Turkish Journal of Agriculture - Food Science and Technology, 11(4): 799-802, 2023
DOI: https://doi.org/10.24925/turjaf.v11i4.799-802.5969

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Association between Stearoyl CoA Desaturase (SCD) Gene Polymorphisms and Milk Production in Holstein Cattle Breed

Mervan Bayraktar[1,a,*], Bahri Devrim Özcan[1,b]

[1]*Department of Animal Science, College of Agriculture, Çukurova University, Adana, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 25-01-2023
Accepted : 17-03-2023

Keywords:
Stearoyl CoA desaturase
Milk production
c.878T>C
Polymorphism
Allele frequency

ABSTRACT

The SCD gene is a significant component of the leptin signaling pathway. The SCD gene has also been suggested as a candidate essential gene that can change the ratio of saturated to unsaturated fatty acids in milk and increase the amount of conjugated linoleic fatty acid, which is thought to have anti-cancer properties. The current research was carried out on Holstein cows to determine the association between SCD (Stearoyl-Coenzyme A Desaturase) gene polymorphism and total milk yield at 305 days (TMY305) and daily milk yield (DMY). The polymorphism in the SCD gene was identified using the PCR-RFLP technique and the *Sat*I restriction enzyme for genotyping at SNP c.878T>C in the exon 5. The TT, TC, and CC genotype frequencies were 0.21, 0.50, and 0.29 respectively. While the allele frequencies of T and C were 0.46 and 0.54, respectively. According to the Chi-square test results, the SCD/c.878T>C distribution was in Hardy-Weinberg disequilibrium (P<0.05). Statistical analysis indicated a significant association between the SCD gene polymorphism and TMY305 (P<0.05). The TC genotypes showed a higher mean TMY305 compared to the TT and CC genotypes.

[a] *mervan.bayraktar@gmail.co* *https://orcid.org/0000-0003-3268-864X* | [b] *bdozcan@gmail.com* *https://orcid.org/0000-0002-9198-656X*

Introduction

The economics of dairy farms are heavily influenced by milk yield and composition. In addition to fat and protein percentages and microbiological quality, the technical features of milk are critical for milk manufacture since cheese has become an essential product in the dairy business, and the enzymatic coagulation of milk is a critical stage in the cheesemaking process. The effect of nutrition on the cheesemaking characteristics of milk is often studied. Other effects on technical features, such as the housing system or lactation stage, have also been studied (Čítek et al., 2021; García-Gómez et al., 2019; Migliorati et al., 2017).

Unsaturated fatty acids (UFA) are considered advantageous for human health; therefore, increasing their concentration in milk would improve its nutritional value. However, in the recent decade, the amount of UFA in milk has declined in the Netherlands, possibly because of changes in the dietary habits of dairy cattle. The level of biohydrogenation of UFA in the rumen, the amount of UFA in the food, and the activity of the stearoyl-CoA desaturase (SCD) enzyme in the mammary gland all play a role in determining the amount of UFA in milk (Heck et al., 2009; Ntambi and Miyazaki 2004; Paton and Ntambi 2009).

Stearoyl-CoA desaturase (SCD), also known as alpha-9-desaturase, is an enzyme found in the endoplasmic reticulum. It is the rate-limiting step in making monounsaturated fatty acids (MUFAs), especially oleate and palmitoleate from stearoyl-CoA and palmitoyl-CoA. The main components of phospholipid membranes, cholesterol esters, and alkyl-diacylglycerol are oleate and palmitoleate in large amounts (Wang et al., 2005; Zhang et al., 1999). The SCD gene is the one that's responsible for encoding the enzyme in humans. Stearoyl-CoA desaturase-1 is an enzyme that plays a vital role in the metabolic process of fatty acids. It is in charge of creating a double bond in Stearoyl-CoA and is accountable for doing so. From the saturated fatty acid known as stearic acid, the monounsaturated fatty acid known as oleic acid may be created using this method (Bartoň et al., 2010; Kgwatalala et al., 2009; Mele et al., 2007; Moioli et al., 2007).

During a chain of redox reactions, two electrons flow from NADH to flavoprotein cytochrome b5, then to electron acceptor cytochrome b5 and molecular oxygen. This creates a double bond in a row of methylene fatty

acyl-CoA substrates. These substrates are used as building blocks for other molecules. The complex enzyme forms a single, double bond between the C9 and C10 of long-chain acyl-CoAs synthesized from scratch (Griinari et al., 2000; Keating et al., 2006; Milannesi et al., 2008). This enzyme is a member of the oxidoreductase family, which includes those that work on paired donors and use oxygen dioxide (O2) as the oxidant. These enzymes may either incorporate or reduce oxygen. The integrated oxygen does not necessarily have to be generated from O2. Instead, the oxygen may be reduced to two molecules of water by the oxidation of a pair of donors. This particular enzyme class is referred to by its scientific name: stearoyl-CoA, ferrocytochrome-b5: oxygen oxidoreductase (9,10-dehydrogenating). Other names in frequent usage include Delta9-desaturase, acyl-CoA desaturase, fatty acid desaturase, and stearoyl-CoA, hydrogen donor: oxygen oxidoreductase. This enzyme is responsible for the production of polyunsaturated fatty acids as well as the PPAR signaling pathway. Iron is the only cofactor used (Dujková et al., 2015; Kaplanova et al., 2013; Oh et al., 2013).

SCD is an enzyme that contains iron and speeds up the rate-limiting step in making unsaturated fatty acids. Stearic acid is broken down to make oleic acid, which is the main product of SCD. Researchers think that the ratio of stearic acid to oleic acid affects how cells grow and change by affecting how fluid their membranes are and how they send signals. Mice have been found to have four different types of SCD, called Scd1 through Scd4. On the other hand, only SCD1 and SCD5 have been found in humans. About 85% of the amino acids in SCD1 are the same as those in all four mouse SCD isoforms and rat Scd1 and Scd2. SCD5, also called hSCD2, has less in common with rodent SCDs and seems unique to primates. SCD-1 is an important control point for metabolism. Stopping its expression may make it easier to treat several metabolic diseases (Gu et al., 2019; Li et al., 2020). The bovine SCD gene is located on the 26th chromosome. The length of the SCD gene is 15210 base pairs (bp), containing six exon and five intron regions. Also, the SCD gene includes a protein with 359 amino acids and an open reading frame consisting of 1,080 nucleotides, while the 3' untranslated region comprises 3,884 nucleotides (Alim et al., 2012; Nergis et al., 2019). The SCD gene is a candidate gene associated with meat, milk, and fatty acid composition. This study aims to determine the association between SCD gene polymorphism and daily milk yield in the Holstein cattle breed.

Materials and Methods

Material

Eighty cows were taken from the Çukurova University Research and Application Farm in Adana, Turkey. The cows were fed concentrated feed, forage, and ad libitum. The ages of the cows ranged from three to four. The total milk yield at 305 and daily milk yield were considered for periods 1. and 2. lactations. DNA was isolated from jugular vein blood samples and kept at 20°C using the salting-out process with minor modifications (Miller et al., 1988). A spectrophotometer was used to determine the DNA content. DNA samples were kept at 20°C until further examination.

Methods

Table 1 shows the SCD locus PCR conditions and primer sequences. The PCR reaction was carried out in a 20 µL volume using 5 µL (50 ng) DNA, 5 µL of PCR Master Mix (Thermo Scientific, USA), 0.5 µL for each primer (forward and reverse), and 9 µL of distilled water. *Sat*I FastDigest Restriction Enzymes were used to digest the PCR products at 37°C for 5-15 minutes (Thermo Scientific, USA). The following ingredients were used in the reaction: PCR products (8 µL), distilled water (4 µL), 10X buffer (2 µL), and restriction enzymes (1 µL). The digested product was separated and stained with ethidium bromide on 5% agarose gel. An imaging system was used to determine the fragment length.

Statistical Analysis

POPGENE software was used to calculate the allele and genotype frequencies of the SCD gene locus and the gene distribution based on the Hardy-Weinberg equilibrium.

Analysis of Associations

The general linear model (GLM) was performed to investigate the relationships between gene polymorphism with TMY305 and DMY. The following linear mathematical model was used:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

Y_{ijk}: phenotypic value, µ: overall means; α_i: effect of genotypes; β_j: effect of parity (one and two); ϵ_{ij}: random errors.

Table 1. The primer sequence and PCR conditions for the SCD locus.

Gene locus	The sequencing of primers	PCR length	Restriction enzyme	Location	Condition of PCR	Reference
SCD	5'-ACCTGGCTGGTGAATAGTGCT-3' 5'-TCTGGCACGTAACCTAATACCCT-3'	170 bp	SatI	5th exon c.878T>C	95°C 5m, 94°C 20s, 62°C 30s, 72°C 40s, 30 cycles 72°C 10m	(Inostroza et al., 2013)

Results and Discussion

*Sat*I was used to digest the 170 bp PCR products, and three genotypes (TT, TC, and CC) were found (Figure 1). The length of the fragment was TT 122, 28 and 20 bp; TC 122, 75, 47, 28 and 20 bp; and CC 75, 47, 28 and 20 bp, respectively (Table 2). χ^2 tests were statistically significant ($P<0.05$). The genotype was 0.21 (TT), 0.50 (TC), and 0.29 (CC), and the allele frequency was 0.46 (T) and 0.54 (C) (Table 2). The analysis results show that the common heterozygote genotype is TC, with a frequency of 0.50 occurrence. Meanwhile, the other two homozygote genotypes, TT and CC, have relatively low frequencies, with 0.21 and 0.29 levels, respectively. This significant result contributes to the analysis of the influence of the SCD/*Sat*I genotype on CLA (c9, t11) content in cow's milk. χ^2 shows that the SCD/*Sat*I genotype frequency is not in equilibrium according to Hardy-Weinberg ($P<0.05$). In other words, the SCD/*Sat*I genotype frequency will change for many generations. This study's results show that the SCD genotype and the T and C allele frequencies are similar to some other studies (Čítek et al., 2021; Inostroza et al., 2013; Kaplanova et al., 2013). The association results demonstrated a significant association between TMY305 and SCD gene polymorphism. SCD was shown to be associated with TMY305 ($P<0.05$) but did not show any association with DMY (Table 3). The genotype means for TMY305 and DMY were TT (7046±457), TC (8139±300), and CC (7021±405); TT (20.10±0.86), TC (22.55±0.48) and CC (19.63±0.68) respectively. Compared to the TT and CC genotypes, the TC genotypes showed a significantly higher TMY305. Also, homozygous TT genotypes showed the lowest TMY305 than other genotypes. Similar results have been found in other studies. Citek et al. (2021) found that the TT genotype was associated with the lowest milk, fat yields, protein, and the highest milk protein percent ($P<0.01$), and except for the fat percentage, the T allele showed higher values than the C allele ($P<0.05$) in Czech Simmental and Holstein cows. Jaleta (2012) determined no association between the SCD gene polymorphism and milk yield at 305 days in Hungarian Holstein Friesian cows. Alim et al., (2012) identified two SNP in introns 3 and 4, and three SNP in exon 5, and showed significant associations between all SNP with milk yield at 305 days, protein and fat, and yields and protein rate in Chinese dairy cattle. Many studies in this field have investigated the effect of SCD gene polymorphism on fatty acids. Taniguchi et al. (2004) showed the valine-to-alanine substitution in the third histidine-rich region. This amino acid substitution may have a crucial impact on the action of enzymes. Thus, genetic differences in SCD may explain differences in fatty acid content in Japanese black steers.

Conclusion

Genetic markers have quickly become essential tools for improving animals because they enable us to choose the best ones quickly. This study determined the association of SCD gene polymorphism at 5th exon c.878T>C locus with TMY305 in Holstein cows. Individuals with TC genotypes showed the highest TMY305 than individuals with TT and CC genotypes. At the same time, individuals with the TT genotype showed the lowest rate of TMY305 than the other individuals. SCD gene polymorphism can be considered a valuable candidate gene for milk production.

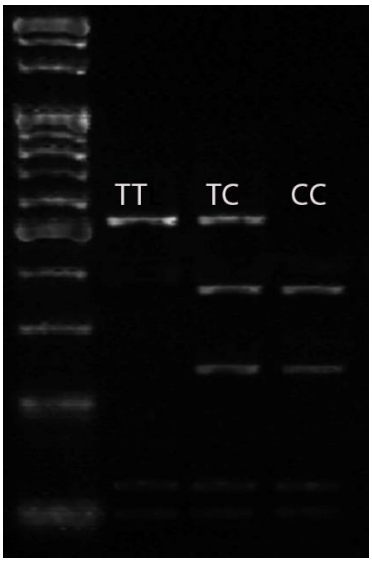

Figure 1. PCR-RFLP results of SCD gene locus c.878T>C, TT (122, 28 and 20 bp); TC (122, 75, 47, 28 and 20 bp); CC (75, 47, 28 and 20 bp)

Table 2. Allele and genotype frequency of the SCD gene locus

Gene locus	Genotype frequency	Allele frequency	χ^2 (HWE)
SCD	TT(0.21) TC(0.50) CC(0.29)	T(0.46) C(0.54)	0.003*

χ^2, Chi-square; HWE, Hardy–Weinberg equilibrium; * $P<0.05$

Table 3. Association of SCD gene polymorphism with TMY305 and DMY

Traits	Genotypes			P value
	TT	TC	CC	
TMY305	7046±457	8139±300	7121±405	0.038*
DMY	20.10±0.86	22.55±0.48	19.63±0.68	0.630

* $P<0.05$

References

Alim M, Fan Y, Wu X, Xie Y, Zhang Y, Zhang S, Sun D, Zhang Y, Zhang Q, Liu L. 2012. Genetic effects of stearoyl-coenzyme A desaturase (SCD) polymorphism on milk production traits in the Chinese dairy population. Molecular biology reports, 39: 8733-8740. doi: 10.1007/s11033-012-1733-6

Bartoň L, Kott T, Bureš D, Řehák D, Zahradkova R, Kottova B. 2010. The polymorphisms of stearoyl-CoA desaturase (SCD1) and sterol regulatory element binding protein-1 (SREBP-1) genes and their association with the fatty acid profile of muscle and subcutaneous fat in Fleckvieh bulls. Meat Science, 85: 15-20. doi: 10.1016/j.meatsci.2009.11.016

Čítek J, Brzáková M, Hanusová L, Hanuš O, Večerek L, Samková E, Křížová Z, Hoštičková I, Kávová T, Straková K. 2021. Gene polymorphisms influencing yield, composition and technological properties of milk from Czech Simmental and Holstein cows. Animal bioscience, 34: 2. doi: 10.5713/ajas.19.0520

Dujková R, Ranganathan Y, Dufek A, Macák J, Bezdíček J. 2015. Polymorphic effects of FABP4 and SCD genes on intramuscular fatty acid profiles in longissimus muscle from two cattle breeds. Acta Veterinaria Brno, 84: 327-336. doi: 10.2754/avb201584040327

García-Gómez B, Vázquez-Odériz ML, Muñoz-Ferreiro N, Romero-Rodríguez MÁ, Vázquez M. 2019. Interaction between rennet source and transglutaminase in white fresh cheese production: Effect on physicochemical and textural properties. LWT, 113: 108279. doi: org/10.1016/j.lwt.2019.108279

Griinari J, Corl B, Lacy S, Chouinard P, Nurmela K, Bauman D. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. The Journal of nutrition, 130: 2285-2291. doi: 10.1093/jn/130.9.2285

Gu M, Cosenza G, Iannaccone M, Macciotta N, Guo Y, Di Stasio L, Pauciullo A. 2019. The single nucleotide polymorphism g. 133A> C in the stearoyl CoA desaturase gene (SCD) promoter affects gene expression and quali-quantitative properties of river buffalo milk. Journal of Dairy Science, 102: 442-451. doi: org/10.3168/jds.2018-15059

Heck J, Van Valenberg H, Dijkstra J, Van Hooijdonk A. 2009. Seasonal variation in the Dutch bovine raw milk composition. Journal of Dairy Science, 92: 4745-4755. doi: org/10.3168/jds.2009-2146

Inostroza KB, Scheuermann ES, Sepúlveda NA. 2013. Stearoyl CoA desaturase and fatty acid synthase gene polymorphisms and milk fatty acid composition in Chilean Black Friesian cows. Revista Colombiana de Ciencias Pecuarias, 26: 263-269.

Jaleta TG. 2012. Effect of Stearoyl-CoA desaturase gene polymorphism on milk production traits of Hungarian Holstein Friesian cows. MSc Dissertation. Debrecen University.

Kaplanova K, Dufek A, Dračková E, Simeonovová J, Šubrt J, Vrtková I, Dvořák J. 2013. The association of CAPN1, CAST, SCD, and FASN polymorphisms with beef quality traits in commercial crossbred cattle in the Czech Republic Original Paper. Czech Journal of Animal Science, 58. doi: 10.17221/7044-CJAS

Keating AF, Kennelly JJ, Zhao F-Q. 2006. Characterization and regulation of the bovine stearoyl-CoA desaturase gene promoter. Biochemical and biophysical research communications, 344: 233-240. doi: 10.1016/j.bbrc.2006.03.133

Kgwatalala P, Ibeagha-Awemu E, Mustafa A, Zhao X. 2009. Influence of stearoyl-coenzyme A desaturase 1 genotype and stage of lactation on fatty acid composition of Canadian Jersey cows. Journal of Dairy Science, 92: 1220-1228. doi: org/10.3168/jds.2008-1471

Li Y, Zhou H, Cheng L, Zhao J, Hickford J. 2020. Variation in the stearoyl-CoA desaturase gene (SCD) and its influence on milk fatty acid composition in late-lactation dairy cattle grazed on pasture. Archives Animal Breeding, 63: 355-366. doi: org/10.5194/aab-63-355-2020

Mele M, Conte G, Castiglioni B, Chessa S, Macciotta NPP, Serra A, Buccioni A, Pagnacco G, Secchiari P. 2007. Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. Journal of Dairy Science, 90: 4458-4465. doi: 10.3168/jds.2006-617

Migliorati L, Boselli L, Pirlo G, Moschini M, Masoero F. 2017. Corn silage replacement with barley silage in dairy cows' diet does not change milk quality, cheese quality and yield. Journal of the Science of Food and Agriculture, 97: 3396-3401. doi: org/10.1002/jsfa.8190

Milannesi E, Nicoloso L, Crepaldi P. 2008. Stearoyl CoA desaturase (SCD) gene polymorphism in Italian castle breeds. Journal of Animal Breeding and Genetics, 125: 63-67. doi: 10.1111/j.1439-0388.2007.00697.x

Moioli B, Contarini G, Avalli A, Catillo G, Orru L, De Matteis G, Masoero G, Napolitano F. 2007. Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. Journal of Dairy Science, 90: 3553-3558. doi:10.3168/jds.2006-855

Nergis H, Koncagül S, Kiraz S. 2019. Polymorphism of stearoyl-CoA Desaturase (SCD) gene in Hereford cattle breed. Harran Journal of Agricultural and Food Science, 23: 247-253. doi: 10.29050/harranziraat.487490

Ntambi JM, Miyazaki M. 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Progress in lipid research, 43: 91-104. doi: 10.1016/s0163-7827(03)00039-0

Oh D-y, Jin M-h, Lee Y-s, Ha J-j, Kim B-k, Yeo J-s, Lee J-y. 2013. Identification of stearoyl-CoA desaturase (SCD) gene interactions in Korean native cattle based on the multifactor-dimensionality reduction method. Asian-Australasian Journal of Animal Sciences, 26: 1218. doi: 10.5713/ajas.2013.13058

Paton CM, Ntambi JM. 2009. Biochemical and physiological function of stearoyl-CoA desaturase. American Journal of Physiology-Endocrinology and Metabolism, 297: E28-E37. doi: 10.1152/ajpendo.90897.2008

Wang J, Yu L, Schmidt RE, Su C, Huang X, Gould K, Cao G. 2005. Characterization of HSCD5, a novel human stearoyl-CoA desaturase unique to primates. Biochemical and biophysical research communications, 332: 735-742. doi: 10.1016/j.bbrc.2005.05.013

Zhang l, ge l, parimoo s, stenn k, prouty sm. 1999. Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem polyadenylation sites. Biochemical Journal, 340: 255-264. doi: 10.1042/bj3400255