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# Evaluation of SCD and FASN Gene Expression in Baluchi, Iran-Black, and Arman Sheep

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## Abstract

***Background:*** With the increasing concern for health and nutrition, dietary fat has attracted considerable attention. The composition of fatty acids in the diet is important because they are associated with major diseases including cancers, diabetes, and cardiovascular disease. The fatty acid synthase (FASN) and stearoyl-CoA desaturase (delta-9-desaturase) (SCD) genes affect fatty acid composition (1). The expression of SCD and FASN genes is related to an increase in conjugated linoleic acid (CLA) in dairy products, which benefits human health.The aim of current study was to investigate expression changes of SCD and FASN genes that resulted from crossbreeding the local Baluchi sheep with alien breeds.

***Methods:*** We collected tissue samples from the mammary glands of 24 single-born ewes from local Baluchi and synthetic Iran-Black and Arman sheep breeds in the Abbas Abad breeding center. After RNA extraction and cDNA synthesis, real-time PCR was performed with all samples in triplicate.

***Results:***The maximum and minimum expression of SCD and FASN genes was in the local Baluchi sheep and the crossbred Arman sheep, respectively.

***Conclusions:*** With the highest SCD and FASN gene expression in local Baluchi sheep and relatively less expression of these genes in synthetic Iran-Black and Arman Sheep breeds, it may be necessary to consider the consequences of crossbreeding local sheep and the fatty acid composition of their dairy products.

**Keywords:** SCD, FASN ,Gene expression, Real-time PCR

## Introduction

There is growing consumer recognition of the link between diet and health. This awareness impacts food choices and the term ‘‘functional food’’ is a generic one often used to describe this concept (1). The functional role of conjugated linoleic acid (CLA) in health has been reviewed by Benjamin and Friedrich (2). Conjugated linoleic acid is a generic term for a range of positional and geometric isomers of linoleic acid that benefit human health. Cis-9, trans-11 CLA is responsible for the anti-carcinogenic properties of CLA, although the mechanisms are still under study (3). Trans-12, cis-10 CLA decreases fat mass in animals (4). Dairy products provide approximately 75% of human CLA dietary intake. The cis-9, trans-11 CLA in milk fat is the major isomer and represents 78–89% of the total CLA in sheep milk fat (5).

Sheep milk fat contains several components that may benefit human health, such as monounsaturated fatty acids (FAs) and CLA. Most of the CLA in ruminant milk is synthesized in the mammary gland by the action of the enzyme stearoyl-CoA desaturase

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(SCD) on circulating vaccenic acid (trans-11 C18:2; VA) (6).

The fatty acid synthase (FAS) enzyme is encoded by the FASN gene. Fatty acid synthase catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA in the presence of NADPH into long-chain saturated FAs (7).

The Baluchi sheep is the most common native breed in the RazaviKhorasan, Sistan and Baluchistan, and Kerman and Yazd provinces of the Islamic Republic of Iran, comprising about 30% of the total sheep population, or approximately 15 million heads (8). The Baluchi sheep breed originated in what is now southwest Pakistan, eastern Iran, and southern Afghanistan, also known as Mengali, Kermani, Naeini, Neini, Yazdi, Araghi, and Khorasani, based on geographic distribution. This breed is well-adapted to a wide range of harsh environments from the northeast to the southeast of the country and commonly reared on low-quality pastures via household extensive systems. The small stature and particular physiological characteristics of Baluchi sheep have enabled them to tolerate unfavorable natural conditions. For this reason, Baluchi sheep are categorized as a high-quality mutton-producing breed with low cost. The breed is fat-tailed with white fleece spots of black markings on their heads and legs. They are primarily employed in meat production with wool suitable for carpets (9, 10). This breed plays an important role in the economy of ranchers and meets protein and dairy needs of society (11).

To increase production in this breed, a crossbreeding program was begun in 1975 with Chios sheep, a breed from the Greek island of Chios, in 1975. The breeding project commenced in the sheep-breeding station of Abbas Abad, located in KhorasanRazavi province in northeast Iran. The result of this breeding project was named Iran-Black (12).

Iran-Black was the first synthetic sheep breed in Iran, developed to increase litter size and improve weaning performance, and tolerate the harsh environmental conditions prevalent in the Baluchi sheep region (12). The milk yield of lactating Iran-Black ewes averages 962 grams per day over a 90-day period (13).

The Arman sheep breed was developed in 1976 by crossbreeding Chios, Suffolk, Ghezel, and Baluchi sheep. This breed was developed for arid regions and is well-adapted to the wide range of harsh environmental conditions found in northeastern Iran (13). The crossbred Arman ewes produce more offspring and milk than the local breed ewes (13). The milk yield of lactating Arman ewes averages 958 grams per day over a 90-day period (13).

Crossbreeding offers two primary advantages over the use of only one breed; crossbred animals 1) exhibit heterosis (hybrid vigor), and 2) combine the strengths of the breeds used to form the cross. The goal of a well-designed, systematic crossbreeding program is to simultaneously optimize these advantages (14). Crossbreeding between sheep breeds is common worldwide; however, to our knowledge, no studies of gene expression changes resulting from crossbreeding in sheep have been reported in Iran.

In lactating ruminants, mammary glands have high levels of SCD mRNA (15, 16) and activity (17-22). The SCD protein has a key role in the synthesis of milk monounsaturated FAs and specific CLA isomers by introducing a cis double bond between carbons 9 and 10 of the FAs (23). In milk, about 60% of oleic (cis-9-18:1; a major milk FA), 50–56% of palmitoleic (cis-9-16:1), and 90% of myristoleic (cis-9-14:1) acids, and > 60 % of the major isomer of CLA are synthesized in mammary glands by the action of the SCD enzyme (23). This action is important due to the impact of milk fat concentration and FA profile in determining milk nutritional quality and human health (23).

Indeed, certain saturated (mainly 12:0, 14:0, and 16:0) and trans FAs are considered to exert negative effects when consumed in excess, whereas others (4:0, anteiso-15:0, cis-9-18:1, 18:3 n -3, and some CLA isomers) have potentially positive effects on human health (24, 25). For example, cis-9, trans-11-18:2, the major isomer of CLA in ruminant milk, exhibits anti-carcinogenic and anti-atherogenic properties in animal models (26). Thus, by contributing to the synthesis of FAs that benefit human nutrition, e.g., cis-9, trans-11-CLA, and to a lesser extent cis-9-18:1, SCD improves the nutrition of milk fat. In addition, by introducing a cis-9 double bond to FAs, SCD decreases the milk fat melting point (24). Differences in SCD protein levels in mammary glands may help to explain the substantial variation in the levels of these FAs in milk fat; thus SCD gene expression in the mammary gland has been of major interest in recent decades.

Another enzyme related to the increase of CLA in dairy products and its beneficial effects on human health is FAS (29).Fatty acid synthase is part of a multifunctional enzyme complex that catalyzes the synthesis of saturated FAs, including myristate, palmitate, and stearate (27- 28).

Analysis of SCD and FASN gene expression on local Baluchi and synthetic breeds are important to understand the unsaturated FA contents of milk. Our hypothesis is that comparing SCD and FASN gene expression in these local and synthetic sheep breeds could offer the effect of crossbreeding on the fat composition of sheep milk.

## Materials and Methods

### *Experimental site and sheep breed*

The experiment was conducted at Abbas Abad breeding center of Mashhad in RazaviKhorasan Province during the spring of 2014. Eight each of Baluchi, Arman, and Iran-Black ewes were selected. All were 3 years old and single-born.All the sheep in this study were reared in the same conditions and had ad libitum access to the same ration of grassland diets and water.

### *Mammary gland biopsy*

In late lactation of the ewes, mammary gland tissue was collected with a biopsy needle. The biopsy site was cleaned with 10% povidone-iodine solution, and 2.5 ml of lidocainechlorhydrate was injected subcutaneously. A 3 cm incision was made approximately 2 cm above the nipple to facilitate insertion of the biopsy needle. After the biopsy, the mammary gland surface was sprayed with Aludemin to protect the wound from dirt. Immediately after biopsy, the mammary tissue sample was stored in RNAlater solution (QIAGEN) and then frozen and stored at −80°C.

### *RNA extraction and cDNA synthesis*

Total RNA was prepared from the mammary gland biopsy with TriPure Isolation Reagent (Roche, Germany) according to the manufacturer's instructions. The quantity and quality of the RNA were determined by agarose gel electrophoresis (0.8%, w/v). To exclude possible amplification of contaminating genomic DNA, samples were treated with DNase. Single-stranded cDNA was synthesized from 1 µg of RNA with the RevertaidcDNA synthesis kit (Termofisher Scientific Litvania) following the manufacturer’s recommendations.

### *Real-time polymerase chain reaction analysis (Real-time PCR)*

The expression pattern of SCD and FASN genes in these sheep was analyzed by real-time PCR. To normalize the results of the target genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene in all samples. Glyceraldehyde-3-phosphate dehydrogenase was previously reported to exhibit low variation and no significant differences in gene expression levels between adipose tissues in different sheep breeds (30-32).

Gene sequences for forward and reverse primers for SCD, FASN, and GAPDH were obtained from a study by Dervishi et al., 2012 (33) (Table 1).Real-time-PCR was performed using a Bio-Rad CFX96 real-time PCR system (USA). Standard curves for genes were generated to calculate the amplification efficiency. The efficiency of PCR amplification for each gene was calculated with the standard curve method (E = 10-1/slope -1). The standard curves for each gene were generated by 4-fold serial dilution of pooled cDNA with 1/10, 1/100, 1/1000, and 1/10000 dilutions. The amplification conditions were an initial step of 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 59 or 60°C for 30 sec. The specificity of the amplification products and the lack of primer dimers were confirmed by melting curve analysis in all cases. To quantify the relative gene expression, the standard curve method was used according to the recommendation of

Larionov et al. (34). Normalized real-time PCR data were transformed to the fold-change relative to the control group. Expression of Baluchi sheep breed considered as control group.

**Table 1.** Sequences of primers used in this study.

| Primername | Primer sequence 5′ →3′ | Amplicon (bp) | GenBank accession numbers | Annealing temperature(ºC) |
|---|---|---|---|---|
| SCD F | atgttgaccacatccccatt | 115 | AJ001048 | 57 |
| SCD R | cccagctgtcagagaaaagg | | AJ001048 | |
| FASN F | gtgtggtacagcccctcaag | 110 | GQ150557 | 57 |
| FASN R | gtgtggtacagcccctcaag | | GQ150557 | |
| GAPDH F | atgcctcctgcaccacca | 76 | HM043737 | 57 |

## Results

### *Real-time PCR data analysis*

The minimum average of cycle threshold for SCD, FASN, and GAPDH genes obtained 24, 25/78, and 23/11, respectively. Figure 1 represents normalized data for SCD and FASN results. The SCD and FASN gene expression in the mammary glands of Baluchi sheep breed was greater than in the synthetic Iran-Black or Arman sheep. The FASN gene expression was four-fold and two-fold less in the synthetic Arman and Iran-Black than in the Baluchi sheep. The SCD gene expression was 7.5 and 3.5 times less in synthetic Arman and Iran-Black than in Baluchi sheep, respectively (Table 2).

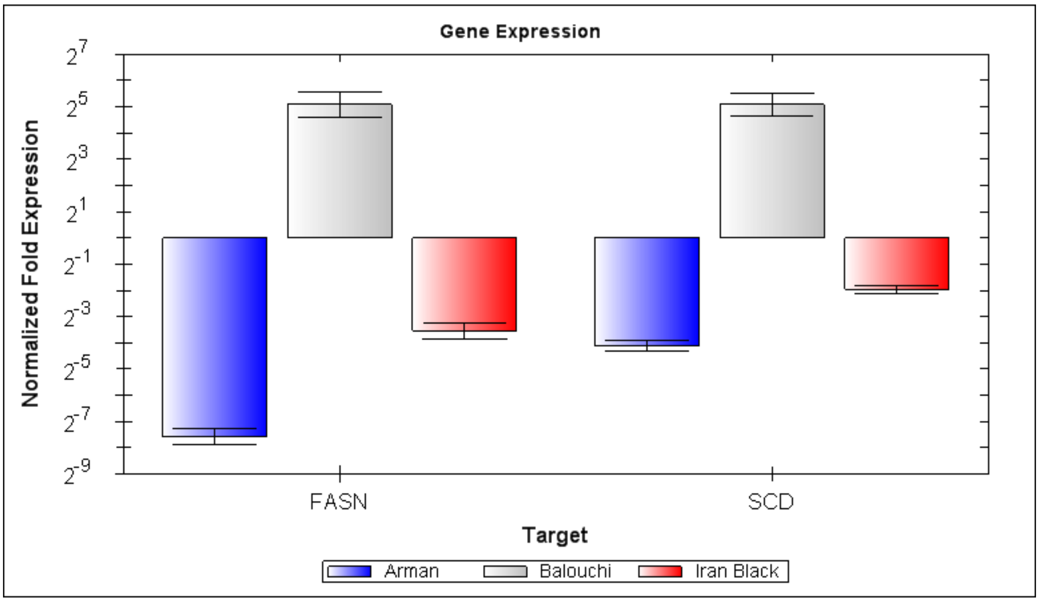


**Fig. 1.** Fold expression for SCD and FASN genes in Baluchi, Iran Black and Arman breeds.

**Table 2.** Dietary Intake among the patients with low vitamin D status, and Control Groups[a]

| Gene | Baluchi | Iran-Black | Arman |
|---|---|---|---|
| **SCD** | 5.08 ± 0.1238 | -3.53 ± 0.029 | - 4.11 ± 0.008 |
| **FASN** | 5.08 ± 0.1429 | -1.95 ± 0.021 | -7.58 ± 0.001 |

## Discussion

Bakhtiarizadeh et al. (35) studied SCD and FASN gene expression in localZel lambs and Lori–Bakhtiari sheep; however, they sampled from the fat-tail and visceral adipose tissues and observed relative expression levels of 5.92 and 11.67 for SCD and FASN genes in the Zel lambs and 0.86 and 1.52 in the Lori–Bakhtiari sheep, respectively. But no study in Iran has as yet been reported that evaluated gene expression changes of SCD and FASN in mammary glands of local and synthetic sheep breeds.

Chen et al. (36) found no significant relationship between FASN gene expression and intramuscular fat (IMF) contents of pigs; however, Guo et al. (37) reported a positive relationship between FASN expression and fat deposits in Xiang pigs. Qiao et al. (31) observed a negative relationship between FASN mRNA expression and IMF content in male Kazak sheep. Cui et al. (38) observed no correlation between the FASN mRNA expression and IMF content in chicken breasts and thighs.

Similar to our findings, Dervishi et al. (33) reported relative expression of SCD and FASN under 2-fold in semitendinous muscle of Rasa Aragonesa lambs.

Gene expression as a result of crossbreeding on different cow populations was studied by Paape et al. (39). They observed less gene expression in crossbred cows than in native cow populations. Also Huang et al. (40) reported that crossbreeding can reduce the expression of some genes. In Iran, Moridi et al. (41) found less IL8RB gene expression in the offspring of crossbred cows than in the native Guilan and Holstein cows used for the crossbreeding.

These studies agree with our results related to decreased gene expression following crossbreeding.

Comparison of the expression of SCD and FASN genes between these local and synthetic sheep breeds is useful in understanding the effect of crossbreeding on FA composition of dairy products.

Until now, no studies have reported that the changes in mammary gland expression of lipid metabolism-related genes can be affected by crossbreeding.The difference we observed may be attributed to a relatively lower contribution of the Baluchi sheep genome than those of the synthetic Iran-Black and Arman sheep.

With consideration of the relatively high SCD and FASN expression in local Baluchi sheep being reduced in the synthetic Iran-Black and Arman Sheep cross-breeds, it may be necessary to consider the effects of crossbreeding on the FA composition of dairy products.

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