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PIT1 gene Polymorphism and its Relationship with Growth Performance of Cross-Bred (Brahman X Lai Sind); (Red Angus x Lai Sind) and (BBB x Lai Sind) Cattle Kept in Dak Lak Province, Vietnam

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Based on the correlation of PIT1 gene polymorphism with cattle fattening (especially local breeds), the aim of this study was to determine the correlation between PIT1 gene polymorphism and growth traits of crossbred cattle. Total 150 cattle from 3 crossbred (Brahman x Laisind, BRL), (Red Angus x Laisind, RAL) and (Blanc Bleu Belge x Laisind, BBL) were collected for ear tissue sampling for DNA extraction to determine the genotype of the PIT1 gene. The body weights at birth, 6, 12, 18 and 24 months of age were recorded, and average daily gains were calculated accordingly. PCR-RFLP analysis was used to analyze PIT1 gene polymorphisms of individual animals. PCR reaction specifically amplified the PIT1 gene segment. The results of genotype/allele analysis of the PIT1 gene of BRL, RAL and BBL cattle showed that the PIT1 gene has two allele forms A and B corresponding to three genotypes AA, AB and BB appearing in all three cattle populations. The actual distribution frequencies of genotypes AA, AB and BB were consistent with the theoretical distribution frequencies according to the Hardy Weinberg Law. PIT1 gene polymorphism is related to body weight and absolute gain (ADG) of crossbred cattle combinations BRL, RAL and BBL. Body weight and average daily gain in BRL, RAL and BBL cattle tend to favor the BB genotype.
Keywords: Average daily gain, body weight, crossbred cattle, local cattle breeds, PIT1 gene polymorphisms.

INTRODUCTION

The development of the meat sector in animal breeding still heavily relies on the production of cattle. Approximately 90% of beef comes from dairy and mixed breed cattle, despite the fact that the number of meat cattle is increasing owing to imported and local specialized breeds (*Sedykh et al.,* 2020). Due to the phenomena of hybrid vigor (heterosis), a crossbred animal offers various benefits over a purebred, particularly in attributes that are significant to commercial cattles; higher fertility, lifespan, feed efficiency, and disease resistance. Additionally, it often yields more growth and carcass than the parents do (Buckley *et al.*, 2014).
Numerous genes regulate the quantitative features of growth and development in cows, and this mechanism will be more important in crossbred cattle (Wu and Zhao, 2021; Michetti *et al.*, 2022). These characteristics of cattle are significant economically. For both farmers and customers, choosing animals with superior carcass composition and strong growth rates is crucial. With the use of modern technologies, scientists can employ selection using genetic markers, or gene polymorphisms, to increase the precision and effectiveness of conventional selection techniques. There is a strong correlation between certain growth and meat yield parameters and genetic variants. Pit-1 was regarded as a genetic polymorphism in this investigation. According to Zhao *et al.* (2004), PIT-1 is a transcription factor that is specific to the pituitary and is in charge of pituitary development and hormone expression in animals. Pit-1 has been linked to ADG, weaning weight, and birth weight in pigs (Yu *et al.*, 1995). Pit-1 has been linked to milk production and body composition in cattle (Renavile *et al.*,1997). Genetic polymorphisms can affect how genes are expressed, and combinations between genetic and environmental factors can

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disguise the effects of a single variation. The pituitary gland expresses growth hormone and pituitary specific transcription factor-1, both of which are essential for animal development and growth. Using PCR-RFLP, genetic polymorphisms of the GH1 and POU1F1 genes were examined and analyzed for statistical correlations with variables related to meat quality in Hanwoo cattle (Han *et al.*, 2010). In HF cattle, Thuy *et al.* (2018) verified the relationship between the PIT1-E2 polymorphism and milk output, with the AA genotype producing more milk than other breeds. The outcomes demonstrated the possible application of PIT1-E2 in MAS for milk production in Vietnamese-bred HF dairy cattle. According to Cohen *et al.* (1996), PIT11 is a transcription factor exclusive to the pituitary that governs pituitary development and hormone expression in mammals.

Zhang *et al.* (2009); Yang *et al.* (2010) showed that polymorphisms of the PIT-1 gene were associated with growth in Nanyang, Qinchuan, Jiaxianhong, Xizhen, Luxi and Holstein cattle in China. Oprządek *et al*. (2003) showed that PIT-1 in cattle is a factor influencing growth and carcass composition.

Production of beef cattle is crucial to household income and sustainable growth in industrial crop-livestock systems in the Vietnamese province of Dak Lak. There are 250 thousand cattle in this province, which makes up 26.7% of all livestock in the Central Highland Region (GSO, 2023). The majority of the cattle are housed on small farms. Many policies on the insemination of specialized beef breeds, such as Red Angus, Charolais, and BBB (Blanc Blue Belgium), for crossbreeding with cross-bred cattle breeds, such as Laisind, have been implemented with the goal of improving beef productivity and quality to meet the growing demand for beef throughout the nation, including the Central Highland region (Quyen *et al.*, 2018). Therefore, the purpose of this study was to screen for polymorphisms in the Pit-1 gene and examine how these polymorphisms related to growth attributes in various crossbred cattle grown in the Central Highland Dak Lak area of Vietnam (Red Angus x Laisind, Charolais x Laisind, and BBB x Laisind).

MATERIALS AND METHODS

***Cattle samplings and growth traits*:** The ear tissue samples were collected from total 150 crossbred cattle from 3 groups (Brahman x Lai Sind, BL), (Red Angus x Lai Sind, RAL) and (Blanc Bleu Belgium x Lai Sind, BBL) for genomic DNA isolation to determine the genotype of the PIT1 gene. The animals were raised in individual farmer households. The body weights at birth, and 6; 12, 18 and 24 months of age were measured. And the average daily gains (ADG) were calculated accordingly.

DNA extraction

Genomic DNA was extracted using Qiagen's kit (QiAmp DNA mini kit, Germany)

***PCR method to amplify PIT1 gene segment*:** Using PCR-RFLP method to analyze PIT1 gene of each collected cattle. PCR reaction specific to PIT1 gene segment was conducted in total 25 µl, including Each PCR reaction was performed on PTC-100® Peltier Thermol Cycler with total volume of 25µl. Including: 12.5µl PCR Master Mix 2X (Fermentas); 1µl each forward and reverse primer; 2µl DNA and 8.5µl sterile purified water. PCR reaction thermal cycle as follows. Denaturation step at 950C for 5 minutes followed by 35 cycles with 94ºC for 45 seconds; 63ºC for 50 seconds; 72ºC for 50 seconds. The final step at 72ºC for 10 minutes.

Primer sequences for amplifying the PIT1 gene segment. We used the primer pair according to Carrijo *et al.* (2008) to amplify the PIT1 gene segment on the collected experimental cattle samples. The primer sequences are as follows:

Forward: 5'-CAATGAGAAAGTTGGTGC-3'

Reverse: 5'-TCTGCATTCGAGATGCTC-3'

***RFLP method using Hinf I enzyme*:** After PCR reaction, we used Hinf I restriction enzyme to cut the PIT1 gene segment to determine polymorphism between cows in each breed group. The enzyme digestion reaction was performed in a volume of 25 µl, including: 2.5 µl of digestion buffer, 15 µl of PCR product, 0.2 µl (5U) of digestion enzyme and 7.3 µl of H_2O, according to Jakaria and Noor (2015). The reaction mixture was incubated in an incubator at 37ºC for 7-10 hours or overnight. Check the enzyme digestion reaction results on a 1.5% agarose gel with a voltage of 100 V for 40-50 minutes on a 1X TBE buffer system; stain with Redsafe and illuminate under UV light to take pictures to read and save the results. Compare the electrophoretic bands of each sample with the 100 bp plus DNA ladder marker to read the genotype. According to theory, genotype AA includes the bands: 617, 379, 260 and 45 bp; genotype BB includes the bands: 617, 424 and 260 bp and genotype AB will have the bands: 617, 424, 379, 260 and 45 bp.

HinfI has a recognition site of:

5´ R | GATCY 3´

3´ YCTAG | R 5´

***Data analysis*:** The basic allele counting approach was used to calculate genotype and allele frequencies. The chi-square test was used to compare the observed and expected genotype frequencies in order to assess the existence of Hardy-Weinberg equilibrium in the population under study. With MINITAB 16.2 software, associations between PIT1 genotypes and growth traits were evaluated through the ANOVA approach.

RESULTS

***PIT1 gene polymorphisms*:** The results of PIT1 gene polymorphism analysis in BRL, RAL and BBL cattle are shown in Figure 1.

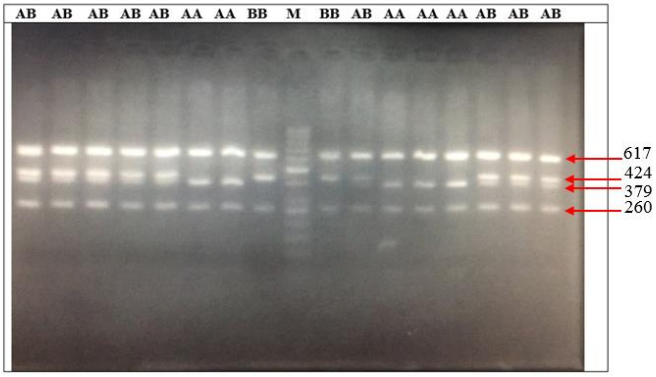

Figure 1. PIT1 gene fragment cleavage product by Hinf1.

In figure 1, M: Standard DAN ladder 100bp (fermentas); and AA, AB, BB are genotypes

Through PCR-RFLP analysis at the polymorphic point of the PIT1 gene, the PCR product incubated with Hinf I enzyme is a specific, clear DNA band with a molecular size corresponding to the theory (Fig. 1). The products cut by restriction enzymes are clear bands with molecular sizes consistent with theoretical calculations.

Genotype AA: 617bp, 379bp, 260bp and 45bp

Genotype AB: 617bp, 424bp, 379bp, 260bp and 45bp

Genotype BB: 617bp, 424bp and 260bp

***PIT1 genotype distribution*:** The results of genotype/allele analysis of the PIT-1 gene of BRL, RAL and BBL cattle are presented in Tables 1, 2, 3, showing that the PIT-1 gene has two allele forms A and B corresponding to the three genotypes AA, AB and BB appearing in all three cattle populations raised in Dak Lak. The results of PIT-1 gene polymorphism analysis in BRL cattle are presented in Table 1, showing that the AA genotype frequency is 0.54, the AB genotype frequency is 0.36 and the BB genotype frequency is 0.1.

Table 1. Frequency of alleles of the PIT-1 gene and actual and theoretical distribution of genotypes, observed and expected numbers of individuals in the BRL cattle (n=50).

Items	Genotype			∑
	AA	AB	BB	
Observed number (head)	27	18	5	50
Expected number (head)	26	20	4	50
Actual distribution	0.5400	0.3600	0.1000	1
Theoretical distribution	0.5184	0.4032	0.0784	1
χ^2				0.574
P-value	0.0450	0.2314	0.2975	0.751
Frequency of alleles	A=0.72		B=0.28	

The theoretical genotype frequencies in the BRL cattle population are AA: 0.5184; AB: 0.4032 and BB: 0.0784. The allele frequencies are: A: 0.72 and B: 0.28. PIT-1 gene polymorphism of RAL cattle (Table 2) shows that the actual genotype frequencies AA, AB, BB are 0.42; 0.46 and 0.12, respectively. The theoretical genotype frequencies are 0.4225; 0.4550 and 0.1225, respectively. The allele frequencies A: 0.65 and B: 0.35. PIT-1 gene polymorphism in BBL cattle (Table 3) showed that the actual genotype frequencies of AA, AB and BB were 0.3; 0.4 and 0.3, respectively, corresponding to the theoretical genotype frequencies of 0.25; 0.5 and 0.25. The frequency of allele A: 0.5 and B: 0.5. These results showed that the population had a high content of heterozygous information and high polymorphism, the population had a high level of genetic variation and more genetic information. In the 3 crossbreds of cattle studied, the homozygous level of BB in BRL and RAL cattle was lower than that of BBL cattle, respectively BRL (0.1), RAL (0.12). The observed and theoretically calculated populations of BRL, RAL and BBL cattle showed that all 3 cattle populations were in genetic equilibrium according to the Hardy Weinberg law.

Table 2. Frequency of alleles of the PIT-1 gene and actual and theoretical distribution of genotypes, observed and expected numbers of individuals in the RAL cattle (n=50).

Items	Genotype			∑
	AA	AB	BB	
Observed number (head)	21	23	6	50
Expected number (head)	21	23	6	50
Actual distribution	0.42	0.46	0.12	1
Theoretical distribution	0.4225	0.4550	0.1225	1
χ^2				0.006
P-value	0.0007	0.0027	0.0026	0.997
Frequency of alleles	A=0.65		B=0.35	

Table 3. Frequency of alleles of the PIT-1 gene and actual and theoretical distribution of genotypes, observed and expected numbers of individuals in the BBL cattle (n=50).

Items	Genotype			∑
	AA	AB	BB	
Observed number (head)	15	20	15	50
Expected number (head)	12.5	25.0	12.5	50
Actual distribution	0.30	0.40	0.30	1
Theoretical distribution	0.25	0.50	0.25	1
χ^2				2.000
P-value	0.50	1.00	0.50	0.368
Frequency of alleles	A= 0.5		B= 0.5	

***Growth traits*:** Body weight and average daily gain (ADG) from birth to 24 old months of BRL, RAL and BBL cattle are presented in Tables 4 and 5. The BB genotype of BRL and RAL cattle tent to have higher body weight and ADG than the AA and AB genotypes, but the difference is at p> 0.05. The AB genotype of RAL cattle has higher ADG than the AA and

Table 4. Relationship between body weight and PIT-1 genotype.

Breed	Genotype	n	Body weight (kg) at different ages				
			Birth	6 month	12 month	18 month	24 month
BRL	AA	27	23.2±2.1	122.3±6.3	186.6±11.5	287.8±12.3	375.0±42.4
	AB	18	23.9±2.0	124.1±7.9	189.4±14.9	287.2±15.0	384.2±39.6
	BB	5	24.4±0.9	122.0±8.9	192.4±11.8	292.6 ±9.50	421.6±13.2
p-value			0.28	0.69	0.58	0.71	0.06
RAL	AA	21	26.0±2.7	126.9±11.4	213.0±10.5	312.9^{b}±18.1	434.0±38.9
	AB	23	24.9±3.1	125.9±10.0	221.3±17.4	336.8^{a}±30.2	428.8±61.4
	BB	6	24.7±3.1	125.5±6.60	211.5±8.80	305.9^{c}±19.1	443.3±54.1
p-value			0.41	0.93	0.11	0.002	0.82
BBL	AA		29.6±3.3	170.7^{c}±7.8	306.3±10.3	444.6^{ab} ±18.3	574.6^{ab}±33.5
	AB		29.1±3.3	173.8^{b}±10.3	311.4±16.0	446.8^{ab}±18.4	577.6 ^{ab}±30.7
	BB		29.7±3.6	179.3^{a}±6.0	311.5±14.4	460.1^{a} ±14,6	601.9^{a}±29.3
p-value			0.86	0.02	0.50	0.04	0.04

The letters a, b, c in the same column are different then their values are statistically significant difference (p<0.05).

Table 5. Relationship between average daily gain and PIT-1 genotype.

Breed	Genotype	n	Average daily gain at different growth periods				
			Birth to 6 months	6 to 12 months	12 to 18 months	18 to 24 months	Average (birth to 24 months)
BRL	AA	27	550.8±34.0	361.3±71.1	557.8±34.9	484.8±219.0	488.7±58.0
	AB	18	556.5±39.3	362.7±78.2	549.4±62.1	539.2±207.5	561.0±83.3
	BB	5	542.2±46.2	391.1±58.5	556.7±101.0	716.7±116.6	551.7±17.4
p-value			0.73	0.70	0.87	0.08	0.07
RAL	AA	21	560.8±61.5	478.5^{b}±47.5	555.1^{b}±76.1	672.8^{b}±145.5	566.8±53.7
	AB	23	561.0±53.2	530.1^{a}±87.9	642.1^{a}±94.3	510.7^{c}±196.9	561.0±83.3
	BB	6	560.1±30.5	477.7^{c}±37.0	524.5^{c}±90.7	763.2^{a}±196.0	581.4±71.2
p-value			0,99	0,04	0.01	0,002	0,82
BBL	AA	15	783.7^{c}±50.0	753.7±29.1	768.1^{b}±48.7	722.2±113.4	756.9^{c}±48.2
	AB	20	803.9^{b}±56.8	764.4±75.0	752.2^{b}±45.5	726.4±130.1	761.7^{b}±41.3
	BB	15	831.5^{a}±35.0	734.1±74.5	825.6^{a}±92.8	787.8±141.8	794.7^{a}±38.9
p-value			0.04	0.39	0.005	0.23	0.04

The letters a, b, c in the same column are different then their values are statistically significant difference (p<0.05).

BB genotypes at 12; 18 and 24 months of age (p<0.05). However, the ADG from birth to 24 months of age in the 3 genotypes AA, AB and BB is not different (p>0.05). Meanwhile, the BB genotype in BBL cattle had higher body weight than the AA and AB genotypes at 6; 12; 18 and 24 months of age (p<0.05) (Table 4). Individuals with the BB genotype had higher ADG than AA and AB individuals at the different growing stages (p<0.05). In general, body weight and ADG in BBL cattle tended to favor the BB genotype.

DISCUSSION

***Gene polymorphisms*:** In this study, the restriction enzyme-cut products were clear bands with molecular sizes consistent with theoretical calculations for three genotypes AA; AB and BB. The results of PIT-1 gene polymorphism analysis in BRL, RAL and BBL cattle are consistent with Carrijo *et al.* (2008) who reported that amplification of the PIT1 gene fragment produced a single product of 1.301 kb in size. Treatment of this product with the HinfI restriction enzyme revealed the HinfI (+) allele with fragments of 260, 617, 379 and 45 base pairs (bp) and the HinfI (-) allele with fragments of 260, 617 and 424 bp. The HinfI (-) allele was less frequent in the two genetic groups (GG1 and GG2). When analyzing the polymorphisms of the PIT-1 gene, it was shown that the BB genotype in BRL cattle had a low frequency of 0.1, whereas the frequency of the AA and AB genotypes appeared with high frequency: AA (0.54); AB (0.36), allele frequency A: 0.72; B: 0.28. RAL cattle with BB genotype appeared with low frequency 0.12 while the frequency of AA and AB genotypes was higher (AA: 0.42; AB: 0.46), allele frequency A: 0.65, B: 0.35). Zhao *et al.* (2004) showed that PIT-1 E6H gene polymorphism in Red Angus cattle distributed genotypes AA; AB and BB respectively 0.11; 0.44 and 0.45. BBL cattle have similar frequencies of 3 genotypes (AA: 0.3; AB: 0.4; BB: 0.3), allele frequency A =B =0.5. Hartati *et al.* (2018) showed that the Pit-1/HinfI gene polymorphism in Grati-Ongole cattle has genotype frequencies AA: 0; AB:

0.009; BB: 0.991, while Hanwoo cattle have genotype frequencies of 0.54; 0.37 and 0.09, respectively. Yan *et al.* (2006) reported that Qinchuan and Chinese Holstein cattle have 3 genotypes AA, AB and BB. Both breeds have allele frequencies A/B of the POU1F1-HinfI locus of 0.232/0.768; 0.132/0.868, respectively; and three genotypes AA/AB/BB have frequencies of 0.030/0.403/0.567; 0.007/0.251/0.742, respectively. BRL cattle had allele frequencies A (0.65), B (0.35), RAL cattle had allele frequencies A (0.72); B (0.28) similar to the results of Carrijo *et al.* (2008) in GG1 cattle (5/8 Charolais, 3/8 Zebu) corresponding to HifI(+) (0.87); HifI(-) (0.27). The frequencies of allele A and allele B obtained in BRL and RAL cattle were similar to the results of ½ Angus cattle with allele A (0.641); allele B (0.359) and ½ Simental cattle with allele A (0.867), allele B (0.133) (Rogério *et al.*, 2006). Kenmenes *et al.* (1999) showed that Caracu cattle had allele frequencies A (0.68), B (0.32) and Canchim cattle had frequencies A (0.63), B (0.37) with allele A frequency higher than allele B frequency. The frequencies of alleles A and B in BBL cattle in the experiment were equivalent to Charolais cattle with allele frequencies A (0.48), allele B (0.52) (Kenmenes *et al.*, 1999). Di Stario *et al.* (2002) showed that Piemontese cattle had POU1FA and POU1FB frequencies of 0.25 and 0.75, respectively. Zabeel *et al.* (2018) showed that the local Iraqi cattle breed had genotype distribution frequencies of AA, AB and BB of 0.081; 0.688 and 0.299, respectively. The frequency of alleles A (0.43), B (0.57), there was no difference in body weight between genotypes. Moody *et al.* (1995) showed that Angus cattle had an allele frequency of A (0.45). Putra *et al.* (2019) suggested that the nucleotide polymorphism g.1256G>A in the bPit-1 gene in Pasunda cattle had low genetic diversity, no AA genotype was found, allele A=0.05; allele B=0.95; allele A in the bPit-1/HinfI gene of Pasunda cattle appeared at a low frequency. Thuy *et al.* (2018) showed that the polymorphism of the PIT1 gene in Holstein Friesian cattle appeared with 3 genotypes AA, AB and BB, genotype frequencies were 0.576; 0.240 and 0.184, respectively. The frequencies of alleles A and B were 0.696 and 0.304, respectively. Prastowo *et al.* (2020) showed that Indonesian Holstein Friesian cattle had genotypes AA, AB and BB with frequencies of 0.21; 0.26 and 0.52, respectively. The frequencies of alleles A=0.35; B=0.65. The genotype BB was associated with milk yield (P<0.05). Trakovická *et al.* (2015) determined that the PIT-1/Hinfl allele B polymorphism (0.7743), the dominant BB genotype (0.7014), BB (PIT-1/Hinfl) had a positive effect on growth. The average values of growth parameters of heterozygous BB and AB genotypes tend to be favorable for growth, body weight gain, and absolute ADG gain, which is favorable for selection in future beef cattle breeding programs. Woollard *et al.* (1994) showed that the genotype frequencies of AA, AB and BB in Angus cattle were 0.11, 0.44 and 0.45 respectively. Rogério *et al.* (2006) POUIFI/Hinfl polymorphism segregated genetically in 4 groups of Nellore, ½ Simental, Canchim and ½ Red Angus cattle. The frequency of allele A was higher than the frequency of allele B in Nellore, Canchim and ½ Simental cattle compared to ½ Red Angus cattle. Ozdemir *et al.* (2012) determined the PIT-1/HINF1 gene polymorphism in Holstein and Ear cattle and showed that the PIT-1 gene had genotype frequencies of AA, AB and BB in Ear cattle of 0.14; 0.54 and 0.32, respectively. The frequency of allele A in Ear cattle was 0.408. Pytlewski *et al.* (2022) showed that the polymorphism of the PIT1 gene at the locus c.1178G>A of Limousine cattle with genotypes AA, AG and GG had frequencies of 0.0957; 0.3913 and 0.5130, respectively.

***Gene polymorphism associated with growth performance*:** In this study, body weights at various growth stages and ADG in BBL cattle were associated with the BB genotype. This result was consistent with a lot of earlier research. The frequency of the A/B allele in the Nanyang population was found to be 0.465/0.535, according to a study on the genetic polymorphism effect of the POU1F1 gene on growth attributes of Nanyang cattle. When it came to the characteristics of birth weight, weight gain, body weight, and size at 12 months, the BB genotype influenced the AB genotype more than the AB genotype (Xue *et al.*, 2006). Di Stasio *et al.* (2002) demonstrated that PIT1 affected body weight and ADG in cattle at 240 days of age (p<0.05). The association between the POU1F1 genotype and carcass qualities of Hanwoo cattle was demonstrated by Sang-Hyun *et al.* (2010). By using molecular markers for bull-based prediction, this genetic technique can be applied to increase the productivity and meat quality of Hanwoo cattle. According to Xue *et al.* (2006), birth weight and ADG at 12 months of age were impacted by the GG genotype of Nanyang bulls. According to these authors, growth performance may be significantly influenced by the G allele. According to Yang *et al.* (2011), growth features in Chinese cattle breeds were linked to polymorphisms of the PIT1 gene. Zhang *et al.* (2009) discovered that Germany Yellow x Qinchua crossbred calves with AG heterozygotes had larger shoulders and heavier bodies than GG homozygotes. In black and white cattle, Oprzqdek *et al.* (2003) discovered that GG homozygotes consumed less feed and its constituents than AG heterozygotes. Animals with the AB x GG genotype had the greatest reported pre-slaughter body weight, whereas those with the BB x GG genotype had the best carcass value and fat content. According to Sang-Hyun *et al.* (2010), body weight and fat content in Hanwoo bulls can be impacted by the polymorphism of the PIT-1 gene in exon 6. According to Yang *et al.* (2011), Chinese cattle with varying genotypes had varying body weights, and at 24 months, cattle with the BB genotype had greater chest girth and body length than calves with the AB genotype. According to Sang-Hyun *et al.* (2010), body weight and fat content in Hanwoo bulls may be impacted by the polymorphism of the PIT-1 gene in exon 6. In 6- and 12-month-old Nanyang cattle, Xue *et al.* (2006) demonstrated a greater favorable effect of the GG genotype on birth weight, body weight gains up to 12 months of age, and body length and girth.

The G allele may be crucial for characteristics related to body growth, according to the scientists' hypothesis. However, in this investigation, the BB genotype in RAL and BRL cattle did not correlate with body weight or ADG. Several investigations concur with this conclusion. Pit1E6H polymorphism in Angus cattle with genotypes AA, AB, and BB did not affect body weight at birth, weaning, carcass weight, or carcass eye area, according to Zhao *et al.* (2004). Pan *et al.* (2008) demonstrated that for various growth stages (6, 12, 18, and 24 months of age), there was no significant correlation between TaqI polymorphism and body weight and ADG in Nanyang cattle. Similarly, the GG genotype did not differ substantially from the AG genotype, according to Putra *et al.* (2019), who hypothesized that the PIT1 polymorphism had no effect on the body weight of Pasudan cattle. Perhaps as a result of the small sample size, there was no correlation found in the study between the bPit-1/HinfI gene polymorphism and body weight. According to Agung *et al.* (2024), body weight and ADG are unaffected by the PIT-1 gene in Sumba Ongole cattle with the BB genotype. Nonetheless, PIT1 gene polymorphism with three genotypes—AA, AB, and BB—appears at frequencies of 0.134, 0.402, and 0.463, respectively, in Holstein cattle, according to Fındık and Özdemir (2022). Genotype BB has no effect on birth weight. According to the authors, allele B has a favorable impact on the traits associated with cattle growth. The IGF1/SnaBI genotype polymorphism between the AA, AB, and BB genotypes in Charolais cattle did not differ in birth weight or body weight at 400 or 600 days of age, according to De la Rosa Reyna *et al.* (2010). However, calves with the BB and AB genotypes had higher weaning weights than calves with the AA genotype.

***Conclusion*:** This study showed that PIT1 gene polymorphism in three cross-bred cattle namely BRL (Brahman x Laisind), RAL (Red Angus x Laisind), and BBL (Blanc Blue Belgium x Laisind) appeared with 3 genotypes AA, AB and BB at different frequencies. Alleles A and B of PIT1 gene in BRL, RAL and BBL cattle appeared with different frequencies depending on the genotype. Genotype BB was associated with body weight and average daily gain in all 3 cross-bred cattle, body weight and average daily gain were biased towards genotype BB. Genotype BB (Pit1/HinfI) had a positive effect on body weight and average daily gain. To increase body weight and average daily gain in the beef cattle breeding selection program, allele B can increase body weight and daily gain.

***Authors contributions statement*:** All authors had similar participation in designing, experiment, analysis and drafting the manuscript.

***Conflict of interest*:** The authors declare no conflict of interest.

***Ethical statement*:** All process of experiments were in according to animal welfare rules under monitoring of university ethical team.

***Availability of data and material*:** Available and can be provided when requested by journal.

***Informed consent*:** N/A

***SDGs addressed*:** Zero Hunger, Decent Work and Economic Growth.

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