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Association of leptin gene polymorphism with growth in crossbred cattle through PCR-RFLP analysis

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Abstract

Efforts to enhance cattle productivity often involve strategic crossbreeding and selection methods. This study investigates the relationship between leptin gene polymorphism and growth characteristics in crossbred cattle, which is crucial for improving livestock performance. Successful crosses were achieved by mating Brahman Cross females with Belgian Blue, Galician Blonde, and Wagyu bulls. The leptin gene plays a pivotal role as a selection marker, influencing body metabolism in cattle. One hundred seventeen crossbred cattle samples were collected from Cianjur, West Java, Indonesia. Using the *HindIII* restriction enzyme, the research method employed PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). Single nucleotide polymorphism (SNP) analysis of the SNP g.3272 T/C in first filial (F1) crossbreeds of Belgian Blue, Galician Blonde, and Wagyu demonstrated Hardy-Weinberg equilibrium (HWE). It exhibited high Polymorphism Information Content (PIC) values, indicating genetic stability and diversity within the populations. The association between SNP g.3272 T/C and growth characteristics was highly significant. Specifically, this SNP showed substantial associations with birth weight, body length, chest circumference, shoulder height, and average daily gain at birth, weaning, and one year of age. It was also associated with average daily gain in F1 Belgian Blue crossbred cattle, particularly among those with the highest frequency of the TC genotype. Similarly, significant associations were observed at weaning and 1.5 years of age and with average daily gain in F1 Galician Blonde crossbred cattle, especially among those with the highest frequency of the CC genotype. This study highlights the importance of genetic markers, like SNP g.3272 T/C in the leptin gene, for understanding and improving growth characteristics in crossbred cattle populations.

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Introduction

In 2023, consumer demand for beef was 583.36 thousand tons, an increase from the previous year of 500.43 thousand tons. Beef production in 2023 is only 583.36 thousand, so there is a deficit between demand and beef production of 322.98 thousand tons. Indonesia imports cattle with an average meat import volume of 19.065.90 tons per month (1). To cover the shortage, it is necessary to increase local beef production. One of the cattle used by the company is Brahman Cross cattle. Brahman Cross cattle have good mothering ability, are resistant to tropical diseases (2), and have tropical environmental resistance (3). However, Brahman Cross cattle have unfavourable traits in

terms of productivity speed (4). One way to increase the productivity of Brahman Cross is by crossing with *Bos taurus* cattle that have greater weight. Crossing local cattle with *Bos taurus* cattle can increase beef cattle's slaughter or body weight because of the genetic combination that combines superior traits (heterosis) of the two types of cattle (5). Breeds can be crossed include Belgian Blue (BB), Galician Blonde (GB), and Wagyu. The Belgian Blue (*Bos taurus*) is known for its large body size and moderate to high growth rates (6). The typical performance of the Galician Blonde breed showcases positive attributes such as a strong growth rate of 1200 grams per day, an efficient feed conversion rate of approximately 5, a carcass yield approaching 60%, and a calving interval slightly exceeding 400 days (7). Wagyu cattle demonstrate a high average daily weight gain (ADG) of 0.916 kilograms daily, indicating strong growth performance in weight gain each day. This ADG value reflects the efficient growth rate characteristic of Wagyu cattle, highlighting their ability to gain weight rapidly during the specified period (8). Selection efforts follow crossbreeding by selecting animals resulting from crossbreeding, breeders can gradually improve the genetics of the livestock population. Choosing parents with desired traits for production can produce the desired genetic changes in the livestock population (9). One method for selecting involves utilizing genetic markers called single nucleotide polymorphisms (SNP). SNP represents the prevalent genetic variation detected in humans and various other organisms. These polymorphisms denote single nucleotide variances at particular positions in the genome, which may arise among individuals within a species or across distinct populations. SNPs can influence physical characteristics or specific traits (10). Gene polymorphisms have been found in the leptin gene (11). The Leptin gene is a protein affecting feed consumption, fat metabolism, energy regulation, and cattle red blood cell formation (haematopoiesis) (12). In cattle, the leptin gene is situated on chromosome 4 and consists of three exons and two introns. The ultimate product of this gene is the leptin protein, which acts autocrinally to impede insulin-stimulated glucose uptake and diminish adipogenesis in adipose tissue (13). A SNP in the leptin gene exon two was identified in Belgian Blue and Wagyu bulls crossed with Brahman Cross females (14) and in exon 3 of Blue-Brahman Cross cattle (15). This study used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) with the restriction enzyme *HindIII* to analyse genetic diversity and identify mutations within the leptin gene. *HindIII* cuts DNA at symmetrical recognition sites, creating uneven incisions with identical single-stranded extensions (16).

A research gap exists regarding the leptin gene's role in growth selection in Brahman crosses with Belgian Blue, Wagyu, and Galician Blonde cattle. This study aims to investigate the association between the leptin gene SNP g.3272 T/C and growth traits in these crossbred cattle, addressing a gap in understanding the genetic factors influencing growth performance in these diverse populations.

Materials and methods

Ethical approve

The Animal Ethics Commission of the Faculty of Veterinary Medicine at UGM University in Yogyakarta, Indonesia, approved all animal-related operations in this study. The approval was granted under ethical number 012/EC-FKH/Eks/2023.

Animals

Total sample 117 Blood Sample. This study used blood samples from the first filial (F1) offspring resulting from the crossbreeding of Brahman Cross cows with *Bos taurus* bulls, specifically Belgian Blue, Galician Blonde, and Wagyu bulls, with a total of 85 samples for F1 Belgian Blue cattle, 20 samples for F1 Galician Blonde cattle, and 12 samples for F1 Wagyu cattle, all raised at PT Widodo Makmur Perkasa livestock industry in Cianjur, Central Java Province, Indonesia. Calves were raised alongside their mothers until they were weaned at six months old, following standard management procedures. They were grouped with their dams in pens based on the breed of their sires. These pens were open-air with concrete floors, monitor roofs, and functional feeding and water systems. Sawdust was spread on the floor for bedding to absorb moisture from faeces and urine. The pens were cleaned every two weeks. Calves were fed calf concentrate and green chop, and nursing cows received lactation concentrate and green chop.

Blood sample

Blood collection was conducted using the prescribed research procedure. Before blood extraction, alcohol was administered into the vein located in the tail of the cow (17). Blood specimens were then obtained from the vein using a needle and transferred into a vacutainer tube containing an anticoagulant (EDTA). Subsequently, the blood samples were preserved in a freezer at temperatures ranging from 4°C for subsequent utilization.

Extraction of DNA

DNA extraction typically includes breaking open the cells to release the DNA, followed by purification steps to remove cellular debris and proteins, resulting in pure DNA for further analysis (18). Bovine blood DNA extraction was performed using the gSYNC^{TM} DNA Extraction Kit (Geneaid, Taiwan) at the Laboratory of Genetics and Animal Breeding, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia. Initially, 200 µl of blood sample and 20 µl of proteinase K were combined in a 1.5

ml tube and thoroughly mixed to ensure homogeneity. The mixture was then incubated at 60°C for 5 minutes. Subsequently, 200 µl of GSB buffer was added to each incubated solution, followed by further homogenization.

The solution was shaken every two minutes during the 5-minute incubation period at 60°C. Next, 200 µl of 100% ethanol was added to the homogenized solution. After transferring the solution to a GD column, it was centrifuged for two minutes at 10,000 rpm. The liquid was discarded, and the column was switched. After adding 400 µl of W1 buffer, the mixture was centrifuged for 30 seconds at 10,000 rpm. After removing the liquid, it was centrifuged for three minutes at 10,000 rpm. The remaining liquid was discarded, and a 1.5 ml tube was used instead of the dye. Subsequently, 200 µl of elution buffer was added, and the centrifuge was operated at 10,000 rpm for 30 seconds.

Amplification of DNA

DNA amplification in cattle increases the DNA obtained from cattle samples through techniques such as polymerase chain reaction (PCR) (19). The DNA extracted was subjected to amplification using the Polymerase Chain Reaction (PCR) method, employing a temperature cycling mechanism. Specifically, the target gene, leptin, was amplified using the forward primer: 5'-AGCTTGGAAACATGGTGGTC-3' and the reverse primer: 5'-CATGATGCTCCCTGGATTCT-3'. The resultant PCR product comprised an 898 bp fragment, encompassing intron 2, exon, and 3'UTR regions. The PCR process involved predenaturation at 94°C, annealing at 55°C, extension at 72°C, and final extension at 72°C.

Agarose gel electrophoresis was performed using a 1.5% agarose gel to facilitate visualization and analysis of the PCR products. The gel was prepared by dissolving 1.5 grams of agarose powder in 100 ml of 1xTBE solution, heating it until completely dissolved, and cooling it to approximately 60°C before adding EtBr. Once evenly mixed, the gel was poured into a casting tray and set for approximately 30 minutes after inserting a comb to create wells.

After the gel solidified, it was stored in 1xTBE buffer. For electrophoresis, 4 µl of ddw, 1 µl of PCR product DNA, and 1 µl of loading dye were added to each well. The gel was subjected to electrophoresis in an appropriate chamber, with the first well reserved for a size marker and subsequent wells for the samples. The chamber was sealed and connected to a power supply, and electrophoresis was conducted at 150 V for 25 minutes. Following electrophoresis, DNA bands of expected lengths, ideally 898 bp or longer, were anticipated. These bands fluoresced under UV light due to the binding of EtBr to nucleic acids. The results were documented using a digital camera for further analysis.

PCR-RFLP

PCR-RFLP is a method used to identify differences in DNA sequences by amplifying specific DNA regions through PCR and then digesting the amplified DNA fragments with restriction enzymes (20). The genotyping of SNP g.3272 T/C was conducted on 117 samples using the PCR-restriction fragment length polymorphism (PCR-RFLP) method. Restriction enzyme digestion was carried out in 9 µl reaction volumes, with approximately four µl of PCR products, 3.3 µl of double distilled water (DDW), 1.5 µl of buffer 1.1, and 0.2 µl of *HindIII* restriction enzyme. The digestion process involved an overnight incubation at 37°C. Subsequently, the digested products were separated by electrophoresis on a 1.5% agarose gel.

Body weight and body measurement

To characterize a set of repeated measures or longitudinal data, live weights were analysed from weaning up to one year, considering various measurements acquired in cattle, including body weight, body length, chest circumference, and shoulder height. These measurements were taken at birth, six months (weaning), one year, and 1.5 years. Average Daily Gain (ADG) (days/kg) was also calculated. The shoulder joint's later tuberosity of the humerus and the edge of the pelvic bone were measured to obtain the data of BL (21). Data for WH were acquired by measuring the distance along a perpendicular line from the withers to the surface. The fourth rib on the chest was used as the measurement point for the measuring tape on a scale of 1 cm. The data for BL and WH were measured using a measuring stick with a 1 cm scale. Body weight (BW) information was gathered using a digital weighing scale.

Data analysis

The resulting SNPs were analyzed for allele frequencies and genetic frequencies. They were analyzed using a Hardy-Weinberg equilibrium to find the complexity balance. The analysis of observed heterozygosity, expected heterozygosity, and polymorphism information content are used in population genetics studies to understand the genetic structure and genetic diversity within these three breeds (22). Growth statistics used birth type correction factor, breeding age correction factor, and gender correction factor (FKJK) (FKTL) (23). Associations between genotypes and growth factors were analyzed using SPSS for ANOVA for SNPs with three genotypes (24). Yik = µ + αi + eik, in this equation, Yik represents the number of observations (such as body weight, body length, chest circumference, shoulder height, ADG), µ denotes the mean of the growth trait, αi represents the treatment effect for the genotype I, and eik stands for the random error.

Results

Polymorphism

PCR amplification of the leptin gene from exon 3 to the 3' untranslated region (3'UTR) yielded an 898 bp fragment, and an SNP g.3272 T/C was identified within exon 3. PCR-RFLP using the *HindIII* enzyme produced the following patterns: the TT genotype showed a 463 bp fragment, the CC genotype exhibited a 435 bp fragment, and the TC genotype displayed two pieces of 463 bp and 435 bp (Figure 1).

The highest frequency of the T allele occurred in the F1 Belgian Blue Crossbred and F1 Galician Blonde Crossbred crosses. Meanwhile, in the F1 Wagyu Crossbred cross, the highest allele frequency was for allele C. The F1 Belgian Blue, F1 Galician Blonde, and F1 Wagyu cattle exhibit genotype frequencies of TT, TC, and CC at the SNP g.3272 T/C locus. Additionally, these populations are in Hardy-Weinberg equilibrium (Table 1).

Genetic diversity was assessed in three crossbred cattle populations: F1 Belgian Blue Crossbred, F1 Galician Blonde Crossbred, and F1 Wagyu Crossbred. The observed heterozygosity (Ho) values varied among the populations, with F1 BB showing the highest Ho (0.36), followed by F1 GB (0.35), and F1 Wagyu displaying the lowest Ho (0.33). Expected heterozygosity (He) values were relatively similar across all populations, indicating a high level of genetic diversity within each group. The values for He was 0.491 (F1 BB), 0.495 (F1 GB), and 0.496 (F1 Wagyu). The polymorphic information content (PIC) was highest in the F1 Wagyu Crossbred population at 0.67, indicating a greater level of genetic polymorphism and discriminatory power among individuals within this population (Table 2).

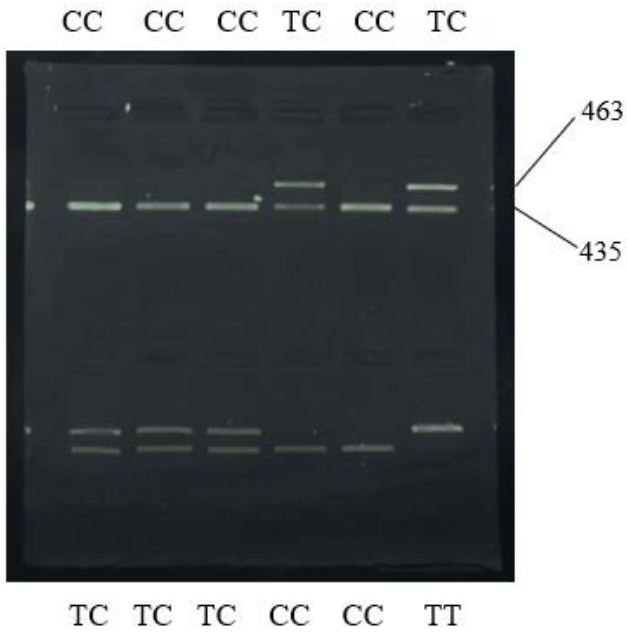

Figure 1: Agarose gel for the differentiation of TT, TC, and CC genotypes from PCR-RFLP using *HindIII* enzyme.

Table 1: Genotype Frequency, Allele Frequency, and Hardy-Weinberg SNP g.3272 T/C in Crossbred

Breed	N	Genotype Frequency			Allele Frequency		X^2 (HWE)
F1 BB Cross	85	TT (25%)	TC (36%)	CC (39%)	T (0.57)	C (0.43)	5.5
F1 GB Cross	20	TT (35%)	TC (35%)	CC (30%)	T (0.525)	C (0.475)	1.7
F1Wagyu Cross	12	TT (25%)	TC (33%)	CC (42%)	T (0.42)	C (0.58)	1.1

BB (Belgian Blue), GB (Galician Blonde).

Table 2: Observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content (PIC) SNP g.3272 T/C in Crossbred

Breed	N	Ho	He	PIC
F1 BB Cross	85	0.36	0.491	0.64
F1 GB Cross	20	0.35	0.495	0.65
F1 Wagyu Cross	17	0.33	0.496	0.67

Association single nucleotide polymorphism with growth in crossbred cattle

The F1 Belgian Blue Crossbred showed significant associations between the SNP g.3272 and body weight, body length, chest circumference, and height at birth, weaning, and one year of age. Cattle with the TC genotype exhibited significantly higher body weight, length, chest circumference, and height at birth, weaning, and one year. However, these associations were not significant at 1.5 years of age for body weight, body length, chest circumference, and height at girth (Table 3).

The F1 Galician Blonde crossbred cattle displayed notable connections between the SNP g.3272 T/C and characteristics such as body weight, body length, chest width, and height at the ages of weaning and 1.5 years. Cattle carrying the CC genotype exhibited notably higher measurements in body weight, body length, chest width, and height at weaning and 1.5 years. Nevertheless, these correlations did not prove significant at the weaning stage or one year of age in terms of body weight, body length, chest width, and height at girth (Table 4). The research findings, showed no significant results in body weight, body length, chest width, and height at the withers in F1 Wagyu Crossbred cattle at birth, weaning, one year of age, and 1.5 years of age (Table 5).

Table 3: The association between SNP g.3272 T/C of leptin gene with growth traits in F1 Belgian Blue Crossbred

Variable	N	Genotype			P-value
		TT (21)	TC (31)	CC (33)	
Birth weight (kg)	85	34.61±3.81^{ab}	35.48±4.46^{b}	32.69±3.14^{a}	0.01
Birth body length (cm)	85	71.61±3.81^{ab}	72.06±4.61^{b}	69.69±3.14^{a}	0.04
Birth chest circumference (cm)	85	68.61±3.81^{ab}	69.06±4.61^{b}	66.69±3.14^{a}	0.04
Birth shoulder height (cm)	85	74.61±3.81^{ab}	75.06±4.61^{b}	72.69±3.14^{a}	0.04
Weaning weight (kg)	85	130.23±32.52^{a}	152.58±33.93^{b}	149.30±33.25^{ab}	0.05
Weaning body length (cm)	85	100.23±6.79^{a}	122.58±33.93^{b}	119.30±33.25^{ab}	0.05
Weaning chest circumference (cm)	85	88.80±17.79^{ab}	98.38±16.64^{b}	86.36±20.53^{a}	0.05
Weaning shoulder height (cm)	85	94.80±17.79^{ab}	104.38±16.64^{b}	92.36±20.53^{a}	0.03
Yearling weight (kg)	85	229.85±58.66^{a}	266.93±59.47^{b}	260.48±45.70^{ab}	0.03
Yearling body length (cm)	85	113.19±10.80^{a}	138.83±10.96^{b}	133.27±9.18^{ab}	0.00
Yearling chest circumference (cm)	85	115.80±17.79^{ab}	125.38±16.64^{b}	113.36±20.53^{a}	0.03
Yearling shoulder height (cm)	85	151.80±17.79^{ab}	161.38±16.64^{b}	149.36±20.53^{a}	0.03
		TT (11)	TC (24)	CC (34)	
Adult Weight (kg)	68	367.90±65.73	373.04±77.38	380.03±72.96	0.87
Adult body length (cm)	68	134.36±7.04	135.54±7.79	137.27±8.97	0.54
Adult chest circumference (cm)	68	135.00±7.15	134.79±6.41	136.54±6.91	0.59
Adult shoulder height (cm)	68	175.00±7.15	174.79±6.41	176.45±7.42	0.64

^{a,b} means different superscripts within the same line show significantly different values at P<0.05.

Table 4: The association between SNP g.3272 T/C of leptin gene with growth traits in F1 Galician Blonde Crossbred

Variable	N	Genotype			P-value
		TT (7)	TC (7)	CC (6)	
Birth weight (kg)	20	25.57±1.27	26.14±2.11	25.66±1.86	0.81
Birth body length (cm)	20	58.14±2.26	60.14±3.71	60.33±3.72	0.41
Birth chest circumference (cm)	20	69.42±2.22	69.57±3.04	69.50±4.03	0.99
Birth shoulder height (cm)	20	69.00±2.70	70.28±2.42	71.50±2.42	0.23
Weaning weight (kg)	20	133.28±7.40^{a}	137.85±8.19^{ab}	148.00±9.77^{b}	0.01
Weaning body length (cm)	20	95.28±7.40^{a}	99.85±8.19^{ab}	108.33±7.76^{b}	0.02
Weaning chest circumference (cm)	20	97.71±5.31^{a}	102.85±8.29^{ab}	108.33±5.46^{b}	0.03
Weaning shoulder height (cm)	20	129.85±5.49^{a}	134.57±7.87^{ab}	140.00±5.17^{b}	0.03
Yearling weight (kg)	20	204.14±32.35	221.42±68.41	253.83±38.98	0.22
Yearling body length (cm)	20	118.57±6.10	112.28±9.84	116.00±8.96	0.39
Yearling chest circumference (cm)	20	121.42±5.50	117.285±7.11	113.50±7.63	0.13
Yearling shoulder height (cm)	20	149.42±13.50	140.57±13.20	139.00±15.83	0.36
Adult Weight (kg)	20	367.57±5.12^{a}	375.00±7.43^{ab}	379.16±8.25^{b}	0.02
Adult body length (cm)	20	118.42±5.06^{a}	126.57±9.86^{ab}	130.83±5.77^{b}	0.02
Adult chest circumference (cm)	20	124.57±5.12^{a}	131.28±6.84^{ab}	136.66±7.81^{b}	0.01
Adult shoulder height (cm)	20	162.57±17.95^{a}	172.57±14.45^{ab}	189.66±12.04^{b}	0.01

^{a,b} means different superscripts within the same line show significantly different values at P<0.05.

The association results of SNP g.3272 T/C indicate significance in F1 Belgian Blue cattle, with the highest average daily gain (ADG) observed in the TC genotype. Similarly, significance was observed in F1 Galician Blonde Crossbred cattle, with the highest average ADG in the CC genotype. However, no significant associations were found in F1 Wagyu Crossbred cattle (Figure 2).

Table 5: The association between SNP g.3272 T/C of leptin gene with growth traits in F1 Wagyu Crossbred

Variable	N	Genotype			P-value
		TT (3)	TC (4)	CC (5)	
Birth weight (kg)	12	26.66±3.05	27.50±1.29	25.80±1.48	0.44
Birth body length (cm)	12	58.00±4.58	64.00±3.55	60.80±5.44	0.29
Birth chest circumference (cm)	12	70.00±4.58	73.75±1.25	72.40±2.88	0.30
Birth shoulder height (cm)	12	69.33±3.51	71.50±2.38	70.20±1.30	0.48
Weaning weight (kg)	12	137.33±59.28	152.50±22.86	145.60±26.57	0.85
Weaning body length (cm)	12	116.00±4.00	116.25±3.50	116.40±3.50	0.98
Weaning chest circumference (cm)	12	115.33±1.52	111.75±3.20	113.00±4.06	0.41
Weaning shoulder height (cm)	12	133.33±3.05	133.50±2.51	137.20±5.40	0.34
Yearling weight (kg)	12	317.33±12.66	294.00±12.80	288.00±54.66	0.57
Yearling body length (cm)	12	121.66±4.72	118.50±3.00	118.20±3.11	0.39
Yearling chest circumference (cm)	12	120.33±4.16	116.25±3.77	116.40±2.40	0.25
Yearling shoulder height (cm)	12	159.33±6.42	155.00±8.24	154.80±4.38	0.59
Adult Weight (kg)	12	455.00±24.26	403.75±22.98	389.20±54.65	0.13
Adult body length (cm)	12	140.00±2.00	143.25±3.77	136.80±8.31	0.32
Adult chest circumference (cm)	12	140.00±5.29	135.25±9.46	135.00±4.94	0.58
Adult shoulder height (cm)	12	179.33±7.57	182.00±6.92	181.60±10.8	0.91

a,b means different superscripts within the same column show significantly different values at P<0.05.

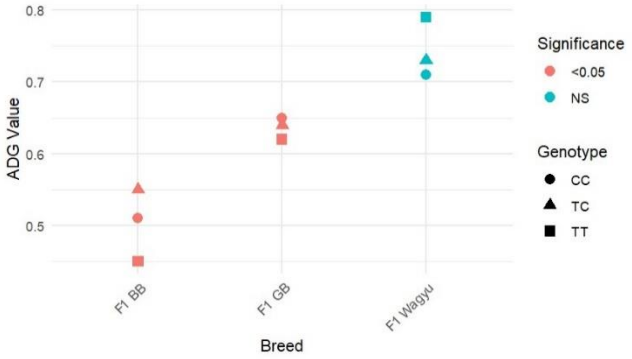

Figure 2: Association of SNP g.3272 T/C with average daily gain (kg/day) in F1 BB Crossbred, F1 GB Crossbred, and F1 Wagyu Crossbred cattle.

Discussion

From this study, the restriction enzyme *HindIII* can cleave at position g.3272 T/C, whether in F1 Belgian Blue, F1 Galician Blonde, or F1 Wagyu crossbred, resulting in 3 genotypes: TT, TC, and CC. *HindIII* is a type II restriction enzyme that recognizes and cuts the palindromic sequence AAGCTT in DNA. This enzyme is crucial in genetic research, particularly in DNA analysis and genetic studies, due to its precise ability to cut at specific DNA sequences (23-25). Based on research, SNP g.3272 T/C found in exon 3 in the F1 Belgian Blue, F1 Galician Blonde, and F1 Wagyu crossbred shows polymorphic allele frequencies. Locus is considered polymorphic if the population at that locus has more than one allele and has an average allele frequency of 0.95 or less, so the allele frequency calculation results in the crossbred cattle population indicate polymorphism and can be used as a marker (26). The variant allele frequencies (VAF) for leptin gene A19G and G2548A were 0.74 and 0.67, respectively, and can be used as markers (27). Based on the study, SNP g.3272 T/C with calculated chi-square (X2) values in the three breeds are in Hardy-Weinberg equilibrium. A population is said to be in Hardy-Weinberg equilibrium when genotype and allele frequencies remain constant from generation to generation (28). Hardy-Weinberg equilibrium exists in alleles and genotypes SNP in p.E115 of the Holstein-Friesian population studied for the distribution of the bovine leptin gene (29).

The SNP g.3272 T/C in F1 Belgian Blue (BB) Crossbred offspring has Ho of 0.36 and He of 0.49, in F1 Galician Blonde (GB) Crossbred offspring has Ho of 0.35 and He of 0.49, and in F1 Wagyu Crossbred offspring has Ho of 0.33 and He of 0.49. Ho values of 0.14-0.70 and He values of 0.30-0.50 indicate high heterozygosity values (30). Due to high levels of inbreeding, low values of heterozygosity jeopardize the survival of a species or population (31). The observed heterozygosity (Ho) in the second-generation Belgian Blue, Wagyu, and Brahman cross was 0.416 which was lower than the expected heterozygosity of 0.4875 (32). This indicates that some degree of endogamy occurs within the population due to the intense selection process. The genetic variation observed in crossbred cattle in this study can be considered significant, as indicated by the high polymorphic information content (PIC) values of 0.64, 0.65, and 0.67 for F1 BB, F1 GB, and F1 Wagyu, respectively, at the SNP position g.3272 T/C. This suggests a rich diversity of alleles within the population. PIC values serve as a measure of genetic diversity, with higher values indicating greater diversity. Additionally, the PIC values fall within the

high polymorphism range (33): 0.35 for Pinzau cattle and 0.25 for Slovak cattle. These values indicate varying levels of genetic diversity among different cattle populations, with Pinzau cattle exhibiting higher polymorphism levels than Slovak cattle (34).

Leptin is essential for maintaining body weight and energy balance by communicating energy storage status to the brain and other tissues (35). It is a hormone produced by the leptin gene, also known as the ob gene, which codes for synthesizing the leptin protein primarily in adipose (fat) tissue (36). The SNP g.3272 T/C is significantly associated with birth weight, body length, chest circumference, and shoulder height in F1 Belgian Blue Crossbred cattle. Cattle carrying the TC genotype showed significantly higher body weight, body length, chest circumference, shoulder height, and average daily gain at birth, weaning, and one year of age. However, these associations were not significant at 1.5 years old. Similar findings regarding the importance of the TC genotype in the leptin gene for body weight, body length, and chest circumference have been reported in Madura cattle and other cattle breeds (37). Additionally, an association between the TC genotype and chest circumference has been observed in Ongole cattle (38), while Nellore cattle carrying the TC genotype displayed higher birth weights and average daily gains (39).

Research findings indicate significant correlations between SNP g.3272 and various characteristics such as body weight, body length, chest width, and height at weaning and 1.5 years in F1 Galician Blonde crossbred cattle. Cattle carrying the CC genotype exhibited higher measurements in body weight, body length, chest width, and height at weaning and 1.5 years, along with increased average daily gain. However, these correlations were insignificant at weaning and one year of age regarding body weight, body length, chest width, and girth height (40). Genotypes containing the C allele (CC or TC) of SNP E2-169 T>C (C57R) were associated with increased dressed weight, loin thickness, marbling score (MCS), fatty acid composition (FCS), intramuscular fat content, and polyunsaturated fatty acid content compared to other genotypes in Chinese Simmental-cross steers (41). Additionally, SNP R25C was associated with carcass traits or fatty acid composition in Japanese Black Cattle, where genotypes carrying the C allele (CC or T) showed significant effects (42). Based on research, in Wagyu cattle, the leptin gene does not significantly influence body weight, body length, chest width, and withers height at birth, weaning, one year, or 1.5 years, nor does it affect average daily gain. Wagyu cattle selection primarily focuses on marbling quality (43).

Conclusion

The significance of SNP g.3272 T/C lies in identifying and utilizing genetic markers to enhance breeding programs and growth performance in crossbred cattle populations at PT Pasir Tengah. Specifically, Belgian Blue cattle with the TC genotype and Galician Blonde cattle with the CC genotype are maintained for breeding purposes. This discovery has the potential to positively impact the beef cattle market by improving production efficiency and livestock quality, which is crucial given the scarcity of production numbers in Indonesia. The benefits of this research and further studies in this field can boost beef cattle productivity, support the sustainability of the livestock industry, and contribute to the national economy through increased yields of high-quality beef.

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Conflict of interest

There is no conflict of interest.

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ارتباط تعدد أشكال جين اللبتين بالنمو في الماشية المهجنة من خلال تفاعل البوليميراز المتسلسل متعدد أشكال طول القطعة المقيدة

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الخلاصة

غالبا" ما تتضمن الجهود الرامية إلى تعزيز إنتاجية الماشية على أساليب استراتيجية للتهجين والانتقاء. تبحث هذه الدراسة العلاقة بين تعدد أشكال جين اللبتين وخصائص النمو في الماشية المهجنة، وهو أمر بالغ الأهمية لتحسين أداء الثروة الحيوانية. تم الحصول على تمريرات عرضية ناجحة من خلال تزاوج إناث براهمان كروس مع الثيران البلجيكية الزرقاء والشقراء الجاليكية والواغيو. يلعب جين اللبتين دورا محوريا كعلامة للاختيار، مما يؤثر على عملية التمثيل الغذائي في الجسم في الماشية. تم جمع مائة وسبعة عشر عينة من الماشية المهجنة من سيانجور، جاوة الغربية في إندونيسيا. باستخدام إنزيم التقييد HindIII، استخدمت طريقة PCR-RFLP(تفاعل البوليميراز المتسلسل متعدد أشكال طول القطعة المقيدة). تحليل تعدد أشكال النوكليوتيدات المفردة لـ SNP g. 3272T/C في التهجين الأول (F1) الأزرق البلجيكي والأشقر الجاليكي والواغيو أوضح توازن هاردي واينبرغ. أظهر قيما عالية لمحتوى معلومات تعدد الأشكال (PIC)، مما يشير إلى الاستقرار الجيني والتنوع داخل المجاميع. كان الارتباط بين SNP g.3272 T / C وخصائص النمو مهما للغاية. على وجه التحديد، أظهر SNP ارتباطات كبيرة مع الوزن عند الولادة، وطول الجسم، ومحيط الصدر، وارتفاع الكتف، ومتوسط الكسب اليومي عند الولادة، والفطام، وعمر سنة واحدة. كما ارتبط بمتوسط الكسب اليومي في الماشية المهجنة F1 البلجيكية الزرقاء، لا سيما بين تلك التي لديها أعلى تواتر للنمط الوراثي .TC ومشابها" لذلك، لوحظت ارتباطات كبيرة عند الفطام وعمر ١,٥ سنة مع متوسط الكسب اليومي في الماشية المهجنة الشقراء الجاليكيةF1 ، خاصة بين تلك التي لديها أعلى تواتر للنمط الوراثيCC. تبرز هذه الدراسة أهمية العلامات الجينية، مثل SNP g.3272 T / C في جين اللبتين لفهم وتحسين خصائص النمو في مجموعات الماشية المهجنة.