the transcriptional regulatory mechanism of the MyoD1 gene in Guanling bovine. RSC advances. 2018; 8(22):12409-12419. https://doi.org/10.1039/c7ra11795g

[6] de las Heras-Saldana S, Chung KY, Lee SH, Gondro C. Gene expression ofHanwoo satellite cell differentiation inlongissimus dorsi and semimembranosus. BMC Genomics. 2019; 20:1-15. doi: 10.1186/s12864-019-5530-7

[7] Chung ER., Kim WT. Association of SNP marker in IGF-I and MYF5 candidate genes with growth traits in Korean cattle. Asian-Australian Journal of Animal Science. 2005; 18:1061-1065.

[8] Zhang RF, Chen H, Lei CZ, Zhang CL, Lan XY, Zhang YD, Zhang HJ, Bao B, Niu H, Wang XZ. Association between polymorphisms of MSTN and MYF5 genes and growth traits in three Chinese cattle breeds. Asian-Australian Journal of Animal Science. 2007; 20:1798-1804.

[9] Çinar MU, Akyűz B, Kiyici JM, Arslan K, Kaliber M., Aksel EG. Effects of GH-AluI and MYF5-TaqI polymorphisms on weaning weight and body measurements in Holstein young bulls. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2018; 24:873-880. doi: 10.9775/kvfd.2018.20193

[10] Nei M, Kumar S. Molecular Evolution and Phylogenetics. New York, USA: Oxford University Press. 2000

[11] Hall T. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences. 2011; 2: 60-61.

[12] Hall BG. Building phylogenetic trees from molecular data with MEGA. Molecular Biology and Evolution. 2013; 30:1229-1235. doi: 10.1093/molbev/mst012

[13] Fadhil M, Zülkadir U. Association between polymorphisms of Myf5 , MSTN and CAST genes and fattening performance in Brown Swiss and Holstein cattle breeds. Animal Biotechnology. 2021; 32(2) :121–129. https://doi.org/10.1080/10495398.2020.1781148

[14] Hilmia N, Noor RR, Sumantri C, Gurnadi RE, Priyanto R. Polymorphism of stearoyl-CoA desaturase (SCD1) gene in Indonesian local cattle. Journal of the Indonesian Tropical Animal Agriculture. 2017; 42:1-5. doi: 10.14710/jitaa.42.1.1-5

[15] Anwar S, Putra AC, Wulandari AS, Agung PP, Putra WPB, Said S. Genetic polymorphism analysis of 5' untranslated region of thyroglobulin gene in Bali cattle (Bos javanicus) from three different regions of Indonesia. Journal of the Indonesian Tropical Animal Agriculture. 2017; 42:175-184. doi: 10.14710/jitaa.42.3.175-184

[16] Anwar S, Wulandari AS, Putra WPB, Said S. The favorable alleles of AKIRIN2:c.*188G>A, EDG1:c.-312A>G and TTN:g.231054C>T as candidate markers for high-marbling are very low in Bali cattle. Biodiversitas. 2019; 20:965-970. doi: 10.13057/biodiv/d200405

[17] Sutikno S, Priyanto R, Sumantri C, Jakaria J. Polymorphism of ADIPOQ and EDG1 genes in Indonesian beef cattle. Journal of the Indonesian Tropical Animal Agriculture. 2018; 43:323-332. doi: 10.14710/jitaa.43.4.323-332

[18] Sutikno S, Munyaneza JP, Asmarasari SA, Jakaria J. Monomorphic allele rs109231213 in 3'UTR PLAG1 gene in purebred of Bali cattle (Bos javanicus). E3S Web of Conferences. 2022; 335:00010. doi: 10.1051/e3sconf/202233500010

[19] Hartati H, Luthfi M, Ratnawati D, Soewandi BDP. Detection of growth hormone (GH|MspI,GHR|AluI, Pit1|HinfI) genes polymorphism and its association with body weight of Grati-Bali cattle (Bos sondaicus). IOP Conf. Series: Earth and Environmental Science. 2021; 788:012010. doi: 10.1088/1755-1315/788/1/012010

[20] Anwar S, Putra WPB, Khaerunisa I, Wulandari AS, Wisnu K, Sutikno S. The 84-bp indel polymorphism of sterol regulatory element-binding protein 1 (SREBP1) gene in several cattle breeds in Indonesia. Jurnal Ilmu Ternak dan Veteriner. 2023; 28:102-111. doi: 10.14334/jitv.v28.i2.3-3046

[21] Shidiq F, Anwar S, Rahmiyati S, Sutikno S, Khaerunnisa I, Furqon A, Irnidayanti Y. The genetic polymorphism of c.-1221A> C and discovery of novel SNPs in the promoter region of Mitochondrial Transcription Factor A (TFAM) gene in Bali Cattle. In IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2024; 1292(1):012001. 10.1088/1755-1315/1292/1/012001

[22] Ujan JA, Zan LS, Ujan SA, Adoligbe C, Wang HB. Back fat thickness andmeat tenderness are associated with a 526 T→ A mutation in the exon 1 promoter regionof the MyF-5 gene in Chinese Bos taurus. Genetics and Molecular Research. 2011; 10:3070-3079. doi: 10.4238/2011.December.12.6

[23] Saputra EA, Ulum MF, Jakaria J. Association of SNP g.643G>A of MYF5 gene polymorphism withbody weight and body measurements in Bali cattle. Journal of the Indonesian Tropical Animal Agriculture. 2020; 45: 1-6. doi: 10.14710/jitaa.45.1.1-6

[24] Khasanah H, Gunawan A, Priyanto R, Ulum MF, Jakaria J. Polymorphism of Myostatin (MSTN) promoter gene and its association with growth and muscling traits in Bali cattle. Media Peternakan. 2016; 39: 95-103. doi: 10.5398/medpet.2016.39.2.95

[25] Dairoh D, Ulum MF, Jakaria J, Sumantri C. Calpain 1 gene expression in liver tissue and the association of novel calpain 1 single-nucleotide polymorphisms (SNPs) with meat quality in Bali cattle. Archives Animal Breeding. 2024: 67:311–321. doi: 10.5194/aab-67-311-2024, 2024.

[26] Pertiwi EA, Ulum MF, Jakaria J. SNP detection in FREM2 gene and its association with carcass quality in Bali beef. Tropical Animal Science Journal. 2024; 47:149-154. doi: 10.5398/tasj.2024.47.2.149

[27] Syarifulaya N, Sriasih M, Maskur M. Polymorphism of Leptin gene in Bali cattle and Kacang goat. Jurnal Ilmu dan Teknologi Peternakan Indonesia. 2015; 1:47-54. doi: 10.29303/jitpi.v1i1.9

[28] Suwiti NK, Suardana IW, Watiniasih NL, Besung INK, Yowani C. Identification of growth hormone gene of Bali cattle with qualitative superior in Bali Province. Journal of Veterinary Medicine and Animal Science. 2017; 1: 36-39.

[29] Rozi T, Maskur M, Sarini N P, Suardana W. Exon 10 polymorphism of the GHR|CviAII gene and its association with growth traits in Bali cattle. Biodiversitas. 2025; 26:6039-6046. doi: 10.13057/biodiv/d261207

[30] Misrianti R, Wijaya SH, Sumantri C, Jakaria J. Phylogenetic tree analysis for Bali Cattle based on partial sequence 16S rRNA Mitochondrial DNA. E3S Web of Conferences. 2022; 335:00014. doi: 10.1051/e3sconf/202233500014

[31] Sudrajad P, Suhada H, Prasetyo D, Gariri PN, Eddianto E, Abiyoga AF, Cahyadi M. Genome-wide Association Study of Birth Weight in Bali Cattle (Bos javanicus). Tropical Animal Science Journal, Tropical Animal Science Journal. 2023; 46(2):151-156 https://doi.org/10.5398/tasj.2023.46.2.151

[32] Putra WPB, Hartati H, Mariyono M, Noor RR, Sumantri C, Margawati ET. Early detection of candidate genes for body weight in Indonesian cattle breeds with genome-wide association study (GWAS). Acta Veterinaria-Beograd. 2024; 74:246-260. doi: 10.2478/acve-2024-0017