Polymorphism of Melanocortin-4 Receptor Gene and Its Association with Growth Traits in Bali Cattle

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ABSTRAK

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Melanocortin-4 receptor (MC4R) merupakan gen yang turut mengontrol sifat-sifat pertumbuhan. Gen MC4R masuk dalam *leptin-melanocortin pathway* yang bertugas mengatur bobot badan. Penelitian sebelumnya telah banyak berhasil dalam mengidentifikasi keragaman genetik gen MC4R yang terkait dengan sifat pertumbuhan. Namun demikian, penelitian serupa pada sapi Bali masih sangat terbatas. Penelitian ini bertujuan untuk mengetahui *marker* SNP gen MC4R dan pengaruhnya terhadap bobot badan lahir, bobot badan sapih, lingkar dada sapih, tinggi pundak sapih, panjang badan sapih, bobot badan *yearling*, lingkar dada *yearling*, tinggi pundak *yearling*, dan panjang badan *yearling* pada sapi Bali (n=43). Genotip gen MC4R diidentifikasi menggunakan metode *sequencing* dan disejajarkan melalui program BioEdit v.7.2.5. Studi asosiasi pengaruh genotip terhadap sifat pertumbuhan dianalisis berdasarkan prosedur GLM dan DMRT pada program RStudio v.2022.02. Empat SNP berhasil teridentifikasi pada wilayah ekson: g.355G>T, g.394C>T, g.463G>A, dan g.682G>A. Berdasarkan uji *Chi-square*, populasi memenuhi kesetimbangan HWE (p>0.05). Asosiasi genotip-fenotip berdasarkan SNP menunjukkan hasil yang tidak signifikan (p>0.05) dimana lokus g.682G>A (AG) memiliki nilai WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), dan YBL (92.83±4.31 kg) lebih tinggi dibandingkan genotip lainnya. Asosiasi berdasarkan tipe haplotip menunjukkan hasil yang signifikan (P<0.05) pada BB, dimana haplotip 2 memiliki BB tertinggi (21.17±0.75 kg). Kesimpulannya, *marker* SNP dalam penelitian ini bersifat polimorfik akan tetapi tidak mempengaruhi sifat pertumbuhan pada sapi Bali.

Kata Kunci: Sapi Bali, Keragaman Genetik, Sifat Pertumbuhan, Gen MC4R, *Single Nucleotide Polymorphism*

ABSTRACT

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The melanocortin-4 receptor (MC4R) is a gene that controls growth traits. This gene is embedded in the leptin-melanocortin pathway and regulates body weight. Previous studies have successfully identified the genetic diversity of the MC4R associated with growth traits. However, studies on Bali cattle are limited. This study aimed to identify the SNP markers of the MC4R gene and its effect on birth body weight, weaning body weight, weaning chest girth, weaning withers height, weaning body length, yearling body weight, yearling chest girth, yearling withers height, and yearling body length in Bali cattle (n=43). The MC4R genotype was identified by sequencing and aligned using BioEdit v.7.2.5. The association between genotype and growth traits was analyzed using the GLM procedure and DMRT in RStudio program v.2022.02. Four SNPs were identified in the exon region: g.355G>T, g.394C>T, g.463G>A, and g.682G>A. Based on the Chi-square test, the population was fitted with HWE (p>0.05). Genotype-phenotype association based on SNPs showed no significant result (p>0.05) where the g.682G>A (AG) locus had values of WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), and YBL (92.83±4.31 kg) higher than the other genotypes. Association based on haplotype type showed significant results (p<0.05) in BB, where haplotype 2 had the highest BB (21.17±0.75 kg). In conclusion, the SNP markers found in this study were polymorphic but did not affect growth traits in Bali cattle.

Key Words: Bali Cattle, Genetic Diversity, Growth Traits, MC4R Gene, Single Nucleotide Polymorphism

INTRODUCTION

Bali cattle *(Bos javanicus)* are known to become the third species of domesticated cattle in addition to *Bos taurus* and *Bos indicus* (Mohamad et al. 2012). Bali cattle have abundant superior traits such as good adaptation to harsh and limited feed resources, tick resistance, a pregnancy rate of up to 88.44% with a birth

rate of 75-85%, a carcass percentage of approximately 53-56%, tick resistance, and low meat fat content (Wawo 2018; Hafid et al. 2019). The increase in meet demand for the market makes the government and stakeholders synergize to improve the genetics of Bali cattle. Improving meat production to meet sufficient market demand also requires good breeding practices for Bali cattle production. Selection based on the genomic level remains a complex study. Marker Assisted Selection (MAS) is a useful and highly efficient method of modern animal selection (Zhao et al., 2020). Detecting single nucleotide polymorphism (SNP) can represent nucleotide variants that serve as genetic markers. In recent years, genetic markers have been the primary criteria used for selection. Genetic markers can resolve traditional selection limitations, which require a relatively long time (Jakaria et al. 2021). In biotechnology, the genetic marker is a DNA fragment associated with a specific location in the genome to identify parts of the DNA sequence in an unknown DNA pool (Singh et al. 2014). The melanocortin-4 receptor (MC4R) is widely used to detect polymorphism by examining SNP as a candidate genetic marker for assessing growth traits.

The melanocortin-4 receptor gene in cattle is located on chromosome 24 with a length of 1,808 base pairs (bp) and consists of only one exon as a region containing coding sequences (CDS) (Liu et al. 2020). The melanocortin-4 receptor is a peptide produced in the hypothalamus of mammals to control food intake and energy expenditure (Ayers et al., 2018). It is one of the smallest G-protein coupled receptor (GPCR) superfamily members, which increases the intracellular level of cyclic AMP (cAMP) and activates protein kinase A (PKA) (Ju et al. 2018). Mutations in the MC4R gene knockout have been implicated in hypophagia in rats and pigs (You et al. 2016; Hao et al. 2019). Thus, the MC4R gene is the key to regulating satiety, energy expenditure, blood pressure, and growth in the leptin-melanocortin signaling pathways (Kühnen et al., 2018). A previous study successfully confirmed the association between MC4R and growth traits in several species, such as pigs, sheep, cattle, and camels (Saini et al. 2018; Shishay et al. 2019; Liu et al. 2020; Al-sharif et al. 2022).

A previous study successfully identified the genotype-phenotype association of several genes in Bali cattle. The SNP g.10428C>T in the stearoyl-CoA desaturase (SCD) gene is associated with marbling score and intramuscular trait (Alwiyah et al. 2016; Karimah et al. 2021). Several SNP in calpain1 (CAPN1) are associated with the carcass, meat characteristics, and backfat thickness, namely g.3669T>C, g.3854G>A, g.3899C>T, and g.15525G>A (Pratiwi et al. 2016; Dairoh et al. 2021). However, the genotype-phenotype association of the MC4R gene in Bali cattle has yet to be reported. Single nucleotide polymorphism detection of the MC4R gene in Bali cattle is needed to determine the population's genetic diversity. Furthermore, information on SNP has become a valuable tool for identifying genetic markers as a characteristic of each individual. This study aimed to analyze SNP markers of the MC4R gene in Bali cattle to map the association between genotype and growth traits.

MATERIALS AND METHODS

Sample collection

This study involved blood and phenotype data from 12 male and 31 female Bali cattle from the Breeding Centre of Superior Livestock and Forage (BPTU-HPT Denpasar), Bali Province. Cattle were kept in a semi-intensive system and maintained under the same feeding system. Collecting 43 blood samples from Bali cattle was performed through the jugular vein with a minimum volume of 3 ml and kept in an EDTA tube. Phenotype data of body weight and body measurements at birth, weaning, and yearling were obtained from the phenotype data record. The phenotype data used in this study included birth body weight (BB), weaning body weight (WB), weaning chest girth (WCG), weaning withers height (WWH), weaning body length (WBL), yearling body weight (YB), yearling chest girth (YCG), yearling withers height (YWH), and yearling body length (YBL).

DNA extraction and amplification

All molecular analyses (except sequencing) were performed at the Breeding and Genetics Laboratory, Faculty of Animal Science, Universitas Gadjah Mada. DNA extraction of 200 µl whole blood was performed using the Geneaid (gSYNC™ DNA extraction kit, Taiwan) protocol. A primer pair (F: 5'-AATGAACTCTACCCAGCCCC-3'; R: 5'-CAGCAGACAACAAAGACCCC-3') of the MC4R gene, located in the exon region was designed based on the GenBank Acc. no. EU366351.1. Amplification of the 774 bp PCR product was performed using a PCR machine (Peqlab Primus 25). The total volume used in the PCR reaction is 25 µl consisting of 9.5 µl DDW, 12.5 PCR kit, 0.5 µl of each primer, and 2 µl DNA extraction. Amplification of the MC4R PCR product was performed under the following conditions: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation, annealing, and extension at 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s, respectively. The final extension was done at 72 °C for 10 min.

Electrophoresis and DNA sequencing

The PCR products were analyzed by electrophoresis before DNA sequencing. The electrophoresis was performed on agarose gel with a concentration of 0.8%

containing 0.25 µl of Ethidium Bromide (EtBr) using a Mupid-EXU electrophoresis machine at 100 volts for 30 minutes. A 1 kb marker was also added to measure the size of the PCR products. The DNA bands were visualized using a UV transilluminator (UVP TEM-40). In total, 43 PCR products of the MC4R gene were sequenced by 1st BASE Malaysia. The reference sequence (EU366351.1, OL623708-OL623717) and sample sequences were analyzed using the BioEdit v.7.2.5. Finally, representative sequences of each haplotype were submitted to GenBank (Submission ID: 2618554).

Data analysis

The genetic polymorphism parameters of allele frequency, genotype frequency, heterozygosity (H), Polymorphic Information Content (PIC), Hardy-Weinberg Equilibrium (HWE), and the association study of genotype-phenotype were analyzed using the RStudio program. The frequency of allele and genotype were calculated using the formula (Nei & Kumar 2000):

$$X_i = \frac{2n_{ii} + \sum_{i \neq j} n_{ij}}{2N}$$

$$X_{ii} = \frac{n_{ii}}{N}$$

where X_i is the frequency of the allele, X_{ii} is the frequency of the genotype, n_{ii} is the number of individuals with genotype ii, n_{ij} is the number of individuals with genotype ij, and N is the total sample.

The HWE was analyzed using the Chi-square test according to Nei & Kumar (2000) as follows:

$$X^2 = \sum \frac{(O - E)^2}{E}$$

where X^2 is the Chi-square, O is the observed value, and E is the expected value.

The association study of genotype-phenotype for each SNP was calculated in one-way Anova using the RStudio program according to the following statistical general linear model:

$$Y_{ij} = \mu + G_i + E_{ij}$$

Where Y_{ij} is the observation of the phenotype, µ is the overall mean, G_i is the effect of the genotype, and E_{ij} is the random error. All data are described as least square means±standard error of means (LSM±SEM). If the Anova value was significant, further testing was performed using Duncan's Multiple Range Test (DMRT).

Correction factor

The phenotype data record was corrected to the parent's age correction factor (FKUI) to reduce environmental errors. The birth body weight data of females were corrected towards males with a correction factor of 1.07 (USDA). Weaning and yearling body weight data were corrected to 205 and 365 days, respectively, according to Hardjosubroto (1994) as follows:

$$WB_{205} = \left[\frac{WBw - BB}{Age} x205 + BB\right](FKUI)$$

$$YB_{365} = [\frac{YBw - WB}{\Delta t} x160 + WB_{205}$$

where WB_{205} is the corrected weaning body weight, YB_{365} is the corrected yearling body weight, WBw is the weaning body weight when weighing, BB is the birth body weight, age is the age at the time of weaning, YBw is the yearling body weight when weighing, WB is the weaning body weight, and Δt is the period from weaning until yearling weighing. The FKUI of Bali cattle followed Pane (1981) for 5-9 years old (1.00). Body size data were corrected by using the body weight correction factor formula.

RESULTS AND DISCUSSION

DNA amplification and SNP identification

The specific DNA fragment of MC4R was successfully amplified, as indicated by clear DNA bands at 774 bp in the exon region (Figure 1). The DNA sequencing results of 43 samples were used for alignment. In total, four SNP markers with a length of 774 bp from gene target: g.355G>T (GG, TG), g.394C>T (CC, CT), g.463G>A (GG, AG, AA), and g.682G>A (GG, AG) were found in this study by comparing the DNA sequencing results with EU366351.1 and OL623708-OL623717, as the GenBank reference (Figure 2). BioEdit showed that there were differences in the nucleotide positions according to Acc. no. EU366351.1 between the sample and the GenBank reference (OL623708-OL623717), whereas they were the same mutation (Table 1). All SNP markers were in the exon region as coding sequences (CDS) that changed nucleotides to proteins during translation. Three SNP (g.394C>T, g.463G>A, g.682G>A) showed a transition mutation, whereas SNP g.355G>T was a transversion mutation that changed the purine to pyrimidine (Table 2). However, all SNPs in this study were classified as silent mutations; therefore, they did not change the amino acid code.

Biomolecular techniques are based on the identification of genetic markers that affect important. traits such as growth traits. A tool such as SNP genotyping helps investigate marker-trait associations (Bali et al. 2018). Single nucleotide polymorphism can be used as markers to determine allele variation as candidate genes in the selection process. One gene related to growth traits is MC4R. Based on the SNP

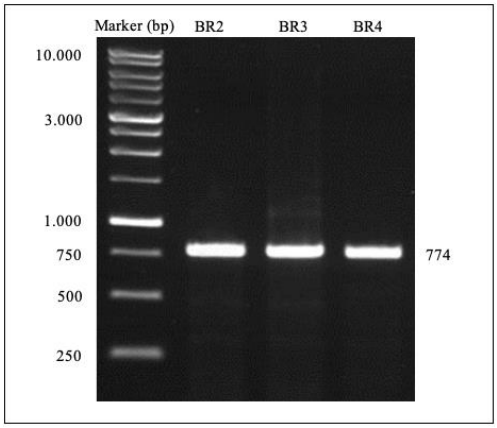

Figure 1. DNA amplification of the MC4R gene on a 1 kb DNA ladder and 0.8% agarose gel. BR2-4: Sample code

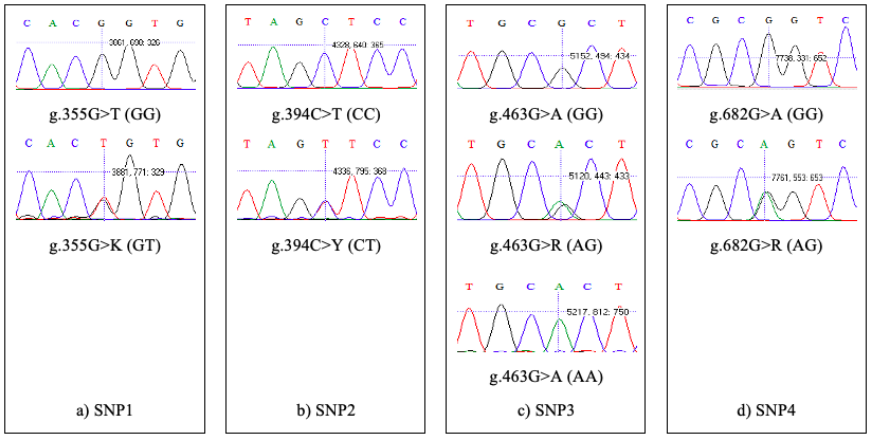

Figure 2. The chromatogram indicates the genotype of four SNP markers in the MC4R gene

Table 1. Nucleotide position between samples and GenBank references

GenBank[a]	GenBank[b]	This study
g.631G>T	g.731G>T	g.355G>T
g.670C>T	g.770C>T	g.394C>T
g.739G>A	g.839G>A	g.463G>A
g.958G>A	g.1058G>A	g.682G>A

[a]EU366351.1; [b](Perdana and Hartatik 2022)

Table 2. Types of the MC4R gene mutations in each SNP

SNP	Mutation	Amino acid change
g.355G>T	Transversion - Silent	ACG (Thr) > ACT (Thr)
g.394C>T	Transition - Silent	AGC (Ser) > AGT (Ser)
g.463G>A	Transition - Silent	GCG (Ala) > GCA (Ala)
g.682G>A	Transition - Silent	GCG (Ala) > GCA (Ala)

genotyping of the MC4R gene (774 bp), this study successfully identified the population balance, including genetic diversity, HWE, and genotype association with growth traits in Bali cattle. Our data showed that the four SNP markers (g.355G>T, g.394C>T, g.463G>A, and g.682G>A) of MC4R were the same as those in a previous study (Perdana and Hartatik 2022). Other studies that have reported MC4R SNP markers in cattle include g.19C>A, g.20A>T, g.83T>C, g.85A>G, g.128G>A, g.129A>G, g.192T>G, g.193A>T,

g.293C>G, g.709G>A, g.927C>T, g.1069C>G, g.1133C>G, g.1343C>A, and g.1786C>T (Seong et al. 2012; Du et al. 2013; Lee et al. 2013; Fathoni et al. 2020; Liu et al. 2020; Utomo et al. 2021).

Genetic diversity

Information about genetic diversity, including the frequency of genotypes and alleles, H, PIC, and HWE of the MC4R gene in Bali cattle, are presented in Table 3. Three of the four loci contained two genotypes: g.355G>T (GG, TG), g.394C>T (CC, CT), and g.682G>A (GG, AG). The locus g.463G>A has three kinds of genotypes: AA, AG, and GG. The loci (g.355G>T and g.394C>T) had the same genotype and allele compositions. As shown in Table 3, the genotypes GG (g.355G>T), CC (g.394C>T), AG (g.463G>A), and GG (g.682G>A) were higher than others. Based on this study's four SNP markers of MC4R, seven haplotype types (five heterozygous and two homozygous) were formed with various percentages (Table 4). Haplotype 1 (GenBank Acc. no. OP376529) had the highest frequency (53%) compared to others. The frequencies of haplotype 2 (Acc. no. OP376530) and haplotype 3 (Acc. no. OP376531) were 14%. Haplotype 4 (Acc. no. OP376532) and haplotype 5 (Acc. no. OP376533) at the same frequency (7%). The lowest frequency (2%) for haplotype 6 (Acc. no. OP376534) and haplotype 7 (Acc. no. OP376535).

Genetic diversity is essential for the adaptation and survival of populations to avoid extinction. For humans, it is important to study population genetics to achieve preservation goals and to perform good breeding practices to maximize genetic potential. There are several methods to calculate genetic diversity, such as heterozygosity (H), runs of homozygosity (ROH), Wright's F-statistic (F_{st}), linkage disequilibrium (LD), and effective population size (N_e) (Al-Mamun et al. 2015). In this study, we used heterozygosity to estim ate genetic diversity. As shown in Table 3, homozygous genotypes (GG and CC) in g.355G>T, g.394C>T, and g.682G>A dominated the genotype (0.95, 0.95 and 0.86), and allele frequency (0.98, 0.98, and 0.93), respectively. In contrast, the heterozygous genotype (AG) at locus g.463A>G (0.63) was higher than that in the homozygous, whereas allele G was still higher than A. A previous study on Bali cattle in BPTU-HPT Denpasar reported that the GG genotype and G allele were the most prevalent (Fahira et al. 2022). According to the genotype and allele information of four loci, the MC4R gene of Bali cattle in BPTU-HPT Denpasar was polymorphic (less than 0.99) (Volkandari et al. 2013). The gene becomes monomorphic if the allele exceeds 0.99 (Putra et al. 2021).

The heterozygosity score of the three SNP markers ranged from 0.05-0.13 with a PIC of 0.04-0.12. The PIC value is closely related to the H score, which depends on the number of alleles; this indicates that the three SNP markers had low polymorphism (PIC<0.25). On the other hand, the SNP g.463G>A, including three genotypes (GG, AG, AA), has almost the same allele (G= 0.55; A= 0.45), while the heterozygosity and PIC values were 0.50 and 0.37, respectively. This g.463G>A SNP marker indicates that the locus was in moderate polymorphism ($0.25 \leq PIC \leq 0.5$) (Botstein et al. 1980).

Table 3. Genetic diversity of the MC4R gene in Bali cattle

Locus	Genotype	N	X_{ii}	X_i	H	PIC	HWE (P-value)
g.355G>T	GG	41	0.95	G: 0.98	0.05	0.04	1
	TG	2	0.05	T: 0.02			
	TT	0	0				
g.394C>T	CC	41	0.95	C: 0.98	0.05	0.04	1
	TC	2	0.05	T: 0.02			
	TT	0	0				
g.463G>A	GG	10	0.23	G : 0.55	0.50	0.37	0.07
	AG	27	0.63	A: 0.45			
	AA	6	0.14				
g.682G>A	GG	37	0.86	G : 0.93	0.13	0.12	1
	AG	6	0.14	A: 0.07			
	AA	0	0				

N= Number of individu; X_{ii}= Frequency of genotype; X_i= Frequency of allele; H= Heterozigosity; PIC= Polymorphic Information Content; HWE= Hardy-Weinberg Equilibrium; α= 0.05

Table 4. Haplotype and frequency of the MC4R gene in Bali cattle

Hap	N	g.355G>T	g.394C>T	g.463G>A	g.682G>A	Frequency (%)	Allele
1	23	G	C	R	G	53	Heterozygous
2	6	G	C	A	G	14	Homozygous
3	6	G	C	G	G	14	Homozygous
4	3	G	C	G	R	7	Heterozygous
5	3	G	C	R	R	7	Heterozygous
6	1	K	Y	G	G	2	Heterozygous
7	1	K	Y	R	G	2	Heterozygous

Hap= Haplotype; N= Number of individu; G, C, A= Homozygous; K= Heterozygous of GT; Y= Heterozygous of CT; R= Heterozygous of AG

A higher PIC value indicates a higher degree of polymorphism (Shan et al., 2020). The low genetic diversity observed in the Bali cattle may be due to selection within a limited area and population. Selection in a limited population can lead to a decrease or loss of one of the minor genes or genetic drift. Mutations and genetic drifts control genetic diversity in populations. Mutation can increase genetic variation, but genetic drift tends to reduce it (Teixeira and Huber 2021). Genetic drift is the leading cause of genetic diversity loss in several cattle breeds, including Canadienne, Milking Shorthorn, Brown Swiss, Guernsey, and Ayrshire (Melka et al. 2013). Genetic drift mainly occurs because of the small effective population size that accumulates over non-founder generations. The Chi-square (X^2) test showed that the genotype distributions of the Bali cattle population were in HWE (P>0.05). The HWE law states that the genotype and allele frequencies will always be the same from generation to generation during random mating (Lachance 2016).

Association of genotype with growth traits

This study analyzes the genotype-phenotype using two analysis approaches based on SNP markers and haplotypes. The values of the genotype-phenotype associations based on the four MC4R SNP markers are presented in Table 5. Statistical analysis of MC4R genotypes and growth traits based on SNP markers revealed no significant association between the four SNP markers (P>0.05). Therefore, the heterozygous (g.682G>A) has a higher body weight and body size than the other three SNP markers, namely, WB (104.33±17.15 kg), WCG (112.83±3.66 kg), WBL (89.50±3.94 kg), YB (124.17±22.44 kg), YCG (120.50±5.50 kg), and YBL (92.83±4.31 kg). The second approach used in this study was based on the haplotype types produced. One-way ANOVA from five haplotypes of body weight and body size showed significant differences in BB (p<0.05) (Table 6). The highest BB was in haplotype 2 (GCAG) with 21.17±0.75 kg. Despite no significant (P>0.05), haplotype type 4 (GCGR) produced the higher body weight and body size of Bali cattle, namely, WB (109.67±21.94 kg), WCG (114.00±5.20 kg), WWH (94.67±4.62 kg), WBL (89.67±2.89 kg), YB (131.33±26.56 kg), YCG (121.33±7.51 kg), and YBL (93.67±1.15 kg).

The genotype-phenotype association study on growth traits of Bali cattle to determine the correlation between SNP markers, body weight, and body size was done. None of the SNP markers in the present study showed significant associations. A previous study found an association between SNP markers and growth traits. However, some did not. Association studies between MYF5 and PLAG1 with body weight and size in Bali cattle from BPTU-HPT Denpasar have shown no significant (Saputra et al. 2020; Fahira et al. 2022). In contrast, the previous studies reported that each genotype has a different effect on the economic feature. For example, the CC genotype in SNP g.1069C>G of Chinese and Korean cattle has better economic features of backfat thickness than the GG genotype (Seong et al. 2012). This study's highest body weight and size scores were for heterozygous SNP marker g.682G>A (AG). The mutation occurring in SNP g.682G>A may affect the increase in body weight and body size at the age of weaning and yearling. As shown in Table 5 and Table 6, the mean birth body weight (BB) was 19.74 kg, and the highest BB was located in haplotype type 2 (21.17±0.75 kg) (P<0.05). This finding was higher than a previous study in which the BB was 17.8 kg (Kaswati et al. 2013). It is conducted that the selection program in BPTU-HPT Denpasar successfully increased the BB of Bali cattle. However, the significant association between haplotype type and BB cannot yet be used as a genetic marker because BB is still highly dependent on the mother (Sulistiyoningtiyas et al. 2017). Selection based on high birth weight is not recommended because of the difficulty of parent-bearing.

Table 5. Association of growth traits with four SNP markers of the MC4R gene in Bali cattle

Locus	X_{ii}	N	BB (kg)	WB (kg)	WCG (cm)	WWH (cm)	WBL (cm)	YB (kg)	YCG (cm)	YWH (cm)	YBL (cm)
g.355G>T	GG	41	19.78±1.96	96.71±13.30	108.76±7.59	92.12±4.61	86.71±5.56	115.61±18.14	117.34±6.97	95.95±4.23	91.19±5.11
	TG	2	19.00±2.83	96.00±4.24	111.00±1.41	92.00±7.07	86.00±4.24	112.00±0.00	119.50±0.71	96.50±2.12	90.00±1.41
	P-value		0.589	0.941	0.682	0.972	0.861	0.782	0.667	0.857	0.746
g.394C>T	CC	41	19.78±1.96	96.71±13.30	108.76±7.59	92.12±4.61	86.71±5.56	115.61±18.14	117.34±6.97	95.95±4.23	91.19±5.11
	TC	2	19.00±2.83	96.00±4.24	111.00±1.41	92.00±7.07	86.00±4.24	112.00±0.00	119.50±0.71	96.50±2.12	90.00±1.41
	P-value		0.589	0.941	0.682	0.972	0.861	0.782	0.667	0.857	0.746
g.463G>A	GG	10	20.20±2.10	100.20±16.38	110.70±5.38	94.50±4.45	87.70±5.75	122.40±22.58	119.10±6.72	97.00±4.50	91.70±5.35
	AG	27	19.26±1.95	96.15±12.21	108.96±8.07	91.18±4.60	86.78±5.54	113.37±15.55	117.26±6.79	95.41±4.18	91.18±5.10
	AA	6	21.17±0.75	93.17±10.81	105.33±7.12	92.33±4.27	84.50±4.85	113.17±18.36	115.50±7.69	96.83±3.49	90.00±4.56
	P-value		0.067	0.555	0.382	0.154	0.53	0.375	0.589	0.514	0.81
g.682G>A	GG	37	19.65±1.99	95.43±12.02	108.22±7.71	91.95±4.80	86.22±5.58	114.03±16.78	116.95±6.94	95.95±4.29	90.86±5.10
	AG	6	20.33±1.86	104.33±17.15	112.83±3.66	93.17±3.60	89.50±3.94	124.17±22.44	120.50±5.50	96.17±3.37	92.83±4.31
	P-value		0.482	0.121	0.16	0.555	0.175	0.197	0.241	0.905	0.377

Values are shown as the least squares means±standard error. X_{ii} = Genotype; N = Number of individu; BB = Birth body weight; WB = Weaning body weight; WCG = Weaning chest girth; WWH = Weaning withers height; WBL = Weaning body length; YB = Yearling body weight; YCG = Yearling chest girth; YWH = Yearling withers height; YBL = Yearling body length; $\alpha = 0.05$

Table 6. Association of growth traits with the haplotype of the MC4R gene in Bali cattle

Hap	N	BB (kg)	WB (kg)	WCG (cm)	WWH (cm)	WBL (cm)	YB (kg)	YCG (cm)	YWH (cm)	YBL (cm)
1	23	19.00+1.81^{b}	95.65+12.61	108.48+8.66	91.30+4.87	86.61+5.65	112.96+15.73	116.83+7.18	95.39+4.48	91.17+5.15
2	6	21.17+0.75^{a}	93.17+10.81	105.33+7.12	92.33+4.27	84.50+4.85	113.17+18.36	115.50+7.69	96.83+3.49	90.00+4.56
3	6	20.83+2.32^{a}	96.67+14.54	109.17+5.64	94.00+5.06	86.50+7.20	119.67+23.60	118.00+7.38	97.00+5.25	90.83+6.91
4	3	20.00+0.00a^{b}	109.67+ 21.94	114.00+5.20	94.67+4.62	89.67+2.89	131.33+26.56	121.33+7.51	96.67+4.62	93.67+1.15
5	3	20.67+2.89^{ab}	99.00+13.00	111.67+1.53	91.67+2.08	89.33+5.51	117.00+19.97	119.67+4.16	95.67+2.52	92.00+6.56
P-value		0.049*	0.493	0.553	0.634	0.668	0.549	0.769	0.900	0.900

Values are shown as the least squares means±standard error. Hap = Haplotype; N = number of individu; BB = Birth body weight; WB = Weaning body weight; WCG = Weaning chest girth; WWH = Weaning withers height; WBL = Weaning body length; YB = Yearling body weight; YCG = Yearling chest girth; YWH = Yearling withers height; YBL = Yearling body length; The different superscripts ($^{a, b}$) in the same column denote significant difference ($\alpha = 0.05$). Only 7% or more was used for an association between growth traits and haplotypes

CONCLUSION

In summary, four SNPs in the exon region (774 bp) of MC4R in Bali cattle were successfully identified. The Bali cattle population in this study had low to moderate polymorphism and fit the HWE. Association analysis of the SNP markers did not show significant results. On the other hand, the association analysis for haplotype showed a significant result for BB. However, this study provides information about the genotype of four SNPs that are useful for reference in subsequent studies and breeding methods based on genetic information.

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