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Association of Stearoyl-CoA Desaturase Expression with Cattle Milk Characteristics

Research Article

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ABSTRACT

Stearoyl-CoA desaturase (SCD) enzyme plays an important role in the metabolism of the lipids, thus the goal of this study was to investigate the influence of canola and soybean oilseeds on gene expression of SCD in adipose tissue, composition and yield of milk, fatty acid profile in Iranian Holstein cattle. Animals (n=20) were randomly selected to test experimental diets. Fatty acid composition was determined. After isolation of total RNA, cDNA was synthesized. Reverse transcription polymerase chain reaction (RT-PCR) was used to amplify SCD and GAPDH. For analyzing the real-time PCR results, LinRegPCR, REST and SPSS softwares was employed. The animals fed canola seed in comparison animals fed soybean seed showed higher gene expression. The milk production, fat percentage, 4% fat corrected milk, body condition score and milk urea nitrogen showed a significant difference between two groups. The amount of a number of fatty acids extracted from adipose tissue including C18:3t, C18:0 and C16:1 in animals fed two different diets was variable and their amount was significantly different. SCD gene expression was not significantly different between animals fed two diets (canola and soybean). This may be due to the similarity of the fatty acid composition of the two compounds and their nutrient balance. Since canola seed are higher in fat and protein than soybean seed, it can be a good substitute for soybean seed in the diet of dairy cows. In addition, canola seed, with the effect of nutrition on the composition of milk fatty acids can be used to improve milk.

KEY WORDS canola, cattle, SCD gene expression, soybean.

INTRODUCTION

According to reports on the undesirable effects of saturated and trans fatty acids on health of consumers, they have expressed concern about the use of these types of fats (Gamarra *et al*. 2018), while, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are useful for human health (Burlingame *et al*. 2009). The melting point of fat is affected by the composition of the fatty acid (FA) of the meat and to increase palatability of meat, ratio of MUFA to saturated FA (SFA) should be increased, therefore increasing the ratio of MUFA can increase the quality and nutritional value of livestock products (Brooks *et al*. 2011). Stearoyl-CoA desaturase (SCD) enzyme converts saturated FAs into MUFA in adipose tissues of mammals and plays an important role in the metabolism of the lipids (Mannen, 2012). Because this enzyme establishes a double bond at the Δ9-position in a wide spectrum of FAs,

also known as Δ9-desaturase (Conte *et al*. 2012). Generally, SCD converts acyl-CoA of myristic, palmitic, stearic acid and C18:1 t11 to C14:1 c9, C16:1 c9, C18:1 c9, and C18:2 c9, t11, respectively (Corl *et al*. 2001). The C18:2 c9, t11 is a conjugated linoleic acid (CLA) isomer that has many advantages in the health of consumers. Some of these benefits for CLA include anti-atherogenic, anti-carcinogenic and anti-diabetic effects (Conte *et al*. 2012). The main method to provide CLA in the human diet is to consume ruminant milk and meat (Ritzenthaler *et al*. 2001). The location of SCD gene is on chromosome 22 of ovine (Conte *et al*. 2012) and 26 of bovine (Alim *et al*. 2012). Cattle SCD gene has 6 exons and 5 introns and its approximate molecular weight is 17 kb (Alim *et al*. 2012). The whole cDNA of cattle SCD was sequenced by Taniguchi *et al*. (2004). They reported that this cDNA has 5331 bp length and its protein-coding sequence (CDS) has 1080 nucleotides that code for 359 amino acids. One SNP at 878 bp in CDS replaces valine with alanine in the SCD protein. PUFA decrease the SCD gene expression and regulate peroxisome proliferator-activated receptor proteins (PPARs) expression (Conte *et al*. 2012). It is reported that modulation of sterol regulator element binding protein (SREBP-1) and PPAR effects on expression of SCD gene (Waters *et al*. 2009). It is demonstrated that the reason for dietary PUFA effect on gene expression and function in ruminants is the applying linseed oil or CLA in beef, lamb and goat (Herdman *et al*. 2010). The most efficient method for decreasing saturated FA and increasing the enrichment with unsaturated FA of milk and meat is adding lipids with high level of PUFA to diets of ruminants (Lanza *et al*. 2011). Unfortunately, there is not enough information on evaluating and selecting suitable source of PUFA.

Jacob and Monod (1961) have shown that bacteria can alter their metabolism by changing the expression levels of enzymes so that they can achieved their nutritional needs. These researchers were the first to report a link between changes in gene expression and altering in enzyme activity. Therefore, changes in the mechanisms that control gene expression may cause various positive or negative alterations (Haro *et al*. 2019). If we can understand the relationship between nutrients and gene expression, we can offer solutions to many of the problems in animal husbandry based on the genetic makeup of the animals. Changes in dietary protein sources have been shown to lead to changes in the expression of different genes (about 300 genes) in liver tissue.

This suggests that the protein components of the diet can affect nutritional function by altering gene expression (Endo *et al*. 2002).

One way to provide the energy and protein needed by dairy cows is to use oilseeds in their diets. Because high-yield dairy cows have limited feed intake, the use of oilseeds in their diets can be helpful. Soybean meal is one of the great protein supplements that is a source of amino acids needed by lactating cows and is widely used in animal nutrition. However, compared to other meals, such as canola meal, it has a higher price (Hosseini *et al*. 2012). Soybean seeds contain high quality protein (33-40%) and a rich source of fat (16-22%), and using soybeans is a suitable method for increasing the energy concentration of dairy cows. Beauchemin *et al*. (2009) reported that canola seeds contain 40% oil and 30% protein, so they can replace soybean seeds in the diets of dairy cows. In addition, this seed can be used for improving the composition of milk FAs.

Although many studies have been performed on Iranian Holstein and native cattle (Alinaghizadeh *et al*. 2007; Ghasemi *et al*. 2010; Ruzina *et al*. 2010; Kharrati Koopaei *et al*. 2011; Mohammadabadi *et al*. 2011; Ebrahimi *et al*. 2015a; Ebrahimi *et al*. 2015b; Pasandideh *et al*. 2015; Barazandeh *et al*. 2016), but association between milk production and SCD gene expression did not study. Therefore, the goal of this investigation was to investigate the influence of soybean oilseed and canola oilseed on gene expression of SCD in adipose tissue, composition and yield of milk, FA profile in Iranian Holstein cattle.

MATERIALS AND METHODS

Animals and diets

In this study 20 animals (2 treatments with 10 repeats) with the same mean body weight (680±80 kg) and gestational age (second pregnancy) were used in the form of a completely random design. After two weeks of adaptation period with experimental diets, animals were tested randomly on the twentieth day of lactation. The two experimental diets were similar in all components (protein, energy, starch, minerals, vitamins and fiber), but in one diet roasted soybean seed (treatment 1) and in the other diet roasted canola seed (treatment 2) was added (Table 1). Diet formulations were performed based on the livestock requirements with a pure protein and carbohydrate system of the Cornel university Cornell Net Carbohydrate and Protein System (CNCPS) for dairy cattle with performance of 42 kg milk with 3.2% fat per day. The cattle were fed total mixed ration (TMR) in the morning and in the afternoon, and the cattle had free access to food and water in groups. During the period of experiment, the milking was performed three times a day and the amount of milk produced was recorded at each 2 weeks.

Table 1 Components of diets fed to the studied animals (% of DM basis)

Components	Diet containing soybean	Diet containing canola
Corn silage	22.0	22.0
Alfalfa hay	17.5	17.5
Barley grain, ground	17.5	17.5
Corn grain, ground	15.7	15.7
Soybean meal	8.7	6.0
Roasted soybean seed	9.0	0
Corn gluten meal	1.5	4.6
Roasted canola seed	0	9.0
Fish meal	1.3	1.3
Fat supplement (prilled)	2.0	1.6
Calcium carbonate	0.8	0.8
Di calcium phosphate	0.2	0.2
NaCl	0.6	0.6
Sodium bicarbonate	1.5	1.5
Vitamins and minerals[1]	1.3	1.3
Magnesium oxide	0.4	0.4
Chemical compositions		
Dry matter (%)	50	51
Crude protein (CP) (%)	16.5	16.1
Ether extract (%)	5.15	5.78
Ash (%)	8.21	8.1
Neutral detergent fiber (NDF) (%)	31.3	29.3
Acid detergent fiber (ADF) (%)	18.4	17.5
Net energy for lactation (NE_L) (Mcal/kg of DM)	1.71	1.72

[1] Vitamins and minerals provided (per kilogram of DM): Zn: 56 mg; Mn: 46 mg; Fe: 22 mg; Cu: 12 mg; I: 0.9 mg; Co: 0.4 mg; Se: 0.3 mg; vitamin A: 6440 IU; vitamin D: 2000 IU; vitamin E: 16 IU and Monensin: 12 mg.

Measurements and analytical methods

Milkoscan (EKOMILK, Milkana Kam 98-2A, Foss Electric, Denmark) was used to analyze samples in terms of protein, solids, fat, and solids without fat. Urea nitrogen levels were determined. Changes in fat body scores and corrected milk for fat were performed. Sampling for studying SCD gene expression was done in the tail region from subcutaneous adipose tissue.

To do this, the animal's skin was first washed and disinfected. A scalpel was then used to make a small incision in the skin. Two small samples of subcutaneous adipose tissue were removed from the site and then the site was sutured. Physiological serum solution was used to wash the samples. After wrapping the washed samples in aluminum foil, they were transferred to -80 ˚C in the lab. A gas chromatography (GC) applying CP-3800 gas chromatograph (Varian, Palo Alto, CA, USA) was used to analyze fatty acid composition. The experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regards care and use of laboratory animals.

RNA expression analysis

The One Step RNA Reagent Kit (Biobasic Co. Ltd., Iran) was used to extract total RNA from tissue samples. As below: any sample was lysed in 1 mL of RNAzol RT, and the homogenate was supplemented with 400 µL of water to precipitate proteins, DNA and polysaccharides, while RNA remained soluble in the homogenate. After 10 sec vortexing and 15 min (500 rpm) shaking, using centrifugation (15 min, 13 000 g) the precipitated compounds were removed from the homogenate. The supernatant was transferred to a fresh tube with 10 µL of 4-Bromoanisol and vortexed for 10 sec. After incubation for 5 min at ambient temperature and 10 min at 13000 g centrifugation, by the addition of 1 volume of 2-propanol pure RNA was precipitated from the resulting supernatant. After mixing by inversion 5 times, the solution was allowed to stand for 20 min at ambient temperature then centrifugation (15 min at 12 500 g) was done to pellet RNA. Before re-suspension in 50 µL of water, the pellet washing was done three times with 1 mL of 75% ethanol.

For defining RNA concentration and quality, spectrophotometry at 260 nm (260 nm:280 nm absorbance ratio) and electrophoresis on 2% agarose gel were used. For preparation of 2% agarose gel, 4.0 g agarose (electrophoresis grade) was added to 200 mL 1X TBE electrophoresis buffer in a 600 mL Erlenmeyer flask and then stirred to suspend agarose. Erlenmeyer flask was covered with aluminum foil, and heated in boiling-water bath (double boiler) until all agarose was dissolved (approximately 10 minutes). Solution was swirled and checked bottom of beaker to insure that all agarose has dissolved. (Just prior to complete dissolution, particles of agarose appear as translucent grains). Reheated for several minutes if was necessary. It was covered with aluminum foil, and hold in a hot-water bath (at about 60 ˚C) until ready for use. Removed any "skin" of solidified agarose from surface prior to pouring. cDNA was synthesized from total RNA using an oligo d(T) primer and

RerertAid™ H Minus First Strand cDNA Synthesis Kit (#K1631, Fermentase Co., Iran), according to manufacturer's protocol. 1 µg total RNA was used in each reaction. Components of used kit were: 25 µL RevertAid H Minus M-MuLV Reverse Transcriptase (200 U*/µL); 25 µL RiboLock RNase Inhibitor (20 U**/µL); 150 µL 5 × Reaction Buffer 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT; 50 µL 10 mM dNTP Mix; 25 µL Oligo(dT)18 Primer 100 µM, 0.5 µg/µL (15 A260 U/mL); 25 µL Random Hexamer primer 100 µM, 0.2 µg/µL (6 A260 U/mL) and 2 × 1.25 mL Water, nuclease-free.

For SCD gene, 5'-CCGCCCTGAAATGAGAGATG-3' and 5'-GCAGGTGGGGATCAATGTG-3' primers (GenBank accession number; AY241932.1) and for GAPDH gene, 5'-GTTCAACGGCACAGTCAAGG-3' and 5'-5'-GTTGATGTTGGCAGGATCTCG-3' primers (GenBank accession number; NM_001034034.2) were synthesized by Bioneer Co (representative in Iran). For amplification of samples, optical 96-well skirted microplates and Power SYBR Green PCR Master Mix kit (Iran) were used. The final PCR reaction volume was 15 µL. The contents of each microtube were: 1.5 µL template cDNA, 7.5 µL from 2X SYBR Green PCR Master Mix, 0.3 µL ROX, 1 µL from 10 µM forward and reverse primers, and 4.7 µL ddH2O. Conditions for performing PCR were: initial denaturation at 94 ˚C for 3 min, 35 cycles with denaturation at 94 ˚C for 60 s, annealing at 60 ˚C for 60 s and extension at 72 ˚C for 60 s. Melting curve analysis was performed for all final PCR products to ensure that we did not have non-specific PCR products such as primer dimers. To analyze the results of real-time PCR and gene expression study, LinRegPCR (11.0), REST (2009), and SPSS 20.0 (SPSS, 2011) were applied.

Relative Expression Software Tool (REST) compare two or more treatments groups or conditions (in REST-MCS), with up to 100 data points in sample or control group (in REST-XL), for multiple reference genes and up to 15 target genes (in REST-384). The mathematical model used is based on the correction for exact PCR efficiencies and the mean crossing point deviation between sample group(s) and control group(s). Subsequently the expression ratio results of the investigated transcripts are tested for significance by a Pair Wise Fixed Reallocation Randomization Test and plotted using standard error (SE) estimation via a complex Taylor algorithm.

LinRegPCR is a program for the analysis of quantitative RT-PCR (qPCR) data resulting from monitoring the PCR reaction with SYBR green or similar fluorescent dyes. The program determines a baseline fluorescence and does a baseline subtraction. Then a Window-of-Linearity is set and PCR efficiencies per sample are calculated. With the mean PCR efficiency per amplicon, the Ct value per sample and the fluorescence threshold set to determine the Ct, the starting concentration per sample, expressed in arbitrary fluorescence units, is calculated.

RESULTS AND DISCUSSION

The results of total RNA electrophoresis on agarose gel (revealed two 18S and 28S bands) and the 260 nm:280 nm ratio (1.77 to 1.90) for extracted RNA from adipose tissue of the Iranian Holstein dairy cattle showed that it has no contamination and its quality is also desirable (Figure 1).

The observation of sharp single peaks in the melting (Figure 2 and Figure 3) and amplification (Figure 4 and Figure 5) curves of SCD and GAPDH PCR products confirmed the absence of primer dimers. Moreover, no observation of amplification product in the negative control samples confirmed that the used primers were specific.

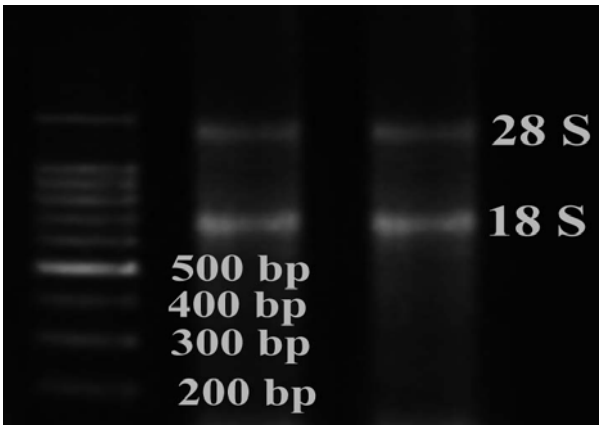

Figure 1 Quality of RNA extracted on agarose gel

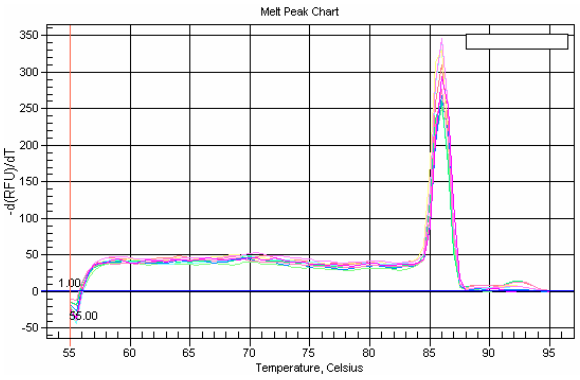

Figure 2 An example of melting curve for SCD gene in the studied tissue of dairy cattle

SCD gene expression in the adipose tissue was not significantly different between animals fed two diets (whole processed canola seed and whole processed soybean seed) (P>0.05), although this expression level for whole processed canola seed treatment was higher.

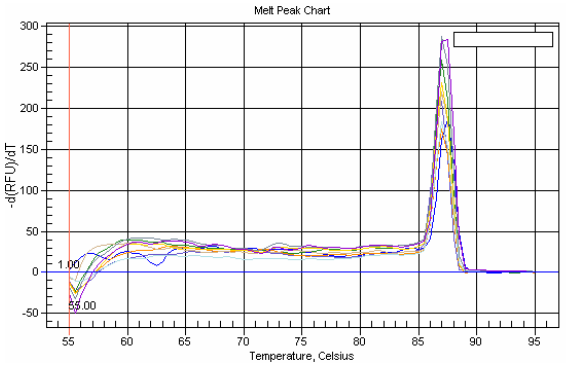

Figure 3 An example of melting curve for GAPDH gene in the studied tissue of dairy cattle

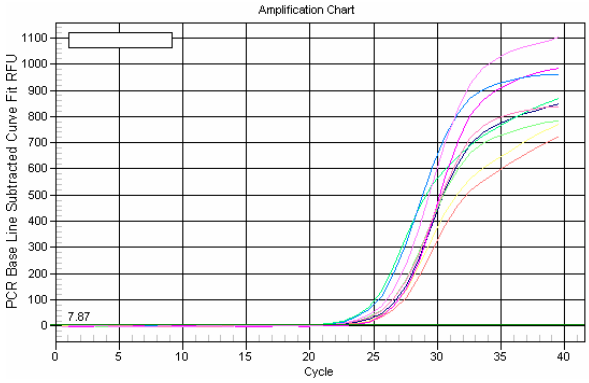

Figure 4 An example of amplification curve for SCD gene in the studied tissue of dairy cattle

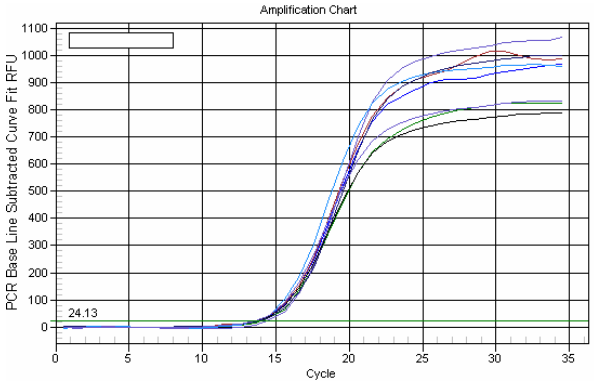

Figure 5 An example of amplification curve for GAPDH gene in the studied tissue of dairy cattle

The milk production, fat percentage, 4% fat corrected milk, body condition score and milk urea nitrogen showed a significant difference between two groups (P<0.05). But, for dry matter and other milk composition was not observed between two treatments whole processed canola seed and whole processed soybean seed (P>0.05) (Table 2).

The amount of a number of FAs extracted from adipose tissue including C18:3t, C18:0 and C16:1 in animals fed two different diets (whole processed canola seed and whole processed soybean seed) was variable (Table 3) and their amount was significantly different (P<0.05). The most different components of the two used diets (roasted canola and soybean seed) were protein and fat percentage (37.6% protein and 21.7% fat for soybean oilseed and 22.9% protein and 37.7% fat canola oilseed).

Crude fat (ether extract), protein, net energy lactation (NEL) (mega calories per kilogram of dry matter= Macl/kg of DM), rumen-undegradable protein (% of total protein), neutral detergent fiber (NDF), metabolisable protein (g/day), ash and acid detergent fiber (ADF) for roasted soybean seed (treatment 1) were 5.15, 16.5, 1.71, 33, 31.3, 2750, 8.21, and 18.4, respectively. These amounts for canola soybean seed (treatment 2) were 5.78, 17, 1.72, 33, 29.3, 2750, 8.10 and 17.5, respectively. Comparing soybean and canola seeds in terms of FA composition, it was found that the amount of oleic acid (C18:1c) in canola seed (65 mol/100 mol of FA) was higher than soybean seed (25 mol/100 mol of FA). In contrast, it was demonstrated that the amount of cis linoleic acid (C18:2c) in canola seed (17 mol/100 mol SA) was lower than soybean seed (49 mol/100 mol FA). In addition, the amount of stearic and palmitic FAs in rapeseed was lower than soybean (Table 4).

In this research, the effect of soybean oilseed and canola oilseed on milk yield and its composition, FA profile and SCD gene expression in adipose tissue of Iranian Holstein cattle was investigated. The results showed that in terms of the milk production, fat percentage, 4% fat corrected milk, body condition score and milk urea nitrogen, there was a significant difference between two groups (whole processed canola seed and whole processed soybean seed). The amount of a number of FAs extracted from adipose tissue including C18:3t, C18:0 and C16:1 in animals fed two different diets (whole processed canola seed and whole processed soybean seed) was variable and their amount was significantly different (P<0.05). Johnson *et al.* (2002) demonstrated that canola seed reduced the C16:0, C14:0, C12:0, and C10:0 milk FAs, and enhanced the C18:1, trans-C18:1, and C18:0 FAs. Studies on sheep with similar criteria to previous criteria have shown that the use of canola seeds reduces methane emissions. In some treatments, canola seeds reduced methane release by up to 27%. When comparing coconut oil and protected fat with canola, they found that coconut oil and protected fat could not reduce methane release compared to the control group, while the use of oilseeds reduced methane emissions compared to the control group (Machmüller *et al.* 2000). In another study, Esmaeili *et al.* (2016) used roasted canola seeds instead of roasted soybean seed in the diet of dairy cows and showed that the use of roasted canola seeds reduces the amount of linoleic acid and linolenic acid in cow's milk fat. However, it did not change the amount of conjugated FAs.

Table 2 Body condition score, production and composition of milk for studied dairy cows comparing diet containing soybean and diet containing canola

Parameter	Soybean seed diet	Canola seed diet	SEM	P-value
Initial body condition score	3.17	3.18	0.03	0.100
Final body condition score	2.92	3.01	0.02	0.010
Dry matter intake (kg/d)	22.1	22.0	-	-
Milk yield (kg/d)	51	53.5	0.61	0.003
4 % fat corrected milk yield (kg/d)	38.4	41.9	0.94	0.005
Milk composition (%)				
Fat (%)	2.36	2.56	0.06	0.010
Protein (%)	3.03	3.04	0.02	0.107
Solid non-fat (%)	9.34	9.35	0.01	0.502
Total solids (%)	11.92	12.05	0.06	0.064
Milk urea nitrogen (mg/dL)	13.25	14.61	0.69	0.060

SEM: standard error of the means.

Table 3 Profiles of fatty acid for studied dairy cows in adipose tissue comparing diet containing soybean and diet containing canola

Fatty acid name based on carbon number	Diet containing soybean (treatment 1)	Diet containing canola (treatment 2)	SEM	P-value
C4:0	0.14	0.02	0.12	0.50
C6:0	1.55	0.02	1.54	0.50
C8:0	0.21	0.04	0.20	0.50
C10:0	0.55	0.05	0.39	0.29
C12:0	0.10	0.12	0.01	0.35
C14:0	2.66	2.95	0.87	0.67
C14:1	1.39	0.64	0.29	0.83
C15:0	0.38	0.58	0.21	0.38
C15:1	0.22	0.23	0.05	0.71
C16:0	22.01	24.40	3.13	0.39
C16:1	8.11	5.90	0.33	0.01*
C17:0	0.78	0.91	0.16	0.65
C17:1	0.83	0.70	0.67	0.14
C18:0	8.44	14.16	1.77	0.05*
C18:1t	1.09	1.35	1.80	0.63
C18:1c	42.04	40.27	0.82	0.94
C18:2t	0.10	0.88	0.32	0.92
C18:2c	1.45	1.80	0.34	0.27
C18:3t	0.01	0.03	0.0001	0.0001*
C18:3c	0.23	0.31	0.07	0.28
C20:0	0.20	0.25	0.02	0.59
C20:1	0.04	0.03	0.001	0.64
CLA c9t11	0.26	0.27	0.08	0.82
CLA t10c12	0.09	0.08	0.005	0.22
C22:0	0.16	0.11	0.03	0.20
C20:4w6	0.16	0.10	0.07	0.23
C20:4w3	0.17	0.14	0.06	0.53
C20:5 EPA	0.02	0.07	0.01	0.30
C22:1	0.06	0.04	0.03	0.32
C24:0	0.23	0.05	0.21	0.46
C24:1	0.30	0.02	0.30	0.45
C22:5 W3	0.07	0.12	0.02	0.90
C22:6 DHA	0.09	0.05	0.07	0.67
C22:2	0.03	0.03	0.04	0.74

SEM: standard error of the means.
* ($P<0.05$).

The results of these researchers confirm the results of our research. Conte *et al*. (2012) studied substitution effect of sunflower with linseed oil on gene expression of SCD in sheep. In their investigation, total FAs composition was determined by gas-chromatograph, while SCD gene expression was assessed by real-time reverse-transcription PCR. They showed that this replacement decreases SCD mRNA level and slightly reduces SCD enzyme activity. They resulted that PUFAs (n-3) are more effective than n-6 on gene expression of SCD in sheep.

Gamarra *et al*. (2018) investigated association of lipogenic gene expression and FA composition of cattle breeds in subcutaneous fat and showed that amount of MUFA, CLA and oleic acid and SCD gene expression is different in various studied cattle breeds. They also reported that there is a relationship between FA desaturation indices and expression of SCD1 and SCD5 genes and demonstrated association between expression levels of SCD1 and SRBEP1 genes and reverse relation between expression levels of SCD1 and SCD5 genes in different studied cattle.

Table 4 Compounds of fatty acid (mole per 100 mole of fatty acid) for roasted canola and roasted soybean seeds

Fatty acid name based on carbon number	Roasted canola seed	Roasted soybean seed	Fatty acid name based on carbon number	Roasted canola seed	Roasted soybean seed
C12:0	0.03	ND	C18:2c	17.03	48.95
C14:0	0.08	0.12	C18:3t	0.11	0.03
C16:0	4.91	12.63	C18:3c	7.88	6.75
C16:1	0.33	0.17	C20:0	0.72	0.41
C17:0	0.06	0.15	C20:1	1.43	0.27
C17:1	0.07	0.07	C22:0	0.32	0.42
C18:0	2.67	4.76	C22:1	0.59	0.03
C18:1t	0.35	0.01	C22:2	0.01	ND
C18:1c	62.82	24.75	C24:0	0.14	0.14
C18:2t	0.04	0.06	C24:1	ND	0.03

ND: no data.

Zheng *et al*. (2001) reported that SCD gene expression in mammals is affected by sex and tissue, but until now no scientist has identified or reported the cause. Although, this can be probably due to the difference in fat type and amount of the various tissues or levels of the hormones, especially sex hormones. This different can be due to the fact that the levels of testosterone are higher in males (Dridi *et al*. 2007). It should be noted that various other hormones such as growth hormone, thyroid hormones, leptin and ghrelin can play a role in this difference (Dridi *et al*. 2007).

They studied the effects of leptin, cerulenin, food deprivation, genotype, and gender on SCD gene expression in chickens and showed that leptin increases plasma leptin levels and decreases food intake, but promotes SCD gene expression in liver, whereas in muscle and hypothalamus this increase was not observed, thus they concluded that leptin controls SCD gene expression in a tissue-specific way. It seems that the effect of leptin on liver gene expression in birds is in contrast to mammals. Adipose tissue is the main place of leptin gene expression in mammals, but in the avian this gene expresses in liver and in adipose tissue. Main reason for these different results can be explained that various factors regulate liver and adipose tissue expression of SCD gene (Dobrzyn and Dobrzyn, 2006). Based on research by Ntambi and Miyazaki (2004) some of these factors are hormonal signals (e.g. glucagon, insulin), fat of diet (e.g. vitamin A, cholesterol, PUFAs), development procedures, environmental elements (e.g. thiazolinediones, alcohol, metals, temperature), and proliferators of peroxisomes. Regulation of translation or post-translation may be influenced by the above factors and thus affect the expression or activity of the enzyme. Jump and Clarke (1999) and Al-Hasani and Joost (2005) showed that L-pyruvate kinase, adipocyte lipid-binding protein (aP2), Acetyl-CoA Carboxylase, L-pyruvate kinase, FAs synthase, Malic enzyme, and SCD that are main effective genes in synthesis of lipid that their transcription may be inhibited by PUFAs. These changes reduce de novo lipogenesis. Miyazaki and Ntambi (2003) demonstrated that expression of SCD gene and other lipogenic genes is reduced by PUFA through suppressing the activity of sterol-binding protein binding protein. In addition, they showed that peroxisome proliferator-activated receptor proteins (PPARs) are activated by PUFA for gene expression modulation in response to active environmental stimulating factors. It is proven that key lipogenic enzymes gene expression is up-regulates with adding corn oil (up to level of 4.94% total FAs) in diet (Joseph *et al*. 2010). However, when the level of these total FAs reaches 99.7 in the diet, genes encoding lipogenic enzymes manufacturing FAs de novo is down-regulated (Joseph *et al*. 2010). For more accurate conclusions, it is better to study the expression of other genes that interact with SCD gene in adipose tissue in the future studies.

CONCLUSION

Overall, SCD gene expression was not significantly different between animals fed two diets (canola and soybean). This may be due to the similarity of the FA composition of the two compounds and their nutrient balance. Since canola seed are higher in fat and protein than soybean seed, it can be a good substitute for soybean seed in the diet of dairy cows. In addition, canola seed, with the effect of nutrition on the composition of milk FAs can be used to improve milk.

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