**J I T A A**
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
48(1):1-9, March 2023
DOI: 10.14710/jitaa.48.1. 1-9

# Association between leptin gene polymorphism and growth traits in Bali cattle

**T. Kurlyana[1], T. Hartatik[2]* and Sumadi[2]**
*[1]Graduate Student at Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada. Jl. Fauna No. 3, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.*
*[2]Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada. Jl. Fauna No. 3, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.*
*[*]corresponding e-mail: tety@ugm.ac.id*

*Received May 25, 2022; Accepted January 4, 2023*

## ABSTRACT

Leptin (LEP) gene produces hormone leptin which is secreted by adipose tissue, and plays an important role in energy balance, regulating feed intake, regulating endocrine function, and immune functions. This study aims to identify molecular markers of LEP gene and its association with growth traits based on SNP in Bali cattle. The blood samples were collected from 16 male and 30 female Bali cattle. The growth data were recorded from 2018 to 2020, consist of body weight and body size. Amplification of leptin gene with polymerase chain reaction (PCR) using pair of primers, Leptin_3Forward: 5'- AGCTTGGAAACATGGTGGTC-3' and Leptin_3Reverse: 5'-CATGATGCTCCCTGGATTCT-3' with DNA target 898 bp. The SNPs were identified by the direct sequencing technique. Genotypes of the SNPs were identified using sequencing method. Association of LEP genotypes with growth traits was performed using oneway ANOVA. Three DNA polymorphisms of the LEP gene were found, namely g.2913C/T, g.3260T/C, and g.3549G/A. SNP g.2913C/T was significantly associated (P<0,05) with weaning shoulder height (WSH), weaning body length (WBL), weaning chest circumference (WCC), yearling shoulder height (YSH), yearling body length (YBL), and yearling chest circumference (YCC). Meanwhile, SNPs g.3260T/C and g.3549G/A were not associated with the body weight and body size in Bali cattle. In conclusion, the SNP g.2913C/T can be used as molecular marker for body size in weaning and yearling of Bali Cattle.

*Keywords*: *Bali cattle, Leptin gene, Growth traits, Molecular markers, SNP*

## INTRODUCTION

Bali cattle are germplasm that was domesticated from descendants of the wild ancestor, Banteng (*Bos javanicus*) around 5000-10000 years before present (Mohamad *et al*., 2011) and grow rapidly in Bali. Despite this, Bali cattle are spread massively in West and East Nusa Tenggara, South Sulawesi, and Sumatra. Bali cattle is one of the native Indonesian cattle that has been

registered through the Ministry of Agriculture, Republic of Indonesia (325/kpts/OT.140/1/2010). Several advantages of Bali cattle are high percentage of carcass, good adaptability in tropical environments, good reproduction (fertility and low calf mortality), and can digest high-fiber feed (Purwantara *et al.,* 2011). These make Bali cattle the perfect indigenous breed in tropical climate like Indonesia. Therefore, Bali cattle must be conserved to maintain genetic diversity, so it is important to select Bali cattle for breeding programs which can be done by quantitative and molecular methods.

Recently, molecular approaches using gene markers have been widely used, namely by detecting single nucleotide polymorphism (SNP) as molecular markers. SNPs indicate genetic diversity in individuals which refers to variations in the DNA sequence. Genes in body affect many traits, one of which is growth traits that are influenced by one of the genes, the leptin gene. LEP gene produces the hormone leptin which is secreted by adipose tissue (Putra and Indriastuti, 2017), and plays an important role in energy balance, regulating feed intake, regulating endocrine function, and immune functions (Javanmard *et al*., 2008; De la Hoya *et al*., 2015). LEP gene is located on chromosome 4 in cattle, sheep, and goat (Gregorio *et al*., 2014). The weight of the leptin gene is 16 KD, which encodes for 167 amino acids of the obese (ob) gene. This gene has 3 exons and 2 introns, even though only partially exon 2 and exon 3 are translated to protein (Haruna *et al*., 2020).

The study association of LEP gene with economic traits showed significant results in several cattle. Shin and Chung (2007) stated that SNP C1180T on exon 2 of LEP gene impacted backfat thickness and marbling score in Korean Cattle, SNP E2FB and T945M were found significantly associated with weight gain in Nellore cattle (da Silva *et al*., 2012), and the SNP A59V was significantly associated with body weight in Limousin cattle (Kulig and Kmieć, 2009). In Indonesian cattle, the SNP 1180C/Y and 1218A/G have found in Sumba Ongole (SO) cattle which is in exon 2 of Leptin gene (Anugratama and Hartatik, 2020). In addition, SNP 1181G/A and 1218A/G have found in Bali cattle (Anugratama and Hartatik, 2020). Fathoni *et al*. (2019) stated that SNP g.1180C/T of LEP gene was found in Ongole Crossbred (PO) cattle and affected weaning chest circumference. The study of Nugroho *et al.* (2022) presented g.1180C/T has an association with growth, carcass, milk, and reproduction trait in Madura cattle. Recently, identification of exon 3 leptin gene polymorphism in Sumba Ongole (SO) cattle has been reported and found SNP g.3260T/C (Putra and Agung, 2020). Based on previous research, SNP in leptin gene polymorphism is potential gene as a selection marker for economic traits. Therefore, this study aimed to identify molecular markers of LEP gene and its association with growth traits based on SNP in Bali cattle.

## MATERIALS AND METHODS

### Samples and Data

The blood samples were collected from 16 male and 30 female Bali cattle at the jugular vein using venoject needle. Approximately about 3 ml

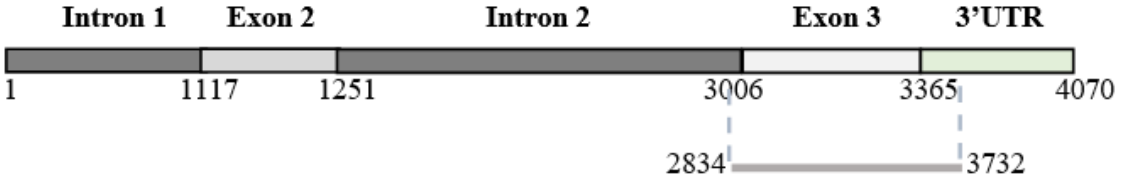


Figure 1. Target sequence of Leptin Gene based on GenBank Acc. No. U50365.1

of blood was collected from each animal and stored at vacutainer containing $K_3$EDTA. All animals were reared with the same maintenance procedures at Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak (BPTU HPT) Denpasar, Bali province and fed with King grass (*Pennisetum purpupoides)* (10% of body weight) and concentrate (2% of body weight). Feeding is done twice a day with ad libitum drinking method.

The growth traits data were recorded from 2018 to 2020 at BPTU HPT Denpasar, consist of body weight and body size such as weaning weight (WW), weaning shoulder height (WSH), weaning body length (WBL), weaning chest circumference (WCC), yearling weight (YW), yearling shoulder height (YSH), yearling body length (YBL), and yearling chest circumference (YCC).

**DNA Extraction and Polymerase Chain Reaction (PCR) Amplification**

The DNA was isolated using a DNA Extraction Kit (Geneaid, New Taipei City, Taiwan) with extraction methods in the Laboratory of Genetics and Animal Breeding, Faculty of Animal Science, Universitas Gadjah Mada. The protocol procedure includes sample preparation, cell lysis, DNA binding, wash, and DNA elution. The quality of DNA extraction was performed by 0,8% agarose gel electrophoresis and observed in a UV Transilluminator. There will be a band indicating the DNA has been isolated successfully.

The primer is designed using Primer3 (http://primer3.ut.ee/) by performing alignment using Genbank Acc. No. U50365.1 *(Bos taurus)*, EU313203 *(Bos indicus),* JQ711179 *(Bos taurus),* EU642566 *(Bos frontalis),* and MN709609 *(Bos javanicus)* from National Center for Biotechnology Information (NCBI). A specific pair of primers design is Leptin_3Forward: 5'-AGCTTGGAAACATGGTGGTC-3' and Leptin_3Reverse: 5'-CATGATGCTCCCTGGATTCT-3' with DNA target sequence 898 bp located in Intron 2, Exon 3, and 3'UTR (Figure 1). Polymerase chain reaction (PCR) was performed in a total reaction of 25 µl per sample containing 9,5 µl aquabidest, 12,5 µl PCR Kit (KAPA BIOSYSTEMS, USA), 0,5 µl forward primer and 0,5 µl reverse primer, and 2 µl of DNA. The PCR protocol is a modification of Anugratama *et al.* (2020), namely predenaturation temperature at 94$^o$C for 1 min, and 30 cycles of denaturation at 94$^o$C for 1 min, annealing at 55$^o$C for 1 min, and extension at 72$^o$C for 1 min, then the final extension at 72$^o$C for 5 minutes. The quality of the PCR products was visualized using agarose gel electrophoresis through 1.5% with ethidium bromide as a fluorescent tag and observed using UV Transilluminator.

**SNP Genotyping and Data Analysis**

PCR products were sequenced in Laboratorium Penelitian dan Pengujian Terpadu Universitas Gadjah Mada (LPPT UGM) and 1$^{st}$ Base Laboratory service, Malaysia. The SNPs were found at the early and middle sequences, so the primer used for sequencing were only the reverse primer (Figure 2). Sequencing results were aligned using BioEdit program (version 7.2) and compared with GenBank Acc. No. U50365.1 to confirm the SNP in samples. The "double peak" that appears on electropherogram indicates that a

Table 1. Single Nucleotide Polymorphism (SNP), and amino acid analysis for Leptin gene in Bali cattle

| SNP | Region | Fragment size (bp) | Amino Acid | Mutation |
|---|---|---|---|---|
| g.2913C/T | Intron 2 | 898 | - | - |
| g.3260T/C | Exon 3 | | Valine/Valine | *Synonymous* |
| g.3549G/A | 3'UTR | | - | - |

bp= base pair

confirmed SNP has been found (Figure 4).

**Statistical analysis**

The data of body weight and body size have been corrected for sex and parent age using formula of Hardjosubroto (1994) as follows:

$$WW_{205} = \left[\frac{WW-BWx}{\text{weaning (days)}} x\ 205 + BWx\right] \left[\frac{WW-BWx}{\text{weaning (days)}} x\ 205 + BWx\right] x \text{ FKUI x FKJK}$$

$$YW_{365} = \frac{YW-WW}{IT\ (days)} \text{ x } 160 + \frac{YW-WW}{IT\ (days)} \text{ x } 160 + WW_{205}$$

Where: $WW_{205}$ = corrected weaning weight at 205 days of age (kg); WW = weaning weight (kg); BWx = birth weight (kg); weaning = time of weaning (days); FKUI = correction factor of parent age; FKJK = correction factor of sex; $YW_{365}$ = corrected yearling weight at 365 days of age (kg); YW = real yearling weight (kg); IT = interval time between WW and YW measurement (days).

The association between genotypes and growth traits were analyzed using one way ANOVA with IBM SPSS Statistics v.26. The mathematical model as follows (Becker, 1992):

$Y_{ik} = \mu + \alpha_i + e_i$

Where $Y_{ik}$ is the observation value (body weight and body size); $\mu$ is the average overall growth trait; $\alpha_i$ is the genotype effect, and $e_i$ is the random error experiment. The significant association was determined by P-value (P<0,05).

## RESULTS AND DISCUSSION

**Polymorphism, Genotype and Allele Frequency of Leptin gene**

This study found three SNPs in target sequence, namely g.2913C/T located in intron 2, g.3260T/C located at exon 3 and g.3549G/A located at 3'UTR based on GenBank Acc. No. U50365.1 (Table 1). The target fragment of leptin gene successfully amplified with the specific primer and the protocol of PCR, the visualization of amplification shown in agarose gel 1,5% in (Figure 3) and the electropherogram sequencing of the leptin gene in Bali cattle are presented in (Figure 4). The obtained SNPs formed seven haplotypes and have been submitted to GenBank with accession number OP748255 (type I), OP748256 (type II), OP748257 (type III), OP748258 (type IV), OP748259 (type V), OP748260 (type VI), and OP748261 (type VII).
In this study, allele and genotype frequencies of Bali cattle were presented in (Table 2). The genotype frequencies of SNP g.2913C/T are CC, CT and TT, which are CC genotype frequency (0.68) had a higher frequency than CT (0.17) and TT (0.15) genotypes. Meanwhile, SNP g.3549G/A had a genotype frequency GG (0.80) was higher than AG (0.02). The SNP g.3260T/C had a higher frequency of CC genotype (0.96) than CT genotype (0.04). In Sumba Ongole (SO) cattle, SNP g.3260T/C showed that TT (0.41) and CT (0.41) genotypes had a higher frequency than CC (0.18) genotype (Putra and Agung, 2020). These differences of genotype distribution are probably due to differences of cattle breeds and the number of samples. However, these SNP needs to

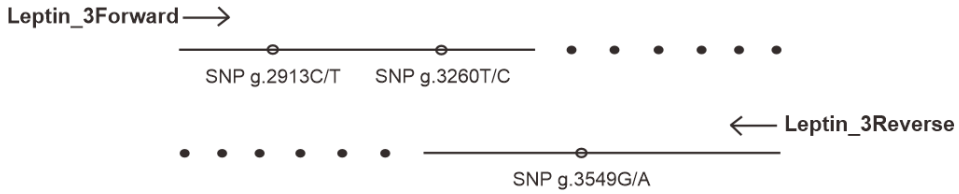


Figure 2. The position of SNP on target sequence of leptin gene

Table 2. Allele and genotype frequency in LEP gene in Bali cattle

| SNP | Genotype | N | Genotype Frequency | Allele Frequency |
|---|---|---|---|---|
| g.2913C/T | CC | 31 | 0.68 | C=0.76 |
| | CT | 8 | 0.17 | T=0.24 |
| | TT | 7 | 0.15 | |
| g.3260T/C | CC | 44 | 0.96 | C=0.98 |
| | CT | 2 | 0.04 | T=0.02 |
| | TT | 0 | 0 | |
| g.3549G/A | GG | 37 | 0.80 | G=0.90 |
| | AG | 9 | 0.20 | A=0.10 |
| | AA | 0 | 0 | |

N= number of animals

confirm through other study with large number of samples in Bali cattle.

**Association of Leptin Gene with Growth Traits in Bali Cattle**

SNP g.3260T/C and g.3549G/A in Bali cattle were not associated with WW, WSH, WBL, WCC, YW, YSH, YBL and YCC (Table 3). Meanwhile, SNP g.2913C/T was significantly associated with body size (WSH, WBL, WCC, YSH, YBL and YCC). SNP g.3260T/C located in exon 3 which codes for amino acid in a protein sequence, but the mutation of SNP g.3260T/C did not change the encoded amino acid, so it did not change its effect on growth traits. It is called a synonymous mutation. A mutation in SNP g.3260T/C encoding the amino acid valine to valine with GTT and GTC codon. In addition, SNP g.3549G/A located in 3' untranslated regions (3'UTR) was not associated with WW, WSH, WBL, WCC, YW, YSH, YBL and YCC in Bali cattle. However, SNP g.15525 G>A in 3'UTR region of CAPN1 gene was associated with a backfat thickness in Bali cattle (Dairoh *et al*., 2022), this proves that 3'UTR region also affects economic traits in cattle but does not occur in SNP g.3549G/A of the leptin gene in Bali cattle. 3' untranslated region plays an important role in regulate mRNA-based processes such as influencing mRNA localization, mRNA stability,

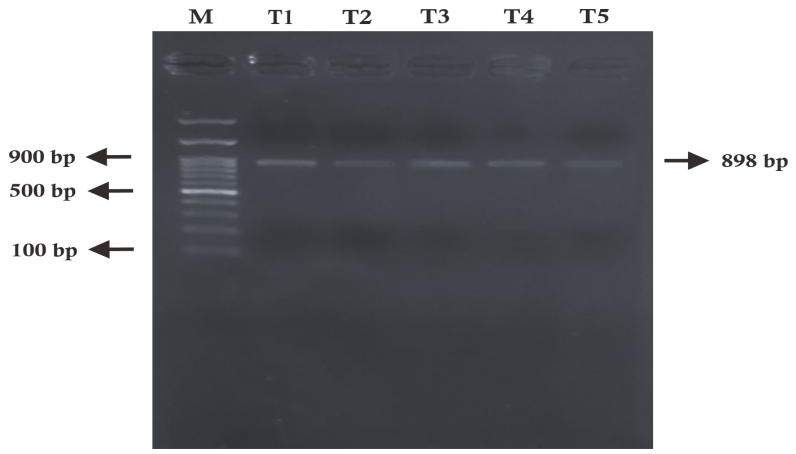


Figure 3. The PCR products performed in 1,5% gel agarose through electrophoresis. M = marker; T1-T5 = samples.

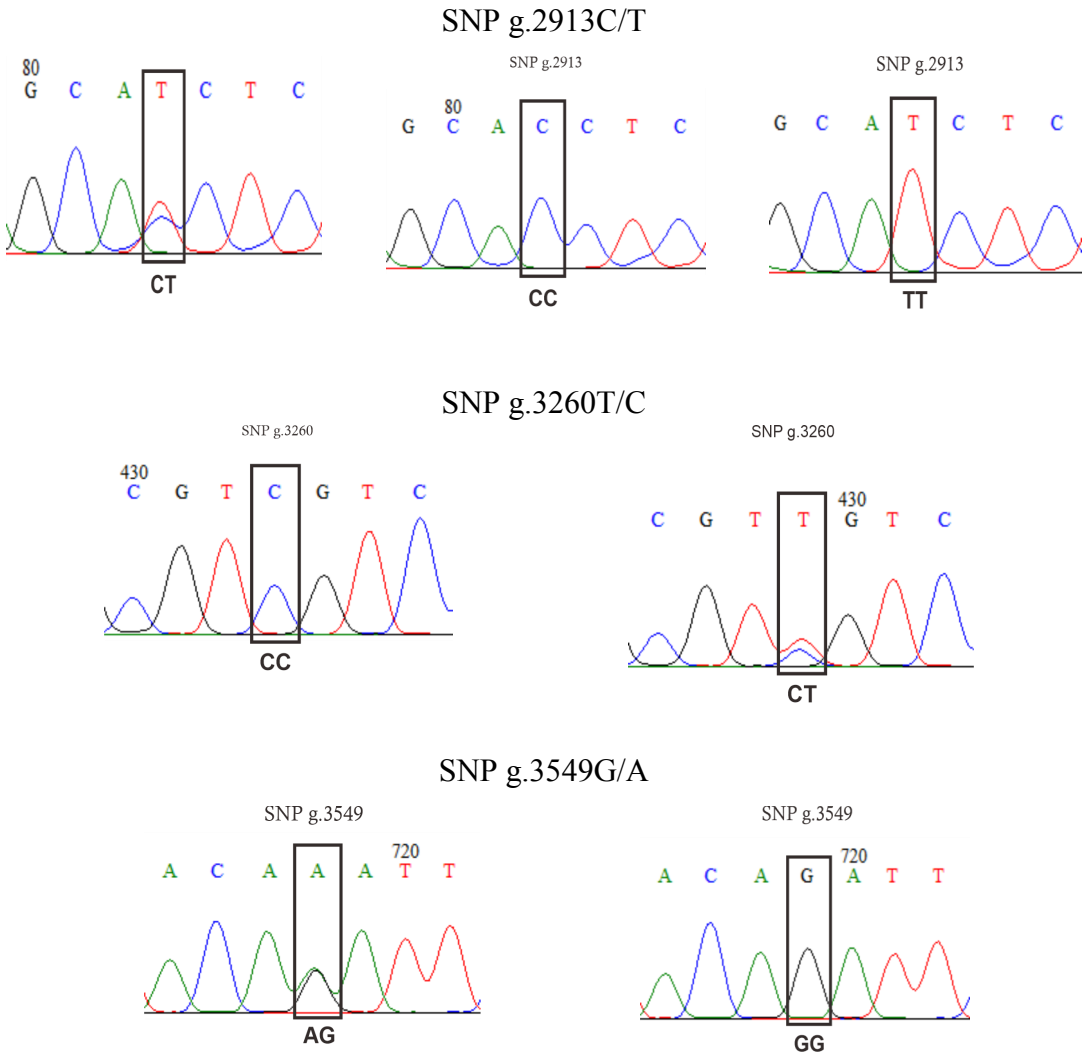


Figure 4. The results of SNPs identification in the Leptin gene based on the electrophoregram using the BioEdit program.

and efficiency of mRNA translation (Mayr, 2019).

The SNP g.2913C/T is located on intron 2 and was significantly associated with body size (WSH, WBL, WCC, YSH, YBL and YCC), although mutation in intron region does not change to amino acid but it can change structural and functional properties (Komar, 2007). Mutation in intron 2 of LEP gene in Bali cattle may help change to amino acid, if it occurs at the initial site of mRNA splicing after transcription (Maskur and Arman, 2014). The function of intron is encoded functional RNA through processing after splicing to produce non-coding RNA molecules before the RNA molecules are translated into proteins. Introns help to produce variations in mRNA molecule to form proteins (Chorev and Carmel, 2012). Moussavi *et al.* (2006) stated that the intron (g.820C/T) in the leptin gene could influence the phenotype in Iranian Holstein cattle. Futhermore, SNP LEP/Sau3AI in intron 2 change position 2059 of protein chain (cytosine, C to thymine, T) and significantly affected protein and fat yield, and age of first calving in Slovak Spotted and Pinzgau cows (Trakovicka *et al*., 2013).

## CONCLUSION

Leptin gene in intron 2, exon 3 and 3' untranslated region (3'UTR) in Bali cattle showed polymorphic SNPs. There are SNP g.2913C/T, g.3260T/C and g.3549G/A. The SNP g.2913C/T was significantly associated with body size

Table 3. The association between SNP of LEP Gene with Growth Traits in Bali cattle

| SNP | Variable | Genotype | | | P-value |
|---|---|---|---|---|---|
| | | CT (n=8) | TT (n=7) | CC (n=31) | |
| g.2913C/T | WW (kg) | 90.42±20.71 | 78.43±8.80 | 100.97±25.68 | 0.06 |
| | WSH (cm) | 91.80±10.21[b] | 78.79±5.63[a] | 94.90±13.97[b] | 0.04 |
| | WBL (cm) | 85.29±9.67[b] | 74.40±7.02[a] | 87.17±13.56[b] | 0.04 |
| | WCC (cm) | 108.85±13.43[b] | 90.70±4.75[a] | 108.58±19.36[b] | 0.04 |
| | YW (kg) | 142.51±20.89 | 134.87±12.94 | 155.53±29.24 | 0.12 |
| | YSH (cm) | 100.05±6.88[b] | 91.15±2.64[a] | 105.23±11.07[b] | 0.00 |
| | YBL (cm) | 96.45±6.65[b] | 85.60±4.41[a] | 101.38±12.79[b] | 0.00 |
| | YCC (cm) | 130.80±16.50[b] | 113.51±5.84[a] | 130.41±16.23[b] | 0.03 |
| | | CT (n=2) | CC (n=44) | | |
| g.3260T/C | WW (kg) | 84.31±2.81 | 96.22±24.66 | | 0.50 |
| | WSH (cm) | 78.23±6.85 | 92.53±13.48 | | 0.14 |
| | WBL (cm) | 75.51±9.51 | 85.32±12.87 | | 0.29 |
| | WCC (cm) | 91.36±9.23 | 106.57±18.06 | | 0.24 |
| | YW (kg) | 156.66±13.97 | 149.82±27.46 | | 0.73 |
| | YSH (cm) | 92.01±4.69 | 102.65±10.77 | | 0.17 |
| | YBL (cm) | 89.67±4.63 | 98.50±12.40 | | 0.32 |
| | YCC (cm) | 120.83±3.28 | 128.22±16.43 | | 0.53 |
| | | AG (n=9) | GG (n=37) | | |
| g.3549G/A | WW (kg) | 89.26±25.20 | 97.27±24.08 | | 0.38 |
| | WSH (cm) | 90.89±18.48 | 92.16±12.37 | | 0.80 |
| | WBL (cm) | 84.49±16.28 | 85.00±12.10 | | 0.91 |
| | WCC (cm) | 104.89±19.91 | 106.15±17.77 | | 0.85 |
| | YW (kg) | 151.00±26.67 | 149.90±27.39 | | 0.91 |
| | YSH (cm) | 101.28±12.53 | 102.41±10.49 | | 0.78 |
| | YBL (cm) | 98.77±17.37 | 97.96±11.01 | | 0.86 |
| | YCC (cm) | 129.12±19.14 | 127.61±15.61 | | 0.80 |

WW = Weaning weight; WSH = weaning shoulder height; WBL = weaning body length; WCC = weaning chest circumference; YW = yearling weight; YSH = yearling shoulder height; YBL = yearling body length; and YCC = yearling chest circumference; [a,b] means different superscripts within the same column show significantly different values at $P<0.05$.

(WSH, WBL, WCC, YSH, YBL and YCC). Although, SNP g.3260T/C and g.3549G/A were not associated with WW, WSH, WBL, WCC, YW, YSH, YBL and YCC. The SNP g.2913C/T could be used as molecular markers in population of Bali cattle at BPTU-HPT Denpasar, however it remains to be seen if this applies to other breeds of cattle.

## ACKNOWLEDGMENTS

This research was financially supported by the Indonesia Endowment Fund for Education (LPDP) in 2019. In this great chance, the researcher would like to offer their gratitude to the government of LPDP, BPTU HPT Denpasar, Mrs. Retno Setyawati as a laboratory assistant, and all my friends for the help in the laboratory.

## REFERENCES

Anugratama, L.E and T. Hartatik. 2020. Short communication: identification of leptin gene in crossbred beef cattle. Biodiversitas. 21

(1):226-230. https://doi.org/10.13057/biodiv/d210129

Becker, W. 1992. Manual of quantitative genetics. Fifth Edition. Academic Enterprises. Pullman. Washington.

Chorev, M and L. Carmel. 2012. The Function of Introns. Front Genet. 3(55):1-15. https://doi.org/10.3389/fgene.2012.00055

Dairoh, D., J. Jakaria, M.F. Ulum and C. Sumantri. 2022. A new SNPs at 3'UTR region of calpain 1 gene and its association with growth and meat quality traits in beef cattle. J. Indonesian Trop. Anim. Agric. 47(1):17-28. https://doi.org/10.14710/jitaa.47.1.17-28

Da Silva, R.C.G., J.B.S. Ferraz, F.V. Meirelles, J.P. Eler, J.C.C. Balieiro, D.C. Cucco, E.C. Mattos, F.M. Rezende and S.L. Silva. 2012. Association of single nucleotide polymorphisms in the bovine leptin and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle. Genet Mol Res. 11(4):3721–3728. https://doi.org/10.4238/2012.august.17.10

De la Hoya, M.P.G., J.A.T. Ramirez, P.R. Contreras, C.E.P. Diaz, F.O.R. Saucedo and J.S.Q. Saucedo. 2015. Role of leptin in cattle production: Review. J. Anim. Vet. Adv. 14(4):18 – 90. http://dx.doi.org/10.3923/javaa.2015.81-90

Fathoni, A., D. Maharani, R.N. Aji, R. Choiri and S. Sumadi. 2019. Polymorphism of the SNP g.1180 C<T in leptin gene and its association with growth traits and linear body measurement in Kebumen Ongole Grade Cattle. J. Indonesian Trop. Anim. Agric. 44 (2):125-134. https://doi.org/10.14710/jitaa.44.2.125-134

Gregorio, P.D., A.D. Trana, P. Celi, C. Salvatore and A. Rando. 2014. Comparison of goat, sheep, cattle, and water buffalo leptin (LEP) genes and effects of intron 1 microsatellite polymorphism in goats. Animal Prod. Sci. 54:1258-1262. http://dx.doi.org/10.1071/AN14101

Hardjosubroto, W. 1994. Aplikasi pemuliabiakan ternak di lapangan. Jakarta: Gramedia Widiasarana Indonesia.

Haruna, I.L., S. A Hadebe, O. J Oladosu, G. Mahmoud, H. Zhou and J. G H Hickford. 2020. Identification of novel nucleotide sequence variations in an extended region of the bovine leptin gen (LEP) across a variety of cattle breeds from New Zealand and Nigeria. Arch Anim Breed. 63(2):241-248. https://doi.org/10.5194/aab-63-241-2020

Javanmard, A., M.R. Mohammadabadi, G.E. Zarrigabayi, A.A. Gharahedaghi, M.R. Nassiry, A. Javadmansh and N. Asadzadeh. 2008. Polymorphism within the intron region of the bovine leptin gene in Iranian Sarabi cattle (Iranian *Bos taurus*). Russ. J. Genet. 44:495-497. https://doi.org/10.1134/S1022795408040169

Komar, A.A. 2007. Silent SNPs: impact on gene function and phenotype. Pharmacogenomics. 8(8):1075-1080. https://doi.org/10.2217/14622416.8.8.1075

Kulig, H. and M. Kmieć. 2009. Association between leptin gene polymorphisms and growth traits in Limousin cattle. Russ. J. Genet. 45:738-741. https://doi.org/10.1134/S1022795409060131

Lee, S., K. Simon, Z. Weng and C.R. Cantor. 2008. Quantitative analysis of single nucleotide polymorphisms within copy number variation. PloS One. 3(12):1-10. https://doi.org/10.1371/journal.pone.0003906

Maskur, R. and C. Arman. 2014. Association of a novel single nucleotide polymorphism in GHR gene with production traits in Bali cattle. Ital. J. Anim. Sci. 13:841-844. https://doi.org/10.4081/ijas.2014.3461

Mayr, C. 2019. What are 3'UTRs doing?. Cold Spring Harb. Perspect. Biol. 11(10):1-15. https://doi.org/10.1101/cshperspect.a034728

Mohamad, K., M. Olsson, G. Andersson, B. Purwantara, HTA.V. Tol, H. Rodriguez-Martinez, B. Colenbrander and JA. Lenstra. 2011. The origin of Indonesian cattle and conservation genetics of the Bali cattle breed. Reprod. Domest. Anim. 47:1-4. https://doi.org/10.1111/j.1439

0531.2011.01960.x

Moussavi, A.H., M. Ahouei, M.R. Nassiry and A. Javadmanesh. 2006. Association of leptin polymorphism with production, reproduction, and plasma glucose level in Iranian Holstein cows. Asian-Australasian J. Anim. Sci. 19**:** 627-631. https://doi.org/10.5713/ajas.2006.627

Nugroho, T., T.S.M. Widi and D. Maharani. 2022. The potency of leptin gene as a selection marker of economic traits for Madura cattle: preliminary study. Yogyakarta, Indonesia: Proceedings of 9th International Seminar on Tropical Animal Production (ISTAP); 2021.

Purwantara, B., R. Noor, G. Andersson and H. Rodriguez-Martinez. 2012. Banteng and Bali cattle in Indonesia: status and forecasts. Reprod. Domest. Anim. 47:2-6. http://dx.doi.org/10.1111/j.1439-0531.2011.01956.x

Putra, W.P.B. and P.P. Agung. 2020. Novel single nucleotide polymorphisms (SNPs) in intron 2 and exon 3 region of leptin gene in Sumba Ongole cattle. Iran. J. Appl. Anim. Sci. 10(2):241-247.

Tambasco, D.D., C.C.P. Paz, M. Tambasco-Studart, A.P. Pereira, M.M. Alencar, A.R. Freitas, L.L. Coutinho, I.U. Packer and L.C.A. Regitano. 2003. Candidate genes for growth traits in beef cattle crosses *Bos taurus* x *Bos indicus*. J. Anim. Breed. Genet. 120(1):51-56. https://doi.org/10.1046/j.1439-0388.2003.00371.x

Trakovická, A., N. Moravčíková and R. Kasarda. 2013. Genetic polymorphisms of leptin and leptin receptor genes in relation with production and reproduction traits in cattle. Acta Biochim. Pol. 60(4):783-787. PMID: 24432332.