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THE POLYMORPHISM OF LEPTIN AND THYROGLOBULIN GENES IN LAI SIND AND LAI BRAHMAN CATTLE

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ABSTRACT: The aim of this study was to investigate the single nucleotide polymorphisms (SNPs) present in intron 2 region of leptin (LEP) and 5' untranslated region of thyroglobulin (TG5) genes in Lai Sind and Lai Brahman cattle populations raised in the Central Vietnam. For each cattle group, fifty hair root samples were collected and extracted genomic DNA. The LEP/*Sau*3AI and TG5/*Psu*I gene polymorphisms were analyzed using PCR-RFLP technique. The results showed that the SNPs of LEP/*Sau*3AI and TG5/*Psu*I were found in the both cattle groups. Three LEP/*Sau*3AI genotypes were detected, including LEP^{AA}, LEP^{AB} and LEP^{BB}. All of the investigated cattle carried $TG5^{CT}$ genotype. The Hardy-Weinberg equilibrium was reached in the both cattle populations for LEP/*Sau*3AI, but not for TG5/*Psu*I. The polymorphisms at these two loci were moderate in the both cattle populations. It can be concluded that the SNPs LEP/*Sau*3AI and TG5/*Psu*I can be used as genetic markers for molecular selection in these cattle groups. A selection program is needed to increase the frequency of $TG5^{T}$ allele in Lai Sind and Lai Brahman cattle groups to improve beef marbling score.

Keywords: Cattle, Lai Brahman, Lai Sind, Leptin gene, Polymorphism, Thyroglobulin gene.

INTRODUCTION

Beef has been the third most consumed meat worldwide after poultry and pork. The beef consumption per person in Vietnam amounted to about 8.73 kilograms in 2023, and was forecast to increase to 9.6 kilograms per capita annually (Statista Research Department, 2023). The Vietnamese yellow cattle is an indigenous breed well adapted to tropical condition and poor nutrition. However, the disadvantages of this cattle breed were small size, low carcass and milk yield. In Vietnam, from the 1960s to 1970s, with the implementation of the program of "Red-Sindhization" and afterward of "Zebuization", the ratio of crossbred cattle was increased. Today, the two cattle groups of Lai Sind (a crossbred breed between Red Sindhi bulls and local cows) and Lai Brahman (a crossbred breed between Brahman bulls and local cows) have been used as dam lines for crossing with beef cattle to improve growth performance and carcass yield in many provinces in Vietnam (Bang et al., 2022).

Breeding values predicted by Best Linear Unbiased Prediction for selection economic indices have become widely applied in animal breeding over the last few decades. The discovery of genetic markers related to the phenotypic expression of particular traits -has raised hopes for successful animal breeding based on candidate genes. Advancements in genotyping technologies for single nucleotide polymorphisms (SNPs) at different loci in the genome to estimate the breeding values are the strong basis for marker-assisted selection (Binh, 2019). Most of the economically important traits in cattle are quantitative traits and environmental factors (Getaneh and Alemayehu, 2022). Until now, many scholars have identified great quantities of candidate genes associated with economically important traits in cattle, such as LEP, TG5, CAST-T1, Calpain 316-T2, Calpain 4751-T3 (De Carvalho et al., 2012; Sedykh et al., 2016; Coria et al., 2018).

The leptin (LEP) gene is located on chromosome 4 in cattle (Pomp et al., 1997). It consists of three exons separated by two introns. The exon 1 and four nucleotides of exon 2 are not translated. The remaining part of exons 2 and 3 are translated into the functional 16kDa leptin protein of 146 amino acids (Haruna et al., 2020). Leptin hormone is produced by adipose tissue and the small intestine, plays an important role in lipid accumulation, and regulates the energy balance by suppressing hunger, resulting in the reduction of fat in adipocytes (Al-hussaniy et al., 2021). Many study results also found leptin roles in regulating body mass, reproductive and immune functions (Santos-Alvarez et al., 1999; Kadokawa et al., 2000; Block et al., 2001).

The SNP LEP/Sau3AI (g.1926C>T) in intron 2 region was changed the amino acid at position 2059 of the chain protein from arginine to cysteine (Moravčíková et al., 2012; Trakovická et al., 2013; Putra et al., 2019). This SNP has been used as molecular marker for selection many economically traits in cattle, such as milk yield (Moravčíková et al., 2012; Trakovická et al., 2013), reproductive performance (Moussavi et al., 2006; Öner et al., 2017; Ferchichi et al., 2018), body

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weight (Almeida et al., 2003; Nobari et al., 2010; Hussain et al., 2017), fat percentage (Sedykh et al., 2016) and feed intake (Liefers et al., 2002).

Thyroglobulin is a glycoprotein hormone synthesized in thyroid follicular cells. It is a precursor of triiodothyronine (T3) and thyroxine (T4) hormones, playing an important role in metabolic regulation and can influence adipocyte development, differentiation and adipose tissue homeostasis (Ailhaud et al., 1992; Casas et al., 2005). Also, Mears et al. (2001) demonstrated that both T3 and T4 are involved in fat marbling in Japanese black cattle. Findings of Barendse et al. (2004) indicated that the SNP TG5/*Psu*I (g.422C>T) had been associated with marbling in cattle. The SNP TG5/*Psu*I has been used for genetic markers for selection marbling index (Barendse, 1999; Barendse et al., 2004; Casas et al., 2005), fat percentage (Sedykh et al., 2016) and backfat thickness (Gan et al., 2008; Mears et al., 2001; Moore et al., 2003) in some beef cattle breeds.

The aim of this study was to identify the LEP/*Sau*3AI and TG5/*Psu*I gene polymorphism of Lai Sind and Lai Brahman cattle. The study result could aid in the marker-assisted selection program to improve productivity and beef quality.

MATERIALS AND METHODS

Sample collection and genomic DNA extraction

A total of 100 hair follicle samples was collected from Lai Sind and Lai Brahman cattle (n=50) raised in Quang Ngai province, Vietnam (Latitude: 14°32'-15°25' North, Longitude: 108°06'- 109°04' East). The samples were kept separately in a plastic bag and transported to the Laboratory of Molecular Biology, College of Agriculture and Forestry, Hue University. For increasing DNA extraction efficiency, the hair follicles were homogenized in the lysis buffer within 5 minutes using Bullet blender (Next Advance, USA). The further steps were followed the kit manufacturer's instruction. Nanodrop system (Thermo Scientific, USA) was used for DNA quantity and quality measurement. The research protocol was approved by the Scientific Committee of Hue University dated 30th September 2021, Decision No: 1472/QĐ-DHH.

Leptin and Thyroglobulin gene polymorphism analysis

The intron 2 fragment of LEP gene and the TG5 gene were amplified by polymerase chain reaction (PCR) technique. Following specific primers described by Barendse (1999) and Liefers et al. (2022) were used in this study, including: LEPF: 5'-TGGAGTGGCTTGTTATTTTCTTCT-3'; LEPR: 5'-GTCCCCGCTTCTGGCTACCTAACT-3'; TG5F: 5'-GGGGATGACTACGAGTATGACTG-3'; TG5R: 5'-GTGAAAATCTTGTGGAGGCTGTA-3'. The PCR reaction was performed in PCR thermal cycler (Axygen® MaxyGene™) in a total volume of 20µL containing 50ng genomic DNA, 1.25µM of each primer, 200µM dNTP, 1× PCR buffer, and 0.75 unit Taq polymerase (Solgent, Korea). The optimized cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 40 sec, 40 sec for primer annealing at 62°C (for LEP gene) or 55°C (for TG5 gene), extension at 72°C for 40 sec, and a final extension at 72°C of 7 min (for LEP gene) or 10 min (for TG5 gene). About 8µL of PCR products were electrophoresed on a 2.0% agarose gel with 6× GelRed (ABT, Vietnam) and then was analyzed using Gel DocTM XR+ (Bio Rad, USA). The LEP or TG5 gene amplification was restricted by *Sau*3AI or *Psu*I endonucleases, respectively. The restriction reaction mixture of 12 µL containing 10 µL of PCR product, 1x buffer and 3 units of restriction enzyme was digested at 37°C overnight. The number and lengths of the restriction fragments were determined by 2%-agarose gel electrophoresis, 6× GelRed staining and UV-visualization, then analyzed by gel-documentation.

Statistical analysis

Genotypic frequency, allele frequency, expected heterozygosity (He), observed heterozygote (Ho) were calculated based on Nei and Kumar (2000). Polymorphic informative content (PIC) was calculated as Roychoudhury and Nei (1988). Chi-squared (χ^2) was used to test for Hardy-Weinberg equilibrium.

RESULTS AND DISCUSSION

Polymorphism of LEP/*Sau*3AI gene in cattle

The 422bp fragment of LEP gene was amplified from genomic DNA of the two cattle populations and digested with restriction enzyme *Sau*3AI. Three genotypes of LEP/*Sau*3AI gene were detected in Lai Sind and Lai Brahman cattle, included LEP^{AA} (390 and 32 bp), LEP^{AB} (390, 303, 88 and 32 bp), and LEP^{BB} (303, 88 and 32 bp) (Figure 1). The observed allelic frequencies of LEP^{A} ranged from 0.64 to 0.67, higher than those of LEP^{B} allele (0.33-0.36) (Table 1). This result agrees with the previous reports in various cattle populations (Sharifzadeh and Doosti, 2010; Jecminkova et al., 2016; Hussain et al., 2017). In the Lai Sind cattle population, the observed frequency of LEP^{AA} genotype was highest, followed by LEP^{AB} and LEP^{BB} genotypes. Meanwhile, the observed genotypic frequencies of LEP^{AA} and LEP^{AB} were similar in Lai Brahman cattle group. Only 7/50 investigated Lai Brahman cattle had LEP^{BB} genotype. Some study results on LEP gene polymorphisms in other cattle breeds also indicated that LEP^{AA} and LEP^{AB} were two main genotypes (Nassiry et al., 2008;

Hussain et al., 2017). According to Trakovická et al. (2013), the SNP LEP/*Sau*3AI had a significant impact on milk yield and first calving age. The highest milk yield, protein and fat content in milk and the lowest age at first calving associated with LEP^{AA} genotype cows. The authors also indicated that the highest age at first calving was found in the LEP^{AB} genotype cattle (Trakovická et al., 2013), but the lowest milk production was observed in the LEP^{BB} genotype cows. However, the cattle carrying LEP^{BB} genotype had superior growth ability (Yang et al., 2007).

The expected genotypic frequencies of three genotypes in the both cattle populations were also calculated based on the Hardy-Weinberg formulas. The expected genotypic frequencies of LEP^{AA} and LEP^{BB} were similar in Lai Sind cattle group (Table 1). Meanwhile, the expected genotypic frequency of LEP^{AB} was higher than those of LEP^{AA} and LEP^{BB}. The expected frequency for LEP^{BB} genotype was lowest in the both investigated cattle populations. The calculated Chi-squared test values in Table 1 indicated that LEP/*Sau*3AI gene polymorphism in the both cattle populations were in Hardy-Weinberg equilibrium ($\chi^2<3.84$). The polymorphism of the LEP/*Sau*3AI gene was moderate (0.25<PIC<0.50) in the both cattle populations. Therefore, LEP/*Sau*3AI polymorphism can be used as molecular selection in these cattle groups.

Polymorphism of TG5/*Psu*I gene in cattle

The genotypes of TG5/*PsuI* gene consisted of $TG5^{CC}$ (295, 178 and 72 bp), $TG5^{CT}$ (473, 295, 178 and 72 bp), and $TG5^{TT}$ (473 and 72 bp; Anwar et al., 2017). All surveyed cattle carried $TG5^{CT}$ genotype (Table 2). Therefore, the allelic frequencies of $TG5^{C}$ and $TG5^{T}$ were equal. The representative result of PCR-RFLP analysis TG5/*Psu*I was shown in Figure 2. On the basis of the Hardy-Weinberg equation, the expected frequency of $TG5^{CT}$ genotype in both crossbred cattle groups were 0.5. The expected heterozygosity coefficient (H_e) was lower than the observed heterozygosity coefficient (H_o) in the both cattle groups. The χ^2 value in Table 2 indicated that Hardy-Weinberg equilibrium was not reached in these two cattle populations for this investigated locus ($\chi^2 > 3.84$). The PIC value of the TG5/*Psu*I gene in both cattle groups was 0.38, indicating that the level polymorphism in genetic marker was moderate. Therefore, TG5/*Psu*I gene polymorphism can be used as molecular selection to improve the productivity and meat quality in Lai Sind and Lai Brahman cattle.

In Hereford and Limousine breeds, Sedykh et al. (2016) indicated that the cattle had significant potential for increased beef taste and nutritional qualities associated with a high proportion of desirable $TG5^{TT}$ genotype. Also, Casas et al. (2005) reported the increase in fat yield in cattle carried $TG5^{TT}$ genotype. Meanwhile, some authors reported that $TG5^{CC}$ genotype cattle had tendency increase pre-slaughter live weight, hot carcass yield, carcass output, dressing weight and slaughter yield (De Carvalho et al., 2012; Sedykh et al., 2016).

Dolmatova et al. (2020) indicated a clear tendency of an effect of the TG5 genotype on milk productivity in dairy cattle. Cows with the $TG5^{TT}$ genotype had the highest milk yield and fat content in milk (Dolmatova et al., 2020), and had significantly higher lipid content in the loin muscle (Thaller et al., 2003) than the $TG5^{CT}$ or $TG5^{CC}$ genotypes. The $TG5^{TT}$ genotype was the only genotype that showed differences in the distribution of marbling, increasing marbling in beef (Burrell et al., 2004).

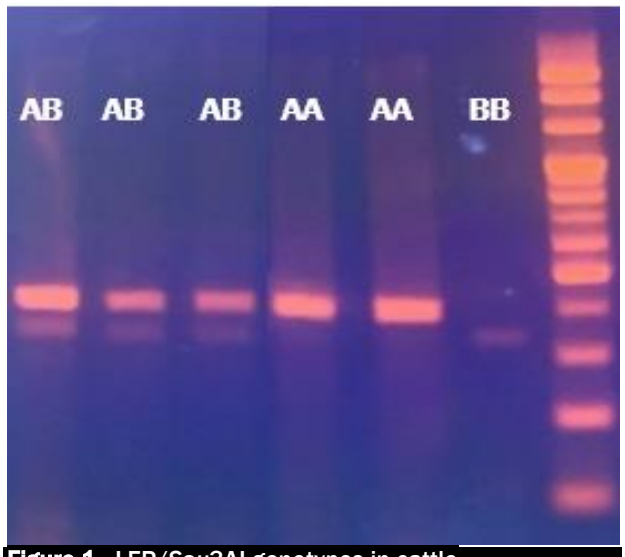

Figure 1 - LEP/*Sau*3AI genotypes in cattle

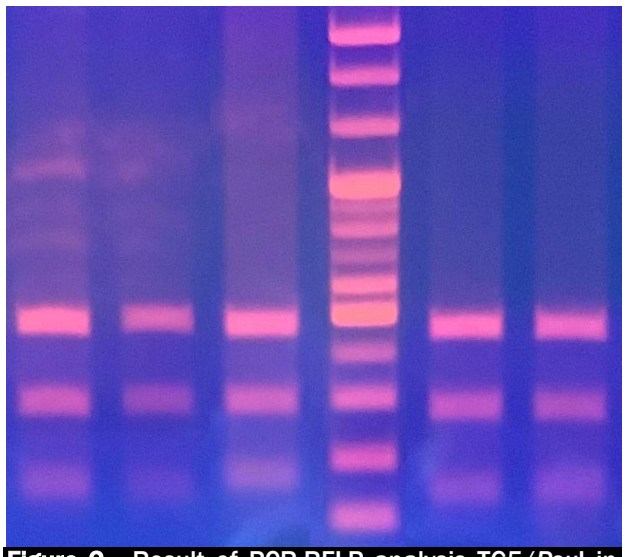

Figure 2 - Result of PCR-RFLP analysis TG5/*Psu*I in cattle

Table 1 - Genotypic and allelic frequency of LEP/*Sau*3AI gene in Lai Sind and Lai Brahman cattle (n=50)

Cattle population	Observed genotypic frequency			Observed allelic frequency		Expected genotypic frequency			H_o	H_e	PIC	HWE (χ^2)
	AA	AB	BB	A	B	AA	AB	BB				
Lai Sind	0.50	0.34	0.16	0.67	0.33	0.45	0.44	0.11	0.34	0.44	0.34	2.67*
Lai Brahman	0.42	0.44	0.14	0.64	0.36	0.41	0.46	0.13	0.44	0.46	0.35	0.10*

H_o: observe heterozygosity; H_e: expected heterozygosity; PIC: polymorphic informative content; HWE: Hardy-Weinberg Equilibrium; χ^2: Chi-square value; *genetic equilibrium ($\chi^2 < 3.84$), $P < 0.05$.

Table 2 - Genotypic and allelic frequency of TG5/*Psu*I gene in Lai Sind and Lai Brahman cattle (n=50)

Cattle population	Observed genotypic frequency			Observed allelic frequency		Expected genotypic frequency			H_o	H_e	PIC	HWE (χ^2)
	CC	CT	TT	C	T	CC	CT	TT				
Lai Sind	0	1.00	0	0.50	0.50	0.25	0.50	0.25	1.00	0.50	0.38	50.00
Lai Brahman	0	1.00	0	0.50	0.50	0.25	0.50	0.25	1.00	0.50	0.38	50.00

H_o: observe heterozygosity; H_e: expected heterozygosity; PIC: polymorphic informative content; HWE: Hardy-Weinberg Equilibrium; χ^2: Chi-square value. $P < 0.05$.

CONCLUSION

It can be concluded that the polymorphisms in LEP/*Sau*3AI and TG5/*Psu*I genes were observed in Lai Sind and Lai Brahman cattle populations. The allelic frequency of LEP^A was higher than LEP^B in both cattle populations. All of investigated cattle had $TG5^{CT}$ genotype. The Hardy-Weinberg equilibrium was reached in the both cattle populations for LEP/*Sau*3AI, but not for TG5/*Psu*I. The SNPs LEP/*Sau*3AI and TG5/*Psu*I can be used as candidate genes for molecular selection in these cattle groups. A selection program is needed to increase the frequency of $TG5^T$ allele in Lai Sind and Lai Brahman cattle groups to improve beef marbling score.

DECLARATIONS

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Authors' contribution

Ho Le Quynh Chau wrote the manuscript, designed the experiment, collected and analyzed data. Duong Thi Huong, Than Thi Thanh Tra and Le Thi Thu Hang collected sample, performed the experiment. Dinh Van Dung designed the experiment, revised the draft of the manuscript. All authors read and approved the final manuscript.

Ethical consideration

The research was approved by the Scientific Committee of Hue University dated 30th September 2021, Decision No: 1472/QĐ-DHH. The experiment was conducted in accordance with ARRIVE guidelines (https://arriveguidelines.org). All methods were performed in accordance with the relevant guidelines and regulations.

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Competing interests

The authors have not declared any conflict of interests.

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