Polymorphism in the SCD gene is associated with meat quality and fatty acid composition in Iranian fat- and thin-tailed sheep breeds

Mohsen Aali a, Hosein Moradi-Shahrbabak a,b, Mohammad Moradi-Shahrbabak a,b, Mostafa Sadeghi a, Hamid Kohram a

a Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
b Excellent Center for Improving Sheep Carcass Quality and Quantity, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

ARTICLE INFO

Article history:
Received 24 April 2014
Received in revised form
12 November 2015
Accepted 5 April 2016

Keywords:
Stearoyl-coenzyme A-desaturase (SCD) gene
Single-strand conformation polymorphism (SSCP)
Fatty acid composition
Meat quality
Fat-tailed lambs
Thin-tailed lambs

ABSTRACT

The enzyme stearoyl-coenzyme A-desaturase (SCD) has been proposed as a key regulator for fat content and fatty acid composition of meat. In this study, a DNA sequence and single-strand confirmation polymorphism analysis were performed in an attempt to investigate the relationship between variation within the SCD gene and fatty acid profiles, physicochemical composition, and the quality characteristics of longissimus dorsi (LD) muscle in two traditional fat- (Chall) and thin- (Zel) tailed Iranian sheep breeds. Only one previously reported SNP (g.379A→T) located in the 5′ untranslated region of the SCD gene was observed. Two previously reported allelic variants, SCD-1 and SCD-2, constituted two previously reported genotypes, “A” and “B”. Based on t-test, no allelic and genotypic frequency differences were observed between Chall and Zel breeds. Lambs with genotype “B” significantly had lower total saturated fatty acid (SFA) level (p<0.05) and more linoleic (p<0.02), arachidonic (p<0.002), and eicosapentaenoic (EPA) (p<0.001) acids and total poly-unsaturated fatty acid (PUFA) (p=0.002) contents and PUFA:SFA ratio (p<0.004) than those with genotype “A”. Meat color of the genotype “B” lambs was lower for a* (p<0.05) and higher for L* (p<0.08) compared to the genotype “A” ones. There were some interactions between breed and SCD gene in terms of palmitic acid (p<0.04), cooking loss (p<0.05), and protein content (p<0.03) of LD muscle. SCD locus explained 17.3%, 15.3%, 13%, and 12% of the phenotypic variation in terms of EPA and arachidonic acid, and PUFA contents and PUFA:SFA ratio, respectively. Interestingly, the results of the correlation analyses showed the significant negative correlations between healthy fatty acid indices (PUFA and PUFA:SFA) and meat color measures of a* and b* (r=-0.26 to r=-0.40, p<0.05) and the significant positive correlations between n-6: n-3 PUFA, one of the main harmful fatty acid indices, and a* (r=0.41, p<0.01) and b* (r=0.27, p<0.05) values which are coordinated with the effect of SCD genotypes on fatty acid composition and color of meat. So, both fatty acid composition and meat color traits should be considered in marker-assisted selection based on SCD gene. Overall, it seems that the genotype “B” of SCD gene is a more desirable genotype when selecting lambs in terms of both fatty acid composition and color of meat, because selecting this genotype would result in higher proportion of healthy fatty acids, lower proportion of harmful fatty acids, and better meat color.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Animal fat plays an important role in the human diet. Red meat (beef and lamb) contains a high level of fat and saturated fatty acids (SFAs) (Doyle, 2004; Wood et al., 2004; Schmid, 2010). Some SFAs are commonly found in meat and meat products, especially myristic and palmitic acids, raise total and low-density lipoprotein (LDL) cholesterol, and are thus the risk factors of coronary heart disease (CHD) (Scollan et al., 2006; Erkkilä et al., 2008; Schmid, 2010). Also, alterations in the ratio of SFAs and mono-unsaturated fatty acids (MUFAs) have been implicated in a variety of diseases associated with modern life, especially in developed countries, which include cardiovascular diseases, obesity, diabetes, neurological diseases, immune disorders, and cancers (Ntambi and Miyazaki, 2004). Further, it should be mentioned that unsaturated fatty acids (UFAs), which have been shown to possess several beneficial effects on human health (Kromhout et al., 1985; Schmid, 2010), predominate in meat fat (Schmid, 2010). Also, beef and lamb are the rich sources of cis-9, trans-11 isomer of conjugated linoleic acid (CLA) which is considered beneficial for human health.
in several aspects (Chin et al., 1992; Fritsche and Steinhart, 1998; O’Shea et al., 1999).

Fatty acids are also involved in various aspects of meat quality (Wood et al., 2004; Webb and O’Neill, 2008). First, because they have very different melting points, variation in fatty acid composition has an important effect on the rates of toughness to firmness and tenderness to softness and color of the inter- and intra-muscular (marbling) fat (IMF) (Enser, 1984; Wood et al., 2004). Second, the ability of UFAs, especially those with more than two double bonds, to rapidly oxidize, is important in regulating the shelf life of meat (rancidity and color deterioration) (Renerre, 2000; Wood et al., 2004) and developing flavor during cooking (Mottram, 1998; Wood et al., 2004).

These issues have been the reasons for increasing interest in recent years in the ways of manipulating the fatty acid composition of meat. The fatty acid composition of ruminant tissues is affected by some factors such as animal species (Schmid, 2010), breed (Schmid, 2010; Yousefi et al., 2012), sex (Clemens et al., 1973), diet (Mandell et al., 1998), and genetic factors (Perry et al., 1998; Inoue et al., 2008). In this regard, advances in molecular genetics have led to the identification of genes or quantitative trait loci (QTL) associated with genes, which have been suggested responsible for fat deposition (lipid synthesis) and fatty acid composition (oxidation of lipids). The enzyme stearoyl-coenzyme A-desaturase (SCD) has been proposed as a key regulator of both of these processes (Warensjö et al., 2007). SCD is a microsomal fatty acid mono-desaturase, which is also commonly known as Δ9-desaturase, and catalyses the committed step in the biosynthesis of MUFAs from SFAs by their Δ9-cis desaturation (Enoch et al., 1976). Another important function of the SCD enzyme is the endogenous production of the CLA (Bauman et al., 1999; Schmid et al., 2006).

Several single nucleotide polymorphisms (SNPs) have been detected in bovine SCD gene (Taniguchi et al., 2004; Kgwatalala et al., 2007) and the SNP at nucleotide 878 in exon 5 has been shown to be associated with fatty acid composition in meat and milk fat (Taniguchi et al., 2004; Mele et al., 2007; Ohsaki et al., 2009; Bartoń et al., 2010). In sheep, some SNPs have been identified within the SCD gene sequence (García-Fernández et al., 2009; Miari et al., 2009; Aali et al., 2014), four of which (SNPs located in intron 4 (3295 C>T) (Miari et al., 2009), intron 2 (g.2893G>A and g.2011T>C), and intron 3 (g.2893 G>A (García-Fernández et al., 2010)) displayed significant association with fatty acid composition of milk fat.

To date, the polymorphism within the ovine SCD gene has been evaluated in terms of its association with fatty acid composition only in the milk fat of Spanish Churra (García-Fernández et al., 2010) and Italian (Miari et al., 2009) sheep breeds and no works have focused on the relationship between variation within the ovine SCD gene and adipose tissue fatty acid composition. Also, no investigations have analyzed the association between genetic variability within the ovine SCD gene and meat quality traits. Therefore, the objective of this study was to determine the existence of previously reported polymorphisms (Aali et al., 2014) in the partial promoter, complete 5′ untranslated region (UTR) and exon 1, and partial intron 1 of the SCD gene in the lambs belonging to two traditional fat- (Chall) and thin- (Zel) tailed Iranian sheep breeds and to assess the relationships between the SCD genotypes and fatty acid profiles, physicochemical composition, and the quality characteristics of their longissimus dorsi (LD) muscle.

Chall is a native breed in a mountainous province of Iran, Qazvin, with a cold, dry weather located in north western part of Iran close to the Elburz Mountain Range (situated at longitude of 48° 45′ to 50° 50′ E, latitude of 35° 37′ to 36° 45′ N and altitude of 1278 m above sea level). Zel is a native breed in two of the northern provinces of Iran, Mazandaran and Golestan, with a wet weather and a heavy rainfall located in south of the Caspian Sea and north of the Elburz Mountain Range (situated at longitude of 50° 34′ to 54° 10′ E, latitude of 35° 47′ to 36° 35′ N and altitude of 54 m above sea level and longitude of 53° 57′ to 56° 22′ E, latitude of 36° 30′ to 38° 08′ N and altitude of 174 m above sea level, respectively). These breeds had different fat metabolisms: Chall is a fat-tailed breed and Zel is a thin-tailed breed. In a recent study by Yousefi et al. (2012), it was demonstrated that the eating quality of Zel lambs was better than Chall lambs. On the other hand, meat color and fatty acid profiles of Chall lambs were more favorable compared to Zel lambs. Therefore, we would expect some interactions between breed and SCD gene effects in terms of some meat quality and fatty acid traits studied in present research. Hence, to investigate this hypothesis, we analyzed the interaction between breed and SCD gene polymorphism for all analyzed traits.

2. Materials and methods

2.1. Experimental animals, slaughter procedures, and DNA extraction

2.1.1. Experimental animals

To obtain genomic DNA and phenotypic data, muscle samples of 59 lambs including 29 Chall (14 males and 15 females) and 30 Zel (15 males and 15 females) lambs previously analyzed by Yousefi et al. (2012) were used.

The selected population was designed using such method that there was no genetic structuration within it, as follows: the Chall and Zel lambs born in January 2009 were selected from native flocks in the Ghazvin and Mazandaran provinces of Iran, respectively. The lambs within each breed were selected from several different flocks. Selection within each flock was done using such method that there was no genetic relationship between any of the selected individuals within each flock (3–5 selected lambs within each flock). For example, all 3–5 selected lambs within each flock were the progeny of the different sires and dams and also there were no relationships between their parents. Selection within each pair of sire and dam was randomly presented.

All lambs form both Chall and Zel breeds were traditionally reared with their mothers on rangeland, suckling milk from their dams and were weaned at 3–4 months of age. After weaning, lambs were raised in a semi-intensive system; where they were partially grain-fed indoors and partially pasture-fed during the winter until they were 10–12 months. The average live weight of lambs, prior to slaughter, was 33–36 kg. To obtain full information about the environmental conditions of lambs raring such as feeding way, dietary components, rearing system and etc, the study by Yousefi et al. (2012) should be referred to.

2.1.2. Slaughter procedure

Lambs were transported (for approximately 75 min) to the commercial abattoirs where they were slaughtered after 24 h fasting. Carcasses were chilled at 4 °C for 24 h. Carcasses were divided into left and right sides.

2.1.3. DNA extraction

Genomic DNA was extracted from meat using a DNA extraction kit according to the manufacturer’s protocol (BioTech, Daejeon, China).

2.2. Phenotypic traits

2.2.1. Meat quality traits

Meat quality traits were ultimate pH, drip loss, cooking loss, shear force, and meat color measurements including L* (lightness), a* (redness), and b* (yellowness) values.
LD muscles were used to obtain instrumental measurements of meat quality. A pH probe was inserted into the LD muscle 24 h after slaughter at the 11th rib, using a Crison, pH-metro 507 and a 52–32 spear electrode. The pH apparatus was calibrated for pH 4 and 7 and also for a temperature of 25 °C. LD muscles were dissected and sliced in half to measure meat color. The half loin was then sliced into three samples for each lamb, and placed on a black foam tray and wrapped with plastic film (3 μm thickness, oxygen transmission rate of 2300–3000 cm3/100 in.2/24 h). The samples were then stored at 4 °C for 72 h. Meat color was estimated using the L*a*b* system with a Chroma meter Hunter Lab. The mobile phase consisted of helium C-50 at a flow rate of 4 ml/min and 20 psi presser at the beginning of the column. The column was 0.22 mm internal diameter, 30 m long and film 0.25 μm thickness. The mobile phase consisted of helium C-50 at a flow of 4 ml/min and 20 psi presser at the beginning of the column. Fatty acids were identified using Sigma reference standards and quantified using an internal standard (pentadecanoic acid, Sigma, St. Louis, MO). The oven temperature program was 160 °C for 5 min then increased at 20 °C/min up to 180 °C and was maintained at this temperature for 9 min then temperature increased at 20 °C/min up to 200 °C and remained at this status until the end of program. Run duration was 40 min. Data arising from the fatty acid determination were expressed as a percentage by weight of the total identified fatty acids and as mg per 100 g of muscle. Cholesterol content of each LD muscle was measured in triplicate according to the method described by Janssen and Meijer (1995) with a slight modification. Briefly, lipid was extracted from 1 g of homogenized sample (Folch et al., 1957), then 0.5 ml of final aliquot was separated and evaporated by N2. After recording the weight of dried material from the last stage, a six fold of Triton X100 chloroform solutions (1:1 volume ratio) based on obtained dried material weights was added. The final product was used to assess the amount of cholesterol using a Pars Azmoon kit (1,500,010, Iran).

2.3. Polymerase chain reaction (PCR)

A region of the ovine SCD gene spanning over the partial sequence of promoter, complete sequence of 5’ UTR and exon 1, and partial sequence of intron 1 was amplified using a set of forward (5’-AAATTCCTCGCCGAATGAC-3’) and reverse (5’-TCTCACCTCCITGTACGCAA-3’) primers described by Aali et al. (2014). The PCR was carried out in 25 μl of reaction volume containing 1X reaction buffer, 0.25 mM dNTP, 1.25 U Taq DNA polymerase, 2.5 mM MgCl2, 0.25 μM of each primer, and ddH2O. The amplifications were performed with a thermal cycler (Bio-Rad, Hercules, CA, USA) with the following thermo-cycling profile: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 63 °C for 40 s, and 72 °C for 40 s. The final elongation was 5 min at 72 °C.

2.4. Single-strand confirmation polymorphism (SSCP)

SSCP is defined as conformational difference of single-stranded nucleotide sequences of identical length as induced by differences in the sequences under certain experimental conditions. In this method, at first stage, a double-stranded DNA sequence is denatured at 95 °C. Two single- stranded DNA sequences are retained as single- strand by means of formamide and fixed on ice. Then, the single- stranded DNA sequences are electrophoresed on polyacrylamide gel. In this condition, the single-stranded DNA undergoes a 3-dimensional folding and may assume a unique conformational state based on its DNA sequence. The difference in shape between two single-stranded DNA strands with different sequences can cause them to migrate differently on an electrophoresis gel, even though the number of nucleotides is the same, which is, in fact, an application of SSCP (Orita et al., 1989). It can be used to preliminary detect point mutations at various positions in a fragment. After that, we can determine the type of each point mutation using DNA sequencing method. Also, SSCP can be used in genotyping to detect homozygous individuals of different allelic states, as well as heterozygous individuals that should each demonstrate distinct SSCP patterns in an electrophoresis experiment (Otto et al., 1993).

For genotyping the SCD locus, 6 μl of each PCR product was diluted with 12 μl of loading dye that included: 98% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. After denaturation at 95 °C for 10 min, the samples were immediately placed on ice and then loaded on 18–20 cm, 12% acrylamide: bisacrylamide (37.5:1) gels. The mixture was electrophoresed using Protein II xi cells (Bio-Rad, Hercules, CA, USA) in
0.5 X TBE buffer for 24 h at 150 V in a 10 °C room with 4 °C water circulating through the cell core. The gels were silver-stained according to the method described by Bassam et al. (1991).

2.5. DNA sequencing

For each of the ovine SCD variants identified by PCR–SSCP, PCR products of two animals were re-amplified in total volume of 25 μl followed by purification from the gel using Accuprep™ PCR purification kit (Bioneer, Daejeon, Korea). Sequencing was performed in both directions with forward and reverse primers using an ABI 3730 XL DNA Analyzer (BioNeer, Daejeon, South Korea).

2.6. DNA sequence analysis

The ovine SCD locus sequences were analyzed using BioEdit software (Hall, 1999). To ensuring that the annotation and variations are not because of PCR or sequencing artifacts, we analyzed sequence chromatograph file for each sequence using Phred quality’s scores presented by ABI 3730 XL DNA Analyzer (BioNeer, Daejeon, South Korea) and filtered them according this score. The observed score for the predicted Phred quality’s score “q” is calculated by the following formula:

\[ q = -10 \log_{10}(p) \]

where p is error probability for each base.

Many sequencing projects use the QV20 rule as a rough measure of the effective length of a DNA read (Nelson and Fridlyand, 2003). Quality values presented by ABI 3730 XL DNA Analyzer (BioNeer, Daejeon, South Korea) are based on the QV20 rule. Based on the study by Li et al. (2004), when QV20 scores were > 55, essentially no error was observed. When scores were < 20, the prediction was fairly consistent. When scores were between 20 and 55, Phred scores overestimate probabilities. Therefore, in the present study, we analyzed the sequences with QV20 > 55 and removed any sequences with QV20 < 55 in SNP and allelic sequence analyses.

Sequences of PCR amplicons, representing the unique PCR–SSCP variants, were analyzed and compared with each other and NCBI reference sequences with the GeneBanks JX944472-JX944475 (Aali et al., 2014), FJ5113370 (García-Fernández et al., 2009), GQ904712.1 (Mari et al., 2009), and AJ001048 (García-Fernández et al., 2010) in order to identify novel allelic variants and SNPs of the ovine SCD gene.

2.7. Statistical analyses

2.7.1. Population genetic analyses

Population genetic parameters including allele and genotype frequencies were calculated in the two studied populations using GenAlEx software (Peakall and Smouse, 2006). Also, t-test was used to analyze frequency difference of alleles and genotypes between two fat- (Chall) and thin- (Zel) tailed breeds. In this test, t-values were obtained by two following formulas:

\[ \sigma^2_p = \frac{p_1q_1 + p_2q_2}{n_1n_2} \]  

\[ t = \frac{p_1 - p_2}{\sigma p} \]

where \( \sigma^2_p \) is variance of allele or genotype frequency between two populations for each allele or genotype, \( p_1 \) is frequency of that allele or genotype in population 1, \( q_1 = 1 - p_1 \), \( p_2 \) is frequency of that allele or genotype in population 2, \( q_2 = 1 - p_2 \), \( n_1 \) is the number of total lambs in population 1 and \( n_2 \) is number of total lambs in population 2. After t-value was calculated for each allele or genotype, it was compared with value of t-student (df=∞ and p-values of 0.05, 0.01, and 0.001).

2.7.2. Association analysis between SCD polymorphism and quantitative traits

Mean, standard deviation (SD), and coefficient of variation (CV) for all phenotypic traits including fatty acids profile, physico-chemical composition, and meat quality traits studied in present work were calculated using Means procedure of SAS (2004). Also, overall (phenotypic) correlation coefficients among the key meat quality and fatty acid traits of 59 lambs studied in present work were previously calculated using Corr procedure of SAS (2004) by Yousefi et al. (2012).

Associations of SCD genotypes with fatty acid profiles, physico-chemical composition, and meat quality traits were investigated by least-squares analysis of variance (P=0.05) using Mixed model procedure of SAS (2004). The statistical model was fitted as follows:

\[ y_{ijkm} = \mu + G_i + S_j + A_k + B_l + G_i \times B_l + s_i \times e_{ijkm} \]

where \( y_{ijkm} \) is phenotypic value of fatty acid profile, physico-chemical composition, and meat quality traits, \( \mu \) is overall mean, \( G_i \) is fixed effect of SCD genotype (i=A or B), \( S_j \) is fixed effect of sex (male and female), \( A_k \) is fixed effect of age (10–12 months old), \( B_l \) is fixed effect of breed (Chall and Zel), \( G_i \times B_l \) is interaction between SCD genotype and breed, \( s_i \times e_{ijkm} \) is random effect of sire, and \( e_{ijkm} \) is random residual error. When SCD genotype x breed was not significant, it was removed from the model. It should be mentioned that in the primary analysis, the fixed effect of herd was entered into the model, but as this factor had no significant effect on any of the traits analyzed in the present study, herd effect was removed from the final model. Furthermore, as the effect of contemporary group overlapped with the effect of breed, its effect was removed from the model.

Also, the percentage of the phenotypic variation explained by the region of the ovine SCD gene was estimated using Mixed model procedure of SAS (2004).

3. Results

3.1. SSCP and DNA sequence analyses

The SSCP analysis revealed two unique banding patterns (SCD-1 and SCD-2) which constituted two different genotypes of “A” (SCD-1/SCD-1) which was the homozygous genotype of “AA” at SNP g.379A > T and “B” (SCD-1/SCD-2) which was the heterozygous genotype of “AT” at SNP g.379A > T in the two studied sheep breeds (Fig. 1).

Sequence analysis of the ovine SCD locus allowed for observation of one SNP (g.379A > T) located in the 5’ UTR (Fig. 2). This SNP constituted two sequences or allelic variants including SCD-1 and SCD-2, corresponding to GenBank IDs of JX944472 and JX944473, respectively (Fig. 2). These two allelic variants constituted two different genotypes of “A” and “B”, which confirmed the results of the SSCP analysis.

3.2. Allele and genotype frequencies

Allele SCD-1 had higher frequency than SCD-2 (0.754 versus 0.246) in the total population (Table 1). Lambs homozygous (two copies) for allele SCD-1 (lambs with genotype “A”) were more frequent than lambs heterozygous for alleles SCD-1 and SCD-2.
3.3. Frequency differences of alleles and genotypes between fat- and thin-tailed breeds

Based on the results of t-test, no allelic frequency differences were observed between Chall (fat-tailed) and Zel (thin-tailed) breeds (t-value for both alleles of SCD-1 and SCD-2 was 0.892) (Table 1). Also, there were no significant differences between Chall and Zel lambs in terms of genotypic frequencies (t-value for both genotypes of A and B was 1.245) (Table 1).

3.4. Descriptive statistics and correlations among traits

Tables S1 and S2 show the means, SD, and CV of the traits analyzed in present study. Fatty acids contents expressed as percentage by weight of total fatty acids were determined in a total of 59 LD muscle samples. Variation coefficients of fatty acid and physicochemical traits had a considerable dispersion, ranging from 4 to 18 and 1–19, respectively, whereas these coefficients had a low dispersion and low values, ranging from 1 to 7 for meat quality traits.

Overall correlation coefficients among some meat quality traits and fatty acid indices previously calculated by Yousefi et al. (2012) are presented in Table S3. The results obtained by Yousefi et al. (2012) showed a moderate, positive correlation between IMF and a* value (r = +0.49, p < 0.01), and there was also a positive correlation between b* and IMF (r = +0.39, p < 0.01). IMF, also, had the moderate, negative correlations with poly-unsaturated fatty acids (PUFA) (r = –0.40, p < 0.05) and PUFA:SFA (r = –0.39, p < 0.05) and a positive correlation with SFA (r = +0.34, p < 0.05). Both of the meat color measures including a* and b* values displayed the moderate, negative correlations with PUFA (r = –0.32, p < 0.05 for a* value; r = –0.26, p < 0.05 for b* value) and PUFA:SFA (r = –0.26, p < 0.05 for a* value; r = –0.28, p < 0.05 for b* value) and a positive correlation with SFA (r = –0.41, p < 0.01 for a* value; r = –0.27, p < 0.05 for b* value).

**Promoter**

**5′UTR**

**Exon 1**

![Fig. 1. PCR-SSCP of the ovine stearoyl-coenzyme A-desaturase (SCD) gene. Two representative genotypes of “A” and “B” for the two unique SSCP patterns corresponding to two allelic variants of SCD-1 and SCD-2 are shown.](image)

(lambs with genotype “B”) (0.508 versus 0.492) in the sum of two studied populations (Table 1). The results for allele and genotype frequencies within each breed were approximately similar to those in the sum of two breeds (Table 1).

![Fig. 2. Nucleotide sequence alignment of the ovine SCD alleles. Allelic variants of SCD-1 and SCD-2 with GenBanks JX944472 and JX944473 were observed in the present study; the sequence with GenBank GQ904712.1 was detected in the study by Miari et al. (2009). Nucleotides in the exon 1 are indicated in upper-case and those in other regions are in lower-case. Amino acids are represented in one-letter code and shown above the corresponding codons. Nucleotides and amino acids identical to the sequence with GenBank GQ904712.1 are presented by dots. The shaded regions indicate the primer-binding regions. Different regions of gene including promoter, 5′UTR, exon 1 and intron 1 were separated by black down arrow (↓). The right arrow (>) in the first of sequence states that the sequence is a part of a larger sequence and its start point is several nucleotides before in 5′ terminal, and the left arrow (<) in the end of sequence indicates that the sequence is a part of a larger sequence and its terminal point is the next several nucleotides in 3′ terminal.](image)
Table 1: Allele and genotype frequencies of SCD locus in Chall, Zel, and total populations.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Allele frequency</th>
<th>Genotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chall</td>
<td>Zel</td>
</tr>
<tr>
<td>SCD-1</td>
<td>46</td>
<td>0.79</td>
</tr>
<tr>
<td>SCD-2</td>
<td>12</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Table 2: Association of SCD genotypes with fatty acid profiles (percentage by weight of total identified fatty acids) and their selected ratios in longissimus dorsi muscle of lambs (mean ± SE) and the percentage of the phenotypic variance explained by SCD locus for traits of interest.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SCD gene effect on traits</th>
<th>Phenotypic variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype A (n=30)</td>
<td>Genotype B (n=29)</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>4.71 ± 0.44</td>
<td>3.85 ± 0.36</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>26.10 ± 0.99</td>
<td>26.63 ± 0.93</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1n-9)</td>
<td>3.88 ± 0.34</td>
<td>4.19 ± 0.38</td>
</tr>
<tr>
<td>Heptadecanoic acid (C17:1n-9)</td>
<td>0.65 ± 0.05</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>15.89 ± 0.69</td>
<td>15.35 ± 0.65</td>
</tr>
<tr>
<td>Oleic acid (C18:1n-9)</td>
<td>39.31 ± 0.93</td>
<td>37.57 ± 0.88</td>
</tr>
<tr>
<td>Linoleic acid (C18:2n-6)</td>
<td>5.51 ± 0.43</td>
<td>6.60 ± 0.40</td>
</tr>
<tr>
<td>Linolenic acid (C18:3n-3)</td>
<td>0.47 ± 0.04</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Arachidonic acid (C20:4n-6)</td>
<td>0.89 ± 0.09</td>
<td>1.65 ± 0.12</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (C20:5n-3)</td>
<td>0.21 ± 0.02</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Docosahexaenoic acid (C22:6n-3)</td>
<td>0.31 ± 0.03</td>
<td>0.51 ± 0.04</td>
</tr>
</tbody>
</table>

Table 3: Least-squares means (± SE) of palmitic acid, cooking loss and protein content in LD muscle, from two sheep breeds, where there was a significant breed x SCD genotype interaction.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chall</th>
<th>Zel</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n=14)</td>
<td>B (n=15)</td>
<td></td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>28.39 ± 1.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.26 ± 1.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cooking loss (g/100 g)</td>
<td>31.05 ± 2.54</td>
<td>33.97 ± 2.59</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20.60 ± 0.61&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.27 ± 0.62&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Note: Means with different superscripts within the same row are significantly different (P < 0.05 for palmitic acid and P < 0.04 for protein content).

3.5. Association of SCD polymorphism with fatty acid composition

Least-square means and standard errors of mean fatty acid composition for genotypes of SCD locus and p-values for the effect of SCD gene on fatty acid composition are shown in Table 2.

Variation in SCD gene had a significant effect on the content of some individual fatty acids including linoleic (C18:2n-6; p < 0.02), arachidonic (C20:4n-6; p < 0.002), and eicosapentaenoic (EPA; C20:5n-3; p < 0.001) acids in the LD muscle of Chall and Zel lambs (Table 2). Although no significant associations were found between SCD genotypes and the content of docosahexaenoic (DHA; C22:6n-3) and oleic (C18:1n-9) acids, SCD polymorphism displayed a weakly non-significant effect on the content of oleic acid (p < 0.08) and DHA (P < 0.09) in the LD muscle of Chall and Zel lambs (Table 2). Genotype “B” of SCD locus resulted in higher contents of linoleic, linolenic, and arachidonic acids, EPA, and DHA and lower oleic acid than genotype “A” (Table 2).

Significant associations were detected between SCD genotypes and PUFA (p < 0.002) and SFA (p < 0.05) levels and PUFA:SFA ratio (p < 0.004) in the LD muscle of Chall and Zel lambs (Table 2). Genotype “B” was associated with higher amounts of PUFA and PUFA:SFA ratio and lower SFA content than genotype “A” (Table 2).

There were no significant interactions between breed and SCD genotype in any of the fatty acid profiles and their selected ratios, except for palmitic acid (C16:0; p < 0.04) (Table 3). Zel lambs with “B” genotype had significantly higher palmitic acid content than lambs with “A” genotype from the same breed (p < 0.05), whereas no significant difference was observed between Chall lambs with “A” and “B” genotypes in terms of palmitic acid content (Table 2). Also, Chall lambs with “A” genotype contained more palmitic acid than Zel lambs with the same genotype (p < 0.05) (Table 3), while no significant difference was identified between the lambs from Chall and Zel breeds with “B” genotype. From this we can hypothesize that the effect of SCD gene on palmitic acid content of LD muscle depended on the breed since in Zel breed, this effect was significant and in Chall breed, it was not significant. Therefore, the effect of breed should be also considered when selecting lambs in terms of palmitic acid content of LD muscle based on the SCD gene.

The percentage of the phenotypic variation explained by SCD locus in terms of fatty acid traits ranged between 0.1% (in terms of palmitoleic acid) and 17.3% (in terms of EPA).

3.6. Association of SCD polymorphism with meat quality traits

Least-squares means and standard errors of instrumental quality characteristics of LD muscle lamb for genotypes of SCD locus and p-values for the effect of SCD gene on instrumental measurements are shown in Table 4.

Based on the present results, meat color of Zel and Chall lambs was influenced by the SCD polymorphism (Table 4). SCD gene had no significant effect on b* value, but a significant difference was
identified between SCD genotypes in terms of a’ value (p < 0.05) (Table 4). Also, a slightly non-significant relationship was observed between SCD gene and L’ value (p < 0.08). The ovine SCD gene did not affect the ultimate pH, shear force, drip loss, and cooking loss (Table 4). However, there was a significant interaction between breed and SCD genotype in terms of cooking loss (p < 0.05) (Table 3).

SCD gene explained 0.1% (in terms of shear force) to 5.3% (in terms of L’ value) of the phenotypic variation for meat quality traits.

3.7. Association of SCD polymorphism with physicochemical composition of LD muscle

There were no significant differences in any of the physicochemical composition of LD muscle including protein, IMF, ash, moisture, triglyceride, and cholesterol contents between “A” and “B” genotypes of SCD locus (Table 5). However, a significant interaction was observed between breed and SCD genotype in terms of protein content (p < 0.03) (Table 3). Zel lambs with “B” genotype had significantly more protein content than lambs with “A” genotype from the same breed (p < 0.04), whereas there was no significant difference between Chall lambs with “A” and “B” genotypes in terms of protein content (Table 3).

The proportion of the phenotypic variation explained by SCD locus in terms of fatty acid traits ranged between 0.3% (in terms of moisture and cholesterol contents) and 1.6% (in terms of ash content).

Table 4

<table>
<thead>
<tr>
<th>Trait</th>
<th>SCD gene effect on traits</th>
<th>Phenotypic variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genotype A (n=30)</td>
<td>Genotype B (n=29)</td>
</tr>
<tr>
<td>Ultimate pH</td>
<td>5.64 ± 0.05</td>
<td>5.62 ± 0.05</td>
</tr>
<tr>
<td>Shear force (kg/cm²)</td>
<td>11.08 ± 0.64</td>
<td>11.27 ± 0.62</td>
</tr>
<tr>
<td>Drip loss (%)</td>
<td>2.70 ± 0.17</td>
<td>2.28 ± 0.16</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>34.67 ± 1.41</td>
<td>34.09 ± 1.77</td>
</tr>
<tr>
<td>L’ (lightness)</td>
<td>40.96 ± 1.05</td>
<td>43.16 ± 1.09</td>
</tr>
<tr>
<td>a’ (redness)</td>
<td>16.00 ± 0.41</td>
<td>15.08 ± 0.43</td>
</tr>
<tr>
<td>b’ (yellowness)</td>
<td>11.10 ± 0.43</td>
<td>10.70 ± 0.41</td>
</tr>
</tbody>
</table>

NS: not-significant.

4. Discussion

The key role of SCD gene in fat metabolism (Ntambi and Miyazaki, 2004) indicated it as a major candidate gene responsible for variation in the phenotypes observed in fat deposition and fatty acid composition of meat and milk of ruminants (García-Fernández et al., 2009) that might impart to gene-assisted selection (GAS).

In this study, the SSCP and DNA sequence analyses revealed one (g.379A > T) of the three previously (Aali et al., 2014) reported SNPs, two (SCD-1 and SCD-2) of the four previously (Aali et al., 2014) reported allelic variants, and two (“A” and “B”) of the four previously (Aali et al., 2014) reported genotypes at ovine SCD locus in Zel and Chall lambs. Aali et al. (2014) detected three SNPs of g.87C > A and g.257G > A located in the promoter, and g.379A > T located in the 5’ UTR and four allelic variants of SCD-1 to SCD-4 with GenBank IDs of JX944472-JX944475 in the ovine SCD locus studied in the present work in three fat- (Lori-Bakhtiar), medium fat- (Zel-Atabay), and thin- (Zel) tailed Iranian sheep breeds. Several other SNPs have been reported in different regions of the ovine SCD gene which include SCD02 (g.1473A > G) and SCD03 (g.2011T > C) within intron 2 (García-Fernández et al., 2009), SCD4 (g.2893G > A) within intron 3 (García-Fernández et al., 2009), and g.3295C > T within intron 4 (Miari et al., 2009).

The present results showed that allele SCD-1 was more frequent than SCD-2, which was in agreement with the result of Aali et al. (2014) in the total of three Iranian sheep breeds (0.754 versus 0.232). Although alleles SCD-3 and SCD-4 were not observed in the present study, it should be mentioned that, in the previous research (Aali et al., 2014), these two allelic variants were only detected in the Lori-Bakhtiar population with very low frequencies of 0.022 and 0.004, respectively, and were not identified in the Zel breed, which was similar to the present results.

Fat-tail is an obvious characteristic for fat deposition in sheep which distinguishes fat- and thin-tailed sheep breeds based on fat metabolism. Frequency difference of SCD alleles and genotypes between two fat- (Chall) and thin- (Zel) tailed breeds was used as a scale for determining the breed-specific alleles and genotypes. Although it was expected to be observed the allelic or genotypic frequency difference between fat- and thin-tailed breeds in one or more loci (SNPs) within the SCD gene, a key gene for fat metabolism (Warenšjö et al., 2007), the results of t-test showed no breed-specific alleles and genotypes related to fat metabolism between these two breeds which may be due to three following reasons:

The major part of SCD locus analyzed in present study (494 bp from all 527 bp) which includes the promoter and 5’UTR regions as the important regulatory regions for gene expression (Lodish et al., 2003) is a conserved locus (Putta and Mitra, 2010) since, in present study, no mutations were detected within partial promoter region (300 bp) and only a single SNP was identified within 5’UTR. 2) It may suggest that the genetic control of fat-tail in sheep is based on the existence of mutations in other regions of the ovine SCD gene such as the other coding (exons) and non-coding (introns) regions, as suggested by the polymorphisms identified in the intron regions in sheep (García-Fernández et al., 2009). 3) The number of animals used in the present study seems to be not big enough to fully determine the allelic and genotypic frequency differences between these two breeds with a high accuracy.

The lambs used in this study were reared in the same environmental condition such as feeding way, dietary components, and rearing system and slaughtered at the closely same age. Still, the variation coefficients of some of the physicochemical composition and fatty acid traits were closely high. In the other researches, CV for the meat quality, physicochemical composition, and fatty acid traits was high in lamb (Mortimer et al., 2014;
Ramírez-Retama et al., 2014; Silva Sobrinho et al., 2014), beef (Matsuhashi et al., 2011), pork (Ovilo et al., 2006), and chicken (Starčević et al., 2014) meat which were in agreement with the present results. Genetic factors are likely to explain a certain amount of this variation for meat quality, physicochemical composition, and fatty acid traits (Barton et al., 2010).

Our results displayed that SCD gene significantly affected several individual fatty acids, fatty acid indices, and their ratios including linoleic and arachidonic acids, EPA, DHA, SFA, and PUFA contents and PUFA:SFA ratio. In agreement with our results, SCD gene showed a significant effect on the proportion of linoleic acid in the milk fat of Spanish Churra sheep (García-Fernández et al., 2010). Similarly, polymorphism within the SCD gene was significantly associated with SFA content in the milk fat of the Spanish Churra sheep (García-Fernández et al., 2010) and LD muscle of Korean (Oh et al., 2011) and Japanese Black (Matsuhashi et al., 2011) cattle breeds. In contrast with the present results, there were no significant associations between SCD polymorphisms and PUFA percentage of milk fat in Spanish Churra sheep (García-Fernández et al., 2010) and Holstein cattle (Rincon et al., 2012).

Several studies have illustrated an inverse association between omega-3 fatty acids, linolenic acid (C18:3n−3), EPA, and docosahexaenoic acid (DHA; C22:6n−3), and CHD, acute myocardial infarction (AMI), and angina pectoris (Kromhout et al., 1985; Wahleqvist et al., 1989). It has been demonstrated that dietary n-6 PUFA promote the growth of pancreatic tumors and n-3 PUFA inhibit them (Pandian et al., 1999; Roebuck, 1992). Higher PUFA:SFA ratio in meat is related to more desirable meat quality characteristics such as meat color, flavor, and shelf life (Wood et al., 2004).

In this study, the lambs with “B” genotype favorably contained more linoleic, linolenic, and arachidonic acids, EPA, DHA, and PUFA and PUFA:SFA ratio and lower oleic acid and SFA than those with “A” genotype. SCD gene demonstrated the strongest effect on EPA (P < 0.001), since genotype “B” resulted in 90% increase in the concentration of EPA in the LD muscle of lambs than the other genotype.

SCD gene also influenced meat color of lambs. It has been demonstrated that meat with a more pink-color is more desirable to consumers in different countries (Santos-Silva and Portugal, 2001; Martinez-Cereo et al., 2005; Ekiz et al., 2009). In present study, the lambs with “A” genotype had greater a’ and lower L’ values than those with “B” genotype, displaying poorer meat color of lambs with genotype “A”. Although there was no significant relationship between the bovine SCD gene variation and beef color standard in Japanese Black cattle, SCD gene had a significant effect on the beef luster (p < 0.05) as a scale for lightness (Matsuhashi et al., 2011), which was in agreement with the present results.

Although SCD enzyme is not directly associated with meat color, the relationship between variation in this gene and this trait might be due to the effect of meat fatty acid profile on meat color (Wood et al., 2004). The effect of fatty acids on meat color is due to two fatty acid characteristics including 1) the different melting points of the fatty acids since groups of fat cells containing solidified fat with a high melting point appear whiter than when liquid fat with a lower melting point is present (Wood et al., 2004), and 2) the propensity of UFAs to oxidise leading to the development of rancidity with the increased display times. The color change is due to the oxidation of red oxymyoglobin to brown metmyoglobin. This reaction generally proceeds in parallel to that of rancidity (Renerre, 2000).

Interestingly, Yousefi et al. (2012) reported the significant correlations between fatty acid indices (PUFA, PUFA:SFA, and n−6:n−3PUFA) and meat color traits (a’ and b’) in 59 Chali and Zel lambs studied in present work. Also, based on their results, the considerable correlations were detected between IMF and meat color traits (a’ and b’). Furthermore, Mortimer et al. (2014) reported the moderate to strong positive genetic correlations between IMF content and meat color measures including a’ (r = 0.26), b’ (r = 0.81), and L’ (r = 0.56) values in Merino and crossbred progeny of Merino. Also, in their study, the moderate genetic correlations were estimated between fatty acid profiles and meat color measures. Similar to these results, Lorentzen and Vangen (2012) and Karamichou et al. (2006) reported the moderate genetic correlations between IMF and meat color measures of a’, b’, and L’ values in lamb meat. Based on considerable values of genetic correlations between these traits (the above-mentioned values), the effect of SCD gene on meat color of lambs identified in present research is likely due to 1) the pleiotropic effect (Stewart et al., 2011; Jiang et al., 2009) of this gene, as a potential candidate gene for fatty acids and IMF contents, on meat color or 2) the linkage disequilibrium (Wu et al., 2007) of the QTL/s within this gene with the QTL/s within the gene/s biochemically influencing color of meat.

Based on the present results, SCD gene did not illustrate any significant effect on the physicochemical composition of LD muscle. However, a significant interaction was observed between breed and SCD genotype in terms of protein content, since Zel lambs with “B” genotype had desirably more protein content than lambs with “A” genotype from the same breed. Similarly, SCD gene displayed no significant effect on moisture, IMF, and crude fat in the LD muscle of Japanese Black cattle (Matsuhashi et al., 2011). Some significant relationships were detected between SCD polymorphisms and milk protein percentage in Chinese Holstein (Alim et al., 2012) cows, which were in agreement with the present results about significant difference between SCD genotypes for the concentration of meat protein.

In present study, SCD locus explained the considerable proportion of the phenotypic variation in terms of some fatty acid contents (15.3% for arachidonic acid and 17.3% for EPA) and their selected ratios (13% for PUFA and 12% for PUFA:SFA). In contrast to our results obtained on “meat” fatty acid profile in Iranian “sheep” breeds, Rincon et al. (2012) illustrated that SCD locus explained a low proportion of the phenotypic variation (between 4% and 7%) of “milk” fatty acid contents and their selected ratios in Holstein “cattle”. This finding displayed that the expression level of the SCD gene and its activity on fatty acids may be depended on the target tissue (meat or milk or etc.) and animal species (sheep or cattle or etc.).

5. Conclusion

In summary, based on the results of the present study, SCD gene influences both fatty acid composition and color of meat, and these traits should be considered in marker-assisted selection (MAS) based on SCD gene. Overall, genotype “B” is a more desirable genotype and results in a higher proportion of healthy fatty acids, lower proportion of harmful fatty acids, and better meat color. The sample size used in present study is not big enough to fully support the results for the effect of SCD polymorphism on traits of interest and the research should be continued on a larger group of animals. Overall, these results represent an opportunity to improve the quality and healthfulness of lamb meat from the perspectives of human nutrition and health by MAS strategy.

Acknowledgements

This work was financially supported by Excellent Center for Improving Sheep Carcass Quality and Quantity, Department of
Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Elburz province, Iran. We appreciate the staff, teachers and graduate students at Laboratory of Bio-technology, Department of Animal Science, University of Tehran, for technical assistance during this research.

Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.livsci.2016.04.003.

References


