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Research Article

Polymorphisms of Snp in Dgat1 and Capn1 Genes in Brahman Cattle and Hybrid Cattle between Brahman Cows with Blanc Blue Belge, Red Angus, and Charolais Cattle

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Abstract: In this study, we conducted a single-nucleotide polymorphism (SNP) survey of DGAT1 and CAPN1 genes on pure Australian imported Brahman (BRA) and crossbred cows between Brahman cows and Blanc Blue Belge bulls (BBB), Charolais (CHA) and Red Angus (RA). The SNP were made using the restriction enzyme shear pcr cleavage (PCR-RFLP). A total of 142 cows including: 37 BRA cows, 34 BRAxBBB crosses, 38 BRAxRA crosses, and 33 BRAxCHA crosses. The results showed that K232A polymorphism of DGAT1 gene allen type mainly K all accounted for 68.42% (BRAxRA) to 93.24% (BRA). The AA genotype only occurred in an individual in the BRAxBBB crossbred group (4.04%). For the g.5709 C> G polymorphism point in the CAPN1 gene, the CC genotype also appeared only in an individual of BRAxBBB cross-bred cow and was not found in other groups. Besides, the G allele frequency is quite high, from 66.73% (BRAxBBB) to 88.64% (BRAxCHA). The present study shows the prevalence of genotypes in imported Brahman and crossbred cattle between Brahman and other foreign beef breeds. The obtained results can be applied in selecting the superior genotype cows in the future.

Keywords: Blanc Blue Belge, Charolais, Red Angus, Brahman; gene marker, meat quality, Imported Cattles.

INTRODUCTION

In present, molecular genetics and new biotechnology techniques have provided insights to help identify gene markers related to yield traits and meat quality in livestock. Therefore, the investigation of dominant gene markers correlating with outstanding traits has been widely applied in animal husbandry (Ardicli *et al*., 2019). In the beef industry, yielding greater meat weight and higher meat quality is the top concern. In the goal of increasing beef value, fat accumulation performance and carcass traits proved to be controlled by multi-genetics (Ardicli *et al*., 2017). These traits play a very important role in the beef industry. The selection of better-performing breeds and higher meat productivity is one of the main goals for husbandry and farms. Genetic characteristics as well as the cultural environment influence meat productivity, therefore if genetic prediction indicators for good traits are identified, they will serve as advantages in livestock production (Wang *et al*., 2019).

Many candidate genes related to beef quality has been identified in recent years, and single nucleotide polymorphisms (SNPs) of these candidate genes have been evaluated to be closely correlated and potential reliable indicators for meat quality traits (Kesek-Wozniak *et al*., 2020). The diacylglycerol gene O-acyltransferase 1 (DGAT1) encodes the microsomal enzyme DGAT1 that catalyzes the final step of triglyceride synthesis. The substitution of lysine for alanine (K232A) in the DGAT1 gene has been shown to be linked to milk fat content (Dokso *et al*., 2015) and muscle fat content (IMF) (Thaller *et al*., 2003). Ease-calpain (CAPN1) have been shown to be involved in meat tenderization (Koohmaraie, 1996). The CAPN1 genes, which encode for enzymes, have been shown to be associated with a change in meat tenderness (Schenkel *et al*., 2006). Moreover, polymorphisms of SNP in the CAPN1 g.5709 C>G gene have been found to correlate with water retention and color in beef (Li *et al*., 2013; Ardıclı *et al*., 2019).

Recently, importing live cattle into Vietnam for breeding and slaughtering has become a regular activity. The importation of cows is carried out by import-export companies based on the criteria of volume and phenotype. However, the imported cows are not often does analyzed for genotypes. Therefore,

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information on the genotypic structure of cattle imported to Vietnam regarding candidate genes of important pig traits is limited. The evaluation of genotypes of imported cattle will provide significant insights into genetic dynamics of foreign breed herds, and therefore it will affect economically important trait frequency in breeding cows in Vietnam. Moreover, evaluations of these genetic markers may help orientating strategies for importing cow breeds towards better management of cattle husbandry industry. The purpose of this study is to evaluate the diversity of meat quality indicating genes in cattle individuals imported from Australia to Vietnam and imported exotic cows.

MATERIALS AND METHODS

Sample collection

The research was conducted on 142 samples, contains: 37 Brahman cows imported from Australia to Vietnam (BRU) and 34 hybrid cattle between BRU x Blanc Bleu Belge (BRU x BBB), 38 hybrid cattle BRU x Red Angus (BRU x RA), and 33 hybrid cattle BRU x Charolais (BRUxCHA). These cattle were collected at ranches in the Saodo husbandry farm, in Dak Lak province, Vietnam. The samples were collected independently and randomly and the individuals were not related in families. We use specialized ear pliers to collect samples of skin tissue, washed them with 96% alcohol, and stored them in the laboratory for DNA extraction.

The 20 mg of tissue sample was taken to conduct DNA separation. The method of DNA extraction was performed according to the steps of the Gene JET Genomic DNA Purification Kit (Thermo Scientific, UK). The extraction procedure was carried out following manufacturer's instructions. OD measurements of DNA samples after separation were made by Nano Drop machine (eppendof, Germany) to evaluate the content and purity; the samples were stored at 20°C.

Multiplication of specific gene fragments

We multiply specific DGAT1 and CANP1 gene fragments for analysis according to primer sequence in Table 1. The PCR method is performed with a MasterMix kit (Thermo scientific, UK), a PCR reaction component with a volume of 25 µl including Buffer 2X, forward bait (10 pmol), reverse bait (10 pmol), DNA sample, and distilled water 2X. The thermal cycle is performed with specialized primers, including (I) 94^{o}C/3 minute (II) periods 35 cycles: 94^{o}C / 30 seconds-Tm^{o} C (according to each specific pair of primers in Table 1) / 45 seconds -72^{o}C/60 seconds (III) 72^{o}C / 5 minutes hold at 4^{o}C. PCR products with the desired tape size are sequenced to confirm the exact gene segment isolate.

Table 1: These primers sequences for amplification

Gene	Primers (5'-3')	Annealing temp (°C)	PCR product size (bp)	Restriction endonuclease	SNP name	References
DGAT1	F-5'-GCACCATCCTCTTCCTCAAG-3' R-5'-GGAAGCGCTTTCGGATG-3'	60	411	*Cfr*I	K232A	(Lacorte *et al.*, 2006)
CAPN1	F-5′-GACTGGGGTCTCTGGACTT-3′ R-5′-GGAACCTCTGGCTCTTGA-3′	50	415	BtgI	G316A	Lisa and Di Stasio (2009)

The PCR product were incubated with restriction endonuclease for PCR-RFLP technique. The PCR-RFLP was conducted included 2.0 µl Buffer 10X, 1.0 µl enzyme, 15 µl PCR product, and 2.0 µl deionized water. The mixture was incubated at 37 ° C (2 hours), thereafter PCR-RFLP products were electroforesed on agarose gel (2%), and results were determined using UV light.

RESULTS AND DISCUSSION

The PCR result has isolated the DGAT1 gene segment, the size corresponding to 411bp with the sequencing results showing that the gene segment corresponds to the DGAT1 gene, code EU348566.1. The genotype SNP of DGAT1 gene fragment by restriction enzyme *Cfr*I is shown in Table 2. The results show that in most cow groups, the rate of KK genotypes is common. The rate of KK genotype was highest in Brahman group (83.78%) and lowest in BraxRA group (36.84%). The common allele type in all groups is the K allele (ranging from 68.42% in BraxRA crosses to 93.24% in Brahman cows). Although the Brahman cross cattle were raised in the region, the genotype KK of them were lower than in our results (respectively, 69.74% & 83.78%) (Ha Thanh Tung *et al*., 2019). Lacorte *et al*. (2006) reported that Holstein cattle breeds in Brazil had the highest frequency for alleles A (73%), which is higher than that on ordinary Holstein cows. in

New Zealand (40%) (Spelman *et al*., 2002) and Holstein cows in Germany (42%) (Kaupe *et al*., 2004). This frequency, however, is lower than the results for Jersey cattle in New Zealand (88%) (Spelman *et al*, 2002) and Brown Swiss cattle in Germany (98%) (Kaupe *et al*., 2004). For Sindhi x Holstein crossbred cows, the allele A frequency is relatively low, corresponding to 4% (Lacorte *et al*., 2006). Whereas for Simmental, Ardicli *et al*. (2017), the rates of AA, AK and KK genotypes were 82.72%, 17.28% and 0%, respectively. With the results, it showed that the type A allen in the cow group in this study had a lower rate than other breeds.

Table 2: Genotype and allele frequencies of the SNP K232A of the bovine DGAT1 gene in the total sample of animals

Cattle breeds	Number (n)	Genotype			Allele	
		AA (n,%)	AK (n,%)	KK (n,%)	A (%)	K (%)
Brahman	37	0 (0.0)	5 (13.51)	31 (83.78)	6.76	93.24
BRA x BBB	34	1 (4.04)	12 (36.40)	19 (56.62)	22.24	77.76
Bra x RA	38	0 (0.0)	24 (63.16)	14 (36.84)	31.58	68.42
Bra x Cha	33	0 (0.0)	14 (41.67)	19 (58.33)	20.83	79.17

The results of electrophoresis of the PCR product gave the tape corresponding to the design. The sequencing showed that the isolated gene segment was corresponding to the CAPN1 gene in cows, code BC112700.1. The genotypic and allelic frequencies of CAPN1 g.5709 C>G polymorphism in the different groups are shown in Table 3. The genotype CC was not present in three of four groups. It also only 4.04% in hybrid cattle BRAxRA. Besides, the frequency of G allele was quite high. It was lowest in Bra x BBB (66.76%) and highest in Bra x Cha (88.64%). On the report, Ardıclı *et al*. (2019) shown the frequency of allen G was the highest in Brahman breed (88.24%) whereas it was lower in Charolais bulls (85.71%) (Ardıclı *et al*., 2019). Previous studies have reported the absence or low frequency of CC genotype in various cattle populations (Allais *et al*., 2011; Curi *et al*., 2010; Li *et al.*, 2013). This results in the present study, indicating that there was no animal with the CC genotype, is in accordance with such previous ones. Nevertheless, in all subject breeds of the present study, the frequency of C allele (~0.26) was rather higher.

Table 3: Genotype and allele frequencies of the SNP g.5709 C>G of the bovine CAPN1 gene in the total sample of animals

Cattle breeds	Number (n)	Genotype			Allele	
		GG (n,%)	GC (n,%)	CC (n,%)	G (%)	C (%)
Brahman	37	26 (70.27)	11 (29.73)	0 (0.00)	85.14	14.86
Bra x BBB	34	14 (40.44)	18 (52.57)	1 (4.04)	66.73	33.27
Bra x RA	38	29 (76.19)	9 (23.81)	0 (0.00)	88.10	11.90
Bra x Cha	33	26 (77.27)	8 (22.73)	0 (0.00)	88.64	11.36

In conclusion: The results analysis of the genotype of the DGAT1 and CANP1 gene polymorphism show that it was similar to these breeds in the EU countries. It also suggests a possible use of these candidate markers in gene-assisted breeding selection programs or orientation breeding import for improvement of beef production traits in cattle in Vietnam.

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