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Original Article

Sumbawa cattle: a study of growth hormone (*GH*) gene variants and their association with biometric traits

Gado Sumbawa: um estudo das variantes do gene do hormônio do crescimento (*GH*) e sua associação com características biométricas

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Abstract

The growth hormone (*GH*) gene plays a vital role in regulating animal metabolism and body size, making it a potential candidate for influencing livestock performance. This study aimed to investigate the polymorphisms within the *GH* gene and their associations with 10 biometric traits in the Sumbawa cattle population of Indonesia. Biometric trait data and blood samples were collected from 112 Sumbawa cattle individuals, and their *GH* gene sequences were analyzed using two sets of primers for amplification. Seven single nucleotide polymorphisms (SNPs) were identified in the *GH* gene: g.442C>T, g.446G>C, g.558C>T, g.649C>A, g.1492C>A, g.1510C>A, and g.1578G>A. All SNPs were located in the intronic region except for SNP g.558C>T, which was found in the coding sequence (CDS) region. The SNP g.558C>T is classified as a synonymous variant. Haplotype analysis revealed a strong linkage disequilibrium between SNPs g.558C>T and g.649C>A. Distributions of genotypes and alleles of all SNPs were in agreement with the Hardy-Weinberg equilibrium ($p > 0.05$, $\chi 2 < 15.56$), except for SNPs g.446G>C and g.1492C>A. The association study showed that the SNP g.442C>T significantly ($p < 0.05$) affected HL, BL, SH, and PH traits in Sumbawa cattle. Additionally, the g.446G>C and g.558C>T were also found to be associated with PH and CC traits, respectively. The polymorphisms detected in the *GH* gene could have implications for selection programs to enhance desired biometric traits in Sumbawa cattle. Improving livestock productivity can be done by understanding genetic diversity and its relationship with phenotypic characteristics.

Keywords: body measurement, genetic diversity, growth hormone, polymorphism, Sumbawa cattle.

Resumo

O gene do hormônio de crescimento (*GH*) desempenha um papel vital na regulação do metabolismo animal e do tamanho corporal, tornando-se um potencial candidato para influenciar o desempenho do gado. Este estudo teve como objetivo investigar os polimorfismos dentro do gene *GH* e suas associações com 10 características biométricas na população de gado Sumbawa da Indonésia. Dados de características biométricas e amostras de sangue foram coletados de 112 indivíduos de gado Sumbawa, e suas sequências genéticas do gene *GH* foram analisadas usando dois conjuntos de *primers* para amplificação. Sete polimorfismos de nucleotídeo único (SNPs) foram identificados no gene *GH*: g.442C>T, g.446G>C, g.558C>T, g.649C>A, g.1492C>A, g.1510C>A e g.1578G>A. Todos os SNPs estavam localizados na região intrônica, exceto o SNP g.558C>T, que foi encontrado na região da sequência de codificação (CDS). O SNP g.558C>T é classificado como uma variante sinônima. A análise de haplótipos revelou um forte desequilíbrio de ligação entre os SNPs g.558C>T e g.649C>A. As distribuições de genótipos e alelos de todos os SNPs estavam de acordo com o equilíbrio de Hardy-Weinberg ($p > 0,05$, $\chi 2 < 15,56$), exceto para os SNPs g.446G>C e g.1492C>A. O estudo de associação mostrou que o SNP g.442C>T afetou significativamente ($p < 0,05$) as características HL, BL, SH e PH no gado Sumbawa. Além disso, o g.446G>C e o g.558C>T também foram encontrados associados às características PH e CC, respectivamente. Os polimorfismos detectados no gene *GH* podem ter implicações para programas de seleção visando melhorar as características biométricas desejadas no gado Sumbawa. A melhoria da produtividade pecuária pode ser feita através da compreensão da diversidade genética e sua relação com as características fenotípicas.

Palavras-chave: medição corporal, diversidade genética, hormônio de crescimento, polimorfismo, gado Sumbawa.

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1. Introduction

Sumbawa cattle, a local Indonesian breed commonly found on Sumbawa Island, West Nusa Tenggara Province, Indonesia, trace their origins back to Hissar cattle imported from India in 1908. Over generations, they have been bred locally (Decree of the Minister of Agriculture No. 2909/Kpts/OT.140/6/2011), resulting in excellent environmental adaptation and commendable reproductive performance for both males and females (Figure 1) (Sutarno et al., 2018). The Sumbawa cattle also support local populations' livelihoods and cultural activities on Sumbawa Island. Besides their excellence, the Sumbawa cattle are currently facing threads on their genetic purity due to crossbreeding and hybridization due to shifting consumer preferences and changing market demands. It has been reported that due to long-term adverse selection, several problems arise, such as decreased body weight, shorter legs and shoulders, and smaller body sizes. The decrease in genetic integrity within the Sumbawa cattle population could lead to significant adverse effects on both their productivity and sustainability. When the genetic makeup of Sumbawa cattle becomes contaminated or deteriorates, it may result in a reduction in the quality of meat or other derived products. Consequently, this decline could negatively impact the economic worth of these cattle and their associated products. Furthermore, diminished genetic purity might hamper the productivity of Sumbawa cattle. Cattle with impure genetics could exhibit lower reproductive rates, slower growth, and increased susceptibility to health issues, thereby reducing meat yield and impeding the overall growth of the Sumbawa cattle population. As genetic purity declines, Sumbawa cattle may become more susceptible to diseases and environmental stressors. Reduced genetic integrity could compromise the resilience of these cattle against certain illnesses, thereby affecting their well-being and long-term survival. In addition to its biological significance, the economic and social impacts of the decline in genetic purity of Sumbawa cattle can have economic ramifications through reduced productivity affecting the livelihoods of local farmers, as well as social implications due to the shift away from traditional farming culture resulting from the decline in the Sumbawa cattle population. Reduced productivity of Sumbawa cattle can lead to reduced income for local farmers who rely on these cattle. Farmers accustomed to practicing traditional Sumbawa cattle farming culture may struggle to adapt to more modern farming practices or switch to alternative sources of livelihood. Therefore, improving the productivity and maintaining genetic purity of local breeds, specifically Sumbawa cattle, is crucial to overcome these issues. Genetic diversity plays a pivotal role in breeding programs, allowing for better livestock improvement in quantity and quality and preserving genetic resources (Eusebi et al., 2019; Hartatik et al., 2018).

One of the approaches to achieving high livestock productivity is Marker-Assisted Selection (MAS) using the molecular approach (Maharani et al., 2019). Genotyping at the DNA level helps identify gene loci responsible for character variations (Quantitative Trait Loci / QTL) with economic value (Meuwissen et al., 2016; Sutarno et al., 2018). In the context of growth, two primary factors influence it: the hormonal system and energy availabiliy (D'Occhio et al., 2019). Candidate genes, like growth hormone (*GH*), insulin-like growth factor 1 (*IGF1*), myoblast determination protein 1 (*MyoD*), myostatin (*MSTN*), myogenic factor 5 (*Myf5*), and calpastatin (*CAST*) (Abousoliman et al., 2020; Dybus, 2002; Eghbalsaied et al., 2016; Fadholly et al., 2024; Gebreselassie et al., 2019), are selected based on known relationships between physiological or biochemical processes and the trait (Sweett et al., 2020).

The growth hormone, also known as somatotrophic hormone (STH), plays a crucial role in stimulating growth in the body, with bones and muscles being the main target organs (Bayraktar and Özdemir, 2022). It significantly influences postnatal development, metabolism, lactation, protein, lipid, carbohydrate metabolism, tissue growth, and fertility in animals and cows (Bordonaro et al., 2020; Pal and Chakravarty, 2020). The insulin-like growth factor I (*IGF-I*) mediates GH's growth-promoting and metabolic effects (Ramesha et al., 2016). The bovine growth hormone gene is located on chromosome 19 at the q26-qtr position (Bayraktar and Özdemir, 2022) and spans 2061 bp, divided into five exons separated by four introns (Introns A, B, C, and D) (Ishag et al., 2011; Musa et al., 2013). Previous research has suggested associations between the *GH* gene, body weight, and biometric traits. For instance, Kayumov et al. (2019) found that allele variants of the *GH* gene in crossbred Red Angus x Kalmyk heifers resulted

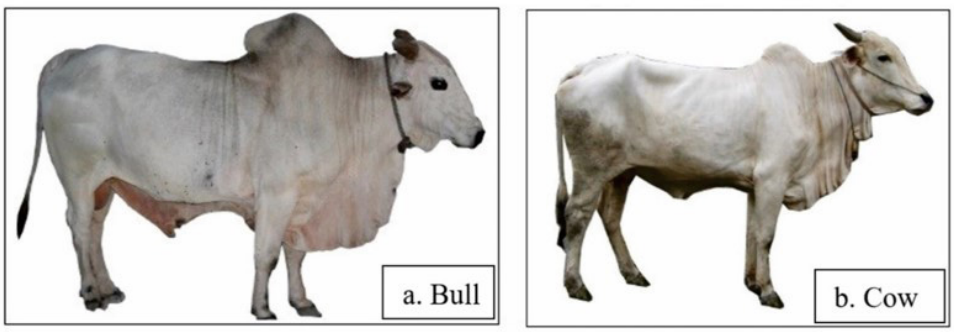

Figure 1. The phenotype of Sumbawa cattle.

in differential expression in 8-month body weight and 18-month linear growth. However, other studies, such as the one by Akcay et al. (Akçay et al., 2015) in Zavot cattle, did not show a significant association between *GH* gene polymorphism and live weight. Similarly, the *GH-Msp*I locus in Sumba Ongole cattle also did not significantly affect some growth parameters (Agung et al., 2017).

Body weight, body size measurements, and weight gains are essential growth traits used as selection criteria in beef cattle breeding programs (Hartati et al., 2021a). However, traditional production systems, like those used for Sumbawa cattle, face challenges in directly measuring body weight with a scale. As a practical and convenient alternative, estimating body weight from body measurements has been adopted (Shoimah et al., 2021). Studies on Nguni cattle, Bali cattle, Holstein-Friesian bulls, West African Shorthorn Somba cattle, and Girolando cattle have reported positive correlations between body measurements (such as hip height, body length, and chest girth) and body weight (Agung et al., 2018; Hlokoe and Tyasi, 2022; Tutkun, 2019; Vanvanhossou et al., 2018; Weber et al., 2020; Widyas et al., 2021). Hence, investigating the genetic factors influencing body conformation becomes crucial.

In light of numerous existing reports focusing on gene polymorphisms related to *GH*, it becomes evident that there remains a noticeable research gap concerning this gene's specific characteristics within the Sumbawa cattle population. Also, the biometric traits were expressions of growth, which could be more accessible to measure and be used as a selection criterion to increase productivity and maintain the genetic purity of Sumbawa cattle. Therefore, this study aims to identify *GH* gene polymorphisms and their relationship with biometric traits (body measurements) in Sumbawa cattle.

2. Materials and methods

2.1. Ethical approval

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Indonesian Agency for Agricultural Research and Development, with the approval number Balitbangtan/Lolitsapi/Rm/16/2021.

2.2. Study areas and sampling methodology

This study was conducted utilizing purposive random sampling, selecting areas with a high concentration of Sumbawa cattle farmers that were easily accessible and had the same rearing system. Sampling was conducted in the Seketeng Village-Sumbawa District and Penyaring Village-Moyo Utara District, Sumbawa Regency, West Nusa Tenggara Province, Indonesia (Figure 2). The Sumbawa cattle selected for data and sample collection were mature (≥ 2 years old), healthy, and non-pregnant.

2.3. Sample collection and DNA extraction

A total of 112 blood samples (23 male and 89 female) of Sumbawa cattle were obtained from the jugular vein using a 20G needle and an EDTA vacutainer tube. The samples were transported to the laboratory and stored in a freezer at -20ºC until DNA extraction. DNA was extracted from 200 µL of whole-blood samples using the gSYNC DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan), following the manufacturer's protocols.

2.4. Phenotype data collection

For the association study, several biometric indicators were collected, including head length (HL), head width (HW), body length (BL), chest circumference (CC), shoulder

Figure 2. The location of the Sumbawa Regency indicates the sampling sites of Sumbawa cattle.

height (SH), chest depth (CD), chest width (CW), pelvic height (PH), pelvic width (PW), and hip width (HIW). All indicators were measured using a measuring stick, except for CC, which used a measuring tape. The illustration of how to measure each indicator is depicted in Figure 3.

2.5. Amplification of DNA

The isolated DNA was used for a Polymerase Chain Reaction (PCR) procedure. The amplification was carried out in a 25 µL total mixed reaction consisting of 2 µL DNA template, 0.5 µL of each primer, 9.5 µL double-distilled water (DDW), and 12.5 µL of MyTaq™ HS PCR Mix (Bioline, United Kingdom) in 0.6 Eppendorf tubes. Two primers were designed to amplify the partial sequence of the cattle's growth hormone gene using a sequence template from *Bos indicus* (GenBank accession No. EF592534). The details of the primers are provided in Table 1. The PCR condition started with pre-denaturation at 94 °C for 5 minutes, followed by a 35-cycle amplification consisting of denaturation at 94 °C for 45 seconds, annealing at a specific temperature for each primer for 45 seconds, and extension at 72 °C for 1 minute. The amplification was concluded with a final extension at 72 °C for 5 minutes. The PCR products were visualized using 2% electrophoresis gel in a UV transilluminator under ultraviolet light.

2.6. Sequencing and genotyping

The unpurified PCR products were sent to the Integrated Research and Testing Laboratory (LPPT) of Universitas Gadjah Mada for PCR clean-up, purification, and sequencing using an automated DNA Sequencer (3500 Genetic Analyzer, Applied Biosystems). The sequencing output was read using the BioEdit version 7.0 program to identify each sample's polymorphic loci and genotype. The sample genotype was determined based on the single or double peaks of the chromatogram in the SNP position (Sari et al., 2021). The NNSPLICE 0.9 Version was used to detect whether the variants may influence splicing (Reese et al., 1997; Riepe et al., 2021).

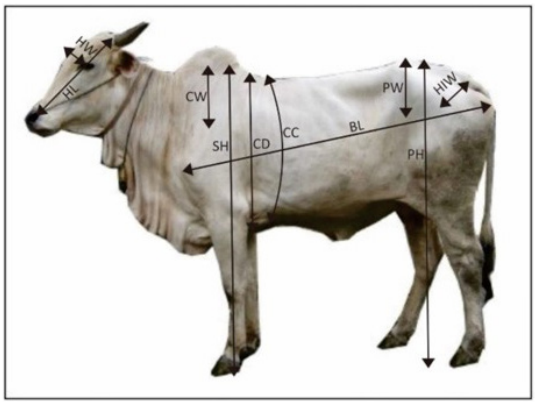

Figure 3. Illustration of body measurement in cattle. Note: HL (head length), HW (head width), BL (body length), SH (shoulder height), CC (chest circumference), CW (chest width), CD (chest depth), PH (pelvic height), PW (pelvic width), and HIW (hip width).

2.7. Data analysis

The genotype data were analyzed using the POPGENE version 3.2 software to compute the allele frequency, genotype frequency, and Chi-square test for Hardy-Weinberg equilibrium (Yeh and Boyle, 1997). The expected genotypic frequencies under the principles of Hardy-Weinberg equilibrium were calculated based on allele frequencies, employing Levene's adjustment for cases involving a limited sample size (Levene, 1949). The haplotype block analysis was conducted using the Haploview program to analyze the linkage of alleles among the SNPs (Taliun et al., 2014). Biometric trait data were corrected to mature cows (>3 years old). The genotype association with biometric trait was performed using a general linear model analysis in IBM SPSS Statistics (Version 23). The mathematical model used was as follows (Equation 1):

$$Yij = \mu + Ai + Bj + \varepsilon ijk \quad (1)$$

where µ is the mean of the population. Ai is the effect of the K-individual genotype, Bj is the effect of K-individual sex, and εij is random error.

The cut-off for statistical significance was $P < 0.05$. After quality control, the SNPs g.649C>A and g.1492C>A were excluded from the association analysis due to several chromatogram errors in their positions and the low observed frequency of some genotypes, making the genotyping of the samples unreliable. The AA genotypes of SNPs g.1510C>A and g.1578G>A were also excluded from the analysis due to the low sample size.

3. Results

3.1. Polymorphic loci within the GH gene and linkage disequilibrium among the SNPs

In this study, seven SNPs of the *GH* gene were identified in Sumbawa cattle through sequence alignment and

Table 1. Details of primers used in this analysis.

Primer's ID	Covering Region*	Nucleotide Base (5' - … - 3')	PCR Product (bp)	Annealing Temp (°C)
GH1-F	407-852	GCAGGAGATCAGGCGTCTAG	446	59.5
GH1-R		GGAAGAACACACCCACCCA		
GH3-F	1461-1916	CCCTGCTCTCTCCCTCTTTC	456	59
GH3-R		AGGAAAGGACAGTGGGAGTG		

*Based on the GenBank acc no. EF592534.

direct chromatogram examination. These SNPs were designated as g.442C>T, g.446G>C, g.558C>T, g.649C>A, g.1492C>A, g.1510C>A, and g.1578G>A, with their nucleotide positions corresponding to GenBank accession number EF592534. Among these SNPs, six were located in the intronic region, specifically g.442C>T and g.446G>C in intron 1, g.649C>A in intron 2, and g.1492C>A, g.1510C>A, and g.1578G>A in intron 4. Only the SNP g.558C>T is located in the coding sequence (CDS) region, but this variant did not alter amino acid (Ser > Ser), as shown in Figure 4. The remaining SNP, g.558C>T, was located in the coding sequence (CDS) region, but it was categorized as a synonymous variant as it did not alter the amino acid (Figure 4). The C and T alleles coded for the same amino acid, Serine (S). SNP g.442C>T, g.446G>C, g.1492C>A, and g.1510C>A exhibited three genotypes (Figure 5), while SNP g.558C>T, g.649C>A, and g.1578G>A had only two genotypes (Figure 6). Figure 7 illustrates the haplotype blocks based on two color schemes: the alternated D'/LOD color scheme (Figure 7a) and the confidence-bound color scheme (Figure 7b). Figure 7 showed that the SNP g.558C>T and g.649C>A had the highest LD.

3.2. Genotype distribution of the GH gene

Table 2 displays the genotype distribution of the *GH* gene in Sumbawa cattle. Among all SNPs, the CC genotype had the highest frequency (ranging from 0.68 to 0.98), except for SNP g.446G>C, where the GG genotype frequency (0.82) was higher than the CC genotype (0.13). The C alleles were more dominant (0.82 – 0.99) in each SNP, except for SNP g.446G>C, where the G allele showed the highest frequency (0.85). The chi-square test revealed that Sumbawa cattle deviated from Hardy-Weinberg equilibrium ($\chi 2 > 15.56$, p-value < 0.05) for SNP g.446G>C and g.1492C>A. Homozygous genotypes (CC and GG) were more prevalent than heterozygous genotypes (CT, GC, and CA) in most loci.

3.3. Association of GH gene variants to biometric traits

Table 3 represents the association study of *GH* gene polymorphisms with body measurements. The SNP

Figure 4. Amino acid translation for SNP g.558C>T.

Table 2. The allele frequency, genotype frequency, and chi-square test value for HWE of *GH* gene SNPs in Sumbawa Cattle.

Locus	Genotype	Genotype frequency	Allele	Allele frequency	χ^2	*p*-value
g.442C>T	CC	0.68	C	0.82	0.13	0.72
	CT	0.29	T	0.18		
	TT	0.04				
g.446G>C	CC	0.13	C	0.15	74.97	0.00
	GC	0.05	G	0,85		
	GG	0.82				
g.558C>T	CC	0.93	C	0.96	0.14	0.71
	CT	0.07	T	0.04		
	TT	0.00				
g.649C>A	AA	0.00	A	0.04	0.12	0.72
	CA	0.08	C	0.96		
	CC	0.92				
g.1492C>A	AA	0.01	A	0.02	66.99	0.00
	CA	0.01	C	0.98		
	CC	0.98				
g.1510C>A	AA	0.01	A	0.09	0.11	0.74
	CA	0.15	C	0.91		
	CC	0.84				
g.1578G>A	AA	0.00	A	0.01	0.004	0.94
	CA	0.02	C	0.99		
	CC	0.98				

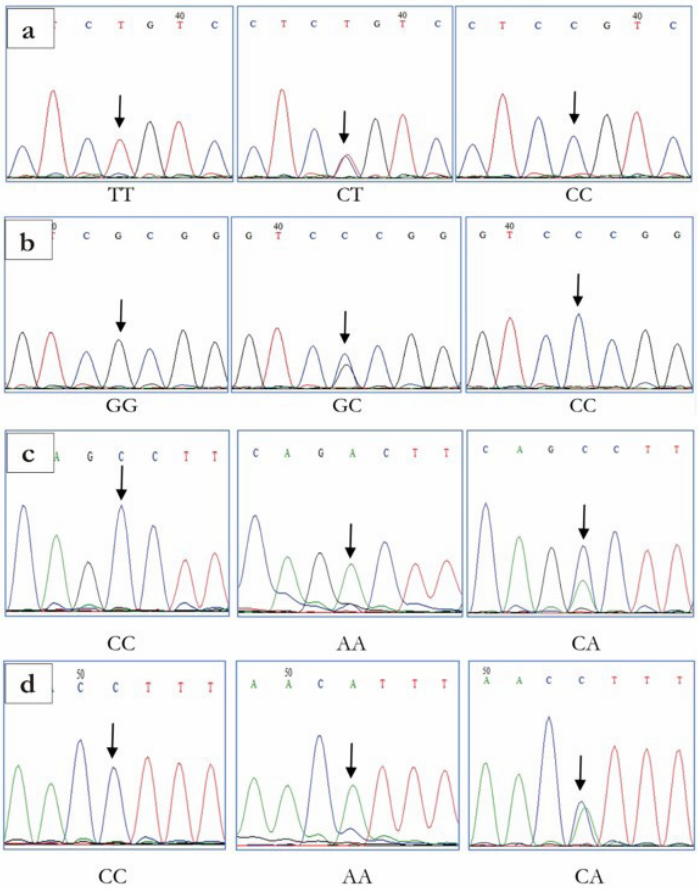

Figure 5. Chromatogram of genotypes for SNPs g.442C>T (a), g.446G>C (b), g.1492C>A (c), and g.1510C>A (d).

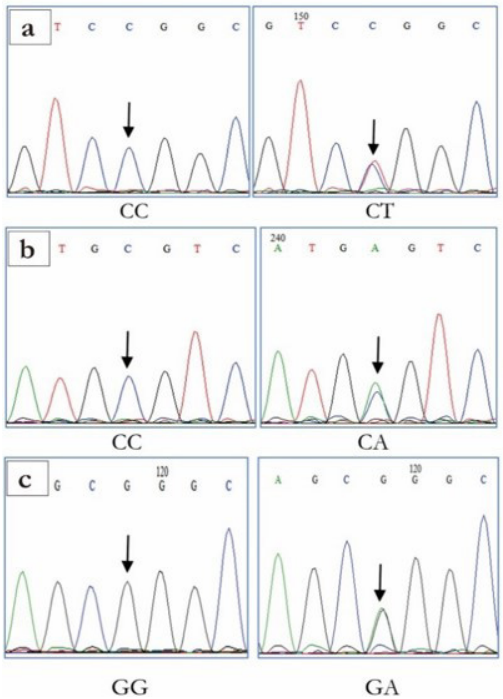

Figure 6. Chromatogram of genotypes for SNPs g.558C>T (a), g.649C>A (b), and g.1578G>A (c).

Table 3. The association study of *GH* gene polymorphisms to several biometric traits (body measurement) in Sumbawa cattle.

Locus	Genotype	HL (cm)	HW (cm)	BL (cm)	SH (cm)	CC (cm)	CW (cm)	CD (cm)	PH (cm)	PW (cm)	HIW (cm)
g.442C>T	CC (71)	45.59 ± 4.31^{b}	19.45 ± 1.65	132.70 ± 11.90^{a}	119.52 ± 9.00^{a}	194.51 ± 30.12	31.41 ± 4.06	64.99 ± 6.57	124.93 ± 10.36^{c}	40.17 ± 4.69	34.55 ± 3.84
	TT (4)	45.96 ± 6.87^{a}	19.53 ± 2.55	132.81 ± 22.58^{a}	118.91 ± 18.17^{b}	194.54 ± 33.62	30.05 ± 5.41	66.08 ± 11.81	126.45 ± 15.66^{a}	38.19 ± 8.20	34.05 ± 4.95
	CT (31)	45.43 ± 5.77^{b}	19.82 ± 2.48	132.29 ± 15.19^{b}	119.80 ± 10.99^{a}	190.91 ± 30.97	30.91 ± 5.38	64.98 ± 8.05	125.21 ± 11.54^{b}	39.19 ± 7.38	33.97 ± 5.21
g.446G>C	CC (13)	45.58 ± 4.98	19.73 ± 1.93	132.00 ± 13.47	119.70 ± 10.36	193.69 ± 31.01	31.38 ± 4.63	65.09 ± 7.52	124.84 ± 10.77^{b}	39.66 ± 5.90	34.14 ± 4.52
	GG (88)	46.98 ± 1.74	18.55 ± 2.06	138.57 ± 5.15	121.51 ± 4.87	200.73 ± 24.00	30.97 ± 1.91	64.38 ± 3.08	130.01 ± 6.26^{a}	41.76 ± 3.29	35.62 ± 2.12
	GC (5)	41.36 ± 5.75	19.22 ± 1.18	127.31 ± 20.08	112.46 ± 9.45	170.50 ± 22.60	28.88 ± 6.67	65.66 ± 9.39	116.28 ± 15.74^{c}	37.44 ± 6.56	34.99 ± 4.16
g.558C>T	CC (98)	45.70 ± 4.77	19.57 ± 1.97	133.06 ± 13.40	119.82 ± 9.94	195.62 ± 29.35^{a}	31.25 ± 4.36	65.25 ± 7.26	125.38 ± 10.82	39.95 ± 5.82	34.47 ± 4.40
	CT (8)	43.81 ± 5.46	19.48 ± 1.66	126.72 ± 9.63	116.57 ± 9.47	166.96 ± 29.96^{b}	30.72 ± 6.22	62.39 ± 5.59	121.22 ± 10.55	38.13 ± 3.60	33.00 ± 2.13
g.1510C>A	CC (85)	45.57 ± 4.50	19.67 ± 1.96	132.33 ± 12.40	118.84 ± 8.70	193.57 ± 29.68	30.96 ± 4.46	64.80 ± 6.84	124.93 ± 9.59	39.28 ± 5.23	33.75 ± 3.80
	CA (15)	45.64 ± 4.78	19.00 ± 1.81	134.41 ± 13.53	118.89 ± 9.18	188.17 ± 29.30	30.83 ± 3.00	63.81 ± 4.44	124.80 ± 12.61	40.60 ± 5.00	34.92 ± 2.96

Notes: Data provided in mean ± standard deviation. HL (head length), HW (head width), BL (body length), SH (shoulder height), CC (chest circumference), CW (chest width), CD (chest depth), PH (pelvic height), PW (pelvic width), and HIW (hip width).

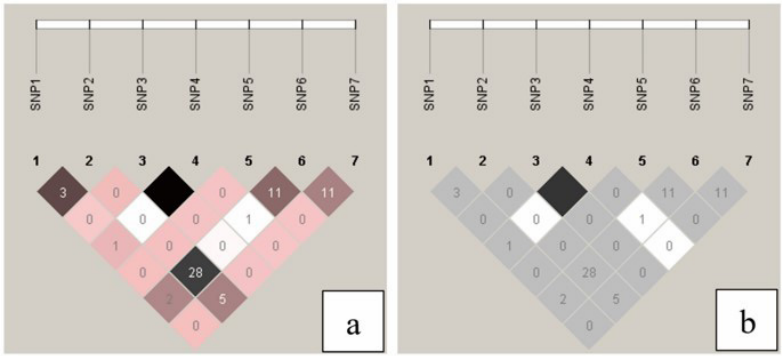

Figure 7. Visualization of LD block based on (a) LOD / D' and (b) confidence bounds.

g.442C>T showed a statistically significant ($p < 0.05$) effect on HL, BL, SH, and PH. Except for shoulder height (SH) (118.91 ± 18.17 cm), the TT genotype showed higher values for HL (45.96 ± 6.87 cm), BL (132.81 ± 22.58 cm), and PH (45.96 ± 6.87 cm) when compared to the CC and CT genotypes. Similarly, the SNP g.446G>C correlated with pelvic height (PH), with the GG genotype (130.01 ± 6.26 cm) showing the highest PH, followed by CC (130.01 ± 6.26 cm) and GC genotypes (116.28 ± 15.74 cm). For the SNP g.558C>T, animals with homozygous CC had a higher chest circumference (CC) than those with the heterozygous genotype.

4. Discussion

In association research, single nucleotide polymorphisms (SNPs) are the most prevalent genetic variants (Meuwissen et al., 2022). In this study, seven SNPs of the *GH* gene were identified in Sumbawa cattle (Figures 5 and 6). The study of *GH* gene polymorphisms has been widely reported in various livestock species, including cattle, pigs, goats, sheep, and chickens. For cattle, several polymorphic sites have been reported at different locations, similar to the SNPs identified in this study. The *GH-Alu*I site, a variant located in exon five, has been reported as polymorphic in Kazakh white-headed cattle, Russian Hereford cattle, Limousin x Madura cattle, Zavot, East Anatolian Red, Simmental, and Brown Swiss cattle in Turkey, and Kerman Holstein cattle (Dzhulamanov et al., 2019; Hartatik et al., 2013; Korkmaz Ağaoğlu and Akyüz, 2013; Mohammadabadi et al., 2010; Selionova and Plakhtyukova, 2020). There were six SNPs, namely g.1304C>T, g.2346C>T, g.2537G>T, g.1059C>T, g.1547C>T, and g.2141C>G (based on their positions in GenBank acc number M57764) reported in Butana and Kenana cattle of Sudan (Musa et al., 2013). However, only the SNP g.1059C>T was similarly found in Sumbawa cattle (named SNP g.558C>T in this study).

The SNPs' frequencies of each genotype and allele were in equilibrium except for SNP g.446G>C and g.1492C>A. Pearson's $\chi 2$ compatibility test assesses whether a sample randomly selected from a population satisfies Hardy-Weinberg equilibrium. However, if the sample size is insufficient, the test may not yield reliable results (Shriner, 2011). Different genotype frequencies have been reported for various SNPs of the *GH* gene in other cattle populations, influenced by genetic backgrounds and breeding practices. In the Fedota et al. (46) study, examining the Aberdeen-Angus cattle population unveiled intriguing insights into specific SNPs' equilibrium and disequilibrium states (single nucleotide polymorphisms). Notably, the research shed light on the equilibrium status of SNPs g.2141C>G and g.257A>G within this cattle population, suggesting a balanced occurrence of these genetic variations. Conversely, SNP g.914T>A exhibited a disequilibrium, indicating a non-random distribution of this particular SNP. A thorough analysis of the researchers revealed the absence of any linkage disequilibrium between the SNPs g.914T>A and g.257A>G, suggesting an independent occurrence of these two genetic markers. Agung et al. (2017) unveiled an allele frequency of 0.87 for the A allele and 0.13 for the B allele at the *GH-Msp*I site. Moreover, the investigation delved into genotype frequencies within the SO cattle population, revealing that the most prevalent genotype is AA, accounting for 0.76 of the population. In contrast, the least common genotype is BB, representing a mere 0.02. In a different population (Pesisir cattle), the allele frequencies of *Msp*I- and *Msp*I+ (*GH-Msp*I site) were 53.3% and 46.7%, respectively (Hartatik et al., 2018). A different result was found in Grati-Bali cattle, which have a monomorphic *GH-Msp*I site genotype (Hartati et al., 2021b).

A solid dark black shade in both representations highlights a distinct genetic block involving SNP g.558C>T and g.649C>A, indicative of a robust linkage disequilibrium between these two specific SNPs (Figure 7). This strong LD is further supported by high r^2 (1.00) and LOD (10.78) values, in line with Yan et al.'s (2017) findings that a positive LOD score indicates the presence of linkages, with a score greater than 3, suggesting evidence of association. This pronounced genetic association potentially signifies shared evolutionary origins or functional interconnections between these markers. Meanwhile, the white color (Figure 7b) represents strong evidence of recombination (Barrett, 2009). Combinations like g.446G>C and g.649C>A, g.649C>A and g.1510C>A, and g.649C>A and g.1578G>A were observed to form blocks displaying strong evidence of recombination. These intriguing findings suggest dynamic genetic interactions within these segments, hinting at complex genetic exchange

processes during evolution and contributing to the diversity of the genetic pool. Al-Thuwaini et al. (2021) state that linkage disequilibrium (LD) refers to the non-random assortment of alleles at different loci.

These findings of genotype-phenotype correlations with body measurements in Sumbawa cattle are consistent with previous reports in other cattle breeds (Table 3). The homozygous dominant genotypes tend to have higher measurement values than the heterozygous and recessive ones. In Nangyang cattle, the BB genotype of the GH-P5 site (located in the promoter region) was associated with higher body length and height from 6 to 18 months (Gao et al., 2006). Differently, in an association study of *GH* polymorphisms with body measurements in crossbred Red Angus × Kalmyk heifers, Kayumov et al. (2019) reported that the GH^{LV} genotype showed higher wither height, hip height, body length, chest width, and chest circumference than the GH^{VV} genotype. The *GH-Alu*I site also correlated with chest girth in Holstein young bulls (Çinar et al., 2018). In Chinese Debian cattle, a noteworthy investigation uncovered correlations between six SNPs (single nucleotide polymorphisms) of the *GHRHR* gene and distinctive body conformation traits. Notably, the Hap3/5 variant (-GCCCCCGGAAGG-) displayed a pronounced association with heightened wither height, hip height, heart girth, and hip width, underlining genetic factors' significance in shaping these anatomical features (Zhao et al., 2022). Intronic mutations within the *SH2B2* gene, which regulates body weight by enhancing GH signaling, were associated with body length and chest circumference in Qinchuan cattle (Raza et al., 2020). Another study by Putra et al. found that the GG genotype of the bovine *GHR* gene SNP g.3338A>G had the lowest body measurements compared to other genotypes. This study's findings confirm Curi et al.'s (2006) statement that the *GH* gene's effect regulates post-natal growth and stimulates the anabolic process of skeletal growth. The animal having a genotype of SNPs with high biometric measurement values could be selected for further mating systems of Sumbawa cattle to pass its superior characteristic to the next generation.

5. Conclusion

The *GH* gene was found to be correlated with biometric traits in Sumbawa cattle, particularly with larger-framed exterior characteristics associated with the presence of the C allele at specific *GH* gene loci. These findings suggest that the polymorphisms detected within the *GH* gene of Sumbawa cattle could hold significance for selection programs aimed at enhancing desirable biometric traits within the population. Enhancing livestock productivity can be achieved through a comprehensive understanding of genetic diversity and its interplay with phenotypic traits.

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