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Exon 10 polymorphism of the *GHR|CviAII* gene and its association with growth traits in Bali cattle

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Abstract. *Rozi T, Maskur, Sarini NP, Suardana W. 2025. Exon 10 polymorphism of the* GHR|CviAII *gene and its association with growth traits in Bali cattle. Biodiversitas 26: 6039-6046.* Bali cattle (*Bos javanicus*) are an important local genetic resource that supports beef production in eastern Indonesia, particularly under smallholder communal breeding systems on Lombok Island. Growth traits in this breed are influenced by both management practices and variation in candidate genes such as the Growth Hormone Receptor (*GHR*). This study aimed to identify exon 10 polymorphisms of the *GHR* gene using the *CviAII* restriction enzyme and to analyze their relationship with major growth traits in Bali cattle. A total of 203 animals, consisting of 106 males and 97 females, were evaluated using Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) and the General Linear Model (GLM). Sequencing detecteded a new synonymous mutation, g.1777T>C, which produced two genotypes, CT and TT, with no CC genotype detected. The T allele showed a higher frequency in both sexes, and observed heterozygosity was higher than expected, indicating heterozygote excess under group-based breeding and sire rotation. Association analysis showed that the CT genotype was linked to superior growth performance compared with TT. In males, CT individuals had a birth weight of 17.22±1.17 kg and a yearling weight of 142.18±6.57 kg, compared with 16.56±1.18 kg and 133.96±5.82 kg for TT. In females, CT individuals had a birth weight of 15.71±0.82 kg and a yearling weight of 133.87±6.18 kg, compared with 15.06±1.10 kg and 126.89±5.26 kg for TT. Weaning weight did not differ among genotypes, suggesting a stronger influence of maternal and management factors. The polymorphic information content indicated moderate informativeness and the need for further validation. These results support the potential of the *GHR|CviAII* locus as a complementary molecular marker for growth-based selection in Bali cattle.

Keywords: Body weight, *Bos javanicus*, Lombok communal breeding, marker-assisted selection, single nucleotide polymorphism

INTRODUCTION

Bali cattle (*Bos javanicus* (d'Alton, 1823)) are one of Indonesia's most important indigenous livestock genetic resources and play a strategic role in sustaining national beef supply and food security. Their strong adaptation to humid tropical climates, efficient feed utilization, stable reproductive performance, and competitive carcass quality make this breed central to beef cattle development programs. In West Nusa Tenggara, particularly on Lombok Island, Bali cattle contribute over ten percent of the national herd, with most animals raised under smallholder communal housing systems managed collectively by farmer groups Sutarno and Setyawan 2015; Dahlanuddin et al. 2014; Directorate General of Livestock and Animal Health Services 2022).

Communal breeding systems typically implement a rotating bull scheme, where a single sire mates approximately seventy to one hundred females over a six-month cycle before being replaced (Fordyce et al. 2003; Dahlanuddin et al. 2014). Although this practice reduces the direct risk of inbreeding by exposing females to diverse sires over time, reliance on a limited number of bulls may decrease the effective population size and produce unequal genetic contributions among individuals. Such conditions can influence allelic distribution and increase vulnerability to genetic erosion (Hall 2016; Sudrajad et al. 2024; Lakotani et al. 2024). Studies across Indonesian islands show that Bali cattle populations differ in genetic diversity, reflecting non-random mating patterns and restricted gene flow among subpopulations (Sutarno and Setyawan 2015).

Growth traits in cattle are quantitative characteristics shaped by interactions between genotype and environment, including feed quality, management, and maternal effects. In local populations, selection based solely on phenotype is often inefficient because performance is heavily influenced by environmental variation. Molecular approaches such as Marker-Assisted Selection (MAS) therefore provide a valuable complement. Genetic markers allow early identification of superior breeding stock, increase selection accuracy, and enhance long-term utilization of local genetic resources (Wakchaure et al. 2015).

The Growth Hormone Receptor (GHR) gene, located on chromosome 20, is a major candidate gene regulating growth in cattle. It mediates the action of growth hormone through the GH-GHR-IGF1 axis, influencing cell proliferation, muscle accretion, tissue differentiation, and metabolic processes (Dehkhoda et al. 2018; Falah et al. 2023). Numerous studies show that nucleotide variation in exon 10 of GHR is associated with growth differences among cattle breeds. For example, an A>G substitution in Peranakan Ongole cattle corresponds with increased body size (Hartati et al. 2021), whereas SNP g.3338A>G is linked to improved growth performance in Indonesian local cattle (Putra et al. 2019). Similar associations have been reported in Indian and Pasundan cattle (Anamika et al. 2016; Putra et al. 2019). These findings indicate that exon 10 polymorphisms can modify receptor function and contribute to phenotypic variation both within and across breeds.

However, information on exon 10 GHR variation in Bali cattle remains scarce. Only a few reports document non-synonymous variants, and no study has thoroughly characterized exon 10 polymorphisms using the restriction enzyme CviAII. Because exon 10 encodes part of the extracellular domain of the receptor, nucleotide substitutions within this region may affect hormone-binding affinity and consequently alter growth performance. Documenting the extent of variation in this exon is therefore essential for understanding the genetic basis of growth traits in Bali cattle.

Comprehensive characterization of GHR polymorphisms is expected to clarify their contribution to birth weight, weaning weight, and yearling weight—traits routinely used for evaluating productivity and selecting replacement stock. Identifying informative variants may improve MAS strategies while ensuring that genetic diversity in local populations is maintained. These insights are particularly relevant for Bali cattle, which are managed primarily in traditional systems and exposed to high environmental variability.

Given this background, the present study aimed to: (i) characterize polymorphisms in exon 10 of the GHR gene in Bali cattle using the restriction enzyme CviAII, and (ii) evaluate the association of these variants with three key growth traits—birth, weaning, and yearling weights. By integrating genotypic and phenotypic data, this study provides a more complete understanding of how GHR variation influences growth performance in Bali cattle and offers a scientific foundation for implementing MAS in adaptive, sustainable breeding programs.

MATERIALS AND METHODS

Experimental animals and DNA isolation

This study identified polymorphisms in exon 10 of the Growth Hormone Receptor (*GHR*) gene and analyzed their corelation with growth traits. Sampling was conducted from January to March 2023 on Lombok Island in Bali cattle raised under communal housing systems. Cattle groups were organized by farmers, and each group practiced natural mating using one selected bull serving approximately 50-70 females for about six months. After one breeding cycle, the bull was replaced to reduce inbreeding and improve gene flow within the population.

Animals were selected using stratified random sampling. First, all eligible animals were listed from communal herds based on farmer records. Inclusion criteria were: purebred Bali cattle, age between 12-18 months, complete growth records, and no history of severe disease or growth disorders. From these lists, individuals were randomly selected within sex and herd groups to ensure representative sampling. A total of 203 animals were included, consisting of 106 males and 97 females aged 1.0-1.5 years.

Blood samples were collected via jugular venipuncture using Kethylenediaminetetraacetic acid (K-EDTA) vacutainer tubes. Approximately 5 mL of blood was drawn from each animal. Samples were stored at -25°C at the Laboratory of Animal Reproduction and Breeding, Faculty of Animal Science, Universitas Mataram, until DNA extraction.

Body weight measurement

Body weight data included birth weight, weaning weight, and yearling weight. Birth weight was recorded within 24 hours after parturition using a portable digital scale. Weaning weight was measured when calves reached 180 days of age using a SONIC AIX digital livestock scale. Yearling weight was recorded at 360 days of age. In addition, animals were weighed monthly at fixed intervals of 30±3 days to monitor growth consistency. All measurements were conducted in the morning before feeding to minimize variation due to gut fill. Data were recorded manually and then tabulated using Microsoft Excel (Microsoft Corp., USA, Version 2016). Males and females were analyzed separately to avoid sex bias.

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) was carried out using a pair of primers specifically designed to amplify exon 10 of the GHR gene. The primers were designed based on the genomic sequence of Bos taurus (GenBank accession EF207442.2). The forward and reverse primers used were: forward: 5”-CTTCATCGTGGACAACGCTT-3” and reverse: 5”-AAAGCTATGGCATGATTTTG-3”. These primers produced a 341 bp amplicon that includes the target polymorphic site within exon 10 (Figure 1).

PCR reactions were prepared in a total volume of 15 µL containing 7.5 µL GoTaq Green Master Mix (Promega, USA), 100 ng of template DNA, 10 pmol of forward primer, and 10 pmol of reverse primer. Amplification was performed using a thermal cycler (Eppendorf Mastercycler, Germany) with the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 45 sec, extension at 72°C for 45 sec, and a final extension at 72°C for 7 min. The expected amplicon size was 341 bp bp were visualized on a 1.5 percent agarose gel and examined under a UV transilluminator.

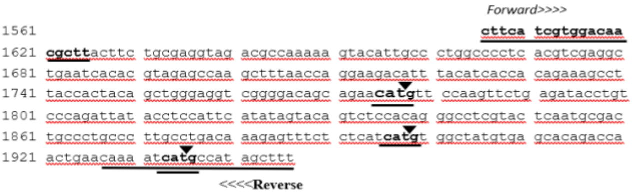


Figure 1. Position of primers (underlined) and the *CviAII* restriction site (underlined arrow) on the *GHR* gene

DNA pooling and sequence alignment

Potential SNPs were identified through pooled DNA sequencing. Three microliters of PCR product from each of ten Bali cattle were combined into one 30 µL DNA pool. Two individual samples were included as controls to validate SNP accuracy. The pooled DNA was sent to a commercial sequencing service and sequenced using an ABI 3730xl platform (PT Genetic Science). Sequence data from each pool and control sample were aligned with the Bos taurus *GHR* reference sequence (GenBank EF207442.2) using BioEdit and MEGA X.

Consensus sequences were generated from high quality forward and reverse reads. Once consensus sequences were obtained, individual sequences were compared with the reference sequence to detect SNPs. Nucleotide differences between the consensus and reference sequences were considered new SNP sites.

Restriction enzyme site analysis

Restriction site analysis was performed using WebCutter and NEBcutter to identify enzymes capable of detecting mutation induced cut sites. The analysis indicated that CviAII (CAT↓G) was suitable for identifying the observed mutation. This enzyme was used in PCR RFLP genotyping for all samples. Allele size and fragment patterns were assessed through agarose gel electrophoresis to determine genotypes.

Data analysis

Genotype and allele frequencies, heterozygosity values, and Hardy-Weinberg equilibrium were calculated using PopGene version 1.31. The Polymorphic Information Content (PIC) was calculated using the GeneCalc tool available at https://gene-calc.pl/pic.

Before statistical modeling, the distribution of growth traits was tested for normality. Birth weight, weaning weight, and yearling weight were evaluated using the Shapiro-Wilk normality test implemented in SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA) through the UNIVARIATE procedure. In addition, residuals from the General Linear Model (GLM) were examined using normal probability plots and residual scatter plots. The Shapiro-Wilk test showed no significant deviation from normality for all traits ($p>0.05$), therefore no data transformation was applied and the data were considered suitable for parametric analysis.

Outliers were screened by visual inspection of boxplots and standardized residual values. Records with biologically implausible values or incomplete information were excluded before analysis. Missing data were handled by listwise deletion, where only animals with complete records for birth weight, weaning weight, and yearling weight were included in the final model.

Body weight traits, including birth weight, weaning weight, and yearling weight, were analyzed using the General Linear Model (GLM) procedure in SAS version 9.1.3. The model included the fixed effects of genotype (*GHR*|CviAII), sex, and their interaction. The statistical model was:

$$Y_{ijk} = \mu + G_i + S_j + (GS)_{ij} + e_{ijk}$$

Where :

Y_{ijk} : Observation of the k-th individual
μ : Overall mean
G_i : Effect of the i-th genotype (CT or TT)
S_j : Effect of sex (male or female)
$(GS)_{ij}$: Interaction between genotype and sex
e_{ijk} : Random error assumed to be normally distributed with mean zero and variance σ^2

Differences among means were tested using the Least Significant Difference (LSD) test at 0.05 and 0.01 significance levels. Results are presented as mean±Standard Deviation (SD). Different superscript letters indicate statistically significant differences among groups.

RESULTS AND DISCUSSION

PCR products of Bali cattle

PCR amplification of the Bali cattle samples yielded clear and distinct bands on a 2% agarose gel, as shown in Figure 2. The fragments corresponded to the targeted region of exon 10 of the Growth Hormone Receptor (*GHR*) gene, amplified using primers derived from the *Bos taurus* reference sequence (GenBank: EF207442.2) and optimized for *Bos javanicus*. The expected amplicon size of 341 bp was consistently obtained across samples, confirming successful amplification of the target locus and suitability of the PCR products for downstream analyses, including sequencing and restriction digestion.

Single Nucleotide Polymorphism (SNP) in exon 10 of the *GHR*|*CviAII* gene

PCR fragments were sequenced to detect genetic polymorphisms in Bali cattle. Sequence alignment was performed using BioEdit and MEGA X software, as shown in Figure 3. The results identified a transition mutation from Thymine (T) to Cytosine (C) at position 172 (g.1777 T>C) of the amplified fragment. Further analysis showed that the restriction enzyme *CviAII* (CAT|G) was able to cut DNA fragments carrying this mutation. This finding confirmed that the mutation can be detected through the PCR-RFLP technique, which can be applied to map genetic variation in Bali cattle populations.

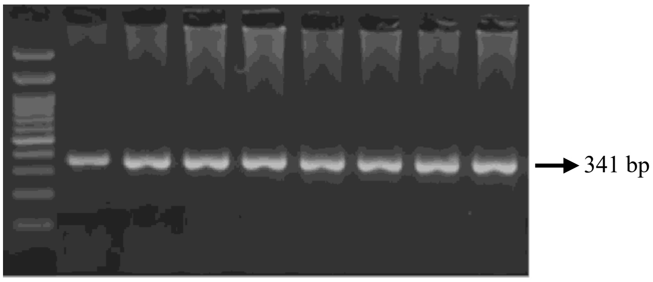
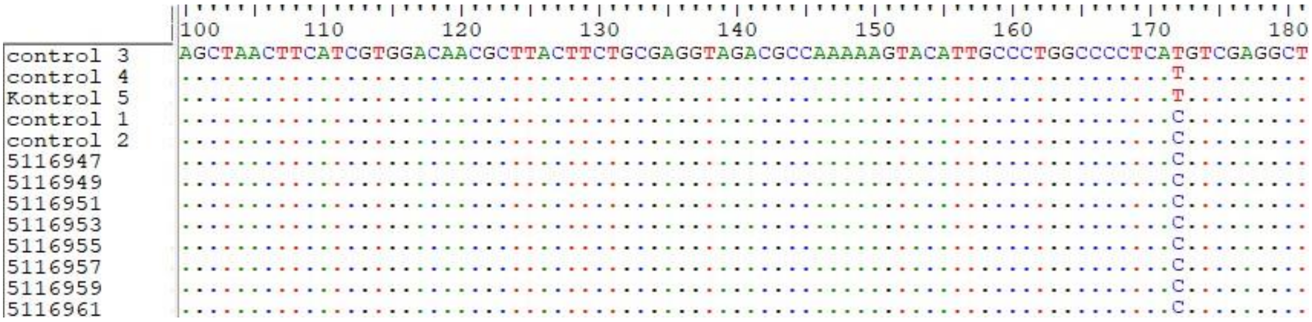


Figure 2. PCR product of the *GHR* exon 10 gene



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             100       110       120       130       140       150       160       170       180
control 3    AGCTAACTTCATCGTGGACAACGCTTACTTCTGCGAGGTAGACGCCAAAAAGTACATTGCCCTGGCCCCTCATGTCGAGGCT
control 4    ........................................................................T.........
Kontrol 5    ........................................................................T.........
control 1    ........................................................................C.........
control 2    ........................................................................C.........
5116947      ........................................................................C.........
5116949      ........................................................................C.........
5116951      ........................................................................C.........
5116953      ........................................................................C.........
5116955      ........................................................................C.........
5116957      ........................................................................C.........
5116959      ........................................................................C.........
5116961      ........................................................................C.........
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Figure 3. Alignment of exon 10 DNA sequences of the *GHR* gene in Bali cattle and the mutation site at nucleotide position 172 (g.1777 T>C) relative to GenBank accession EF207442.2

The T→C substitution identified in exon 10 of the *GHR* gene represents a synonymous mutation, in which the codon changes from CAU to CAC while still encoding histidine. This type of mutation is defined as a silent mutation because it does not alter the amino acid sequence. Although it does not change the primary protein structure, such substitutions can influence gene function through codon usage bias and post-transcriptional regulation. Increasing evidence shows that synonymous mutations may affect mRNA secondary structure, translational efficiency, and co-translational protein folding (Buhr et al. 2016; Quax et al. 2015).

Sequence analysis revealed that the T→C substitution modified codon usage bias at the affected site. This change may potentially influence the translational dynamics of the Growth Hormone Receptor (*GHR*) protein by altering ribosomal pausing patterns and mRNA stability. These effects can contribute to phenotypic variation in growth traits even without changes in amino acid sequence (Quax et al. 2015).

The T-C substitution was confirmed as a silent mutation in the present study. However, the findings indicate that this variation may still have functional consequences by affecting translational efficiency or mRNA stability through altered codon preference. Such molecular effects may indirectly modulate *GHR* gene expression and contribute to variation in growth performance among Bali cattle (Wu et al. 2019).

Restriction site analysis using NEBCutter indicated that this mutation can be detected through PCR RFLP with the CviAII enzyme (CAT↓G). This enzyme cuts at the mutation site and produces two distinct alleles. The T allele is cut and generates fragments of 172 bp, 293 bp, and 330 bp. The C allele is not cut at position 172 bp and maintains fragments of 293 bp, 37 bp, and 11 bp. These digestion patterns reveal two genotypes, TT and CT. Their distribution shows considerable genetic variation within the Bali cattle population. This variation is important for association analysis between *GHR* genotypes and growth traits, as shown in Figure 4.

Previous studies in several cattle populations consistently showed associations between exon 10 polymorphisms in the *GHR* gene and growth or milk production traits. In Peranakan Ongole cattle, Hartati et al. (2021) reported an A to G substitution in exon 10 that changes the amino acid serine (AGC) to glycine (GGC). Putra et al. (2019) found that the SNP g.3338A>G in exon 10 relates to body size and body weight. The A allele supports better growth. Putra et al. (2019) observed that *GHR* polymorphisms show significant associations with body length, height, and body weight in Pasundan cattle. In Indian local cattle, Anamika et al. (2016) identified polymorphisms in exon 10 and the 5' non coding region of *GHR* linked to growth and milk traits. Fedota et al. (2017) also reported that SNPs F279Y and S555G in exons 8 and 10 correlate with increased body weight and daily weight gain.

Genetic differences among cattle breeds, including Bali cattle, may reflect adaptation to tropical environments. Maskur and Arman (2014) detected a novel SNP in intron 8 of *GHR* in Bali cattle associated with weaning weight. This finding indicates that *GHR* variation may influence energy metabolism efficiency in hot environments. The T to C mutation identified in Bali cattle in this study, although synonymous, may represent a local adaptive response to extreme tropical conditions. Similar findings have been reported in other breeds. Anamika et al. (2016) in Tharparkar cattle also found that synonymous polymorphisms in *GHR* show significant associations with body weight and growth efficiency. In contrast, Cobanoglu et al. (2021) reported that GHR polymorphisms in Holstein cattle did not show significant associations with weaning weight, suggesting that the phenotypic impact of synonymous substitutions may differ across breeds and production systems. This suggests that genotype effects can be breed specific and influenced by distinct genetic backgrounds and environmental pressures. These patterns support the idea that the biological impact of *GHR* polymorphisms may involve mechanisms beyond amino acid changes. Regulatory differences in translation and gene expression may contribute to diverse phenotypic outcomes in each cattle population.

Polymorphism of the *GHR|CviAII* gene in Bali cattle

The genetic diversity analysis at the GHR|CviAII locus shows clear differences in genotype distribution between male and female Bali cattle on Lombok Island, as shown in Table 1. In both sexes, the heterozygous CT genotype was predominant, with frequencies of 0.745 in males and 0.649 in females. The TT genotype occurred at 0.255 in males and 0.351 in females, while the CC genotype was absent from all sampled individuals. These patterns indicate that the T allele is the major allele in the population, with frequencies ranging from 0.627 to 0.675, and the C allele functions as the minor allele. The absence of the CC genotype and the dominance of CT suggest non-random mating dynamics and potential selection or gene-flow effects within communal breeding systems typical of Bali cattle on Lombok.

The dominance of the heterozygous CT genotype in both sexes is consistent with findings in Pasundan cattle (Putra et al. 2019). Similar patterns have been reported in Madura cattle, where heterozygous genotypes at growth related loci are common due to limited sire numbers in smallholder breeding systems (Agung et al. 2019). Aceh cattle also show a high frequency of major alleles and very low frequency of homozygous genotypes for minor alleles (Said et al. 2020). In contrast, Holstein cattle exhibit more balanced genotype distributions and greater genetic variability, reflecting structured breeding programs typical of *Bos taurus* breeds (Cobanoglu et al. 2021). These comparisons indicate that Indonesian local cattle generally present reduced diversity at the *GHR* locus relative to imported breeds.

The observed heterozygosity exceeded expected values, confirming heterozygote excess and deviation from Hardy-Weinberg equilibrium. Similar pattern has been documented in Pesisir cattle (Strucken et al. 2021). Such patterns are often associated with group mating systems, strong sire selection, and restricted effective population sizes, which can eliminate certain homozygous genotypes and reinforce non random mating (Hall 2016). The moderate PIC values indicate that the *GHR|CviAII* locus remains informative for association studies and marker assisted selection. Comparable PIC values in Brahman and Brangus cattle show that moderate polymorphism at growth related loci can still support effective genetic analyses (Agung et al. 2019). The low Ne values observed here align with reports from several Indonesian local cattle populations, underscoring the risk of genetic narrowing in herds with small effective population size (Sutarno and Setyawan 2015).

The consistent superiority of the CT genotype in growth traits provides a strong basis for considering the *GHR* locus as a marker for selection. However, implementing marker assisted selection requires breeding strategies that maintain genetic diversity. Structured sire rotation, controlled sire composition, and monitoring of effective population size remain essential to preventing genetic bottlenecks, as recommended in conservation studies of Indonesian cattle (Sutarno and Setyawan 2015).

Body weight performance of Bali cattle

The body weight performance shows clear differences between males and females at all growth stages, as shown in Table 2. For birth weight, male calves were heavier (16.62±1.32 kg, n = 106) than females (15.45±1.00 kg, n = 97), and this difference was highly significant ($F_{1,264}$ = 56.6, p<0.0001). At weaning, males also had higher body weight (81.18±6.40 kg, n = 106) than females (75.30±6.83 kg, n = 97), with a significant sex effect ($F_{1,202}$ = 40.2, p<0.0001). At yearling age, males maintained this advantage, reaching 140.14±7.30 kg (n = 106) compared with 131.38±6.74 kg in females (n = 97), and the sex effect remained highly significant ($F_{1,201}$ = 78.7, p<0.0001). These results demonstrate that sex has a strong influence on variation in growth performance of Bali cattle.

These findings align with the biological characteristics of Bali cattle and several other local breeds. Males grow faster than females. Physiological differences in growth hormone production, *GH* receptor sensitivity, and *IGF1* secretion are key factors that explain the higher growth potential in males (Hartati et al. 2021). In Bali cattle, these differences appear more clearly because feeding and management are relatively uniform. This condition makes it easier to link growth variation to biological factors rather than to environmental factors.

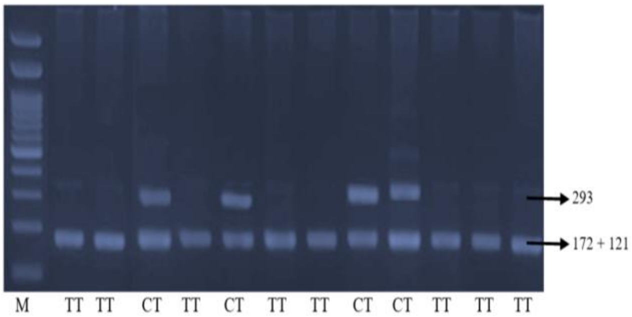

Figure 4. Digestion with the CviAII enzyme produced fragments of 293 bp and 172 + 121 bp, which appeared as a single thick band, as well as fragments of 37 + 11 bp that were not detectable

Table 1. Frequency of alleles, genotypes, and heterozygosity of the *GHR|CviAII* gene in Bali cattle

Gen	Sex	N	Allele frequency		Genotype frequency			χ²Test (HWE)	He	Ho	Ne	PIC
			C	T	CC	CT	TT					
GHR	Male	106	0.37	0.627	0	0.745	0.255	37.40	0.47	0.75	1.88	0.355
	Female	97	0.33	0.68	0	0.65	0.35	22.43	0.44	0.65	1.78	0.344
Total		203	0.35	0.65	0	0.70	0.30	58.73	0.45	0.70	1.83	0.349

Note: N: Number of individual, X^2 test: Chi-square value, He: Heterozigosity expected, Ho: Heterozigosity observed, PIC: Polymorphic Information Content, Ne: Number of effective allele

The average performance of the population shows that weaning weight (78.24±6.62 kg) and yearling weight (135.76±7.02 kg) fall within the range reported for Bali cattle raised under traditional systems. These values are consistent with reports from Gunawan and Jakaria (2011) and Said et al. (2020), which state that Bali cattle usually reach 70 to 85 kg at weaning and 130 to 145 kg at one year of age depending on feed quality and group management. These results support the idea that Bali cattle growth is polygenic and strongly influenced by environmental conditions. The similarity with previous reports indicates that the study environment reflects common management practices on Lombok. The observed performance can therefore be considered representative of the local population.

From a breeding perspective, the significant difference between males and females has practical implications for selection and grouping. Higher growth in males shows their potential as selection candidates. This potential must be balanced with genetic diversity monitoring to avoid a reduction in effective population size, as shown in the genetic diversity analysis in Table 1. Improved feeding and a planned bull rotation system can increase growth performance without reducing population genetic variation.

These results show that Bali cattle growth performance is multifactorial. It depends on interactions among sex, feeding management, and environmental conditions. *GHR* gene polymorphisms contribute to growth variation but do not act as the only determinant. A breeding approach that integrates phenotypic selection, nutrition management, and marker validation will provide more accurate and sustainable improvement of Bali cattle productivity.

Association between *GHR|CviAII* polymorphism and body weight in Bali cattle

The association analysis between the *GHR|CviAII* polymorphism and growth performance in Bali cattle shows a consistent pattern in both sexes, as shown in Table 3. The CT genotype shows a clear advantage over the TT genotype for birth weight and yearling weight. This pattern is not observed for weaning weight. In males, CT animals have higher birth weight (17.22±1.17 kg) than TT animals (16.56±1.18 kg. The difference remains at one year of age, with CT individuals weighing almost 9 kg more. A similar trend is observed in females. The CT genotype shows higher birth weight and higher yearling weight than the TT genotype. The absence of the CC genotype in the population reflects the low frequency of the C allele. This pattern aligns with allele distribution results from the population genetic analysis.

These patterns indicate that the *GHR* locus influences growth most strongly after the pre-weaning period, when the calf becomes less dependent on the dam and endocrine regulation takes over as the main driver of tissue deposition. The lack of significant genotype effects on weaning weight indicates that body weight up to about 180 days is shaped mainly by environmental and maternal factors, such as milk yield, early-life management and feeding strategy, which can obscure the expression of genetic differences in growth (Senkal et al. 2022; Said et al. 2020).

By contrast, the clear association between *GHR* genotype and yearling weight shows that the *GHR-IGF1* axis has a greater influence on growth after weaning, in line with the physiological shift from maternally supported growth to growth that is regulated predominantly by metabolic and endocrine processes in the post-weaning phase (Dehkhoda et al. 2018; Falah et al. 2023).

Evidence from other cattle breeds supports this pattern. In Japanese Black cattle, GHR polymorphisms influence growth and carcass traits (Waters et al. 2011). Genome-wide studies have also shown that GH-IGF1 pathway genes affect feed efficiency and energy utilization (Serão et al. 2013). Similar associations were reported in Indonesian local breeds, where GHR variation in Pasundan and Peranakan Ongole cattle was significantly related to growth performance and linear body measurements (Putra et al. 2019; Hartati et al. 2021). These collective findings underscore the functional role of GHR in regulating muscle deposition, metabolic efficiency, and growth rate.

Table 2. Performance of Bali cattle

Sex	N	Birth weight (kg)	Weaning weight (kg)	Yearling weight (kg)
Male	106	16.62±1.32^{a}	81.18±6.40^{a}	140.14±7.30^{a}
Female	97	15.45±1.00^{b}	75.30±6.83^{b}	131.38±6.74^{b}
Total n	203	16.11±1.16	78.24±6.62	135.76±7.02

Note: Different superscript letters within the same column indicate a significant difference ($p<0.05$) or a highly significant difference ($p<0.01$) between males and females

Table 3. Association between *GHR/CviAII* gene polymorphism and body weight in Bali cattle

Sex	Genotype	N	Birth weight (kg)	Weaning weight (kg)	Yearling weight (kg)
Male (M)	CT	79	17.22±1.17^{a}	81.80±6.68^{a}	142.18±6.57^{a}
	TT	27	16.56±1.18^{b}	79.37±5.20^{a}	133.96±5.82^{b}
Female (F)	CT	63	15.71±0.82^{a}	75.45±6.59^{a}	133.87±6.18^{a}
	TT	34	15.06±1.10^{b}	75.01±7.37^{a}	126.89±5.26^{b}
Male + Female	CT	142	16.55±1.27^{a}	78.99±7.42^{a}	138.49±7.60^{a}
	TT	61	15.72±1.35^{b}	76.94±6.81^{a}	129.90±6.50^{b}
Total n	-	203	16.30±1.35	78.38±7.29	135.91±8.28

Note: Different superscript letters within the same column indicate significant differences at $p<0.05$ or highly significant differences at $p<0.01$

The present results are also in accordance with studies of tropical and commercial breeds. Agung et al. (2019) found that specific *GHR* variants in Brahman cattle correlate with improved body weight during key growth stages. In Pasundan cattle, *GHR* genotypes show significant associations with linear body measurements and live weight (Putra et al. 2019). Comparable trends are documented in Holstein cattle, where exon-level *GHR* polymorphisms influence milk yield, nutrient partitioning, and metabolic balance (Abeygunawardana et al. 2024). This cross-population consistency suggests that *GHR* plays a conserved biological role in growth regulation, regardless of breed or production environment. Such convergence strengthens the potential application of *GHR* polymorphisms as genetic markers for selection in Bali cattle.

Biological interpretation: A synonymous but functional mutation

Although the synonymous transition T→C (g.1777T>C) in exon 10 does not change the encoded amino acid histidine several molecular mechanisms explain how this mutation may still influence growth-related phenotypes. Codon usage bias can alter translation kinetics, where differences in tRNA availability affect the speed and accuracy of protein synthesis. More optimal codons may promote efficient co-translational folding of the *GHR* protein, ultimately affecting receptor performance (Plotkin and Kudla 2011). Nucleotide substitutions can also modify mRNA secondary structure, influencing transcript stability and altering the amount of functional mRNA available for translation (Chamary and Hurst 2005). Furthermore, synonymous mutations may affect splicing regulation by altering exonic splicing enhancers, potentially influencing the abundance of stable *GHR* isoforms (Sauna and Kimchi-Sarfaty 2011). Another possibility is that the observed association reflects broader genomic background effects rather than direct functional consequences of the synonymous mutation itself. Genome-wide analyses have demonstrated that growth-related loci in Indonesian cattle are embedded within complex patterns of admixture, introgression, and population structure, which may influence marker-trait associations across local breeds (Wang et al. 2025).

The present results support the potential use of the CT genotype as a candidate molecular marker in Marker-Assisted Selection (MAS) programs for Bali cattle. The superior performance of CT individuals in birth and yearling weight reinforces its practical applicability in improving growth traits. Incorporating *GHR*-based markers into breeding programs could increase selection accuracy and accelerate genetic improvement, provided that implementation remains aligned with sustainable breeding principles (Rezende et al. 2012). Effective application of MAS must also consider environmental influences on early growth, the need to maintain genetic diversity, and the consequences of group mating systems and sire rotation on population structure. Validation across multiple loci and environments is necessary to ensure consistency and robustness of the *GHR* effects in Bali cattle.

Further studies on Bali cattle from different regions are recommended to verify allele frequencies and the stability of *GHR* genotype effects. Sire based breeding systems also require improved management through planned sire rotation and monitoring of effective population size to maintain genetic diversity. Marker assisted selection should be implemented gradually after this marker is validated in several independent populations.

In conclusion, the results support several conclusions:

This study identified a synonymous substitution, g.1777T>C, in exon 10 of the *GHR* gene of Bali cattle, producing CT and TT genotypes with the CT genotype being the most frequent. Genetic diversity analysis showed excess heterozygosity and deviation from Hardy-Weinberg equilibrium, likely influenced by the group-based mating system used in communal breeding. The CT genotype was consistently associated with higher birth and yearling weights compared to TT, while weaning weight remained more affected by environmental and maternal factors. Overall, the *GHR|CviAII* polymorphism shows potential as a complementary marker for growth-based selection in Bali cattle, although broader validation in other populations is recommended.

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