Research Article

Integration of Leptin Gene Polymorphism and Expression with Essential Amino Acid Supplementation in Relation to Growth, Feed Efficiency, Meat Quality, and Fatty Acid Composition in Bali Cattle

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Abstract | The role of leptin in regulating energy balance and lipid metabolism is crucial for improving meat quality and production efficiency in livestock. This study aimed to examine the genetic variation and mRNA expression of the leptin gene (LEP) in Bali cattle and its relationship with growth traits, feed efficiency, carcass quality, and fatty acid composition. A total of 30 male Bali cattle were supplemented with essential amino acids lysine and methionine throughout the study. LEP genotypes (AA, AG, GG) were identified using PCR-RFLP, and mRNA expression was measured via qRT-PCR in cattle stratified by residual feed intake (RFI). The GG genotype showed the best growth performance and lowest RFI, suggesting better feed efficiency. The AG genotype had the highest redness (a*) value, indicating a more desirable meat color. The AA genotype had higher levels of polyunsaturated fatty acids (PUFAs), such as linoleic acid, EPA, and DHA, which are beneficial to human health. LEP gene expression was higher in animals with high RFI, especially those with the AG genotype, indicating a potential regulatory response to lower metabolic efficiency. These results suggest that the combination of LEP genotype and amino acid supplementation influences performance, meat quality, and fatty acid traits in Bali cattle. The LEP gene may serve as a useful genetic marker in precision breeding programs for Bali cattle populations, serving as a valuable genetic resource for developing high-nutritional, sustainable meat production in tropical regions.

Keywords | Bali cattle, Essential amino acids, Fatty acids composition, Feed efficiency, Leptin

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INTRODUCTION

Bali cattle (*Bos sondaicus*) constitute are indigenous genetic resource in Indonesia. They are derived from domesticated wild bulls and are officially recognized as local livestock by the Ministry of Agriculture of the Republic of Indonesia (Hikmawaty *et al*., 2020; Kurlyana *et al*., 2023). These cattle are known for their high adaptability to tropical environments, efficient utilization of fibrous feeds, and superior carcass yield, making them highly relevant for sustainable livestock systems (Jakaria *et al*., 2017; Purwantara *et al*., 2012). To improve the productivity of local breeds, the integration of molecular genetics offers a promising strategy to enhance economically important traits such as growth, feed efficiency, and meat quality (Syarifulaya and Sriasih, 2015).

Meat quality is a multifactorial trait that encompasses sensory attributes (e.g., tenderness, marbling, color, flavor, and water binding) and nutritional component such as fatty acid composition (Gunawan *et al*., 2021; Listyarini *et al*., 2022). The composition of unsaturated fatty acids, specifically monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), plays a vital role in enhancing flavor, oxidative stability, and promoting human health (Gunawan *et al*., 2021; Muroya *et al*., 2020).

A dual approach incorporating genetic markers alongside optimized nutritional strategies, particularly the supplementation of key amino acids, is essential. Lysine functions as a primary precursor in protein synthesis, promoting the growth of body tissues and enhancing nitrogen retention, thereby directly contributing to metabolism and feed efficiency (Jin *et al*., 2022; Liao *et al*., 2015). Methionine plays a crucial role in transmethylation reactions, lipid metabolism, and antioxidant defenses that collectively enhance feed efficiency and growth (Martínez *et al*., 2017; Zhou *et al*., 2016). Supplementing these amino acids has been shown to improve growth, feed efficiency, and intramuscular fatty acid composition, characterized by increased MUFAs and PUFAs and reduced saturated fats (Alfonso et al., 2023; Yi *et al*., 2025). These effects are associated with upregulation of lipid metabolism genes like FADS1 and FADS2, which are also known to modulate leptin expression (Jump, 2002).

Leptin is a protein hormone with a molecular weight of 16 kDa, comprising 167 amino acids (Kurlyana *et al*., 2023; Nugroho *et al*., 2022). Leptin, secreted by adipose tissue, regulates homeostatic energy balance, body weight, feed intake, and lipid metabolism (Anugratama and Hartatik, 2019; Avondo *et al*., 2019; Friedman, 2014; Münzberg and Heymsfield, 2019). Furthermore, leptin is also present in epithelial cells within the stomach, constituting a significant factor associated with growth rate, body mass, and meat quality, such as carcass fat deposition in cattle (Zalewska *et al*., 2021). Genetic polymorphisms in the LEP gene have been linked to variations in feed efficiency, growth rate, marbling, and fatty acid profiles in several cattle breeds (Kawaguchi *et al*., 2017; Sedykh *et al*., 2020; Wang *et al*., 2022).

The LEP gene is of particular interest due to its pleiotropic effects on growth, fat metabolism, and overall production efficiency (Mukherjee *et al*., 2023). Certain genotypes, such as GG or AG, are often associated with lower residual feed intake (RFI), a trait reflecting better feed conversion and energy efficiency (Mota *et al*., 2017; Nugroho *et al*., 2022). Despite extensive research on LEP polymorphisms in Bos taurus cattle, their relevance and biological impacts in tropical indigenous breeds like Bali cattle remain poorly understood. This gap is particularly evident in the context of essential amino acid supplementation. The absence of integrative studies combining genetic and nutritional approaches in Bali cattle highlights a key limitation in the current literature. This study addresses this gap by investigating the genetic variation and mRNA expression of the LEP gene in Bali cattle and assessing their association with growth, feed efficiency, meat quality, and fatty acid profile under essential amino acid supplementation. This integrated approach provides a novel perspective on how molecular markers and nutritional strategies can be utilized synergistically to enhance performance and meat quality in tropical cattle. The outcomes are expected to contribute to the development of marker-assisted selection (MAS) and nutrigenomic tools suitable for local breeding programs aimed at sustainable and high-nutritional beef production in tropical environments.

MATERIALS AND METHODS

This study aimed to analyze the polymorphism and mRNA expression of the leptin gene (LEP) and their associations with growth performance, feed efficiency, meat quality, and fatty acid composition in male Bali cattle. As part of a precision nutrition strategy, essential amino acid supplementation specifically lysine and methionine was applied to evaluate its influence on these parameters. The supplementation was administered as a daily premix consisting of lysine and methionine with bran, each package 150 grams per head. It was provided each morning before basal feeding with elephant grass forage (*Pennisetum purpureum*), which contained 53.1% total digestible nutrients (TDN) and 11.4% crude protein. This approach aimed to support the anabolic phase and enhance protein synthesis and energy utilization efficiency. The commercial amino acid products used were Smartlys 300 (30% rumen-protected lysine) and SmartMet 600

(60% rumen-protected methionine) by Zhejiang Vega Bio-technology Co., Ltd. All experimental protocols were approved by the Animal Ethics Committee of Hasanuddin University (No. UH21070474).

Animal and experimental design

A total of 30 bulls of Bali cattle, with an average age of 27.10 ± 0.69 months and an initial body weight of 196.78 ± 5.12 kg. were housed for 70 days in individual enclosures measuring 1.5 x 2m at the Maiwa Breeding Center, Hasanudddin University, Barru Regency (Longitude 4°11'38.3" S, 119°40'18.2" E). All subjects received the same feed and were maintained under consistent environmental conditions. Their main diet consisted of elephant grass forage (*Pennisetum purpureum*), provided ad libitum, with free access to water throughout the day. The supplementation included essential amino acids, specifically lysine (13.3 g/head/day) and methionine (6.7 g/head/day), in addition to a mixture of bran (130 g/head/day), totaling 150 g/head/day, administered each morning before the basal feeding.

Dry matter intake (DMI) was calculated based on the average actual consumption, calibrated to the weekly dry matter content. The average DMI from elephant grass alone was 4.71±0.61 kg/head/day. Body weight was measured weekly before feeding, and water intake was monitored during the study period. Metabolic body weight (MBW) was calculated as $BW^{0.75}$ (Kleiber, 1932). The feed conversion ratio (FCR) was calculated by dividing the total feed consumption (dry matter intake, DMI/kg) by the body weight gain (kg) (National Research Council, 2000). Residual Feed Intake (RFI) was determined as the residual from a linear regression model of dry matter intake (DMI) against average daily gain (ADG) and metabolic body weight (MBW) (Koch *et al*., 1963). To determine representative samples for advanced analysis, individual weekly data on body weight and feed intake were collected over the 10th week (70th day) period. These data were used to calculate individual RFI values, based on which two groups were established: high RFI (HRFI; n=3) and low RFI (LRFI; n=3). At the end of the study, these six animals were selected for slaughter and further analysis, including meat quality analysis, fatty acid composition analysis using GC-MS, and qRT-PCR for LEP mRNA expression.

The digestibility of dry and organic matter is calculated based on the acid-insoluble ash content method acid-insoluble ash (AIA) (Van Keulen and Young, 1977). All animals received the same supplementation; thus, the study design did not include a control group without supplementation. Additionally, the limited sample size used in the qRT-PCR analysis (n=3 per RFI group) is recognized as a constraint, which may influence the generalizability of the meat quality findings and gene expression.

Meat quality analysis

Out of the total population, 27 animals that met the inclusion criteria were selected for further analysis. The slaughtering process was conducted under the SNI good slaughtering practice standard 01-6159-1999, by halal-certified slaughterers. Samples of the longissimus dorsi muscle were collected and subjected to color analysis based on the CIE-LAB system (L*, a*, b*) after the blooming process, utilizing a colorimeter (Van Laack et al., 2000). Measurements included pH (24 hours *postmortem*), tenderness (raw meat and cooked meat) assessed by Warner Bratzler Shear Force (WBSF)(Shackelford *et al*., 1997), cooking loss, and water holding capacity (WHC) (Dagong *et al*., 2012; Hamm, 1961).

Fatty acid composition analysis

The composition of fatty acids was analyzed utilizing the gas chromatography-mass spectrometry (GC-MS) technique. Lipids were extracted employing the Soxhlet extraction method (Bligh and Dyer, 1959), and subsequently methylated into FAMEs utilizing methanol sulfate (Morrison and Smith, 1964). Compound identification was conducted using the NIST library, and quantification was determined based on the peak area relative to internal standards (Kind and Fiehn, 2010). The fatty acids identified include saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The analysis was performed in a laboratory adhering to Good Laboratory Practice standards (OECD, 1997).

Polymorphism determination of leptin gene

This study employed the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method to identify polymorphisms in the leptin (LEP) gene with high accuracy. Blood samples were collected from thirty bulls, and genomic DNA was extracted using the Geneaid Genomic DNA MiniKit following the manufacturer's protocol. A novel polymorphism within the LEP gene was identified through high-throughput sequencing, and specific primers were designed based on the Bos taurus reference sequence (GenBank HE605298), as reported by Choudhary *et al*. (2005). The complete primer sequences are listed in Table 1. PCR amplification was conducted in a 25 µL reaction volume containing 2 µL of genomic DNA, 2 µL of forward and reverse primers, 12,5 µL of MyTaq Red Mix, and 8,5 µL of nuclease-free water. The thermocycling conditions on the Sensoquest PCR system were as follows: initial denaturation at 94°C for 5 minutes; 35 cycles of 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 1 minute; and a final extension at 72°C for 5 minutes. The PCR-RFLP approach involves digesting PCR amplicons with appropriate restriction enzymes to produce distinct polymorphic fragments used as species identification markers (Wolf et al., 2000).

Table 1: Genbank accession number and primer sequences of the Leptin gene (PCR-RFLP and qRT-PCR).

Gene name	Accesion number	Primer sequence	Application	Size (bp)	TA (°C)	Restriction enzyme	Digested fragments length (bp)
Leptin	HE605298	F:5'-GTC TGG AGG CAA AGG GCA GAG T-3'	PCR-RFLP	522	60	BsaAI	AA: 522; AG: 522, 441 and 81; GG: 441 and 81
		R: 5'-CCA CCA CCT TGG AGT AG-3'					
Leptin	NM_173928.2	F: 5'- CTACTTGTGCTCAGCCCCAA -3'	qRT-PCR	155	55		
		R: 5'- TGCTCAGTTGCCATGGTGAA -3'					
GAPDH	NM_001034034.2	F: 5'- ACAGTCAAGGCAGAGAACGG -3'	qRT-PCR	235	55		
		R: 5'- GGTTCACGCCCATCACAAAC -3'					

PCR-RFLP analysis involved digesting 5 µL of PCR product with 0.3 µL of B*sa*AI restriction enzyme (New England Biolabs, Catalog No. R0109S), 0.7 µL of buffer, and 1 µL of nuclease-free water, in a final volume of 7 µL. The reaction was incubated at 37 °C overnight. Genotyping was based on fragment length: AA genotype yielded a single 522 bp band; AG genotype yielded three bands (522, 441, and 81 bp); and GG genotype yielded two bands (441 and 81 bp).

Leptin gene mRNA expression analysis

Total RNA was extracted from liver tissues of six Bali cattle exhibiting the highest (n=3) and lowest (n=3) residual feed intake (RFI) values. RNA extraction yielded 1 µg of total RNA per sample. First-strand cDNA was synthesized using the First Strand Transcriptor Synthesis Kit (Thermo Fisher Scientific, Vilnius, Lithuania). One microliter (1 µL) of synthesized cDNA was used per 20 µL qRT-PCR reaction. Quantitative real-time PCR (qRT-PCR) was performed using gene-specific primers for LEP (NM_173928.2) and the iTaq SYBR Green Master Mix with Rox PCR core reagents (Bio-Rad). GAPDH was used as the reference gene for normalization. Relative gene expression was calculated using the ΔCt method, which is the difference in threshold cycles (Ct) between the LEP gene and GAPDH (Harahap *et al*., 2024; Listyarini *et al*., 2022; Silver *et al*., 2006).

Statistical analysis

Genotype and allele frequencies were calculated using Nei and Kumar (2000) method, and Hardy-Weinberg equilibrium was assessed using the chi-square test (Hartl and Clark, 1990). Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test before analysis of variance (ANOVA). Associations between leptin genotypes and variables related to growth performance, feed efficiency, meat quality, and fatty acid composition were analyzed using the General Linear Model (GLM) procedure in SAS software version 9.2. Post-hoc comparisons among genotypes were performed using Duncan's multiple range test, which was selected due to its appropriateness for small sample sizes. However, the limitations of Duncan's test are acknowledged, and alternative post-hoc methods such as Tukey's HSD are recommended for future studies to improve robustness. mRNA expression levels were compared using a two-tailed independent t-test. Additionally, Pearson's correlation analysis was conducted to evaluate the relationships between gene expression levels and residual feed intake (RFI) to explore the potential regulatory role of the LEP gene in metabolic efficiency.

RESULT AND DISCUSSION

Polymorphism of the leptin gene with essential amino acid supplementation

PCR-RFLP amplification of the leptin gene using the BsaAI restriction enzyme produced clear fragment patterns: 522 bp for genotype AA; 522, 441, and 81 bp for genotype AG; and 441 and 81 bp for genotype GG (Figure 1). The figure illustrates representative PCR-RFLP results from a subset of the samples. Genotype distribution showed a predominance of AG (57%), followed by GG (33%) and AA (10%). Allele G had a higher frequency (62%) compared to allele A (38%). The chi-square value was 1.193, indicating that the leptin gene in this Bali cattle population is in Hardy-Weinberg equilibrium. Detailed genotype and allele frequencies are presented in Table 2.

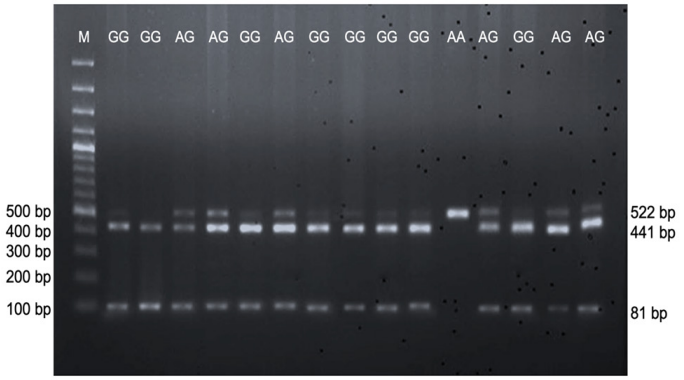

Figure 1: Amplification result of PCR-RFLP genotyping for the Leptin (LEP) gene with *Bsa*AI restriction enzymes. M: The DNA ladder 100 bp size standard; AA, AG, and GG are genotypes; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.

Table 2: The number of animals per genotype and allele frequency in the leptin gene of Bali cattle.

Gen	N	Genotype frequency			Allele frequency		Chi-square (χ^2)
		AA (n)	AG (n)	GG (n)	A	G	
Leptin	30	0.10 (3)	0.57 (17)	0.33 (10)	0.38	0.62	1,.193

Table 3: Association of LEP gene polymorphism related to growth and feed efficiency with supplementation of essential amino acids in Bali cattle.

	Genotype (μ±STD) (n=30)			
Parameter	**AA**	**AG**	**GG**	**P-value**
	(n = 3)	**(n = 17)**	**(n = 10)**	
Growth				
IBW (kg)	152.74±6.37[b]	196.54±29.41[a]	211.46±18.88[a]	**0.006
FBW (kg)	190.88±1.23[b]	235.00±29.89[a]	249.16±21.57[a]	**0.008
ADG (kg/day)	0.48±0.11	0.55±0.12	0.54±0.07	0.632
MBW ($kg^{0.75}$)	47.45±0.61[b]	56.20±5.78[a]	59.08±3.88[a]	**0.006
Feed Efficiency				
ADFI (kg DM/day)	4.63±0.49[b]	5.39±0.75[a]	5.45±0.44[a]	0.149
FCR (kg DM/kg gain)	9.95±2.67	10.25±2.62	10.24±1.16	0.977
RFI (kg DM/day)	0.06±0.55	0.05±0.46	-0.10±0.40	0.685
AIA (g DM/day)	42.57±2.14	44.04±4.56	44.16±5.95	0.881

**significant at p<0.01; [ab] Means with the same letter are not significantly different.

Association of the leptin gene-related growth and feed efficiency with essential amino acid supplementation

The results demonstrated significant associations between leptin genotypes and several growth and feed efficiency parameters (Table 3). Initial body weight (IBW) differed significantly among genotypes (p= 0.006), with GG (211.46 ± 18.88 kg) > AG (196.54 ± 29.41 kg) > AA (152.74 ± 6.37 kg). Final body weight (FBW) also showed a significant difference (p = 0.008), with GG (249.16 ± 21.57 kg) higher than AG (235.00 ± 29.89 kg) and AA (190.88 ± 1.23 kg). Metabolic body weight (MBW) was significantly different (p= 0.006), with GG (59.08 ± 3.88 kg) > AG (56.20 ± 5.78 kg) > AA (47.45 ± 0.61 kg).

Although average daily gain (ADG) did not differ significantly among genotypes (p = 0.632), the AG genotype recorded the highest value (0.55 ± 0.12 kg), followed by GG (0.54 ± 0.07 kg) and AA (0.48 ± 0.11 kg). Similarly, average daily feed intake (ADFI) did not differ significantly (p = 0.149), though GG (5.45 ± 0.44 kg) and AG (5.39 ± 0.75 kg) showed numerically higher values than AA (4.63 ± 0.49 kg). Feed conversion ratio (FCR) also showed no significant difference (p = 0.977) across genotypes: GG (10.24 ± 1.16), AG (10.25 ± 2.62), and AA (9.95 ± 2.67). Residual feed intake (RFI) did not differ significantly (p = 0.685), with the lowest (i.e., most efficient) value in GG (-0.10 ± 0.40), followed by AG (0.05 ± 0.46) and AA (0.06 ± 0.55). Dry matter digestibility as estimated by the AIA index also showed no significant difference (p = 0.881) among genotypes: GG (44.16± 5.95), AG (44.04±4.56), and AA (42.57±2.14). Overall, the GG genotype was associated with more favorable values in several key growth and feed efficiency traits, though not all comparisons were statistically significant. Full results, including exact p-values for all parameters, are provided in Tables 3 and 4.

Association of leptin-related meat quality with essential amino acids supplementation

Meat quality analysis revealed a statistically significant difference in the red color component (a*) among genotypes (p = 0.049), with AG (12.87 ± 1.57) showing the highest value, followed by AA (12.07 ± 2.64) and GG (10.95 ± 1.74). No significant differences were observed for lightness (L*, p = 0.665) and yellowness (b*, p = 0.689). The ultimate pH also did not differ significantly among genotypes (p = 0.552). Measurements of tenderness did not show significant differences in either raw (p = 0.975) or cooked meat (p = 0.179) shear force. AG genotypes showed the highest raw shear force (1.03 ± 0.19), while cooked meat shear force was highest in GG (2.05 ± 0.23). Cooking loss values were also statistically non-significant (p = 0.640), with AG (30.73 ± 3.22), GG (32.00 ± 2.62), and AA (31.41 ± 4.50). Water holding capacity (WHC) did not significantly vary (p = 0.244) between genotypes: GG (29.99 ± 2.91), AG (27.46 ± 3.26), and AA (30.48 ± 9.03).

Table 4: Association of LEP gene polymorphism related to meat quality with supplementation of essential amino acids in Bali cattle.

	Genotype (μ±STD) (n=27)			
Traits	**AA (n=3)**	**AG (n=15)**	**GG (n=9)**	**P-value**
Colour				
L*	37.38±6.04	39.25±3.95	40.12±5.06	0.665
a*	12.07±2.64[a]	12.87±1.57[a]	10.95±1.74[a]	*0.049
b*	7.78±1.87	6.99±1.11	7.07±1.78	0.689
pH	5.44±0.40	5.37±0.24	5.51±0.34	0.552
Tenderness				
Raw meat	1.00±0.07	1.03±0.19	1.02±0.08	0.975
Cooked meat	1.68±0.51	2.05±0.23	1.95±0.36	0.179
Cooking loss (%)	31.41±4.50	30.73±3.22	32.00±2.62	0.640
WHC (%mgH_2O)	30.48±9.03	27.46±3.26	29.99±2.91	0.244

*significant at $p<0.05$; [ab] Means with the same letter are not significantly different.

Association of leptin-related fatty acid composition with essential amino acids supplementation

Significant differences in fatty acid composition were observed among genotypes. Within the saturated fatty acid (SFA) group, myristic acid (C14:0) differed significantly (p= 0.005), with highest levels in GG (15.29 ± 5.46). Heptadecanoic acid (C17:0) also differed significantly (p= 0.042). Lauric acid (C12:0) and heneicosanoic acid (C21:0) approached significance (p= 0.051). No significant differences were found for palmitic acid (C16:0, p= 0.140) or arachidic acid (C20:0, p= 0.120), though AA had numerically higher levels. In the MUFA group, *cis*-11-eicosenoic acid (C20:1) showed a significant difference (p= 0.024), while total MUFA content was not significantly different (p= 0.074). For PUFA, linoleic acid (C18:2n6c) (p = 0.002), EPA (C20:5n3, p = 0.045), DHA (C22:6n3, p = 0.006), and *cis*-13,16-docosadienoic acid (C22:2, p= 0.038) all differed significantly. Total PUFA content also showed a significant difference among genotypes (p= 0.044), with the highest in GG, followed by AG and AA. To further illustrate these findings, a heatmap visualization was generated to represent the distribution of fatty acid concentrations across the three leptin genotypes (Figure 2). The heatmap highlights distinct clustering patterns and fatty acid profiles that vary with genotype.

Messenger RNA (mRNA) expression of the leptin gene by qRT-PCR

Analysis of mRNA expression using qRT-PCR revealed a statistically significant difference between residual feed intake (RFI) groups (p= 0.041). Cattle with high RFI (HRFI), primarily of the AG genotype, exhibited higher leptin mRNA expression levels compared to low RFI (LRFI) cattle, which were predominantly of the GG genotype. The AA genotype was not represented in either group. Although only six animals (n= 3 per group) were analyzed, the observed differences suggest a potential regulatory response related to feed efficiency. Gene expression data corresponding to RFI groupings are visually presented in Figure 3. These results should be interpreted with caution due to the limited sample size, and further studies with larger cohorts are recommended to validate the findings.

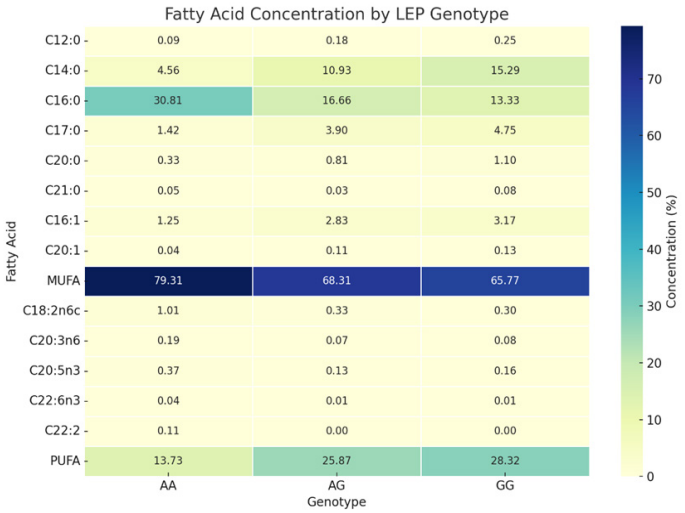

Figure 2: Heatmap visualization of fatty acid concentration (% of total fat) across LEP genotypes (AA, AG, GG). Color intensity represents relative concentration levels for each fatty acid. Data reflects mean values per genotype based on GC-MS analysis.

Leptin (LEP) gene polymorphism plays a pivotal role in regulating energy balance and lipid metabolism, which directly influences growth performance and meat quality in livestock. The present study identified three genotypes AA, AG, and GG with AG being the most prevalent in Bali cattle. This genetic diversity corresponds with previous findings in tropical cattle populations and reinforces the LEP gene's utility as a molecular marker for breeding programs (Kurlyana *et al.*, 2023; Putra and Indriastuti, 2018).

Bali cattle with the GG genotype showed significantly higher initial, final, and metabolic body weights compared to other genotypes, suggesting a growth performance advantage. These findings are in line with prior studies linking the GG genotype to improved growth metrics in various beef cattle breeds (Choudhary *et al.*, 2005; Nkrumah *et al.*, 2005). The enhanced growth performance may be attributed to the influence of leptin on energy metabolism and its downstream activation of growth-related signaling pathways, including JAK/STAT and mTOR (Hamrick, 2017; Wen *et al.*, 2021; Zhou *et al.*, 2016).

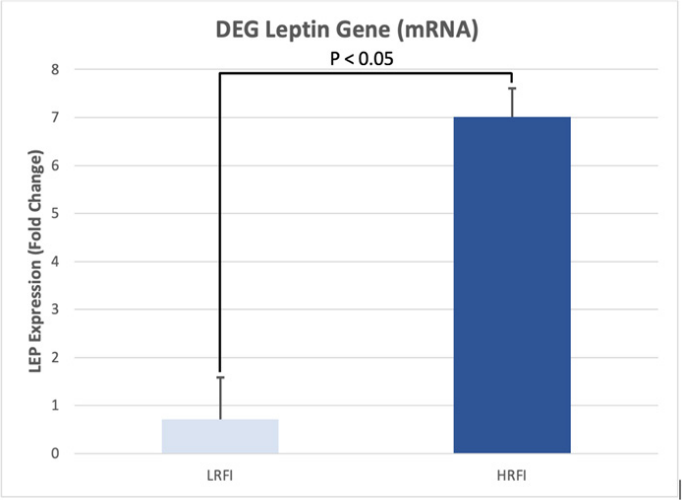

Figure 3: Relative mRNA expression of the leptin (LEP) gene in liver tissue of Bali cattle divergently selected for feed efficiency (High RFI and Low RFI), measured at day 70 of the experiment. Gene expression levels were normalized to GAPDH. Error bars represent standard deviation. A significant difference was observed between groups ($P < 0.05$).

Despite non-significant differences in average daily feed intake (ADFI) and feed conversion ratio (FCR), the GG genotype consistently displayed the lowest residual feed intake (RFI), indicating greater metabolic efficiency. This aligns with findings by Mota *et al.* (2017) and Ujan *et al.* (2024), highlighting RFI as a robust indicator of feed efficiency. The upregulation of metabolic genes induced by lysine–methionine supplementation is thought to support this efficiency by improving nutrient absorption and utilization (Zhao *et al.*, 2019; Mota *et al.*, 2022).

Feed digestibility measured using the acid-insoluble ash (AIA) method did not vary significantly across genotypes, suggesting that post-absorptive metabolic processes are likely responsible for differences in feed efficiency. While the AIA method is commonly employed, its limited sensitivity to post-absorptive variation is recognized, suggesting future studies should consider using multi-marker digestibility systems for improved precision (Cantalapiedra-Hijar *et al.*, 2018; Connor *et al.*, 2013; Kim *et al.*, 2020).

Regarding meat quality, only the a* value (redness) differed significantly among genotypes, with AG showing the highest value. This parameter, associated with myoglobin content and oxidative stability, is a critical attribute influencing consumer preference (Henriott *et al.*, 2020; Yu *et al.*, 2017). Other meat quality traits such as pH, tenderness, cooking loss, and water holding capacity showed no significant differences, indicating similar muscle structure and postmortem metabolism across genotypes (Wu *et al.*, 2020; Zalewska *et al.*, 2021).

Fatty acid profile analysis revealed significant genotype-dependent differences in lipid metabolic profiles. The GG genotype exhibited higher concentrations of saturated fatty acids, particularly myristic (C14:0) and heptadecanoic acids (C17:0), implying increased lipogenesis and intramuscular fat accumulation. This is consistent with leptin's regulatory role in activating lipogenic enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (He *et al.*, 2020; Park and Ahima, 2014).

Supplementation with lysine and methionine further enhances these pathways, promoting intramuscular fat deposition (Haruna *et al.*, 2021; Otto *et al.*, 2022; Zhao *et al.*, 2019). This observation may reflect a metabolic inclination of GG genotypes toward enhanced fat synthesis under targeted amino acid feeding regimes. These patterns align with the known role of leptin in adipogenesis and energy balance, especially when nutrient input modulates gene expression (Mota *et al.*, 2022). Conversely, Bali cattle with the AA genotype showed higher levels of polyunsaturated fatty acids (PUFAs), including linoleic acid (C18:2n6c), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These long-chain PUFAs contribute to improved nutritional value of meat and are associated with cardiovascular and anti-inflammatory benefits in human health (Djuricic and Calder, 2023; Wu *et al.*, 2020).

The enrichment of these PUFAs in AA genotypes suggests more efficient desaturation and elongation processes, which may be regulated through genotype-specific LEP expression pathways interacting with nutritional signals (Vailati-Riboni *et al.*, 2019; Wood and Enser, 2017). These results suggest increased desaturase and elongase activity, potentially regulated by LEP gene expression and influenced by essential amino acid availability (Lee *et al.*, 2017). Detailed findings for 43 fatty acids across genotypes (AA, AG, GG) are presented in Table 5, which reports mean values ± standard deviation (% total fat) alongside ANOVA-derived p-values. Additional, profiles of fatty acid composition by genotype are provided in Table 6, which further corroborates the existence of genotype-specific variations. Fatty acids are grouped by

Table 5: Association of LEP gene polymorphism related to Fatty acid composition with supplementation of essential amino acids in Bali cattle. The table includes 43 identified fatty acids categorized into saturated, monounsaturated, and polyunsaturated groups. Statistical differences were tested using ANOVA with p-values presented for each comparison.

Fatty acid	Genotype (μ ± STD) (n=27)			P-value
	AA (n=3)	AG (n=15)	GG (n=9)	
Fat content	15.76±4.92	26.76±8.44	29.31±9.06	0.132
Saturated fatty acid	4.34±3.77	16.13±31.78	2.87±4.38	0.408
Capric acid, C4:0	0.00±0.00	0.00±0.00	0.00±0.00	0.000
Capric acid, C6:0	0.003±0.005	0.01±0.01	0.01±0.01	0.288
Capric acid, C8:0	0.02±0.01	0.02±0.01	0.02±0.02	0.702
Capric acid, C10:0	0.07±0.03	0.15±0.09	0.16±0.08	0.223
Capric acid, C11:0	0.00±0.00	0.001±0.003	0.002±0.004	0.437
Lauric acid, C12:0	0.09±0.02^{b}	0.18±0.09^{ab}	0.25±0.11^{a}	0.051
Tridecanoic acid, C13:0	0.06±0.05	0.04±0.02	0.09±0.09	0.179
Myristic acid, C14:0	4.56±0.83^{b}	10.93±4.37^{a}	15.29±5.46^{a}	**0.005
Pentadecanoic acid, C15:0	4.03±5.37	1.73±0.86	2.11±1.41	0.172
Palmitic acid, C16:0	30.81±2.87^{a}	16.66±14.35^{ab}	13.33±11.18^{b}	0.140
Heptadecanoic acid, C17:0	1.42±0.63^{b}	3.90±1.58^{a}	4.75±2.42^{a}	*0.042
Stearic acid, C18:0	37.73±4.64	33.63±20.08	28.29±15.85	0.672
Arachidic acid, C20:0	0.33±0.06^{b}	0.81±0.65^{ab}	1.10±0.43^{a}	0.120
Heneicosanoic acid, C21:0	0.03±0.01^{b}	0.05±0.03^{ab}	0.08±0.05^{a}	0.051
Behenic acid, C22:0	0.07±0.02	0.07±0.04	0.11±0.05	0.068
Tricosanoic acid, C23:0	0.03±0.01	0.04±0.02	0.05±0.02	0.129
Lignoceric acid, C24:0	0.04±0.02	0.07±0.16	0.04±0.03	0.824
Unsaturated acid	2.04±1.41^{a}	0.89±0.39^{b}	0.99±0.76^{b}	*0.034
Monounsaturated acid	79.31±4.94^{a}	68.31±8.48^{b}	65.77±9.12^{b}	0.074
Myristic acid, C14:1	0.09±0.02	1.01±2.46	0.43±0.22	0.645
Pentadecanoic acid, C15:1	0.003±0.006	0.01±0.01	0.40±1.16	0.382
Palmitoleic acid, C16:1	1.25±0.46^{b}	2.83±1.31^{a}	3.17±1.38^{a}	0.100
cis-10-Heptadecanoic acid, C17:1	0.22±0.21	0.41±0.38	0.45±0.38	0.635
Elaidic acid, C18:1n9t	0.18±0.31	2.15±4.50	1.38±0.42	0.632
Oleic Acid, C18:1n9c	11.94±3.30	19.34±9.12	22.42±9.11	0.221
cis-11-Eicosenoic Acid, C20:1	0.04±0.01^{b}	0.11±0.05^{a}	0.13±0.04^{a}	*0.024
Erucic acid, C22:1	0.003±0.006	0.01±0.02	0.01±0.01	0.533
Nervonic acid, C24:1	0.003±0.006	0.01±0.03	0.00±0.00	0.723
Polyunsaturated acid	13.73±3.88^{b}	25.87±8.26^{a}	28.32±9.11^{a}	*0.044
Linolelaidic acid, C18:2n9t	0.03±0.06	0.11±0.21	0.13±0.11	0.696
Linoleic acid, C18:2n6c	1.01±0.77^{a}	0.33±0.15^{b}	0.30±0.20^{b}	**0.002
v-Linolenic acid, C18:3n6	0.01±0.01	0.01±0.02	0.01±0.01	0.764
Linolenic acid, C18:3n3	0.00±0.00	0.02±0.02	0.02±0.03	0.024
cis-11,14-Eicosedienoic acid, C20:2	0.00±0.00	0.003±0.011	0.01±0.02	0.437
cis-8,11,14-Eicosetrienoic acid, C20:3n6	0.19±0.13^{a}	0.07±0.04^{b}	0.08±0.10^{b}	0.074
cis-11,14,17-Eicosatrienoic acid, C20:3n9	0.00±0.00	0.001±0.003	0.00±0.00	0.687
Arachidonic acid, C20:4n6	0.37±0.26	0.20±0.12	0.26±0.29	0.369
cis-13,16-Docosadienoic acid, C22:2	0.01±0.02^{a}	0.00±0.00^{b}	0.002±0.007^{b}	*0.038
cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3	0.37±0.23^{a}	0.13±0.09^{b}	0.16±0.18^{b}	*0.045
cis-4,7,10,13,16,19-Docosahexaenoic acid, C22:6n3	0.04±0.03^{a}	0.01±0.01^{b}	0.01±0.02^{b}	**0.006
Total Fat	95.00±0.00	94.68±0.51	94.66±0.49	0.540

*significant at p<0.05; ** significant at p<0.01; ^{ab} Means with the same letter are not significantly different.

Table 6: Profile of fatty acid composition (mean ± STD, % total fat) by LEP genotype (AA, AG, GG) in Bali cattle under essential amino acid supplementation.

Fatty acid	AA (n=3)	AG (n=15)	GG (n=9)	P-value
Lauric acid, (C12:0)	0.09±0.02	0.18±0.09	0.25±0.11	0.051
Myristic acid, (C14:0)	4.56±0.83	10.93±4.37	15.29±5.46	0.005
Palmitic acid, (C16:0)	30.81±2.87	16.66±14.35	13.33±11.18	0.140
Heptadecanoic acid, (C17:0)	1.42±0.63	3.90±1.58	4.75±2.42	0.042
Stearic acid, (C18:0)	37.73±4.64	33.63±20.08	28.29±15.85	0.672
Arachidic acid, (C20:0)	0.33±0.06	0.81±0.65	1.10±0.43	0.120
Heneicosanoic acid, (C21:0)	0.03±0.01	0.05±0.03	0.08±0.05	0.051
Palmitoleic acid, (C16:1)	1.25±0.46	2.83±1.31	3.17±1.38	0.100
cis-11-Eicosenoic Acid, (C20:1)	0.04±0.01	0.11±0.05	0.13±0.04	0.024
Total MUFA	79.31±4.94	68.31±8.48	65.77±9.12	0.074
Linoleic acid, (C18:2n6c)	1.01±0.77	0.33±0.15	0.30±0.20	0.002
Eicosetrienoic acid, (C20:3n6)	0.19±0.13	0.07±0.04	0.08±0.10	0.074
EPA, (C20:5n3)	0.37±0.23	0.13±0.09	0.16±0.18	0.045
DHA, (C22:6n3)	0.04±0.03[a]	0.01±0.01	0.01±0.02	0.006
cis-13,16-Docosadienoic acid, C22:2	0.01±0.02	0.00±0.00	0.002±0.007	0.038
Total PUFA	13.73±3.88	25.87±8.26	28.32±9.11	0.044

class SFAs, MUFAs, and PUFAs enabling structured comparisons and interpretation. Including the full dataset within the main manuscript enhances data accessibility and scientific transparency, in line with publishing best practices (Wadood et al., 2025). The observed genotype-specific FA distribution underscores the influence of genetic variation on nutrient-driven lipid metabolism. Structured reporting of these findings strengthens reproducibility and aligns with evidence from nutrigenomic studies that emphasize the dynamic relationship between gene variants, dietary modulation, and metabolic phenotype (Zhang *et al*., 2022). This approach supports more targeted investigation into LEP-driven lipid pathways and their broader implications for beef quality in tropical cattle breeds.

LEP gene expression analysis revealed significantly higher mRNA levels in the HRFI group (p= 0.041), which consisted entirely of AG genotype individuals. In contrast, the LRFI group was predominantly GG. The elevated LEP expression in HRFI cattle may indicate a compensatory regulatory response to energy imbalance. This is consistent with studies in cattle and other species showing increased LEP expression in animals with higher adiposity and lower feed efficiency (Liu *et al*., 2023; Mota *et al*., 2017; Pérez-Montarelo *et al*., 2013). Although the sample size for qRT-PCR analysis was small (n= 3 per RFI group), the observed gene expression trends are biologically meaningful. These results suggest leptin expression could serve as a dynamic biomarker of energy status and feed efficiency, though protein-level validation is needed.

CONCLUSION

This study confirms that leptin (LEP) gene polymorphisms and mRNA expression are significantly associated with key production traits in Bali cattle, including growth, feed efficiency, meat quality, and fatty acid composition. Genotypic differences influenced both energy utilization and lipid metabolism, with the GG genotype favoring improved feed efficiency, and the AA genotype linked to higher levels of beneficial polyunsaturated fatty acids (PUFAs), such as DHA, EPA, and linoleic acid. Elevated LEP expression in high-RFI cattle (primarily AG genotype) suggests a compensatory mechanism associated with reduced metabolic efficiency. These results underscore the relevance of the LEP gene as a candidate marker for precision breeding programs aimed at enhancing both productivity and meat quality in tropical cattle systems. Future work should include functional validation through LEP knockdown or overexpression, protein-level assessment, and expanded sampling to capture broader phenotypic variability.

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NOVELTY STATEMENT

This study is the first to integrate leptin (LEP) gene polymorphism and mRNA expression analysis with the supplementation of essential amino acids (lysine–methionine) to evaluate their combined effects on growth traits, feed efficiency, meat quality, and fatty acid composition in Bali cattle. It presents novel insights into the nutrigenomic interactions between genetic markers and amino acid-driven metabolic regulation, especially in an indigenous tropical cattle breed. The findings contribute a unique basis for the development of precision breeding and nutritional strategies aimed at enhancing livestock productivity and meat quality within tropical production systems.

AUTHOR'S CONTRIBUTION

H: Collecting data, animal maintenance, and data analysis.
LR: Assembled research design and review manuscript
MIAD: Assembled research design, data analysis, and manuscript writing
AN: Assembled research design and reviewed the manuscript.
AG: Reviewed and advised on manuscript improvement and data analysis.
SRAB: Advised on fine-tuning the manuscript.
IS: Contributed to manuscript refinement.
MH: Provided suggestions for manuscript enhancement.

Generative AI and AI-assisted technology statement

AI-assisted technologies were used solely for non-scientific purposes, such as grammar correction, reference formatting, and minor figure editing (e.g., improving resolution). No AI tools were used for data analysis, interpreting results, or scientific writing. All findings and conclusions in this manuscript are the authors' sole responsibility

Conflict of interest

The authors have declared no conflict of interest.

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