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The diversity of dgat1 and scd1 gene in Vietnamese native, Sindhi and Brahman crossbred cattle

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Abstract
The nucleotide variation of Diacylglycerol acyltransferase 1 (DGAT1) and Stearoyl-CoA desaturase 1 (SCD1) were reported to correlate to beef quality parameter. This study aimed to investigate and analyze the Single nucleotide polymorphism (SNPs) of DGAT1 and SCD1 in Vietnamese native cattle, Sindhi cattle and Brahman crossbred cattle. The PCR-RFLP was applied to analyze SNPs of DGAT1 for total 209 cattle (including 84 Vietnamese native cattle, 69 Sindhi cattle and 56 Brahman crossbred cattle). The results demonstrated that the Single nucleotide polymorphism of DGAT1 was located at K232A position. The frequency of A allele in Vietnamese native cattle was 56.03% which was lower than Sindhi cattle (62.83%).

Keywords: Beef quality, molecular marker, DGAT1, SCD1

1. Introduction
Lipid accumulation and beef characteristics were controlled by genetic variation. These traits play a very important role in the beef cattle industry. The selection of cattle sources with better performance and higher meat productivity is one of the main objectives for producers and farms. Meat productivity is influenced by genetic characteristics as the breeding environment [1]. The cattle productivity improvement program in Vietnam has been implemented since 1990, the program's objective is to improve the performance and beef quality of Vietnamese cattle by crossing with Sindhi or Brahman bulls. Genetic mapping studies have found many mutations that cause bovine genome polymorphism. This correlates with productivity traits which can help evaluate genetic potential for superiority selection in the breeding program [1].
The research on DGAT1 gene variation can also evaluate genetic relationship in cattle populations [2]. In cattle, the DGAT1 gene is considered to be a closely correlated with the ratio of milk fat and meat [3, 4]. This gene is located at the centromeric end of chromosome 14 (BTA14), harboring QTLs which greatly affect to traits of milk fat [5, 6] and fat percentage [7]. The DGAT1 gene encodes a membrane enzyme, the mutations of this enzyme prevent the final step in triglyceride synthesis [5, 8] studied polymorphisms of this gene in Bos taurus and Bos indicus. They reported that K allele of DGAT1 is a primitive type and A allele is a mutation of this gene in Bos taurus and Bos indicus [5]. On cows, the mutation K232A is located on exon 8. The substitution mutation of AA nucleotides (Alanin-K synthesis regulation) by GC nucleotides (Leucin-A synthesis regulation) has affected the ratio of fat content (IMF) [9]. Thus, AK genotype showed the higher IMF index than AA genotype [9].
In cattle, the SCD1 gene is 15-24 kb in length, including six exons and five introns [10]. The mutation of the SCD1 gene (mutation T878C, located on exon 5), caused the replacement of the amino acid Valin by Alanin in the SCD1 protein which has been identified as related to the unsaturated single fatty acid concentration in milk of Holstein cows [11], beef cattle [12] or fatty acid components in Japanese Black beef [13]. In this study, we conducted a polymorphic survey of DGAT1 and SCD1 genes in a herd of cattle in Vietnam. The data collection on the current state of polymorphism of meat quality indicators will be a suggestion for the orientation of selecting suitable hybrid formulas.

2. Materials and Methods

2.1 Sample collecting

The study was conducted on 84 Vietnamese yellow cattle, 69 Sindhi crossbred cattle and 56 Brahman crossbred cattle collected at farms in the Central Highlands, Vietnam. The samples were randomly collected and individuals have no relationship at all. The ear samples were collected, then kept in absolute ethanol and transferred to laboratory.

2.2 DNA extraction

The Gene JET Genomic DNA Purification Kit (Thermo Scientific) was applied for genomic DNA extraction. The 20 mg of tissue was homogenized and incubated with 180 µL of Digestion Solution in 1.5 ml tube. Adding 20 µL of Proteinase K Solution and mix thoroughly by vortexing to obtain a uniform suspension. Adding 20 µL of RNase A Solution, mix by vortexing then incubate for 10 min at room. Adding 200 µL of Lysis Solution and adding 400 µL of 50% ethanol and vortexing. Centrifuging the column for 1 min at 6000 x g. Discarding the collection tube containing the flow-through solution. Placing the Gene JET Genomic DNA Purification Column into a new 2 mL collection tube. The Column was washed twice by 500 µL of Wash Buffer I and II. Genomic DNA was eluted by 50 µL Elution Buffer and kept in -20ºC until use.

2.3 PCR

DGAT1 and SCD1 were amplified using primers as described in table 1. The PCR Master Mix Kit (Thermo Scientific) was used for sequence amplification. The PCR was conducted in 25 µl for each reaction, including 12.5 µl Buffer 2X, 1 µl forward primer (10 pmol) and 1 µl reverse primer (10 pmol), 1 µl template, 9.5 µl dH_2O. The PCR reactions were performed by one cycle of 94°C for 3 min, 35 cycles of 94°C for 30 sec, annealing for 30 sec (followed table 1), 72°C for 60 sec, holding sample at 4ºC. The PCR products were electrophoresed on a 2% agarose gel and stained with Gel Red (Biotium, United States). The gel was exposed to UV light and the picture taken with a gel documentation system.

Table 1: The primer sequences for amplification

Gene	Sequence	Product size (bp)	Tm (ºC)	Enzyme	Reference
DGAT1-*Cfr*I-F	5'-GCACCATCCTCTTCCTCAAG-3'	411	60	*Cfr* I	(Lacorte *et al.*, 2006) [14].
DGAT1-*Cfr*I-R	5'-GGAAGCGCTTTCGGATG-3'				
SCD-*Nco*I-F	5'-CCCATTCGCTCTTGTTCTGT-3'	400	59	*Nco*I	(Nanaei *et al*., 2013) [4].
SCD-*Nco*I-R	5'-CGTGGTCTTGCTGTCGAC-3'				

2.4 PCR-RFLP

The PCR products were treated with *Nco*I and *Cfr*I. The PCR-RFLP was conducted by 2.0 µl Buffer 10X, 1.0 µl enzyme, 15 µl PCR product and 2.0 µl dH_2O. The reactions were incubated at 37ºC for 2 hours. Then the reaction products were electrophoresed on a 2% agarose gel and stained with GelRed (Biotium, United States). The gel was exposed to UV light and the picture taken with a gel documentation system.

3. Results and Discussion

The Table 2 showed that the lowest frequency of the AA genotype for DGAT1 (39.66%) was observed in the Vietnamese native yellow cattle by *Cfr*I enzyme analysis, while the common genotype of Sindhi crossbred cattle and Brahman crossbred cattle were AK genotype (57.52%) and KK (69.74%), respectively. The A allele frequency of the Vietnamese yellow cattle, Sindhi crossbred cattle, and Brahman crossbred cattle were 54.04%; 63.04% and 22.32%, respectively.

The previous study demonstrated that the Holstien cattle in Brazil showed a highest frequency of A allele in (73%) [14]. This frequency is higher than Holstein herds from New Zealand (40%) [15] and Germany (42%) [5]. However, these frequency were lower than Jersey herds from New Zealand (88%) [15] and Brown Swiss herds from Germany (98%) [5]. The A allele frequency is very low from cattle crossbred (4%) between Sindhi x Holstein [14]. Our results revealed that the Sindhi crossbred cattle and Brahman crossbred showed the higher A allele frequency than Vietnamese native yellow cattle.

Table 2: SNPs of DGAT1 gene from cattle in the Central Highlands

Cattle breeds	Number (n)	Genotype			Allele	
		AA (n, %)	AK (n, %)	KK (n, %)	A (%)	K (%)
Vietnamese native	84	33 (39.66)	28 (32.76)	33 (27.58)	54.04	43.96
Sindhi crossbred	69	24 (34.78)	39 (57.52)	6 (8.70)	63.04	37.96
Brahman crossbred	56	8 (14.29)	9 (16.07)	39 (69.74)	22.32	77.68

The results of SCD1 analysis demonstrated that the common AA genotype was observed in Vietnamese native yellow cattle (88.10%), while the AV genotype was determined in Sindhi crossbred cattle (45.72%) and Brahman crossbred cattle (66.07%), respectively (Table 3). The A allele frequency of the Vietnamese yellow cattle, Sindhi crossbred cattle, and Brahman crossbred cattle were 94.05%, 40.00% and 62.00%, respectively.

According to Lacorte *et al*. (2006) [14], the A allele frequency in Sindhi crossbred heards from Brazil was 2%. Nanaei *et al*. (2013) [4] reported that the frequency of A allele and V allele were 0.58 and 0.42, respectively. The highest frequency was observed in the AV genotype (0.65), while the frequency of VV genotype is 0.09. In this study, the A293V homogenous mutation has not been observed in Vietnamese native yellow cattle, but detected in some Sindhi crossbred cattle.

Table 3: SNPs of SCD1 gene from cattle in the Central Highlands

Cattle breeds	Number (n)	Genotype			Allele	
		AA (n, %)	AV (n, %)	VV (n, %)	A (%)	V (%)
Vietnamese native	84	74 (88,10)	10 (11,90)	0 (0,00)	94,05	5,95
Sindhi crossbred	69	12 (17,14)	32 (45,72)	26 (37,14)	40,00	60,00
Brahman crossbred	56	16 (28.57)	37 (66.07)	3 (5.36)	62.00	38.00

4. Conclusion

In this study, Single nucleotide polymorphism (SNPs) of DGAT1 and SCD1 in Vietnamese native cattle, Sindhi cattle and Brahman crossbred cattle were demonstrated by the various distributions of A allele and V allele in cattle population. The A allele showed an dominance in Vietnamese cattle population.

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