Diversity of Myostatin Gene SNPc.267G>A and SNPc.111G>C as Candidate Genetic Markers for Growth Traits in Ongole Grade Cattle

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ABSTRAK

Naufal SM, Noor RR, Jakaria. 2025. Keragaman gen meostatin SNPc.267G>A dan SNPc.111G>C sebagai kandidat marka genetik sifat pertumbuhan pada sapi Peranakan Ongole. JITV 30(1):1-7. DOI:http//dx.doi.org/10.14334/jitv.v30.i1.3417.

Penelitian ini bertujuan untuk menganalisis keragaman gen MSTN dan asosiasinya terhadap bobot lahir, bobot sapih, dan laju pertumbuhan pada sapi PO. Sebanyak 77 sampel darah sapi PO dari Balai Perbibitan dan Pengembangan Inseminasi Buatan Ternak Sapi Perah (BPPIB-TSP) Ciamis yang terdiri dari 38 sapi betina dan 39 sapi jantan digunakan dalam penelitian ini. Keragaman SNPc.267G>A dan c.111G>C dianalisis dengan teknik PCR-RFLP menggunakan enzim restriksi berupa HaeIII dan AluI. Frekuensi genotipe, frekuensi alel, nilai heterozigositas, dan keseimbangan Hardy-Weinberg dihitung menggunakan program PopGen32. Metode uji-*t* digunakan untuk menganalisis asosiasi keragaman dari SNPc.267G>A dan c.111G>C terhadap bobot badan. Hasil penelitian menunjukan bahwa frekuensi genotipe AA (0,38), AG (0,56), and GG (0,06) pada SNPc.267G>A dengan frekuensi alel A (0,66) dan G (0,34). Pada SNPc.111G>C, frekuensi genotipe CC (0,16), CG (0,29), dan GG (0,56) dengan frekuensi alel C (0,30) dan G (0,70). Kedua SNP tersebut tidak berada dalam keseimbangan Hardy-Weinberg. SNPc.267G>A tidak berasosiasi dengan bobot badan sapi PO jantan dan betina. SNPc.111G>C berasosiasi nyata dengan bobot sapih dan laju pertumbuhan hanya pada sapi PO jantan.Hasil penelitian menunjukkan bahwa SNPc.111G>C genotipe GG dapat dijadikan kandidat marka genetik untuk seleksi bobot sapih dan laju pertumbuhan pada sapi PO jantan.

Kata Kunci: Bobot Badan, Gen Myostatin, Sapi PO, SNP

ABSTRACT

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This study aims to analyze the diversity of the MSTN gene and its association with birth weight, weaning weight, and growth rate in PO cattle. A total of 77 blood samples from PO cattle, including 38 cows and 39 bulls, were obtained from the Balai Perbibitan dan Pengembangan Inseminasi Buatan Ternak Sapi Perah (BPPIB-TSP) in Ciamis. The diversity of SNP c.267G>A and c.111G>C was analyzed using the PCR-RFLP technique and the restriction enzymes HaeIII and AluI. Genotype frequencies, allele frequencies, heterozygosity values, and Hardy-Weinberg equilibrium were computed using the PopGen32 program. The t-test method assessed the association of SNP c.267G>A and c.111G>C with body weight. The results indicated genotype frequencies of AA, AG, and GG at 0.38, 0.56, and 0.06, respectively, for SNPc.267G>A, with allele frequencies of A and G at 0.66 and 0.34. For SNPc.111G>C, the genotype frequencies of CC, CG, and GG were 0.16, 0.29, and 0.56, respectively, while the allele frequencies for C and G were 0.30 and 0.70. Both SNPs were not in Hardy-Weinberg equilibrium. The SNPc.267G>A did not show an association with the body weight of male and female PO cattle. However, SNPc.111G>C was significantly associated with weaning weight and growth rate in male PO cattle. These findings suggest that the GG genotype of SNPc.111G>C may be a candidate genetic marker for selecting weaning weight and growth rate in PO bulls.

Key Words: Body Weight, Myostatin Gene, PO Cattle, SNP

INTRODUCTION

The potential impact of this research on the livestock industry is significant, as it could lead to more efficient breeding programs and enhanced meat production. Domestic meat demand can be met by utilizing the genetic resources of local livestock, including Ongole grade cattle, also known as PO cattle. The PO cattle are a local dual-purpose breed used for both meat and as working animals, making them popular among farmers and livestock keepers in Indonesia (Kusuma et al. 2017). PO cattle were developed in 1930 by breeding Javanese cattle with Sumba Ongole cattle (Rohayati and Christi 2017). The Minister of Agriculture Decree Number 2841/Kpts/LB.430/8/2012 designates the Peranakan Ongole Cattle as local livestock genetic resources to be preserved in Indonesia, recognizing PO cattle as a valuable local genetic resource that should be conserved (Kepmentan 2012). According to estimates by Adinata et al. (2022), the total population of PO cattle in Indonesia was approximately 2.8 million head in 2020, accounting for about 16% of the total beef cattle

population, and they can be found in nearly all provinces. Farmers favor PO cattle due to their high adaptability to tropical environmental conditions, which supports efficient growth (Kurniawan et al. 2021). Research conducted by Ngadiyono et al. (2015) indicates that the average live weight of adult PO cattle is 363 kg, with a carcass percentage of 53.83%. This high carcass percentage suggests that PO cattle possess good economic value and muscle growth.

It is stated that muscle growth is a quantitative trait influenced by various pairs of genes and environmental factors (Hilmia et al., 2019). The myostatin gene is essential for regulating muscle development and growth traits (Konovalova et al. 2021). The myostatin gene (MSTN), also referred to as growth and differentiation factor-8 (GDF-8), is part of the transforming growth factor-β (TGF-β) family, which oversees muscle development (Chen et al. 2021). The MSTN gene is located on chromosome 2, consisting of three exons and two introns, and encodes 375 amino acids (Jakaria et al. 2021). As a result, mutations or deficiencies in the MSTN gene impact muscle growth, particularly hypertrophy, which is the excessive growth of muscle tissue (Ayuti et al. 2024). This phenomenon has been applied in livestock breeding, especially beef cattle, as it can increase their body weight.

The practical implications of this research for livestock breeders are significant, as it could provide them with valuable insights for their breeding programs. One method to increase local livestock productivity is through a breeding program for beef cattle. The breeding of PO cattle is generally still based on a phenotyping approach, which has a relatively long generation interval compared to other livestock, resulting in slow genetic improvement. Today's breeding technology includes genome mapping through Single Nucleotide Polymorphism (SNP) to determine the relationship between quantitative traits and DNA variations (Sutikno et al., 2020).

This technology will enhance the exploration of genomic data, particularly in PO cattle. The PCR-RFLP method can evaluate the diversity of polymorphism within a population. This technique was chosen because it identifies genotypes, revealing the genes that encode essential traits (Jakaria et al. 2007).

A study on the genetic diversity of MSTN related to growth traits such as birth weight, weaning weight, and growth rate has been conducted. Previous research using sequencing techniques on PO cattle identified SNPc.111G>C and SNPc.267G>A in the MSTN gene within exon 1 (Jakaria et al. 2021). However, further validation through PCR-RFLP is essential to confirm these findings and evaluate their potential as candidate genetic markers for growth traits in PO cattle. Therefore, this study employed the PCR-RFLP technique to identify and analyze the diversity of SNPc.111G>C and c.267G>A in the MSTN gene within the exon 1 region of PO cattle. The observed diversity is also linked to PO cattle's birth weight, weaning weight, and growth rate.

MATERIALS AND METHODS

Phenotypic data and blood sample collection

A total of 77 blood samples from PO cattle were obtained from the Balai Perbibitan dan Pengembangan Inseminasi Buatan Ternak Sapi Perah (BPPPIB-TSP) in Ciamis, consisting of 38 females and 39 males. The PO calves in this study were produced through artificial insemination using semen collected from bulls chosen based on their weight performance, employing a purebred mating system. The phenotypic data were sourced from records at Balai Perbibitan dan Pengembangan Inseminasi Buatan Ternak Sapi Perah (BPPIB-TSP) in Ciamis. The selection criteria for the cattle were established from their recorded birth weights, weaning weights, and the dates of these measurements. Birth weight data were recorded and measured within 24 hours postpartum. Their dams raised the calves until they reached 180 to 205 days of age to ascertain each calf's weaning weight. The calves received 10% forage and 1% concentrate at this breeding station based on their total body weight. Birth weight data were collected using a digital scale, while weaning weight was measured with a Rondo measuring tape, which was subsequently converted into body weight. Blood samples of five milliliters were drawn via the jugular vein using a 21G needle, placed into a vacutainer with EDTA, and stored in a refrigerator at 4°C until analysis.

DNA extraction and genotyping of MSTN gene using PCR-RFLP Method

A blood sample was collected for DNA isolation using a gSYNCTM DNA extraction kit from GeneAid (Taiwan) following a new blood protocol at the Laboratory of Molecular Genetics, Faculty of Animal Science, IPB University. It was stored at -20°C until analysis. Primers were utilized to amplify the MSTN gene exon 1 according to the PCR procedure specified by Jakaria et al. (2021) with access number AY794986. A 608 bp fragment was amplified using the forward primer 5′-CAA GTT GTC TCT CAG ACT GG-3′ and the reverse primer 5′-CTC CTC CTT ACA TAC AAG CC-3′. A total of 15 µL of PCR amplification mix was prepared by combining 1.0 µL of DNA isolation, 6.1 µL of nuclease-free water (Qiagen, Germany), 0.2 µL of forward primer, 0.2 µL of reverse primer, and 7.5 µL of Red Mix. The reaction was performed in the Esco Swift Maxi SWT-MX-BLC-1 Thermal Cycler (Esco Technology, Inc., USA) under the following conditions: pre-denaturation at 95°C for 1 minute (1 cycle);

denaturation at 95°C for 15 seconds (35 cycles); annealing at 59°C for 20 seconds (35 cycles); extension at 72°C for 10 seconds (35 cycles); and final extension at 72°C for 5 minutes (1 cycle). The PCR product was visualized by electrophoresis in a 1.5% agarose gel (Vivantis Inc., USA), stained with Florosafe DNA (Axil Scientific Pte Ltd., Singapore), with a 100 bp marker (Geneaid, Taiwan), and viewed using a UV transilluminator (AlphaImager; Alpha Innotech, USA).

The MSTN gene was analyzed using the PCR-RFLP method. The PCR product (608 bp) of the MSTN gene was digested at 37°C for 4 hours with two restriction enzymes: *Alu*I (AG│CT) for SNPc.267G>A and *Hae*III (GG│CC) for SNPc.111G>C, as determined by the Neb-cutter program (https://nc3.neb.com/NEBcutter/). Both *Alu*I and *Hae*III used the same reaction mix volume, which consisted of 5.0 µL of PCR product, 1.0 µL of nuclease-free water (Qiagen, Germany), 0.7 µL of enzyme buffer, and 0.3 µL of restriction enzyme. The restriction products were electrophoresed on a 2% agarose gel at a voltage of 100V for 45 minutes and then visualized using a UV transilluminator. The lengths of the DNA bands that appeared were determined by comparing them to a 100 bp DNA ladder, which enabled the determination of the DNA band length and the MSTN gene genotype.

Data analysis

Data on birth weight in PO cattle were available. In addition, several weaning weight measurements were adjusted to 205 days of age using the formulas of Jakaria et al. (2019):

$$WW205=\left[\frac{(WW\text{-}BW)}{\text{actual age}}\times 205\text{ days}\right]+BW$$

where WW205 is corrected weaning weight at 205 days, WW is actual weaning weight, and BW is birth weight. The growth rate, or average daily gain from birth to weaning in kg/day, was estimated using the following formula (Amiano et al. 2020):

$$P=\frac{WW-BW}{\text{Cattle ages}}$$

where P is weight gain (kg/day), WW is weaning weight, and BW is birth weight.

Using Popgene32 software, we conducted data analysis for genotype frequency, allele frequency, heterozygosity value, and Hardy-Weinberg equilibrium. We also performed an association analysis of SNPc.111G>C and c.267G>A diversity concerning birt h weight, weaning weight, and growth rate. Genotype and allele frequencies for SNPc.111G>C and c.267G>A were analyzed using the relevant formulas (Nei & Kumar 2000):

$$X_{ii}=\frac{n_{ii}}{N} \qquad X_i=\frac{\left(2n_{ii}+\sum_{i\neq j} n_{ij}\right)}{2N}$$

where Xi refers to the frequency of allele I, Xii refers to the frequency of genotype ii, nii denotes the number of genotypes ii, nij indicates the number of genotypes ij, and N represents the total sample size. Observed and expected heterozygosity were determined using the formulas (Eugenia & Victoria 2021):

$$H_o=\sum_{i\neq j}^{N}\frac{n_{ij}}{N} \qquad H_e=1-\sum_{i=1}^{n}P_i^2$$

where Ho denotes observed heterozygosity, He represents expected heterozygosity, N signifies the number of observed individuals, nij represents the count of heterozygous individuals, q indicates the number of alleles, and Pi denotes allele frequency. The Hardy-Weinberg equilibrium was calculated using the following formulas (Láruson & Reed 2021):

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

where χ^2 is chi-square value, O: number of observed genotypes; E: number of expected genotypes. The association between SNPc.111G>C and c.267G>A genotype diversity with birth weight, weaning weight, and growth rate was analyzed using a two-sample t-test and calculated using the SAS Demand for Academic program. The mean and standard deviation of birth weight, weaning weight, and growth rate were calculated for each genotype.

RESULTS AND DISCUSSION

Description of body weight and growth in PO cattle

Table 1 presents data on birth weight, weaning weight, and growth rate of PO cattle at UPTD Balai Perbibitan dan Pengembangan Inseminasi Buatan Ternak Sapi Perah (BPPIB-TSP) Ciamis, obtained through descriptive analysis.

In this study, the mean birth weight for female and male PO cattle calves was 21.75±2.81 kg and 23.39±3.33 kg, respectively. The mean weaning weights for female and male calves were 89.89±15.08 kg and 96.81±20.56 kg, respectively. The birth and weaning of PO cattle in

Table 1. Mean and standard deviation for birth weight, weaning weight, and growth rate of PO cattle

Sex	N	Birth Weight (kg)	Weaning Weight (kg)	Growth Rate (kg/day)
Female	38	21.76±2.81	89.89±15.08	0.33±0.07
Male	39	23.39±3.33	96.81±20.56	0.35±0.09

N= number of individuals

SPR Tanjungsari, which reported values of 23.81±2.01 kg and 24.27±2.56 kg for female and male birth weights, respectively, and a weaning weight of 122.40 kg (Kurniawan et al. 2021).

Generally, male cattle weigh more than female cattle due to differences in steroid hormones, which result in faster growth rates for males (Setiyono et al. 2017). Variations in genetic potential, geographic origin, management practices, feed quality, and breeding techniques can lead to differences in livestock weaning weight and overall body size (Hikmawaty et al. 2014).

Polymorphism of SNPc.111G>C and SNPc.267G>A in the MSTN gene

The diversity of the MSTN gene was analyzed using the PCR RFLP method with *Hae*III and *Alu*I restriction enzymes, which produced three genotypes (Figure 1). Two alleles and three genotypes were identified for SNPc.267G>A after digestion with the AluI enzyme, resulting in AA, AG, and GG genotypes. The AA genotype displayed three bands with 322 bp, 261 bp, and 25 bp lengths. The AG genotype showed four bands measuring 322 bp, 199 bp, 261 bp, and 25 bp. The GG genotype exhibited four bands with lengths of 322 bp, 199 bp, 62 bp, and 25 bp. Similarly, the *Hae*III enzyme, when applied to SNPc.111G>C, generated two alleles (G and C) and three genotypes: CC, CG, and GG. The CC genotype revealed three bands with lengths of 380 bp, 173 bp, and 55 bp. The CG genotype displayed three bands measuring 380 bp, 435 bp, and 173 bp. The GG genotype presented two bands with lengths of 435 bp and 173 bp. DNA bands shorter than 100 bp may be invisible due to their short lengths.

The results of the genotype frequency, allele frequency, heterozygosity, and chi-square analysis of the MSTN gene are shown in Table 2. The diversity analysis for SNPc.267G>A revealed that the AG genotype had the highest frequency at 0.56, followed by the AA and GG genotypes with frequencies of 0.38 and 0.06, respectively. The allele frequency distribution displays a higher value for allele A compared to allele G. The highest genotype frequency for SNPc.111G>C was observed for the GG genotype, which had a frequency of 0.56, followed by the CG and CC genotypes with frequencies of 0.29 and 0.16. The distribution of the G allele exceeded that of the C allele, with respective frequencies of 0.70 and 0.30. Genotype frequency represents the ratio of a genotype in a population by comparing a particular genotype to the total population. In contrast, allele frequency indicates the ratio of an allele to the total number of alleles in that population (Noor 2010).

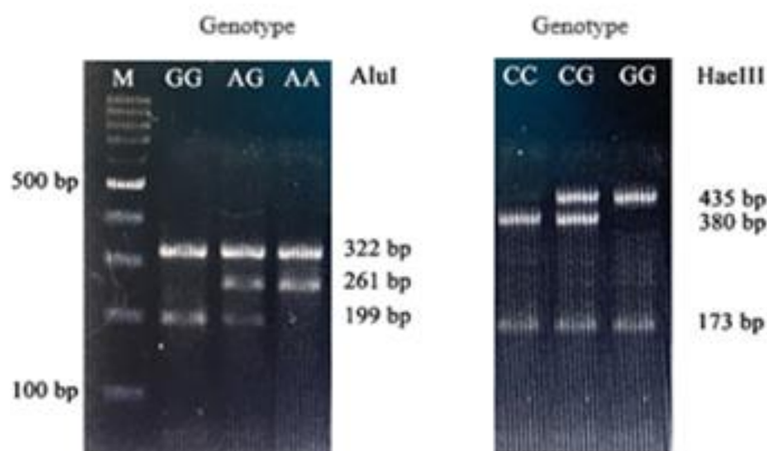

Figure 1. Visualization of PCR-RFLP for the MSTN gene exon 1 following digestion by *Alu*I and *Hae*III restriction enzymes

Table 2. Genotypic and Allelic Frequencies on c.111G>C and c.267G>A at MSTN Gene

SNP	N	Genotypes Frequencies			Alleles Frequencies		Ho	He	χ^2
267G>A	77	AA	AG	GG	A	G	0.56	0.45	4.327*
		0.38 (29)	0.56 (43)	0.06 (5)	0.66	0.34			
111G>C	77	CC	CG	GG	C	G	0.28	0.42	7.788*
		0.16 (12)	0.29 (22)	0.56 (43)	0.30	0.70			

N= Number of individual, Ho= Observed heterozygosity, He= Expected heterozygosity, χ^2= Chi-square test, χ^2 table (0.05;1)= 3.841, (*) significant differences (P<0.05)

Genetic diversity in a population is considered polymorphic when two or more alleles are present, and no single allele exceeds a frequency of 0.99, or 99 percent (Karki et al. 2015). Therefore, SNPc.267G>A and SNPc.111G>C in this study are classified as polymorphic. Extensive genetic diversity creates opportunities for selecting desirable genotypes, thus streamlining the selection process (Mardi et al. 2022).

The equality of alleles in a population, known as the Hardy-Weinberg Equilibrium, can be assessed using the chi-square (χ^2) value, which is calculated based on the differences between observed and expected genotype frequencies (Putra et al. 2017). The results of the population equilibrium analysis are presented in Table 2. The research findings indicated that SNPc.267G>A had a χ^2 value of 4.327, while SNPc.111G>C had a χ^2 value of 7.788. Both SNPs have χ^2 values greater than the critical value presented in the chi-square table, indicating that the observed frequency significantly differs ($P<0.05$) from the expected genotype frequency (Hartwig 2014). Abramovs et al. (2020) noted that several factors, including mutation, natural selection, non-random mating, genetic drift, and gene migration, can lead to deviations from Hardy-Weinberg equilibrium. However, a population can achieve Hardy-Weinberg equilibrium if mating occurs randomly, allowing allele and genotype frequencies to remain constant in the subsequent generation (Smith and Baldwin 2015). The observed heterozygosity and expected heterozygosity values serve as indicators for estimating the level of inbreeding and explaining genetic diversity within a livestock population (Agustina et al. 2021). The study results revealed that SNPc.267G>A had Ho > He values of 0.56 and 0.42, whereas SNPc.111G>C had Ho < He values of 0.28 and 0.42 (Table 2). Similar findings were reported in the study by Jakaria et al. (2021), where the average Ho and He values were 0.42 and 0.44. These Ho values suggest that the genetic variation in PO cattle is high, which is beneficial for fostering genetic development. (Chesnokov and Artemyeva 2015) explained that Ho and He values approaching 0 indicate a lack of heterozygosity, while values nearing 1 imply a more significant presence of alleles with balanced frequencies or heterozygous allele variation.

Association of the MSTN gene with body weight and growth traits

The association of SNPc.111G>C and c.267G>A in the MSTN gene with birth weight, weaning weight, and growth rate are examined. The associations of SNPc.267G>A in MSTN exon 1 with birth weight, weaning weight, and growth rate in PO cattle for both male and female calves are presented in Tables 3 and 4. In contrast, the association of SNPc.111G>C can be found in Tables 5 and 6. Table 3 indicates that female calves with the AA genotype have higher birth weight, weaning weight, and growth rate than those with the AG and GG genotypes. Conversely, male calves with the AG genotype display greater birth weight, weaning weight, and growth rate than the AA and GG genotypes (Table 4). However, the analysis shows that SNPc.267G>A in female and male PO cattle has no significant association ($P>0.05$) with birth weight, weaning weight, and growth rate.

The results of the association analysis of SNP c.111G>C diversity in female cattle presented in Table 5 indicate that cattle with the GG genotype had the lowest birth weight, weaning weight, and growth rate compared to those with the CC and CG genotypes. However, the analysis showed that the SNP c.111G>C diversity did not

Table 3. Association of SNPc.267G>A MSTN Gene on female PO calves

Trait	Genotypes (N)		
	AA (16)	AG (19)	GG (3)
BW (kg)	22.42±2.54	21.24±3.19	21.51±0.88
WW (kg)	93.38±11.30	87.96±18.16	83.45±9.43
GR (kg/day)	0.35±0.05	0.32±0.09	0.30±0.04

N= Number of individual, BW= birth weight, WW= weaning weight, GR= growth rate

Table 4. Association of SNPc. 267G>A MSTN Gene on male PO calves

Trait	Genotypes (N)		
	AA (13)	AG (24)	GG (2)
BW (kg)	22.85±2.40	23.78±3.85	22.27±0.38
WW (kg)	93.85±21.39	99.47±20.60	84.17±14.38
GR (kg/day)	0.34±0.09	0.37±0.09	0.30±0.07

N= Number of individual, BW= birth weight, WW= weaning weight, GR= growth rate

Table 5. Association of SNPc.111G>C MSTN Gene on female PO calves

Trait	Genotypes (N)		
	CC (7)	CG (8)	GG (23)
BW (kg)	22.58±1.64	21.57±1.74	21.56±3.37
WW (kg)	90.76±15.77	96.25±13.54	87.41±15.33
GR (kg/day)	0.33±0.08	0.36±0.07	0.32±0.08

N= Number of individual, BW= birth weight, WW= weaning weight, GR= growth rate

Table 6. Association of SNPc.111G>C MSTN gene on male PO calves

Trait	Genotypes (N)		
	CC (5)	CG (14)	GG (20)
BW (kg)	20.32±1.53[a]	24.75±3.17[b]	23.21±3.30[ab]
WW (kg)	69.60±10.31[a]	99.43±19.76[b]	101.78±18.16[bc]
GR (kg/day)	0.24±0.05[a]	0.36±0.09[b]	0.38±0.08[b]

N= number of individuals, BW= birth weight, WW= weaning weight, GR= growth rate. Superscripts with different letters on the same line signify significant differences ($P<0.05$)

significantly differ among the three genotypes. Additionally, the results indicated a significant association ($P<0.05$) between SNP c.111G>C and the ability of male calves to reach weaning weight and growth rate (Table 6). Therefore, SNP c.111G>C could be a genetic marker for selecting superior bulls, significantly enhancing early growth efficiency in PO cattle. Moreover, the GG genotype exhibited a higher weaning weight and growth rate than the CG genotype. Still, there was no significant difference in body weight between the CG and GG genotypes.

CONCLUSION

The MSTN gene variation at SNPc.267G>A and SNPc.111G>C in PO cattle was polymorphic, and the allele frequencies were not in Hardy-Weinberg equilibrium. SNPc.267G>A displays three genotypic variations: AA, AG, and GG, while SNPc.111G>C has three genotypic variations: CC, CG, and GG. The diversity of SNPc.267G>A among female and male calves did not correlate with birth weight, weaning weight, or growth rate. In female calves, SNPc.111G>C was not associated with growth traits but was significantly linked to weaning weight and growth rate in males. Therefore, the GG genotype of SNPc.111G>C can be considered a potential genetic marker for selecting weaning weight and growth rate in PO bulls.

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