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Association of SNP g.3272 T/C in the leptin gene with reproductive traits in Brahman Cross and Belgian Blue Crossbred

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ABSTRACT

This study investigated the role of single nucleotide polymorphism (SNP) g.3272 T/C in the leptin gene as a genetic marker for enhancing reproductive efficiency in Brahman Cross and Belgian Blue crossbred cattle in Indonesia. A total of 27 Belgian Blue crossbred, and 10 Brahman Cross cows were evaluated for reproductive traits, including age at puberty, age at first mating, postpartum estrus (PPE), postpartum mating (PPM), and service per conception (S/C). SNP analysis was performed using PCR-RFLP with the HindIII restriction enzyme, and verification was carried out by sequencing. Visual estrus symptoms, estrogen levels, vaginal epithelial cell distribution, and reproductive performance were all found to be significantly (P<0.05) correlated with SNP g.3272 T/C in three genotypes (TT, TC, and CC). PPE (78.50±1.29 days), age at first mating, and the youngest age at puberty (19.50±1.29 months) were all improved in Brahman Cross cattle with the TT genotype. The TC genotype of F1 Belgian Blue cattle performed the best, with the youngest PPE (75.80±1.92 days), age at first mating, and puberty (18.85±0.56 months). Both populations were in Hardy-Weinberg equilibrium, Both Brahman Cross and Belgian Blue crossbred cattle had the same Polymorphic Information Content (PIC) values, which were 0.37. These findings suggest that SNP g.3272 T/C of the leptin gene is a promising marker for genetic selection intended to improve the reproductive efficiency of crossbred cattle and advance Indonesia's sustainable livestock productivity.

Introduction

Indonesia must enhance cattle output to fulfill the country's growing demand for beef, which rose by 500.43 thousand tons from the previous year to 1,083.79 thousand tons in 2023. However, only 583.36 thousand tons of beef were produced in Indonesia, leaving a 322.98 thousand ton deficit that necessitates the country's monthly average import of 19,065.90 tons of beef (Statistics Indonesia, 2023). This persistent gap, which has gotten worse over the past five years, not only creates financial hardship but also emphasizes reproductive efficiency as a major obstacle to increasing cow output. Enhancing reproductive performance is therefore a strategic way to increase productivity and reduce reliance on imports (Sutarnoand Setyawan, 2016). Reproductive efficiency, which includes age at puberty, age at first mating, postpartum estrus (PPE), postpartum mating (PPM), and service per conception (S/C), is a crucial factor in the sustainable growth of the beef cow population (Diskin and Kenny *et al.*, 2016). Brahman Cross cattle, which are widely farmed in Indonesia, suffer reproductive issues even though they are adapted to tropical temperatures. The issues include poor conception rates and lengthy calving intervals (Rafsanjani *et al.*, 2023). Crossbreeding with *Bos taurus* cattle, such as Belgian Blue, has been tried to address this because of their advantages in body weight and growth rate (Panjono *et al.*, 2022). However, this crossbreeding often encounters reproductive problems, including low conception rates, high calf mortality, and calving issues. Therefore, improving the reproductive performance of crossbred cattle is essential. One such tactic is selection based on genetic markers, such as Single Nucleotide Polymorphism (SNP). Single nucleotide polymorphisms are genetic variations at a single site in the genome that might impact reproductive traits as pregnancy rate, calf survival, and calving interval (Sharma *et al.*, 2016). Single nucleotide polymorphisms are genetic variations at a single site in the genome that may impact reproductive factors like pregnancy rate, calf survival, and calving interval (Sharma *et al.*, 2016). Research on the leptin gene, a potential gene for reproductive trait selection, has taken center stage in order to improve this technique. The leptin gene encodes a protein that is involved in energy regulation, fat metabolism, and reproductive function (Foote *et al.*, 2015). Genetic markers and molecular or genetic techniques can be used to select cattle. This technique allows for quicker and more efficient selection while improving the genetic quality of the animals. The use of genetic markers in selection reduces maintenance costs since animals can be selected from birth instead of waiting for performance selection at weaning or one year of age. Additionally, marker identification can be done in a laboratory, which makes it acceptable to the appropriate organizations. One popular molecular method for choosing cattle is polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) (Igawa *et al.*, 2023). Leptin was chosen over other genes because it directly affects the success of reproduction by changing hormonal pathways, such as the production of gonadotropin releasing hormone (GnRH), which regulates ovarian function and fertility. Furthermore, leptin's sensitivity to nutritional status makes it particularly relevant for cattle in tropical environments where feed availability varies, in contrast to other candidate genes with less direct links to fertility. This ensures better reproductive outcomes under a variety of conditions (Childs *et al.*, 2021).

Adipocytes produce the hormone leptin, which is encoded by the obesity gene (Ob or Lep). By regulating reproductive hormones and interacting with the brain about energy reserves, this hormone influences reproduction (Denver *et al.*, 2011). Swieten *et al.* (2014) claim that the leptin gene in cattle regulates energy metabolism and food intake through complex neural networks in the hypothalamus, namely in neurons of the Arcuate Nucleus (ARC). Previous studies have shown that SNP variations in the leptin gene significantly affect cow reproductive traits. The SNP g.92450765 has been associated with calving interval (CI) and service per conception (S/C) in Sahiwal cattle (Yadav *et al.*, 2023). SNPs such as UASMS1, UASMS2, A1457G, and A59V are associated with the age at puberty and first mating in Friesian Holstein cattle (Clempson *et al.*, 2011). The age of first calving in the Slovak Spotted and Pinzgau cattle groups is significantly correlated (p<0.01) with the LEP/Sau3AI SNP. There is currently comparatively little study on SNP detection in the leptin gene in Indonesian cattle, even though this gene has a lot of potential for use in molecular selection to improve reproductive performance. Exam-

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ining the leptin gene's potential as a genetic selection tool is essential to enhancing the productivity and reproductive potential of Indonesian cattle. Research on leptin gene SNPs in Indonesian cattle is lacking, despite the possibility that molecular selection could enhance livestock productivity and reproductive efficiency. Due to variations in their habitats, management practices, and genetic backgrounds, research conducted in other countries might not have an immediate positive impact on Indonesian cattle. The lack of studies and direct use in Indonesian selection programs indicates the need for more investigation into the leptin gene's potential as a genetic tool for improving cattle reproduction and productivity locally.

Materials and methods

Animals

Reproductive characteristics such age at puberty, age at first mating, postpartum estrus (PPE), postpartum mating (PPM), and service per conception (S/C) were assessed in this study using 27 Belgian Blue crossbred cows. Ten of these cows exhibited signs of estrus during the observation period, and their ophthalmic symptoms, vaginal cytology, and estrogen hormone levels were further evaluated. Ten Brahman Cross cows were also examined as a reference group in order to compare the reproductive traits of the two genetic groups.

The observation of single nucleotide polymorphism (SNP)

Blood samples from 37 Cross cattle were taken as the first step in the investigation's single nucleotide polymorphism (SNP) monitoring methodology. The gSYNCTM DNA extraction kit was used to extract DNA before PCR amplification targeting the leptin gene with specific primers (Leptin_3 region, exon 3 to 3' UTR). The 898 bp DNA fragment generated by the PCR was examined using 1.5% agarose gel electrophoresis stained with ethidium bromide. To identify polymorphisms, PCR products were digested using the HindIII restriction enzyme and then reanalyzed by electrophoresis. DNA bands were visualized using a UV transilluminator and documented. For SNP confirmation, sequencing was conducted at the LPPT, Universitas Gadjah Mada. Sequence alignment and SNP identification were performed using BioEdit software, followed by silico restriction enzyme analysis to predict fragment sizes. Genotypes were confirmed by comparing band patterns after restriction fragment length polymorphism (RFLP) analysis with HindIII enzyme digestion.

Estrus detection before artificial insemination (AI)

Estrus detection was carried out visually at specific observation times: 06:00, 09:00, 12:00, 16:00, and 21:00. Behavioral indicators of estrus were scored based on established criteria, including signs such as vulva swelling and redness, groaning, restlessness, sniffing of other cows, chin resting, and standing heat. Each sign was assigned a score, with standing heat receiving the highest score (100), as it is a definitive indicator of estrus readiness for insemination.

Vaginal smear examination

Before AI, vaginal smear samples were collected using sterile cotton buds moistened with 0.9% NaCl. The cotton bud was inserted approximately 5 mm into the vagina and rotated in both clockwise and counterclockwise directions to collect epithelial cells. The sample was smeared onto glass slides, airdried, fixed in 70% alcohol for 7 minutes, and stained with 3% Giemsa for 45 minutes. The slides were examined under a microscope to identify the estrus stage based on the proportion and types of cells: parabasal, intermediate, and superficial cells.

Estrogen hormone measurement

Blood samples were collected from the coccygeal vein after disinfection with 70% alcohol. Approximately 3 ml of blood was drawn into EDTA tubes, labeled, and centrifuged at 3000×g for 5 minutes to separate the plasma. The plasma was stored at –4°C until further analysis. Estrogen levels were later measured using the enzymelinked immunosorbent assay (ELISA) method with a Fine Test ELISA Kit (Wuhan, China). Standard solutions with graded concentrations (1000 to 0 pg/ml) were prepared, and 50 µl of each standard and sample was pipetted into wells. A biotin-labeled antibody was added and incubated, followed by washing steps, addition of SABC solution, and substrate incubation. A stop solution was added after color development, and OD values were measured at 450 nm.

Artificial insemination (AI)

Artificial insemination was performed at 06:00 using thawed semen prepared in 37°C water for 15 seconds and loaded into an insemination gun. The procedure followed natural estrus detection without synchronization protocols, in accordance with current regulations prohibiting hormonal synchronization for future reproduction management. Insemination was timed within 12–24 hours after standing estrus, aligning with optimal fertilization timing.

Post-AI evaluation at 30 days

Thirty days after AI, visual estrus signs were re-evaluated to assess whether cows returned to estrus, indicating failed conception. Vaginal smears and blood samples were also collected again to monitor cytological and hormonal changes. Estrogen levels were expected to decrease in pregnant cows, while cell proportions on vaginal smears would shift toward increased intermediate and parabasal cells.

Rectal palpation

Pregnancy confirmation was performed on Day 30 post-AI using rectal palpation by a trained veterinary technician. The examination involved inserting a gloved hand into the cow's rectum to palpate reproductive organs such as the uterus and ovaries. Indicators of pregnancy included a heavier and larger uterus and the detection of a small fetal sac. Changes in uterine tissue texture were also considered during evaluation.

Observation of reproductive traits

Reproductive performance was evaluated based on critical traits: age at puberty, age at first mating, postpartum estrus (PPE), postpartum mating (PPM), and service per conception (S/C). These parameters provide insight into the efficiency and reproductive potential of the cattle under study, allowing comparison between the Belgian Blue crossbred and the Brahman Cross cattle.

Analysis of data

The analysis of the relationship between SNPs and reproductive traits was conducted using analysis of variance (ANOVA) with a one-way completely randomized design (CRD), formulated as follows:

$$Y_{ij} = \mu + a_i + €_{ij}$$

Description:
Y_{ij}: Phenotypic value (growth/reproductive trait) of the ith individual
μ: Overall mean
a_i: Effect of the ith genotype
$€_{ij}$: Random error

Results

First, the SNP g.3272 T/C of the leptin gene was identified using the chromatogram (Fig. 1). The study of SNP g.3272 T/C in the leptin gene using the PCR-RFLP method with the HindIII restriction enzyme on 10 Brahman Cross and 27 Belgian Blue Crossbred samples revealed three genotypes: TT (465 bp, 435 bp), TC (898 bp, 465 bp, 435 bp), and CC (465 bp, 898 bp) (Fig. 2).In Brahman Cross, genotype frequencies were TT (0.40), TC (0.30), and CC (0.30), with allele frequencies of T (0.52)and C (0.48). In Belgian Blue Crossbred, genotype frequencies were TT (0.26), TC (0.52), and CC (0.22), with allele frequencies of T (0.55) and C (0.45) (Table 1). Both populations were in Hardy Weinberg equilibrium. Observed heterozygosity (Ho) and expected heterozygosity (He) in Brahman Cross were 0.30 and 0.49, respectively, while in Belgian Blue Crossbred, both were 0.52 and 0.49. The Polymorphic Information Content (PIC) was 0.37 in both populations, indicating a moderate level of polymorphism (Table 1).

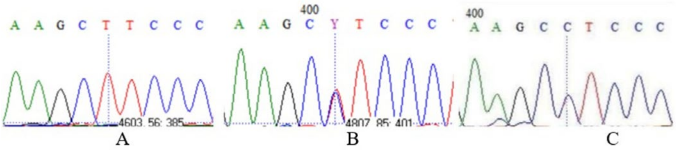

Fig. 1. Sequencing chromatogram of the leptin gene SNP g.3272 T/C. (a) homozigot TT, (b) heterozigot (TC), (c) homozigot (CC).

The study showed that the SNP g.3272 T/C in the leptin gene was significantly associated with visual estrus signs, estrogen levels, vaginal epithelial cell distribution, and reproductive performance in Brahman Cross and Belgian Blue crossbred cattle. In Brahman Cross cattle during artificial insemination (AI), the TT genotype had the highest visual estrus score (129.25±3.50), followed by CC (120.33±2.08) and TC (119.33±1.52), with a significant difference (P<0.05). After AI, the CC genotype showed the highest score (119.18±2.08) (Table 2). Conversely, in Belgian Blue crossbred cattle during AI, the TC genotype had the highest score (131.50±2.64), while after AI, the TT genotype recorded the highest score (121.00±1.00).Regarding estrogen levels, Brahman Cross cattle with the TT genotype showed the highest value during AI (547.13±28.81 pg/mL), while in Belgian Blue crossbred, the TC genotype had the highest level (552.09±25.02 pg/mL). Thirty days post-AI, estrogen levels significantly decreased in both groups, with the TC genotype in Brahman Cross recording the highest value (386.08±49.07 pg/mL) and the TT genotype in Belgian Blue crossbred (395.99±57.23 pg/mL) (Table 3).

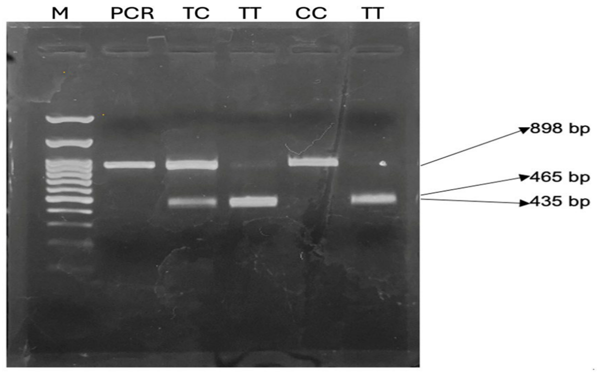

Fig. 2. Visualization of PCR-RFLP for SNP g.3272 T/C: genotype CC has a band size of 898 bp, TC has three fragments at 898 bp, 465 bp, and 435 bp, and TT has two fragments at 465 bp and 435 bp.

Vaginal epithelial cell distribution in Brahman Cross cattle during the estrus phase showed that the TT genotype had the highest percentage of superficial cells (59.50±1.29%), while during the diestrus phase, the TT genotype dominated in intermediate (57.00±2.16%) and parabasal cells (21.50±0.91%). In Belgian Blue crossbred cattle, the TC genotype had the highest percentage of superficial cells during estrus (75.50±1.29%) and the highest percentage of intermediate and parabasal cells during diestrus (62.37±1.49% and 20.25±0.64%, respectively (Table 4).In terms of reproductive performance, Brahman Cross cattle with the TT genotype had the earliest age at puberty (19.50±1.29 months), age at first mating (20.50±1.29 months), and post-partum estrus (PPE) (78.50±1.29 days)

Table 1. Genotype frequency, allele frequency, hardy Weinberg equilibrium, observed heterozigosity, expected heterozigosity, and polymorphic informed content.

Breed	N	Genotype frequency			Alelle frequency		HWE	Ho	He	PIC
Brahman Cross	10	TT (0.40)	TC (0.30)	CC (0.30)	T (0.52)	C (0.48)	0.03	0.30	0.49	0.37
BB crossbred	27	TT (0.26)	TC (0.52)	CC (0.22)	T (0.55)	C (0.45)	1.65	0.52	0.49	0.37

Table 2. Association between SNP g.3272 T/C and visual estrus signs in Brahman Cross and Belgian Blue cattle.

Breed	N	Phase	Visual estrus signs based on Genotype			P-Value
			TT	TC	CC	
Brahman Cross	10	IB	129.25±3.50^{b}	119.33±1.52^{a}	120.33±2.08^{ab}	0,00
BB crossbred	10		119.33±1.52^{a}	131.50±2.64^{b}	120.33±2.08^{ab}	0,00
Brahman Cross	10	after IB	115.25±1.70^{a}	118.33±1.52^{ab}	119.18±2.08^{b}	0,00
BB crossbred	10		121.00±1.00^{b}	115.25±1.70^{a}	118.21±2.03^{ab}	0,00

BB crossbred (Belgian Blue crossbred). The table shows the average scores of visual estrus signs based on the SNP g.3272 T/C genotype in Brahman Cross and Belgian Blue (BB) cattle, both during and after artificial insemination (IB). Different superscript letters (a,b) in the same row indicate significant differences (P<0.05).

Table 3. Association between SNP g.3272 T/C and estrogen levels in Brahman Cross and Belgian Blue Crossbred cattle.

Breed	N	Phase	Estrogen level (pg/ml) based on Genotype			P-Value
			TT	TC	CC	
Brahman Cross	10	IB	547.13±28.81^{b}	414.64±34.11^{a}	423.85±35.37^{ab}	0
BB crossbred	10		400.25±46.26^{a}	552.09±25.02^{b}	408.87±24.68^{ab}	0
Brahman Cross	10	after IB	169.41±9.38^{a}	386.08±49.07^{b}	312.84±131.39^{ab}	0
BB crossbred	10		395.99±57.23^{b}	161.89±12.31^{a}	309.60±107.13^{ab}	0

BB crossbred (Belgian Blue crossbred). a,b Different superscript letters in the same column indicate significantly different values at P<0.05.

compared to TC and CC genotypes. In Belgian Blue crossbred cattle, the TC genotype showed the best performance with the earliest age at puberty (18.85±0.56 months), age at first mating (19.97±0.56 months), and PPE (75.80±1.92 days) (Table 5).

Discussion

The PCR-RFLP method with the HindIII enzyme has proven flexible for genotyping SNPs in the leptin gene, as demonstrated in studies on pigs (Park *et al*., 2015) and Holstein cattle (Miluchová *et al*., 2018), despite differences in the analyzed genetic loci. The SNP g.3272 T/C in both populations is polymorphic (allele frequency ≤ 0.95), supporting its potential as a genetic marker (Igoshin *et al*., 2022). The Hardy Weinberg Equilibrium (HWE) in both populations indicates genetic stability due to random mating without interference from selection or mutation (Graffelman and Weir, 2016). The high heterozygosity in Belgian Blue crossbred suggests a higher rate of recombination, while the lower observed heterozygosity (Ho) in Brahman Cross indicates possible inbreeding (Albakri *et al*., 2022). Similar levels of genetic variation were indicated by the Belgian Blue and Brahman Cross crossbreds' identical PIC values (0.37). This value is comparable to that of Pinzau cattle (PIC=0.35) and greater than that of Slovak cattle (PIC=0.25), according to Ussenbekov *et al*. (2022). As a result, both Indonesian groups exhibit a considerable degree of variance, indicating a comparable probability of marker-assisted selection (Ussenbekov *et al*., 2022).

In Belgian Blue Crossbred, the TC genotype demonstrated a significant advantage in accelerating the reproductive stages when compared to other genotypes, indicating a higher potential for improving reproductive efficiency. Nonetheless, in the Brahman Cross group, cattle with the TT genotype outperformed animals with other genotypes in terms of reproduction. The superiority of the TC genotype in Belgian Blue crossbreds can be explained by the genetic inheritance from the cross between Brahman Cross (Bos indicus), which mostly carries the T allele, and Belgian Blue (*Bos taurus*), which primarily carries the C allele. Choudhary *et al*. (2015) found that the C allele is more prevalent in *Bos taurus* cattle at specific nucleotide positions in the leptin gene. However, Bos indicus cattle are more tolerant to harsh environmental circumstances and frequently carry the T allele, which is associated with slower rates of reproduction (Al-Shuhaib *et al*., 2018). These findings support earlier studies that demonstrated the heterozygous TC genotype dramatically accelerates reproductive stages, enabling earlier puberty and shorter calving intervals. On the other hand, progeny from Brahman Cross cattle with the TT genotype are consistently produced (Chebel *et al*., 2011).

Nellore cows with the TC genotype were younger at birth and more

Table 4. Association between SNP g.3272 T/C and superficial, intermediate, and parabasal cells in Brahman Cross and Belgian Blue crossbred cattle.

Phase	Breed	N	Genotype	Vaginal Smear for Estrus		
				Superfisial (%)	Intermediet (%)	Parabasal (%)
Estrus	BX	10	TT	59.50±1.29[b]	15.75±0.64[b]	9.87±0.85[b]
			TC	54.33±2.08[a]	12.33±1.52[a]	7.00±1.00[a]
			CC	54.66±2.08[ab]	13.00±0.50[ab]	7.26±1.07[ab]
				0,01	0	0
	BBC	10	TT	68.33±4.16[a]	8.00±1.32[a]	3.66±1.04[a]
			TC	75.50±1.29[b]	10.50±1.08[b]	6.20±0.72[b]
			CC	69.00±1.00[ab]	8.43±0.40[ab]	3.83±1.04[ab]
				0.01	0.02	0.01
Diestrus	BX	10	TT	13.12±0.85[b]	57.00±2.16[b]	21.50±0.91[b]
			TC	9.66±1.52[ab]	50.00±2.00[a]	18.16±1.25[a]
			CC	9.00±0.50[a]	53.00±2.00[ab]	18.50±1.80[ab]
				0	0	0.02
	BBC	10	TT	5.26±0.20[ab]	59.00±1.00[ab]	17.50±0.50[a]
			TC	5.75±0.12[b]	62.37±1.49[b]	20.25±0.64[b]
			CC	5.23±0.20[a]	58.43±1.25[a]	18.76±0.25[ab]
				0.01	0.01	0

[a,b]Different superscript letters in the same column indicate significantly different values at P<0.05. BX (Brahman Cross), BBC (Belgian Blue crossbred)

Table 5. Association between SNP g.3272 T/C of the leptin gene and reproductive traits in Brahman Cross and Belgian Blue crossbred cattle.

Variable	Breed	N	TT	TC	CC
Age at puberty (months)	Brahman Cross	10	19.50±1.29[a]	22.00±1.00[ab]	23.00±1.00[b]
	Belgian Blue crossbred	27	22.00±0.53[b]	18.85±0.56[a]	21.48±0.23[ab]
Age at first mating (months)	Brahman Cross	10	20.50±1.29[a]	22.66±1.53[ab]	24.00±1.00[b]
	Belgian Blue crossbred	27	22.90±0.51[b]	19.97±0.56[a]	22.53±0.28[ab]
PPE (days)	Brahman Cross	10	78.50±1.29[a]	87.33±2.08[ab]	88.33±1.52[b]
	Belgian Blue crossbred	27	82.58±1.66[b]	75.80±1.92[a]	82.50±1.87[ab]
PPM (days)	Brahman Cross	10	89.50±1.29	89.00±1.00	90.00±1.00
	Belgian Blue crossbred	27	87.85±1.77	85.33±3.29	87.66±2.06
S/C (times)	Brahman Cross	10	2.12±0.80	2.00±0.89	2.12±0.67
	Belgian Blue crossbred	27	2.12±0.80	2.00±0.89	2.12±0.67

[a,b]Different superscript letters in the same row within each breed indicate significantly different values at P<0.05. PPE: Postpartum Estrus; PPM: Postpartum Mating; S/C: Services per Conception.

productive, according to another study (Fernandes *et al*., 2019). Additionally, it has been shown that the TC genotype of the leptin gene (LEP/BsaAI) has a substantial impact on the gestation period in Hariana cattle (Rambachan *et al*., 2017). Additionally, it was discovered that the SNP rs135560721 significantly altered the service per conception (S/C) of Holstein cattle (Hill *et al*., 2016). Reproductive characteristics like S/C and calving interval have also been linked to a number of additional genes, such as CAST and COQ9 (Regaldo *et al*., 2020).The Belgian Blue C allele's dominance effect increased the F1 offspring's reproductive efficiency, as seen by earlier age at first mating and shorter calving intervals, but it was unable to match the purebred Belgian Blue's peak performance. The leptin gene affects reproductive factors differently in *Bos taurus* and Bos indicus due to their varied physiological characteristics and environmental adaptations. The TT genotype in Bos indicus provides better resilience to environmental stresses but is often associated with lower reproductive performance, whereas the TC genotype in F1 progeny boosts reproductive efficiency. Findings from *Bos taurus* may not apply to Bos indicus, or vice versa, due to differences in genetic background, interactions with the environment, and livestock management practices. Therefore, genetic selection methods need to be tailored to specific cattle populations (Hernandez *et al*., 2016). Utilizing genotype-based selection methods to increase growth and reproductive efficiency has several advantages. Farmers can employ more precise selection in breeding programs to increase the likelihood of producing individuals with enhanced reproductive performance by determining the most advantageous genotype for each crossbreed. Because the TC genotype is linked to better reproductive success than the TT genotype in Brahman Cross, it should be given priority in crossbreeding efforts, particularly Belgian Blue. But we also stress that, given variations in genetic background, environment, and management systems, population specific methods are required.

Conclusion

In Brahman Cross and Belgian Blue crossbred cattle, the leptin gene's SNP g.3272 T/C has a considerable impact on reproductive efficiency and offers clear genotypic advantages. While the TC genotype is better in Belgian Blue crossbred cattle, especially in terms of speeding up puberty and postpartum estrus, the TT genotype improves reproductive performance in Brahman Cross cattle. F1 Belgian Blue calves have a higher potential for genetic improvement through marker-assisted selection due to their high levels of genetic diversity and polymorphism. These results highlight the leptin gene's potential as a useful instrument for molecular breeding initiatives meant to enhance reproductive characteristics and boost Indonesian cattle productivity. To confirm these findings in bigger populations and investigate more SNPs to improve breeding tactics, more research is advised.

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Conflict of interest

The authors have no conflict of interest to declare.

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