Short note [Nota corta]

GENETIC VARIATION IN TWO PHENOTYPES OF BALI CATTLE (*Bos javanicus*) INFERRED BY $(AG)_9C$ ISSR MARKER †

[VARIACIÓN GENÉTICA EN DOS FENOTIPOS DE GANADO DE BALI (Bos javanicus) INFERIDA POR EL MARCADOR $(AG)_9C$ ISSR]

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SUMMARY

Background. Bali cattle (*Bos javanicus*) are Indonesian native cattle that are kept as beef cattle. Among Bali cattle, however, there are white Bali cattle, called Taro cattle which are found in small numbers of the population and used only for religious ceremony needs. **Objective.** To quantify the genetic variation in two phenotypes of Bali cattle (wildtype and white colors) with inter-simple sequence repeat (ISSR) primer of $(AG)_9C$. **Methodology.** Forty Bali cattle, 20 wildtype bulls, and 20 white Bali cows were used. The blood samples were taken from the jugular vein of each animal using venoject vacutainer tube containing EDTA. The DNA extraction was performed using DNA extraction kit following the manufacturer's protocol. Number of DNA fragments, number of effective alleles (n_e), and polymorphic informative content (PIC) were calculated to describe the genetic diversity in animals under study. **Results**: Results showed that three haplotype (H) types were observed in the animals under this study based on the ISSR fragments. The polymorphic informative content (PIC) in the white animals was higher than in the wild-type animals (0.62 vs 0.18). According to $(AG)_9C$ ISSR marker, mostly white (50%) and wildtype (90%) animals were classified into H2 type. Therefore, a total of 25% of white and 10% of wild-type animals were classified into H3 type. While 25% of white animals were classified into H1 type. **Implications.** the information generated in this study can be used as early information to investigate the molecular genetics of Taro cattle. **Conclusions**: This study suggests that many white animals have a specific locus which is absent in wild-type animals.

Key words: Bali cattle; haplotype; ISSR marker; phenotypes.

RESUMEN

Antecedentes. El ganado de Bali (*Bos javanicus*) es un ganado nativo de Indonesia que se cría como ganado de carne. Entre el ganado de Bali, sin embargo, hay ganado blanco de Bali, llamado ganado Taro, que se encuentra en pequeñas cantidades entre la población y se utiliza sólo para necesidades de ceremonias religiosas. **Objetivo.** Cuantificar la variación genética en dos fenotipos de ganado Bali (tipo salvaje y colores blanco) con el cebador de repetición entre secuencias simples (ISSR) de $(AG)_9C$. **Metodología.** Se utilizaron cuarenta animales, 20 toros de tipo salvaje y 20 vacas blancas de Bali. Las muestras de sangre se tomaron de la vena yugular de cada animal utilizando un tubo vacutainer Venoject que contenía EDTA. La extracción de ADN se realizó mediante un kit de extracción de ADN siguiendo el protocolo del fabricante. Se calculó el número de fragmentos de ADN, el número de alelos efectivos (n_e) y el contenido informativo polimórfico (PIC) para describir la diversidad genética en los animales en estudio. **Resultados:** Los resultados mostraron que se observaron tres tipos de haplotipos (H) en los animales de este estudio según los fragmentos ISSR. El contenido informativo polimórfico (PIC) en los animales blancos fue mayor que en los animales de tipo salvaje (0.62 frente a 0.18). Según el marcador $(AG)_9C$ ISSR, la mayoría de los animales blancos (50%) y de tipo salvaje (90%) se clasificaron en el tipo H2. Por lo tanto, un total del 25% de los animales blancos y el 10% de los animales de tipo salvaje se clasificaron en el tipo H3. Mientras

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que el 25% de los animales blancos se clasificaron en el tipo H1. **Implicaciontes.** La información generada en este estudio se puede utilizar como información de base para investigar la genética molecular del ganado Taro. **Conclusiones:** Este estudio sugiere que muchos animales blancos tienen un locus específico que está ausente en los animales de tipo salvaje.
Palabras clave: ganado de Bali; haplotipo; marcador ISSR; fenotipos.

INTRODUCTION

Bali cattle (*Bos javanicus*) are one of Indonesian native beef cattle that originated from Bali Island. Bali bulls at 2.5 years of age fed with forage ration (17 % of crude protein) were able to reach the slaughter weight and carcass weight of 228.60±8.65 kg and 123.64±7.89 kg, respectively (Tahuk *et al.*, 2018). However, crossbreeding and inbreeding can affect the various coat colors in Bali cattle such as white (albino), roan and white spotted (Tabun *et al.*, 2022). Interestingly, the white Bali cattle or Taro cattle were found at Bali Island with a total number of 57 individuals in the year 2020. It was reported that white Bali cattle can reach a body weight of about 227.40±66.20 kg for males and 174.40±31.30 kg for females (Oka e*t al.*, 2020). While Bali cattle at the breeding station were able to reach 17.80±1.08 kg of birth weight; 88.59±16.15 kg of weaning weight at 205 days of age and 131.12±25.50 kg of yearling weight at 365 days of age (Kaswati *et al.*, 2013). Some studies reported the genetic diversity in White Bali cattle based on mitochondrial gene polymorphism. According to the mitochondrial COI gene study, white Bali and wildtype Bali cattle have a close genetic relationship with a genetic distance of 0.000 - 0.001 (Susari e*t al.*, 2021). However, the preliminary study reported that white Bali cattle have the MC1R genotype of EE which expresses the black color (Tabun *et al.*, 2013). Moreover, a recent study reported that mutation in the MC1R and KIT genes did not affect coat color abnormalities in Bali cattle (Jakaria *et al.*, 2023). Inter-simple sequence repeat (ISSR) can be used as the genetic marker in livestock. ISSR primers were reported to detect genomic loci that were important for genomic mapping, fingerprinting (DNA barcoding), and gene tagging (Ye *et al.*, 2005; Pashaei *et al.*, 2009). PCR-ISSR analysis was used to characterize many cattle breeds (Ghasemi *et al.*, 2010; Stolpovsky *et al.*, 2011; Sulimova *et al.*, 2016; Yu *et al.*, 2016; Maherovska, 2021). In addition, many previous studies used the $(AG)_9C$ ISSR marker to characterize many Russian cattle breeds (Sulimova *et al.*, 2016) and Tuvinian Short-Fat-Tailed sheep (Stolpovsky *et al.*, 2010). Currently, there no reports on the molecular marker that causes the white color in Bali cattle including the study of PCR-ISSR in Indonesian native cattle. This study was aimed to quantify the genetic variation in two phenotypes of Bali cattle that important for conservation programs in the future.

MATERIAL AND METHODS

A total of 40 adult animals consisting of 20 wildtype Bali bulls and 20 white Bali cows were used in the present study. The sample size in this study represents about 50% of the animal population in each location. The wildtype Bali bulls originated from the National Artificial Insemination Center (NAIC) of Singosari, East Java, Indonesia. This place is located at latitude 7.0°44’55.11” - 8.0°26’35.45” S and longitude 112.0°17’10.90” - 112,0°57’00” E (440-667 m above sea level) with 22.7-25.1°C of air temperature; 79-86% of relative humidity and 501-2000 mm/year of rainfall. While, white Bali cows originated from Tegalalang District, Gianyar Regency, Bali Province of Indonesia. This place is located at latitude 08°18’48” - 08°38’58” S and longitude 115°13’29” – 115°22’23” S (0-950 m above sea level) with 23-29°C of air temperature; 82% of relative humidity and 2,098.50 mm/year of rainfall.

An amount of 3 µL blood samples was collected from each animal using venoject tubes containing EDTA. The DNA extraction was performed using Genomic Extraction Kit (Geneaid, Taiwan). The amount of 10 µL of PCR reaction consisted of 3 µL of DNA template; 0.5 µL of $(AG)_9C$ ISSR primer; 5 µL PCR master mix (Bioline, USA), and 1.5 µL of free-nuclease water. The PCR-ISSR analysis was performed for 35 cycles with pre-denaturation at 95 °C for 5 minutes, denaturation at 95 °C for 15 seconds; annealing at 54 °C for 15 seconds; extension at 72 °C for 30 seconds, final extension at 72 °C for 3 minutes. The electrophoresis was performed at 100 V for 30 minutes with 2% agarose gel. The DNA visualization was performed using G-box Documentation System (Syngene, UK). The genetic diversity parameters of the number of effective allele (Hartl and Clark, 2007), Shannon index (Shannon and Weaver, 1949), and polymorphic informative content (Weir, 1990) were calculated to evaluate the genetic diversity of animals under study using the mathematical formulas as follow:

$$n_e = 1 / \sum P_{ij}^2$$

$$I = -\sum P_i \ln(P_i)$$

$$PIC = 1- \sum P_i^2$$

where: n_e is the number of effective alleles; I is the Shannon’s diversity index, PIC is the polymorphic

informative content and P_i is the frequency of the j^{th} pattern in the i^{th} band.

RESULTS AND DISCUSSION

The PCR-ISSR in the present study was successfully amplified in 2% agarose gel with the presence of three DNA fragment types (Figure 1). Three haplotype (H) variations were classified according to the typical DNA fragment of each animal *i.e.* H1 (550 bp); H2 (550 bp; 1000 bp) and H3 (550 bp; 700 bp; 1000 bp) as shown in Table 1. Previous studies reported that the $(AG)_9C$ ISSR marker was polymorphic with ten (10) haplotypes in Iranian local cattle (Askari *et al.,* 2011) and thirty-eight (38) haplotypes in 21 cattle breeds in the world (Stolpovsky *et al.,* 2011). Furthermore, total 4 specific DNA fragments of ISSR $(AG)_9C$ were detected in the Ukrainian cattle breeds *i.e.* 250 bp; 350 bp; 500 bp; 580 bp, and 680 bp (Maherovska, 2021).

The n_e, I and PIC values in $(AG)_9C$ ISSR marker of white animals were higher than wildtype animals as shown in Table 2. However, The PIC value of $(AG)_9C$ ISSR marker in wildtype Bali cattle was higher than in Yakutian (0.05), Zeboid (0.07), and Aysire (0.12) cattle breeds (Yu *et al.,* 2016). In contrast, the PIC value for $(AG)_9C$ ISSR marker in Tagil (0.20), Estonian Red (0.25), and Holstein (0.21) were higher than in wild-type Bali cattle (Yu *et al., 2*016). Moreover, Sulabda *et al.* (2022) obtained a close PIC value with the pool animal under study based on two microsatellite markers of DRB3 (0.41) and BM1815 (0.40). Therefore, the I and n_e values of $(AG)_9C$ ISSR marker from 21 cattle breeds in the world was about 0.05 to 0.25 and 1.09 to 1.28, respectively (Stolpovsky *et al.,* 2011) and lower than in pool animals of the present study. In many Russian cattle breeds, the I and n_e values for $(AG)_9C$ ISSR marker were lower than in animals under study *i.e.* Kazakh Whitehead (0.08 and 1.08); Hereford (0.04 and 1.05); Aberdeen Angus (0.04 and 1.03) and Kalmyk (0.08 and 1.10) as reported by Sulimova *et al.* (2016). The n_e, I, and PIC values are three parameters used to evaluate the genetic diversity of animals. Hence, the higher these values the larger the genetic diversity in the observed animals.

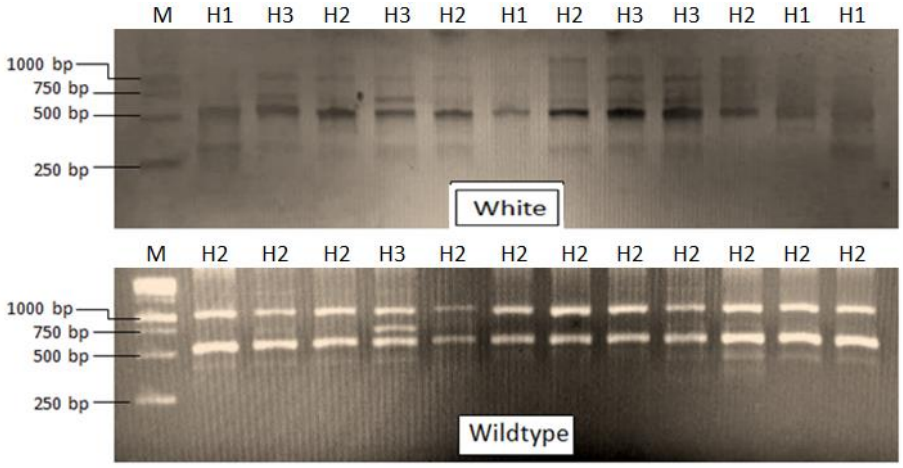

Figure 1. Pattern for $(AG)_9C$ PCR-ISSR markers on 2% agarose gel in two phenotypes of Bali cattle (*Bos javanicus*). M: DNA ladder 1 kb. H: haplotype; Line 1-12: DNA sample

Table 1. Haplotype diversity in two phenotypes of Bali cattle (*Bos javanicus*) based on $(AG)_9C$ ISSR marker.

Haplotype	Number of DNA fragments	Size* (bp)	Frequency (N)		
			White	Wildtype	Total
1	1	550	0.25 (5)	0.00 (0)	0.12 (5)
2	2	1000; 550	0.50 (10)	0.90 (18)	0.70 (28)
3	3	1000; 700; 550	0.25 (5)	0.10 (2)	0.18 (7)

N: number of animals; *Ghasemi *et al.* (2010).

Table 2. The genetic diversity in two phenotypes of Bali cattle (*Bos javanicus*) based on $(AG)_9C$ ISSR marker.

Phenotype	n_e	I	PIC
White	2.67	1.04	0.62
Wildtype	1.22	0.33	0.18
Total	1.86	0.81	0.46

n_e: number of effective alleles; I: Shannon index; PIC: polymorphic informative content.

Interestingly, a specific DNA fragment of 550 bp (H1) was detected in white Bali cattle based on $(AG)_9C$ ISSR marker. Thus, a specific DNA fragment was also reported in two microsatellite primers of ILSTSO45 (188 bp and 180 bp) for polled Bali cattle and ILSTS017 (125 bp and 119 bp) for horned Bali cattle (Zulkharnaim *et al.*, 2023). In this present study, the genetic diversity of $(AG)_9C$ ISSR marker in white Bali cattle was higher than in wild-type Bali cattle. The wild-type Bali bulls of this present study were kept at the breeding station and originated from Bali Island. Hence, those cattle have low genetic variation. While high genetic variation in white Bali cattle can be caused by crossbreeding. As the endangered cattle breeds, the inbreeding depression in white Bali cattle must be prevented since it can cause a genetic mutation with many negative impacts. It was reported that the inbreeding depression of 0.125 in wildtype Bali cattle could able to reduce the body conformation (Putra and Muzawar, 2020). Despite inbreeding, the geographical separation between populations also affects the genetic diversity of animals (Hentati *et al.*, 2019).

In sheep, the $(AG)_9C$ ISSR marker has a significant effect on body weight (Zamani *et al.*, 2015; Mohammadabadi, 2016). Thus, this marker has an I value of 0.25 for Mehraban sheep (Zamani *et al.*, 2011) and 1.03 for Kermani sheep (Mohammadabadi *et al.*, 2017). Moreover, the $(AG)_9C$ ISSR marker of Tuvinian short-fat-tailed sheep reveals the n_e, I, and PIC values of about 1.24; 0.22, and 0.45, respectively (Stolpovsky *et al.*, 2010). However, further study to detect single nucleotide polymorphism (SNP) in the functional genes of coat color in cattle with the genome-wide association study (GWAS) method is important to get the results accurately (Chhotaray *et al.*, 2021). Therefore, genetic conservation in wildtype and white Bali cattle through pure breeding with a good breeding system must be implemented to protect the genetic origin of both cattle.

CONCLUSION

In Bali cattle breed, the $(AG)_9C$ ISSR marker was polymorphic with the presence of three DNA fragment types and three haplotype variations. The second haplotype (H2) variation was observed in most of the animals under study. Therefore, white Bali cattle have higher genetic diversity rather than wild-type Bali cattle. Otherwise, the first haplotype (H1) variation was observed in 5 heads of white animals and its absence in wild-type animals.

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Conflict of interest statement. The authors declare there is no conflict of interest.

Compliance with ethical standards. The experimental procedures were presented and approved by the Animal Welfare Committee of the Udayana University, Bali of Indonesia (Animal Ethical Clearence No: B/41/UN14.2.9/PT.01.04/2023)

Data availability. Data are available from the corresponding author upon reasonable request

Author contribution statement (CRediT): W.P.B Putra - Conceptualization, methodology and writing an original draft., **E.T Margawati -** Investigation, validation, project administration., **A. Furqon –** Writing, review and editing., **I.K. Puja –** Data curation., **H. Hasbi –** Investigation and validation.

REFERENCES

Askari, N., Abadi, M.M. and Baghizadeh, A., 2011. ISSR markers for assessing DNA polymorphism and genetic characterization of cattle, goat and sheep populations. *Iranian Journal of Biotechnology*, 9(3), pp.222-229.

Chhotaray, S., Panigrahi, M., Bhushan, B., Gaur, G.K., Dutt, T., Mishra, B.P. and Singh, R.K., 2021. Genome-wide association study reveals genes crucial for coat color production in Vrindavani cattle. *Livestock Science*, 247, pp.104476. http://doi.org/10.1016/j.livsci.2021.104476

Ghasemi, M., Baghizadeh, A. and Abadi, M.R.A., 2010. Determination of genetic polymorphism in Kerman Holstein and Jersey cattle population using ISSR markers. *Australian Journal of Basic and Applied Science*, 4(12), pp.5758-5760.

Hartl, D.L. and Clark, A.G., 2007. *Principles of Population Genetics*, 4th ed, Sunderland: Sinauer Associated.

Hentati, H.E., Thamri, N., Derouich, W., Hadhli, M. and Boukhorsa, T., 2019. Study of genetic diversity in Tunisian local cattle populations using ISSR markers. *Journal of Animal & Plant Science*, 42(3), pp.7296-7302. http://doi.org/10.35759/JAnmPlSci.v42-3.2

Jakaria, J., Kholijah, K., Darwati, S., Rahman, Q., Daulay, W.L., Suhendro, I., Londra, I.M., Ulum, M.F. and Noor, R.R., 2023. Lack of association between coat color abnormalities in Bali cattle (*Bos javanicus*) and the coding regions of the MC1R and KIT genes. *Veterinary World*, 16(6), pp.1312-1318. http://doi.org/10.14202/vetworld.2023.1312-1318

Kaswati, K., Sumadi, S. and Ngadiyono, N., 2013. The heritability estimation for birth weight, weaning weight and yearling weight of Bali cattle at *Balai Pembibitan Ternak Unggul. Buletin Peternakan*, 37(2), pp.74-78.

Maherovska, O.M., 2021. Selection and assessment of ISSR-markers for analysis of separate cattle populations. *Animal Breeding and Genetics*, 61, pp.137-145. http://doi.org/10.31073/abg.61.15

Mohammadabadi, M., 2016. Inter-simple sequence repeat loci associations with predicted breeding values of body weight in Kermani sheep. *Genetic in 3rd Millennium*, 14(4), pp.4383-4390.

Mohammadabadi, E., Esfandyarpoor, E. and Mousapour, A., 2017. Using inter simple sequence repeat multi-loci markers for studying genetic diversity in Kermani sheep. *Journal of Research and Development*, 5(2), pp.1000154. http://doi.org/10.4172/2311-3278.1000154

Oka, A.A., Putra, I.A.A., Suarna, I.W., Doloksaribu, L. and Puja, I.K., 2020. Productive potency of the endangered Taro white cattle population reared under conservation management system in Bali, Indonesia. *Journal of Animal Health and Production*, 8(4), pp.193-198. http://doi.org/10.17582/journal.jahp/2020/8.4.193.198

Pashaei, S., Azari, M.A., Hasani, S., Khanahmadi, A. and Rostamzadeh, J., 2009. Genetic diversity in Mazandaranian native cattle: A comparison with Holstein cattle using ISSR marker. *Pakistan Journal of Biological Science*, 12(9), pp.717-721. http://doi.org/10.3923/pjbs.2009.717.721

Putra, W.P.B. and Muzawar, M., 2020. The inbreeding case of Bali cattle (*Bos javanicus*) at breeding station. *Kocatepe Veterinary Journal*, 13(4), 1-4. http://doi.org/10.30607/kvj.733991

Shannon, C.E. and Weaver, W., 1949. *The Mathematical Theory of Communication*, 1st ed, Urbana: University of Illinois Press.

Stolpovsky, Y.A., Azari, M.A., Evsukov, A.N., Kol, N.V., Ruzina, M.N., Voronkova, V.N. and Sulimova, G.E., 2011. Comparison of ISSR polymorphism among cattle breeds. *Russian Journal of Genetics*, 47(2), pp.189-200. http://doi.org/10.1134/S1022795410121051

Stolpovsky, Y.A., Kol, N.V., Evsyukov, A.N., Ruzina, M.N., Shimiit, L.V. and Sulimova, G.E., 2010. Analysis of the genetic structure of Tuvinian Short-Fat-Tailed sheep populations with the use of the ISSR-PCR method. *Russian Journal of Genetics*, 46, pp.1462-1470. http://doi.org/10.1134/S1022795410120094

Sulabda, I.N., Wandia, I.N. and Puja, I.K., 2022. Allelic diversity of the endangered Taro white cattle population from Bali using BoLA microsatellite loci. *Journal of Animal Health and Production*, 10(1), pp.16-20. http://doi.org/10.17582/journal.jahp/2022/10.1.16.20

Sulimova, G.E., Voronkova, V.N., Perchum, A.V., Gorlov, I.F., Randelin, A.V., Slozhenkina, M.I. and Zlobina, E.Y., 2016. Characterization of Russian beef cattle breed gene pools using inter simple sequence repeat DNA analysis (ISSR analysis). *Russian Journal of Genetics*, 52(9), pp.963-968. http://doi.org/10.1134/S1022795416090143

Susari, N.N.W., Suastika, P. and Agustina, K.K., 2021. Molecular analysis of Taro and Bali cattle using cytochrome oxidase subunit I (COI) in Indonesia. *Biodiversitas*, 22(1), pp.165-172. http://doi.org/10.13057/biodiv/d220122

Tabun, A., Hartatik, T. and Sumadi, S., 2013. Identification of melanocortin 1 receptor (MC1R) gene based on coat color of Bali cows of Kupang by using the PCR-RFLP method. *Indonesian Journal of Tropical Animal and Agriculture*, 38(2), pp.86-91. http://doi.org/10.14710/jitaa.38.2.86-91

Tabun, A.C., Bidura, I.G.N.G., Putra, I.G.A. and Warmadewi, D.A., 2022. The body dimentions and body weight gain on Bali calf and cows with different coat colors on the semi-intensive maintenance system in

Kupang, Indonesia. *GSC Biological and Pharmaceutical Sciences*, 19(2), pp.187-195. http://doi.org/10.30574/gscbps.2022.19.2.0193

Tahuk, P.K., Budhi, S.P.S., Panjono and Baliarti, E., 2018. Carcass and meat characteristics of male Bali cattle in Indonesian smallholder farms fed ration with different protein levels. *Tropical Animal Science Journal*, 41(3), pp.215-223. http://doi.org/10.5398/tasj.2018.41.3.215

Weir, B.S., 1990. *Genetic Data Analysis. Methods for Discrete Data.* Sunderland: Sinauer Associates Inc.

Ye, C., Yu, Z., Kong, F., Wu, S. and Wang, B., 2005. R-ISSR as a new tool for genomic fingerprinting, mapping and gene tagging. *Plant Molecular Biology Reporter*, 23, pp.167-177. http://doi.org/10.1007/BF02772707

Yu, K.G., Glazko, T.T., Arkhipov, A.V., Khovankina, A.V., Babii, A.V., Kornienko, E.V., Kovalchuk, S.N. and Glazko, V.I., 2016. The use of ISSR markers for characterization of genetic differentiation of cattle breeds. *Problems of Productive Animal Biology*, 3, pp.91-97.

Zamani, P., Akhondi, M. and Mohammadabadi, M., 2015. Association of inter-simple sequence repeat loci with predicted breeding values of body weight in sheep. *Small Ruminant Research*, 135, pp.123-127. http://doi.org/10.1016/j.smallrumres.2015.10.018

Zamani, P., Akhondi, M., Mohammadabadi, M.R., Saki, A.A., Ershadi, A., Banabazi, M.H. and Abdolmohammadi, A., 2011. Genetic variation of Mehraban sheep using two inter-simple sequence repeat (ISSR) markers. *African Journal of Biotechnology*, 10(10), pp.1812-1817. http://doi.org/10.5897/AJB10.1986

Zulkharnaim, M., Baco, S., Rahim, L and Yusuf, M., 2023. Level of DNA similarity the horned and polled Bali cattle using microsatellite approach. *Hasanuddin Journal of Animal Science*, 4(2), pp.125-135. http://doi.org/10.20956/hajas.v4i2.21798