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Exterior characteristics and coat colour marker of MC1R gene in Bali Crossbred cattle

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ABSTRACT

Crossbreeding between Bali cattle with exotic bulls (such as Simmental and Limousin) has been widely developed in several regions in Indonesia. This study aims to determine changes in exterior characteristics and differences in vital statistics of Bali cattle and their crosses in West Lombok Regency. A total of 91 female cattle aged 1 to 5 years were sampled, consisting of 5 Bali cattle (100%Bali), 54 F1 (50%Bali), 21 G2 (75%Bali/25%Bali), and 11 G3 (12.5%Bali) cattle. Qualitative (color and shape) and quantitative cattle data (vital statistics) were collected through observation and measurement. Identification of Bali crossbred cattle genotypes that have different color patterns using molecular markers of the MC1R gene with forward primer MC1R: 5'-GGTGAGTCTCGTGGAGAACG-3' and reverse primer MC1R: 5'-CGTAGAAGATGGAGATGTAGCG-3', at a target DNA sequence size of 308 bp. The dominant color results of Bali cattle showed brick red (60%) and brown (20%), and there was a dark brown and black color change of up to 100% in the crosses. DNA-pool sequencing of 14 samples indicated individual sequence differences, indicated by overlapping nucleotide arrays after the 174 sequence. Furthermore, sequencing of different color representatives from 12 individual samples shows 4 SNPs, namely g.64C>T, g.147C>T, g.159C>T, dan g.235A>C in Bali crossbred cattle. The measurement of chest circumference of Bali cattle, crossbred F1, G2, and G3 aged 3 to 5 years were 151.80±9.60cm, 175.69±12.63 cm, 175.50±11.04cm, and 173.17±15.06cm, respectively.The result indicate the significant differences of body measurement ((P<0.01). In conclusion, the F1 (50% Bali) crossbred produced superior performance. Single nucleotide polymorphism (SNP) can be used as a genetic marker for colour pattern the dark brown-black in crossbred Bali cattle. However, the comprehenshive study in the future is still needed in the future for cattle crossbred Bali cattle at different generation.

Keywords: Bali cattle, crosses, exotic cattle, exterior characteristics, MC1R

INTRODUCTION

Bali cattle are one of the beef cattle from Indonesia. Based on data from the Ministry of Agriculture in 2021, the total population of Bali cattle is estimated to reach 5,621,940 heads which have spread throughout all provinces of Indonesia. Bali cattle are small in stature but have high adaptability to temperature, feed quality, and fertility, and have a high carcass percent-

age with low fat content (Tabun *et al*., 2013).

Bali cattle crosses that have been developed in Indonesia are crosses with exotic cattle breeds, such as Simmental, Limousin, Brahman, Brangus, and Angus cattle. Crossbreeding is done through Artificial Insemination (AI) program where the population of Bali cattle and crossbred cattle, especially in West Nusa Tenggara Province is quite large and is one of the sources of cattle for Eastern Indonesia (Chusna *et al.,* 2022). Crossbreeding in West Nusa Tenggara (NTB) according to the Livestock and Animal Health Service Office was implemented in 2015 and continues to grow through government programs.

Improved performance of Bali cattle crosses is indicated by body measurements, such as body length, chest circumference, gumba height, hip height, and body weight (Islami *et al.,* 2023). Meanwhile, body color changes in crossbred Bali cattle can be seen immediately. Body color changes in Bali cattle crosses include the muzzle color, horn color, legscolor, buttocks color, and dorsal line color (Warman *et al*., 2024). Bali cattle are characterized by horns, reddish-brown fur color and will turn black in Bali bull, while in female Bali cattle it remains reddish-brown, white legs and hamstrings and black dorsal lines (eel lines) (Tabun *et al.,* 2013; Suhendro *et al.,* 2024). Meanwhile, the characteristics of the color will change due to crossbreeding. Differences in body color in cattle are caused by pigments influenced by the *Melanocortin 1 Receptor* (MC1R) gene. Mishra *et al.* (2017) suggested that the *Melanocortin 1 Receptor* (MC1R) gene is responsible for the main transition of pheomelanin (red-yellow) and eumelanin (brown -black), which can be a body color marker gene. The *Melanocortin 1 Receptor* (MC1R) gene is important to study because it plays an important role in the pigmentation process in skin and coat color differences (Sarini *et al.,* 2023).

The F1 crosses have a genetic proportion of 50% exotic bull:50% Bali. The G2 offspring which backcross to the same exotic bulls have a genetic proportion of 75% exotic bull and 25% Bali, while G2 crossbred with two different bulls have a proportion of 25% of F1 bull:25% Bali:50% the last bull. The G3 crossbred which BC to the same bulls have a genetic proportion of 87,5% bull:12,5% Bali, while the G3 crossbred which derived from three breed has a proportion of 62,5% Simmental:25% Limousin:12,5% Bali. Crossbreeding Bali cattle with several semen exotic bulls by AI produce F1, G2, and G3 offspring. The result of cross G3 has a genetic proportion of 12.5%:25%:62.5%. This was also stated by Quenon and Magne (2021) that the genetic composition of cattle consists of 100% purebred cattle, 50% F1 crossbred cattle, and 25% G2 crossbred cattle. This crossbred is intended to increase the gene composition of one parent in the population. Therefore, crosses up to G3 were conducted to see the impact of crosses that changed the exterior characteristics and body size.

Based on the exterior characteristics of Bali cattle and their crosses in West Lombok Regency, the body color and shape of cattle vary due to the traits passed on by the elders to their offspring and the *Melanocortin 1 Receptor* (MC1R) gene as a marker of body color. The purpose of this study was to determine changes in exterior characteristics and differences in vital statistics of Bali cattle and their crosses with exotic bulls in West Lombok Regency.

MATERIALS AND METHODS

The methods used in this study were surveys and direct interviews. Interviews were conducted with animal health officers and 65 farmer respondents with 3 to 40 years of farming experience. Determination of survey locations for data collection was based on recommendations from the Livestock and Animal Health Service Office of West Lombok. Data collected included cattle ownership, cattle age, and information on the elders of the observed cattle to categorize F1, G2, and G3 offspring.

Collection of Qualitative Data

Observations were conducted in West Lombok Regency consisting of the southern region (Lembar and Gerung) and the central region (Narmada) with 91 female cattles. The cattles consisted of 5 Bali cattle (100% Bali), 54 F1 crosses (50% Bali), 21 G2 cattle (BC/3 offspring, 25% Bali and 75% Bali), and 11 G3 cattle

Bali cattle (100% Bali) | F1 crosses (50% Bali), | G2 cattle (BC/3 offspring, 25% Bali and 75% Bali), | G3 cattle (12.5% Bali)

Figure 1. Representative sample of Bali cattle and their crosses

(12.5% Bali) as presented in Figure 1. The percentage of genetics composition according to the information from Livestock and Veterinary Services. Ages used ranged from 1 to 5 years. The research was conducted from August 2024 to November 2024. Qualitative data characteristics include: 1) Body color, 2) Horn shape, 3) Back line, 4) Rump color, 5) Leg color, and 6) Tail tip color. Data collection is done by observing the color and shape of the cattle's body whose criteria have been determined.

Collection of Vital Statistics Data

Quantitative trait data were collected from 67 head out of 91 female cattles. The cattle consisted of 5 Bali cattle (100%Bali), 36 F1 crosses (50%Bali), 20 G2 cattle (BC/3 breed, 25%Bali), and 6 G3 cattle (12.5%Bali). The samples measured for quantitative traits were the same as the samples observed for qualitative traits, exclude <3 years of age. Data collection was done by measuring using FHK brand measuring ruler with 0.2 cm accuracy and FHK brand measuring tape with one cm accuracy. Measurements with the measuring ruler included: 1) Body length, 2) Body width, 3) In the chest, 4) Gumba height, 5) Hip height, and 6) Hip width. Measurements with a measuring tape include: 1) Head length, 2) Head width, 3) Ear length, 4) Ear width, 5) Horn length, 6) Horn circumference, 7) Chest circumference, 8) Eel length, 9) Eel width, and 10) Head index. Age estimation of livestock was obtained from observing tooth development and interviews with farmers.

Blood Sampling and DNA Extraction

The representative of different coat colour of Bali crossbred cattle were selected for further experiment. A number of 26 samples were analyzed to identified molecular genetic marker of MC1R gene. Blood samples were taken from the jugular vein using a vacuntaier containing EDTA which was preserved under -20°C. DNA was extracted from the blood samples using proteinase-K according to the Gsync^{TM} DNA Extraction Kit instructions. DNA sample analysis was conducted at the Animal Breeding Laboratory, Faculty of Animal Science, Gadjah Mada University.

Polymerase Chain Reaction (PCR)

The MC1R gene was amplified using MC1R forward primer: 5'-GGTGAGTCTCGTGGAGAACG-3' and MC1R reverse primer: 5'-CGTAGAAGATGGAGATGTAGCG-3' (Li *et al*., 2008), with a target DNA sequence size of 308 bp. The total reaction volume of 25 µl MC1R gene amplification consisted of 9.5 µl DDW, 12.5 µl PCR Kit, 0.5 µl forward and reverse primers, and 2 µl sample DNA. Amplification cycle conditions were pre-denaturation of 95°C for 5 minutes, 30 cycles at 94°C for 30 seconds, anneling 57°C for 30 seconds, extention 72°C for 30 seconds, and final extention 72°C for 10 minutes using a PCR machine. PCR products were examined through electrophoresis using a 1.5% agarose gel.

Pool-DNA Sequencing

The PCR product results in the form of Pool -DNA consisting of 3 pools. Pool 1 includes 6 samples with dark brown color. Pool 2 includes 5 samples with brown and light brown colors. Pool 3 includes 3 samples with white and black color shades. Pool 1 samples are 17BLL, 62BBrS, 65BSSS, 66BSSS, 82BSSS, and 95 BSL. Pool 2 samples are 12BS, 56BL, 60BBrS, 79BLS,

103BSSS. Pool 3 samples are 1BS, 18BA, and 23BL. PCR products were then sequenced at the Integrated Research and Testing Laboratory of Gadjah Mada University.

Individual Sequencing Sample

PCR products from 12 samples were sequenced individually based on body color. Samples represented dark brown, light brown, black, dark brown with white markings, and black with white markings. The samples included 7BL, 8BL, and 23BL (dark brown), 12BS, 16BS, and 20BS (light brown), 18BA, 26BA, 36BA, and 58BA (black), 1BS (dark brown with white markings), and 19BS (black with white markings). The sequencing process was carried out at Genetics Science.

Weight Estimation

Body weight estimation through age grouping ranges from 3 to 5 years. This grouping is then calculated by estimating body weight using the *Lambourne* formula which has a percentage error of 8.73%, below 10% (Septyan *et al.*, 2023). The *Lambourne* formula is as follows.

$$W = \frac{L \times G^2}{10840}$$

Description :
W = Weight
L = Body lenght (cm)
G = Chest circumference (cm)

Data Analysis

Data on exterior characteristics were analyzed descriptively quantitatively. Quantitative descriptive analysis by calculating the percentage of exterior characteristics. Vital statistics data were calculated as mean and standard deviation by age group and nation combination. Quantitative descriptive data analysis was carried out with the help of SPSS 16 for windows. Sequencing results were carried out with the help of BioEdit software for sequence alignment to determine the position of SNP (Single Nucleotide Polymorphism).

RESULTS AND DISCUSSION

Genetic Composition and Crossbred Type

The entire genetic composition of Bali cattle and crossbred types in West Lombok Regency is presented in percentage form shown in Figure 2. The genetic composition of Bali cattle and crossbred types in Figure 2 shows the most variation is seen in F1 crosses (50%Bali) at 59%, which in total there are 19 kinds of Bali crossbred cattle. Crosses of Bali cattle with exotic bulls are most common in SimmentalxBali at 27%. Adelia *et al.* (2020) found that Simbal crosses are most common because of the proven increase in body weight growth and body size.

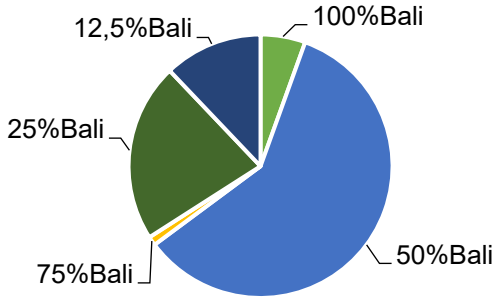
Composition of Bali and Crossbred Cattle Samples per Location

The composition of Bali crossbred cattle and crosses per location obtained data as presented in Table 1. Table 1 shows the region with the most F1 (50%Bali) crosses in Gerung region at 86%. BC (75%Bali), G3 (25%Bali), and G3 (12.5%Bali) crosses were highest in the Narmada region, at 2%, 32%, and 23%, respectively. Widi *et al.* (2015) suggested that the distribution of crosses is influenced by the objectives and demands of smallholders as the Indonesian government has implemented crossbreeding with European beef cattle through artificial insemination to improve local beef performance in response to this increasing demand.

Exterior Characteristics

Comparison of the exterior characteristics of Bali cows and 19 crosses aged 1 to 5 years are presented in Table 2. The comparison of the exterior characteristics of Bali cows and 19 crosses in Table 2 shows differences in body color, horn shape, snout color, back line, rump color, foot color, and tail tip color. The dominant body color in Bali cattle is brick red and brown, while for Bali crossbred cattle it ranges from brown to black, where Bali cattle with Simmental have a characteristic white spot on the head. The same observation results were also shown in the research of Anggraini *et al.* (2021) that Bali cattle have a characteristic brick red body color, and the buttocks and lower legs are white. According to Adelle *et al*., 2020, the cross of Bali cattle with Simmental has a dominant coat color of brown with white stripes and horns. The combination of colors from two different breeds produces different colors in 19 crosses. The body

A)



B)

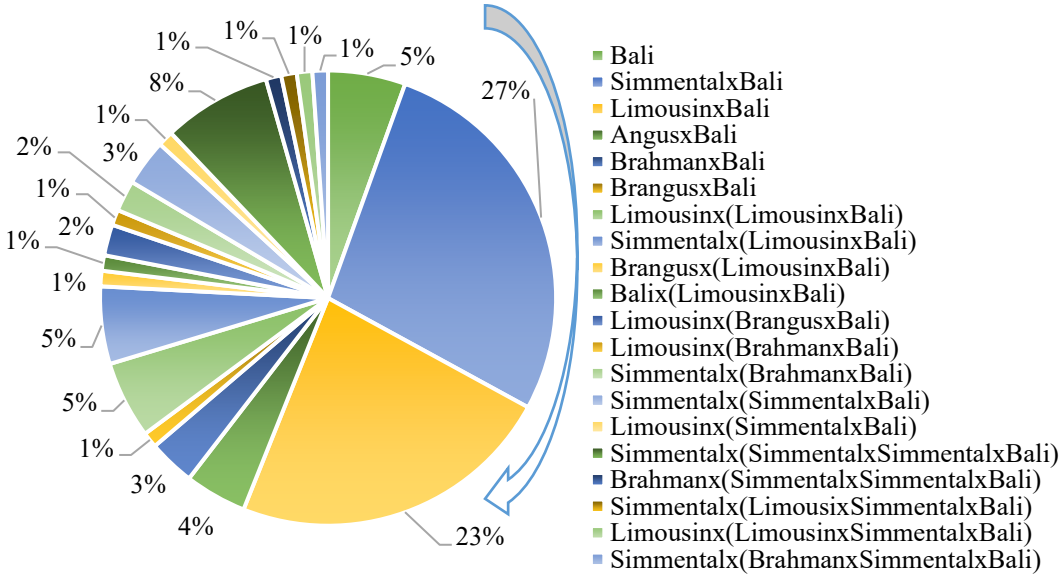


Figure 2. Genetic composition and types of crosses. A) Genetic composition of Bali cattle, B) Bali cattle with studs forming crosses

Table 1. Composition of research samples of Bali cattle and their crosses per location

Location	Bali(100%Bali) n=5	F1(50%Bali) n=54	BC(75%Bali) n=1	G2(25%Bali) n=20	G3(12,5%Bali) n=11	Total
Lembar (n=15)	13%	73%	0%	13%	0%	100%
Gerung (n=29)	0%	86%	0%	14%	0%	100%
Narmada (n=47)	6%	38%	2%	32%	23%	100%

coloration pattern of these cattle is regulated by the MC1R gene presented in Figure 3, Figure 4, Figure 5, Table 3, and Table 4.

The sequencing results of 14 DNA-pool samples and 12 individual samples representing different colors, as shown in Figure 4 and Figure 5, indicate that there is an overlapping nucleotide arrangement after the 174 position (after GCCG sequences), indicating individual sequence differences. The sequencing results of pool-DNA were predicted to be heterozygous at Pool 1 and Pool 2, while Pool 3 sequence show noisy result. The overlapping is thought to be due to deletions.The individual sequencing results of 12

Table 2. Color and body shape characteristics of Bali Crossbred cattle

Exterior Characteristics	Types of Cattle (%)																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Body color																				
Brick red	60.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Light Brown	-	12.00	4.80	-	50.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown	40.00	12.00	90.50	10.00	50.00	-	100.00	-	100.00	-	-	-	100.00	-	-	-	-	100.00	-	-
Dark brown	-	76.00	4.80	-	-	50.00	-	50.00	-	100.00	100.00	100.00	-	100.00	100.00	100.00	100.00	-	100.00	100.00
Black	-	-	-	90.00	-	50.00	-	50.00	-	-	-	-	-	-	-	-	-	-	-	-
Horn shape																				
Curved	100.00	84.00	71.40	50.00	100.00	100.00	60.00	-	100.00	100.00	100.00	100.00	100.00	100.00	100.00	42.90	100.00	100.00	100.00	100.00
None	-	16.00	28.60	50.00	-	-	40.00	-	-	-	-	-	-	-	-	57.10	-	-	-	-
Dorsal line																				
Black	100.00	76.00	57.10	50.00	100.00	100.00	80.00	40.00	100.00	100.00	100.00	100.00	100.00	66.70	-	28.60	100.00	100.00	-	100.00
None	-	24.00	42.90	50.00	-	-	20.00	60.00	-	-	-	-	-	33.30	100.00	71.40	-	-	100.00	-
Butt color																				
White polish	-	52.00	62.00	-	100.00	100.00	100.00	100.00	-	100.00	100.00	-	100.00	100.00	100.00	100.00	-	-	100.00	100.00
White	100.00	-	14.00	25.00	-	-	-	-	100.00	-	-	-	-	-	-	-	-	-	-	-
Brown	-	48.00	24.00	75.00	-	-	-	-	-	-	-	100.00	-	-	-	-	100.00	100.00	-	-
Black	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leg color																				
White polish	-	72.00	76.20	-	100.00	100.00	100.00	100.00	-	-	-	-	100.00	-	-	100.00	100.00	-	-	-
White	100.00	-	4.80	-	-	-	-	-	100.00	-	-	100.00	-	-	-	-	-	-	-	-
Brown	-	28.00	19.00	75.00	-	-	-	-	-	100.00	100.00	-	-	100.00	100.00	-	-	100.00	100.00	100.00
Black	-	-	-	25.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tail tip color																				
Brown	-	24.00	9.50	-	-	-	40.00	100.00	-	-	-	-	-	-	-	-	-	-	-	-
Black	100.00	76.00	90.50	100.00	100.00	100.00	60.00	-	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Description : 1) Bali, 2) SimmentalxBali, 3) LimousinxBali, 4) AngusxBali, 5) BrahmanxBali, 6) BrangusxBali, 7) Limousinx(LimousinxBali), 8) Simmentalx(LimousinxBali), 9) Brangusx(LimousinxBali), 10) Balix(LimousinxBali), 11) Limousinx(BrangusxBali), 12) Limousinx(BrahmanxBali), 13) Simmentalx(BrahmanxBali), 14) Simmentalx(SimmentalxBali), 15) Limousinx(SimmentalxBali), 16) Simmentalx(SimmentalxSimmentalxBali), 17) Brahmanx(SimmentalxSimmentalxBali), 18) Simmentalx(LimousinxSimmentalxBali), 19) Limousinx(LimousinxSimmentalxBali), 20) Simmentalx(BrahmanxSimmentalxBali)

A)

```
                 60                 150        160        170        230
Target MC1R      ACCGCAAC  GAGACGGCAGTCATGCCGCTGCTGGAGGCCGGTG  CTCATCTGC
POOL1 F          ....Y...  ..............T...................  .........
POOL2 F          ....Y...  ..............T...................  .........
POOL3 F          ........  ..............T................G.  G.TC.T.TG
```

B)

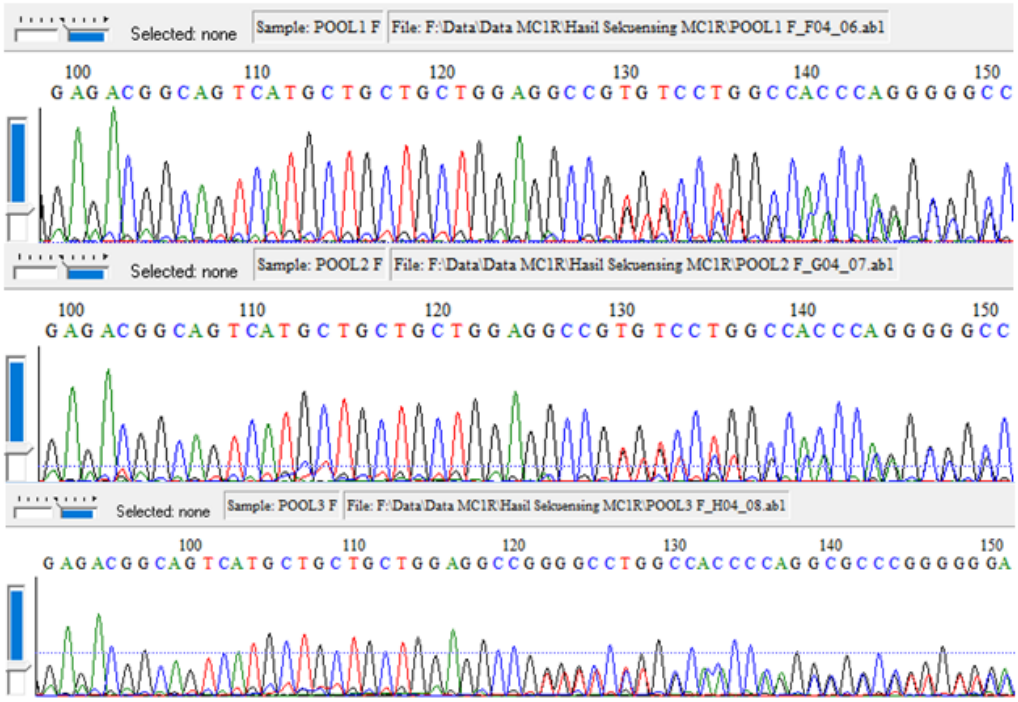


Figure 3. Alignment results and pool-DNA sequencing electrophoregram. A) Alignment result, B) Electrophoregram

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                 60                 150        160        170        230
Target MC1R      ACCGCAAC  GAGACGGCAGTCATGCCGCTGCTGGAGGCCGGTG  CTCATCTGC
5280506 07 BL    ....Y...  ..............T...................  TC.T.T...
5280507 08 BL    ....Y...  ..............T................TGT  TC.TCA...
5280508 12 BS    ....Y...  ....Y.........T................TG.  TC.TCA...
5280509 16 BS    ....Y...  ..............T...................  .....A...
5280510 18 BA    ........  ..............T................TG.  TC.TC.G..
5280511 19 BS    ....Y...  ..............T...................  TC.TCA...
5280512 20 BS    ....Y...  ....Y.........T................TGT  TC.TCA...
5280513 23 BL    ....Y...  ..............T................TG.  TC.TCA...
5280514 26 BA    ....Y...  ..............Y...................  .....M...
5280515 36 BA    ....Y...  ..............T................TGT  TC.T.A...
5280516 58 BA    ........  ..............T................TG.  TC.T..G..
5289410 01 BS    ....Y...  ..............T................G.  ...T.A...
                   ↑          ↑            ↑                      ↑
                g. 64T>C   g. 147T>C    g. 159T>C              g. 235A>C
```

Figure 4. Individual alignment results

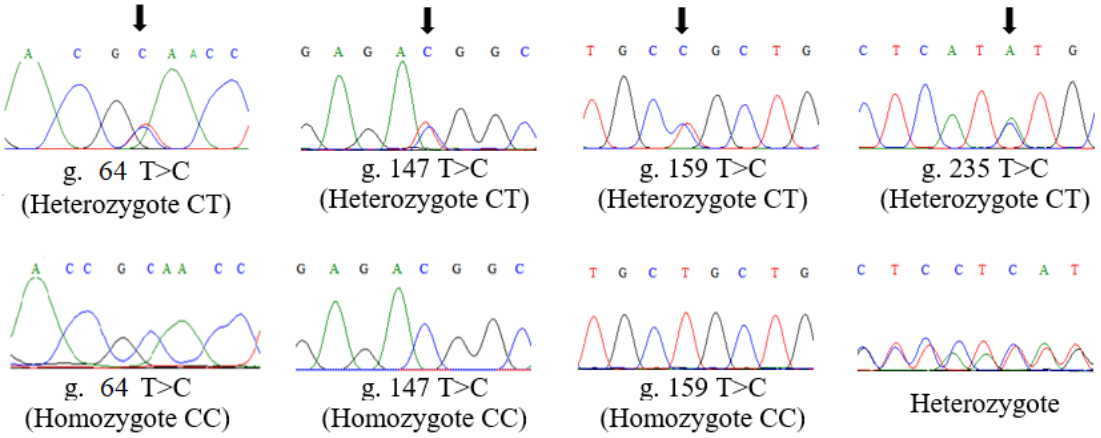

Figure 5. The example of individual sequencing electrophoregram result of Bali Crossbred cattle

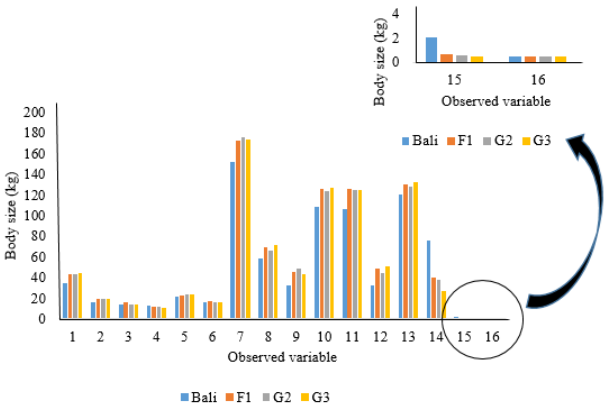

Figure 6. Body size graph of Bali Crossbred cattle. 1) Head length, 2) Head width, 3) Horn length, 4) Horn circumference, 5) Ear length, 6) Ear width, 7) Chest circumference, 8) Chest depth, 9) Body width, 10) Gumba height, 11) Hip height, 12) Hip width, 13) Body length, 14) Eel length, 15) Eel width, 16) Head index

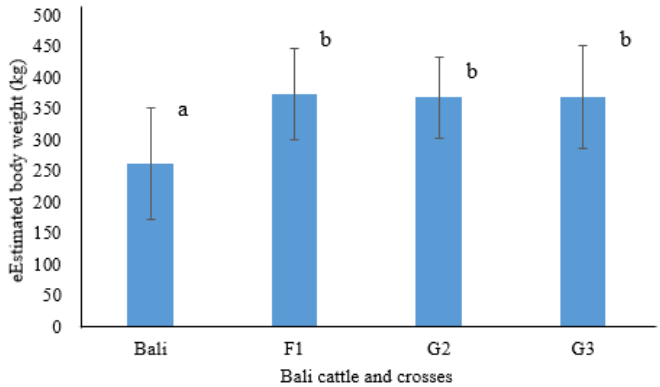

Figure 7. Estimated body weight of Bali Crossbred cattle aged 3 to 5 years by Lambourne formula. abSuperscripts that are not the same indicate significant differences ($p<0.05$)

Table 3. SNP and restriction enzyme data

SNP	Enzyme	Recognition	Frequency	Position
g. 159 T>C	AciI	C'CG_C	5	62, 159, 192, 238, 287
	Fnu4HI	GC'n_GC	7	46, 98, 159, 162, 193, 204, 207
g. 235 A>C	NdeI	CA'TA_TG	1	234

Table 4. Genotype and coat colout of Bali Crossbred cattle

Genotype	Sample Code	Color Body
YCTN	1BS, 7BL, 8BL, 18BA, 19BS, 23BL, 36BA	Dark brown, Black, blackish brown
YYYN	12BS, 20BS	Light brown, dark brown
CCTN	16BS, 58BA	Light brown, black
YYYM	26BA	Black
CCCY	Pool 1, Pool 2, Pool 3	Dark brown, brown, black

Table 5. Mean and standard deviation of body size of 3 to 5 years old Bali Crossbred cattle

No	Variable	100%Bali (n=5)	50%Bali (n=36)	25%Bali (n=10)	12,5%Bali (n=11)	Description
1	Head Length(cm)	34,00±1,41^{a}	42,67±2,65^{b}	43,10±3,21^{b}	44,67±2,94^{b}	**
2	Head Width(cm)	16,40±1,14^{a}	18,83±1,50^{b}	19,20±2,25^{b}	19,67±1,50^{b}	**
3	Horn Length(cm)	13,60±3,85	15,56±10,60	13,50±10,37	13,33±11,55	ns
4	Horn Width(cm)	12,80±1,10	11,83±7,74	12,10±7,49	10,33±8,26	ns
5	Ear Length(cm)	21,00±3,81	22,67±4,86	23,10±2,51	23,50±2,51	ns
6	Ear Width(cm)	15,80±2,77	16,50±2,20	16,30±1,95	15,83±1,60	ns
7	ChestCircumference(cm)	151,80±9,60^{a}	175,69±12,63^{b}	175,50±11,04^{b}	173,17±15,06^{b}	**
8	Inside Chest(cm)	58,40±5,13^{a}	69,92±9,68^{b}	66,40±4,97^{ab}	71,33±7,34^{b}	*
9	Body Width(cm)	32,80±4,97	44,53±28,62	49,10±7,62	43,00±9,94	ns
10	Gumba Height(cm)	108,40±8,62^{a}	125,78±6,37^{b}	123,90±6,49^{b}	126,50±5,13^{b}	**
11	Hip Height(cm)	106,60±7,09^{a}	125,38±6,03^{b}	125,10±4,31^{b}	124,17±4,79^{b}	**
12	Hip Width(cm)	32,40±8,50^{a}	48,83±5,24^{bc}	43,90±5,49^{b}	50,33±6,98^{c}	**
13	Body Length(cm)	120,20±26,27	129,92±11,26	128,20±10,48	132,00±16,50	ns
14	Eel Length(cm)	76,00±7,18^{b}	38,64±35,97^{a}	37,70±41,97^{a}	27,00±32,47^{a}	ns
15	Eel Width(cm)	2,00±0,35^{b}	0,62±0,59^{a}	0,60±0,74^{a}	0,50±0,63^{a}	**
16	Head Index	0,48±0,04^{b}	0,44±0,03^{a}	0,45±0,06^{ab}	0,44±0,03^{a}	ns
17	Body Weight (kg)	260,87±89,31^{a}	373,74±72,81^{b}	367,98±64,43^{b}	368,68±82,63^{b}	*

^{ab}Unequal superscripts in one column indicate there is a significant difference ($p<0.05$); ** = highly significant ($P<0.01$); * = significant ($P<0.05$); ns = not significant ($P>0.05$)

samples consisted of two CC homozygotes at SNP g.64C>T (sample codes 18BA and 58BA) derived from the crossbreediing of Bali cattle with Angus. The other ten samples showed heterozygotes at SNP g.64C>T, g.147C>T, g.159C>T, and g.235C>A derived from the crossbreeding of Bali cattle with Simmental, Limousin, Brahman, and Angus. One sequence out of twelve sequences showed undeleted heterozygote in sample 26BA, and has been submited to GenBank with accession number PV191324. The SNPs found have 5 types of genotypes that have been presented in Table 4, with dominant body colors of light brown, brown, dark brown to black. The SNPs that have been found indicate that the MC1R gene recognizes light brown, brown, dark brown and black colors, this reinforces the findings of Tabun *et al*. (2013) related to the MC1R gene with the MspI enzyme recognizing brown color. Jakaria *et al.* (2023) suggested that the frequency of homozygous genotypes is highest in Bali cattle. Similarly, Hartatik (2017) stated that there is lower heterozygosity in Bali crossbred cattle. SNP posi-

tion g.159C>T recognizes restriction enzymes AciI and Fnu4HI, respectively cutting 5 and 7 with CT genotype. SNP position g.235A>C recognizes restriction enzymes NdeI, cutting 1 with CA genotype. According to Tabun *et al.* (2013) that the MC1R gene SNP can recognize the MseI enzyme at position 296 bp and found 3 SNPs, namely p.52 T>C, p.223 C>A and p.147 C>A.

Vital Measures of Body Statistics

Measurement of crossbred cattle was selected according to the age of 3 to 5 years in accordance with Setiawan *et al*. 2019 which revealed that cattles reach adulthood at the age of 2 to 3 years, indicating that cattles experience peak productivity. Body size can be used as a selection criterion to improve the production performance of Bali cattle and Bali crossbred cattle (Rusdin *et al.,* 2025). The measurement results show a significant increase in body size in Bali cattle with F1 cross (50%Bali) presented in Table 5 and Figure 6. This indicates that Bali cross cattle have better growth than pure Bali cattle. Rusdin *et al.* (2025) stated that there is a change in body size in the offspring of crossbred cattle.

Estimated Body Weight

From the measurement of vital statistics data, body weight estimation for Bali cattle and their crosses can be seen in Figure 7. The body weight estimation used the Lambourne formula. The results showed that body weight estimation showed a significant increase in body weight from Bali cattle (100%Bali) to their crosses ($P<0.05$). However, body weight among crosses F1 (50%Bali), G2 (25%Bali), and G3 (12.5%) were not significantly different ($P>0.05$).

CONCLUSION

The results showed that the monitoring cross that produced superior performance was 50%Bali. The difference is clearly seen in the dominant color change towards darker colors and increased body size, with the discovery of 4 SNPs in dark brown-black body color patterns. However, a more comprehensive study is still needed.

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