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Investigation of (Stearoyl-CoA Desaturase 1) SCD1 Gene Polymorphism in Khuzestan Buffalo Population Using PCR-RFLP Method

Short Communication

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ABSTRACT

Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFA). A number of studies support the hypothesis that SCD gene regulation and polymorphism may affect fatty acid composition and fat quality in meat and milk. Single nucleotide polymorphisms in the coding region of the bovine stearoyl-CoA desaturase gene have been predicted to result in p. 293A (alanine at amino acid 293) and p. 293V (valine at amino acid 293) alleles at the stearoyl-CoA desaturase1 locus. The objective of this study was to characterize polymorphisms of stearoyl-CoA desaturase 1 (SCD1) gene in Khuzestan buffalo population. This is the first study of SCD1 gene polymorphism in Iranian buffalo. The blood samples were taken from 85 buffalo of Khuzestan-Iran population. Genomic DNA was extracted using a kit DIAtom DNA prep. After this, PCR reactions were made by using primers that encloses exon V. A 400 bp fragment amplified with standard PCR method. The PCR products were digested by *NCO1* restriction enzyme with PCR-RFLP method. All samples were genotyped on 3% agarose gels stained with ethidium bromide. Electrophoretic mobility shift determined that there were not genetic differences between these animals in the studied region by using *NCOI* enzyme. Also, this study showed that genotype frequencies were not in Hardy-Weinberg equilibrium for SCD1 gene.

KEY WORDS buffalo, Hardy-Weinberg equilibrium, MUFA, PCR-RFLP, SCD1 gene.

INTRODUCTION

The milk of ruminants is one of the most important food in the human's nutrition that it is characterized by the presence of high proportions of short-chain fatty acids (fewer than eight carbons), probably resulting from efficient extraction of their precursors from the plasma where high concentrations are due to rumen fermentation (Moore *et al.* 1979) Milk fatty acid (FA) composition is an important trait for the dairy cattle industry because of its influence on cheese yield and the organoleptic properties of dairy products (Dobrzyna and Ntambi, 2005). Given the importance of produced milk in provide human needs, it is necessary to study genes influencing this trait. Due to key role of SCD1 gene on fat milk yield, suggested as a candidate gene for fat milk trait. In ruminant species, stearoyl-CoA desaturase 1 (SCD1), a membrane bound protein of the endoplasmic reticulum (Mauvoisin and Mounier, 2011) catalyzes the desaturation of trans-11 18:1, producing cis-9, trans-11 CLA (Ntambi and Miyazaki, 2004). Although SCD1 produces CLA in several species (Keating *et al.* 2006). This enzyme is mainly responsible for catalyzing the desaturation of saturated fatty acyl-CoA substrates, including palmitoyl-CoA and stearoyl-CoA, at the delta-9 position resulting in the synthesis of palmitoleoyl-CoA and oleoyl-CoA (Ntambi *et al.* 2002). It is a critical control point regulating hepatic lipogenesis and lipid oxidation (Mainieri *et al.* 2006).

Several investigations have demonstrated that consumption of saturated fatty acids tend to increase plasma total and low density lipoprotein cholesterol concentrations, and constitutes a risk factor for coronary and heart diseases (Grummer, 1991). Hypercholesterolemic effects of saturated fats in human diets are largely due to lauric (C12:0), myristic (C14:0) and palmitic (C16:0) fatty acids.

These three fatty acids constitute more than 40% of total fatty acids in ruminant milk. From the point of view of human health, the ideal nutritional fatty acid composition for milk fat would contain 82% of monounsaturated fatty acids (MUFA) rather than 25%; the increase would occur at the expense of saturated fatty acids (SFA) (Hu *et al.* 2001). Thus, the increase of unsaturated fatty acids (UFA) proportion in milk, mainly on polyunsaturated fatty acids (PUFA), constitutes an important selection objective to make a healthier product.

In rodents, the SCD1 enzyme is predominantly located in the liver. In contrast, adipose tissue is the major source of SCD1 in growing ruminants and mammary tissue is the major site in lactating ruminants (Kgwatalala *et al.* 2009). The mammalian SCD1 gene, isolated in several species including mouse, cattle and human, show a conserved genomic structure, spanning approximately 15-24 kb and consisting of six exons and five introns. SCD1 gene has a point mutation A293V in exon 5, which is its position on chromosome

BTA26 (Conte *et al.* 2010). Taniguchi *et al.* (2004) detected a single nucleotide polymorphism (SNP) [C/T] in the coding region of bovine SCD1 that causes an amino acid replacement from valine (type V) to alanine (type A), and they found that the SNP allele C or type A was associated with higher percentage of mono unsaturated fatty acid (MUFA) in intramuscular fat of Japanese black cattle. From a genetic perspective, Arnuold and Soyeurt (2009) have reported moderate heritability values for dairy cattle milk SFA (h^2=0.36), MUFA (h^2=0.09-0.17) and PUFA (h^2=0.25), indicating that there is a considerable amount of additive variance for these traits.

Additionally, multiple QTL for milk FA composition phenotypes have been reported in cattle and sheep (Schennink *et al.* 2008). Candidate gene studies have also shown the existence of significant associations between specific genotypes and milk FA content (Moioli *et al.* 2007).

These results suggest that milk FA composition can be modified through selection. Studies indicate that buffalo have higher levels of UFA in milk, especially linoleic acid that could be due to different levels of the SCD1 enzyme mammary gland, existence gene polymorphism SCD1 or changes in enzyme structure and function (Campbell *et al.* 2001).

MATERIALS AND METHODS

In this study, blood samples were randomly collected from 85 buffalo in the Khuzestan province including 20, 20, 20 and 25 heads from Shoshtar, Shadegan, Dezful and Dasht-e-Azadegan cities, respectively. From each animal, about 5 cc of blood was collected from the jugular vein with vaccum tubes coated with ethylenediaminetetraacetic acid (EDTA) transported to the Central Laboratory of the University of Khuzestan Ramin Agriculture and Natural Resources and stored at 4 ˚C until DNA extraction. Genomic DNA was isolated by using DNA extraction kit DIAtom DNA Prep 100.

Spectrophotometer was used investigating quality and quantity. Samples show an optical density (OD) ratio (260 nm/280 nm) ranging between 1.6 and 1.8. The PCR amplification of specific fragment DNA included SNP in 400 bp region of fifth exon (Schennink *et al.* 2008) which is characterized by substitution of C/T (Ala/Val) in position 10329 of the bovine *SCD1* gene sequence (*Accession no.* AY241932) at GenBank. The sequence for using PCR primers were designed by Kgwatalala *et al.* (2007). Forward primer was:

5′-CCCATTCGCTCTTGTTCTGT-3′

and reverse primer was:

5′-CGTGGTCTTGCTGTGGACT-3′.

The PCR reaction volume of 25 µL contained approximately 33.3 ng of genomic DNA, 1.25 m*M* Taq DNA polymerase, 2.5 µL of 1x PCR buffer, 1.5 m*M* $MgCl_2$, 0.2 m*M* dNTP and 10 p*M* of each primer. Amplification conditions included an initial denaturation at 95 ˚C for 5 min, followed by 35 cycles at 94 ˚C for 30s, 58 ˚C for 1 min and 72 ˚C for 1min, followed by a final extension at 72 ˚C for 10 min.

The PCR products were separated by 1% (w/v) agarose gel electrophoresis. The PCR products of 400 bp were digested with the *NCOI* restriction enzyme (Fermentas). The digestion with restriction enzyme was performed with 15 µL of PCR product mixed with 10 units of the restriction enzyme and then was incubated at 37 ˚C for 18 hours. Restriction digestion fragments were loaded on 3% agarose gel electrophoreses containing DNA safe stain and the gels were analyzed by UV rays. The banding was visualized and a documentation system was used. Gen Alex software was applied to estimate the gene and genotype frequencies, the heterozygosity and effective number of alleles. Expected theoretical heterozygosity from Hardy-Weinberg assumption was calculated.

RESULTS AND DISCUSSION

Figure 1 shows the results of PCR amplification of SCD1 gene that is consisting of 400 bp long with 100 bp DNA lader in one sides of the gel.

The pair of primers used for SCD1 was designed to amplify the exon 5. After amplification of the fragment, all samples were digested with PCR-RFLP technique using *NCOI* restriction enzyme. This enzyme splits A allele into two 200 bp fragments. All buffalo animals investigated in the present study were genotyped as VV homozygous genotype where all tested buffalo DNA amplified fragments were digested with *NCOI* endonuclease and gave one undigested fragment at 400-bp (Figure2).

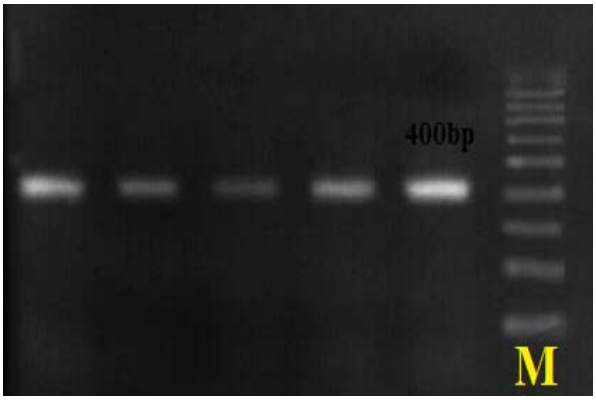

Figure 1 PCR product analyzed by electrophoresis in a 1% agarose gel. M: 100 bp Ladder

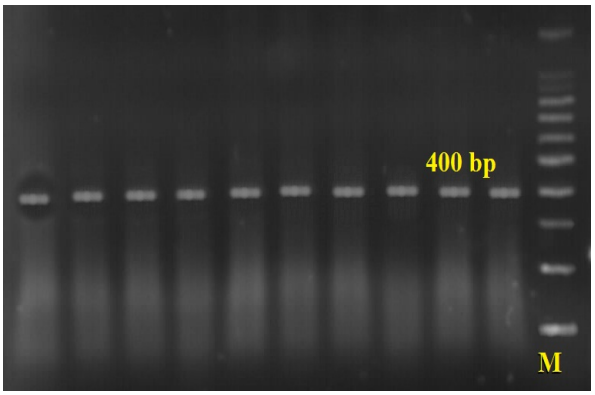

Figure 2 Representatively results of analysis PCR-RFLP for SCD1 gene (400 bp) by restriction enzyme *NCOI* on 3% agarose gel

Based on the results, the frequency of genotype VV was 100% and genotypes AV and AA were 0%. V allele frequency in this population was 1 and A allele was zero. In a study of polymorphism on 55 lactating bubaline breed Murrah by PCR-RFLP method and with using PstI and SmaI enzymes determined that there were no polymorphisms in the region of the gene of SCD analyzed and no correlation with CLA production could be done (Camargo *et al.* 2007).

In another study on Italian Holstein cattle, the genotypic and allelic frequencies were 0.13, 0.27 and 0.60 for the AA, VV and AV genotypes and 0.57 and 0.43 for the A and V alleles, respectively (Keating *et al.* 2006). A study on Jersy cattle in Canada showed that the genotypic and allelic frequencies were estimated 0.68, 0.07 and 0.24 for the AA, VV and AV genotypes and 0.8 and 0.19 for the A and V alleles, respectively (Kgwatalala *et al.* 2009). Also, a study on Italian cattle brown was conducted and the genotype frequencies of AA, VV and AV were 0.67, 0.04 and 0.29, respectively, and the frequencies were of 0.82 for the V allele and 0.18 for the A allele (Conte *et al.* 2003).

CONCLUSION

The differences observed in this study and in other studies could be due to sample size and genetic background of the animals. Monomorphic pattern for SCD1 gene, unlike what has been reported in cattle is considered a unique feature that may be related to the characteristic species in buffalo. Also, due to selection of population size, mutation, migration and genetic drift the Hardy-Weinberg equilibrium in the buffalo herd was not established.

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