Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

The relationship of DGAT1 polymorphisms and milk fatty acids production of cows bred in Iraq (Local, cross and Holstein-Friesen)

M.Y. Yousief[1], H.A. Al-Galbi[1] and H.A. Al-Bataat[2]

[1]Animal Production Department, College of Agriculture, University of Basrah, [2]Agriculture Department of Basrah, Basrah, Iraq

Article information

Article history:
Received 29 March, 2023
Accepted 15 July, 2023
Available online 12 December, 2023

Keywords:
Cattle
DGAT1
Milkfat composition
Milk-free fatty acids

Correspondence:
H.A. Al-Bataat
hadaralbataat@gmail.com

Abstract

This study was conducted on a sample of 41 cows (6 local, 21 crosses, and 14 Holstein Friesen) raised in Iraq aged 3-4 years. The study included blood and milk samples. The sequencing technique was used to determine mutations in the DGAT1 gene to determine the effect of genotypes on the quality of fatty acids in cow's milk for all breeds. The GC-MS device was used to measure the levels of milk fatty acids. The study showed the presence of a replacement mutation, as the change was in bases 148 and 149 in the entire coding region of the DGAT1 gene. Where the two bases AA in (Allele 1) changed to GC in (Allele 2) and then to GA in (Allele 3). This mutation led to the change from lysine (K) in allele 1 to alanine and glutamine in Alleles 2 and 3, respectively. The local breed outperformed the percentage of saturated fatty acids over the cross and Holstein-Friesian. While monounsaturated and polyunsaturated, crosses and Holstein-Friesian significantly outperformed the local breed. The first allele significantly exceeded ($p<0.01$) the other alleles of all breeds in the percentage of saturated fatty acids. In comparison to the first allele, the second allele of cross cows displayed higher levels of monounsaturated and polyunsaturated fatty acids. Regarding unsaturated fatty acids, the third allele of the Friesian breed outperformed the other alleles. Therefore, individual patterns of the DGAT1 gene can be relied upon as markers in the selection process for milk quality purposes.

DOI: 10.33899/ijvs.2023.139337.2929, ©Authors, 2024, College of Veterinary Medicine, University of Mosul.

Introduction

The primary goal of studies on quantitative trait loci is to identify genetic markers related to milk production (1). The gene has been identified in cows, as the gene DGAT1 (diacylglycerol O-acyltransferase 1) consists of 14,117 PB, comprises 17 exons and 16 introns, and is located on chromosome 14 in cattle (2,3). The DGAT1 gene has been proposed as a functional candidate gene for milk production traits (4-7). Milk fat composition significantly impacts dairy products, as more unsaturated milk fat is preferred in terms of human nutrition and health (8-10). However, this may make the milk fat more susceptible to oxidation, giving the milk an off-flavor (11-13). A study conducted on Sahiwal cows showed that the quantitative trait locus (QTL) significantly impacts milk production and composition, as the DGAT1 gene is responsible for fatty acid composition in milk (14,15). DGAT1 is a protein that catalyzes the final step in the formation of triglycerides by encoding the enzyme Acyl-CoA -diacylglycerol acyltransferase, which plays a critical role in converting diglycerol to triglycerol (16-18). Additionally, the DGAT1 gene synthesizes triglycerides deposited in the small intestine, liver, adipose tissue, and mammary gland (19). Agrawel *et al.* (20) indicated that the main cause of many genetic variations in milk production and its components, and for many breeds worldwide, is the resulting mutation in the eighth code of the DGAT1 gene. Furthermore, Banos *et al.* (21) stated that DGAT1 is responsible for 29% of milk fat proportion variations and cattle production. The production of milk and its components depends on the nutrients available in the blood, which are highly correlated with each other. In this regard, traits with high heritability, such as fat and protein percentages, come into play (22,23). On the other hand, the genotypes of this gene were thought to represent indicators of the relative variations in the preceding attributes between individuals. These markers were incorporated into commercial genotype designs for markers-assisted selection (MAS) in many farm animal species (13,24).

The current study aimed to find DGAT1 gene polymorphisms in cattle raised in Iraq. Together with this, information about how these polymorphisms relate to milk fat compositions was presented.

Materials and methods

Ethical approve

The study has been approved by the Animal Ethics committee of Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq.

Sampling

Forty-one cows were used, divided into 6 local, 21 cross, and 14 Holstein Friesian cows reared in Iraq, whose ages ranged between 3 and 4 years. It's worth noting that the cows eat whatever green alfalfa, wheat bran, or flour is available, and pastoral plants like reeds and papyrus are also available. The cows are milked twice (automatically) a day, at six o'clock in the morning and four o'clock in the evening.

Fatty acid estimation

Milk fat was extracted by collecting the fatty layer (cream) by centrifugation at 1100 rpm for 20 minutes at a temperature of 4°C. The fatty layer was collected and stored at 20°C for a day. The frozen fat layer was placed at a temperature of 60°C for 10 minutes, then the centrifugation was carried out at a speed of 2000 rpm for 7 minutes. The surface layer, which represents milk fat, is removed and stored at a temperature of -20°C until the esterification process occurs (25). The esterification process was done by reacting glycerides with methyl potassium hydroxide prepared by dissolving 11.2 g of potassium hydroxide in 100 ml of methanol. Esterification was carried out by weighing 1 g of the sample in a tube of 15 ml capacity and adding 5 ml of methyl potassium hydroxide. Shake the tube for 5 minutes. 5 ml of pure hexane was added, and the contents were shaken and left until two layers were separated. The upper layer contains the methyl esters of the fatty acids in hexane, and the saponified material is in the lower layer.

Half an ml of the esterified fat sample was taken and placed in the injection tube of the device. Then 1 ml of pure hexane was added to it, shaken well, and placed in the sample holder of the gas chromatography-mass spectrometer as the injection process was carried out using the automatic injector. The total fatty acids of cow's milk fat samples were determined in the central chromatographic laboratory of the Ministry of Science and Technology, Department of Environment and Water, at the University of Baghdad, using the gas chromatography device type GC-QP210 Ultra equipped by the Japanese company SHIMADZU with methylpolysiloxane, methylpolysiloxane, and 5% phenyl (BD-GC). As a still phase, the dimensions are 30 meters long and 0.32 in diameter; the thickness of the still phase is 0.25 microliters, and the carrier gas is high-purity helium gas. The separation process was carried out according to the thermal program at 40 meters for a minute. Then it is raised to 150°C for a minute at a rate of 5°C per minute, then to 280°C at a rate of 5°C per minute, and then the temperature is fixed at 280°C for a minute (26). Total fatty acids were calculated as indicated by Oh *et al.* (27) using the following: SFA= C12:0+ C14:0+C15:0+C16:0+C17:0 + C18:0. MUFA= C14:1 c9+ C16:1 c9+ C18:1 c9. PUFAs=C18:2 c9,c12+C18:3n-3+C18:2 c9,t11+ C20:3n-6+ C20:4+ C20:5+ C22:5. USFA= MUFA+ PUFA. IC14=(C14:1 c9/ C14:0 + C14:1 c9)*100. IC16= (C16:1 c9/ C16:0+ C16:1 c9)*100. IC18= (C18:1 c9/ C18:0+ C18:1 c9)*100.

The DNA extraction

This study was conducted in the genetic engineering laboratory of the College of Agriculture, University of Basrah. Blood samples (n = 41) were collected from the studied cows. Blood samples were drawn from the jugular vein at the rate of one sample for each animal, at 5 ml for each sample. The extraction was carried out using a refrigerated centrifuge and a special DNA extraction kit produced by the company (Geneaid).

Primer and PCR amplification

The primers were forward 5'-AAGGCCAAGGCTGGTGAG-3'; reverse 5'-GGCGAAGAGGAAGTAGTAG-3' (28). The optimized thermal profile includes an initial denaturation at 94°C for 3 minutes, 30 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 45 seconds, elongation at 72°C for 1 minute, and a final extension at 72°C for 7 minutes (28). Samples of 20 microliters of amplified gene segments (PCR product) were sent to Yang Ling Tianrun Aoka Biotechnology Company in China to obtain the nitrogen base sequences of the desired gene segments. As the sequencing process was performed for one strand of DNA, which is forward, and according to our request from the company to identify genetic mutations, After the results were received, the sequence identity in GenBank was reviewed using bioinformatics techniques and algorithms such as the Blast search tool. This tool helped to determine the similarity between the records of the sent samples and the records in the gene bank and the extent of their conformity with the studied species. It was identical to a large number of species but in different proportions. This match reflects the similarity in structure and function with the studied genes, after which an alignment was made between the samples, and the sequences of each sample were cut into the same length and alignment, and the differences between them were identified using specialized software.

Statistical analysis

A completely randomized design (CRD) was used to analyze the effect of genotypes on the concentration of hormones and blood biochemical components using the statistical program (29) Version 24. Means were compared using the revised least significant difference test ($P<0.05$) within the program and according to the following mathematical *model* (*Yij* =μ+ Ti+ eij), where, Yij is the value of j observation that belongs to I treatment, μ is the common man, Ti is the genotype effect, and it is the error associated with each observation, which is randomly and normally distributed with a mean of zero and variance of σ^2e.

Results

Polymorphisms

The local breed and crosses both showed two alleles, H1 and H2, while the Holstein breed exhibited three alleles, H1, H2, and H3. Three alleles were obtained when analyzing three SNPs (SNP1-SNP3) of the bovine DGAT1 gene located on the centromeric region of the bovine chromosome 14, having 17 exons with 14,117 bp and 18 introns. The change was in two consecutive bases, numbers 148 and 149, of the entire coding region of the DGAT1 gene. The two bases changed from AA in (Allele 1) to GC in (Allele 2) and then to GA in Allele 3. This mutation led to an amino acid change from lysine (K) in Allele 1 to alanine and glutamine in Allele 2 and Allele 2, respectively (Figure 1).

```
Allele1 SAGLGPRPRLVRAASGWGHWAATCLGTGRGSAHPRPAPCRLLVALAGKKANGGAAQRTVS 28
Allele2 SAGLGPRPRLVRAASGWGHWAATCLGTGRGSAHPRPAPCRLLVALAGKAANGGAAQRTVS 28
Allele3 SAGLGPRPRLVRAASGWGHWAATCLGTGRGSAHPRPAPCRLLVALAGKEANGGAAQRTVS 28
        ****************************

Allele1 YPDNLTYRGEDPAGGWGLPGGLACPRPPFQISTTSSSPPPCATSSTSPAPPA 64
Allele2 YPDNLTYRGEDPAGGWGLPGGLACPRPPFQISTTSSSPPPCATSSTSPAPPA 64
Allele3 YPDNLTYRGEDPAGGWGLPGGLACPRPPFQISTTSSSPPPCATSSTSPAPPA 64
        ************************************
```

Figure 1: Amino acids, K(Lysine), A(Alanine), E(Glutamine) of different alleles.

It is noted that the number of animals carrying the second allele, H2, was greater than the number of animals carrying the first allele (24 and 15, respectively), with a frequency of 0.59 and 0.37 for the two alleles (Table 1). Especially in the cross cows (12 and 9, respectively), with a frequency of 0.57 and 0.43 for the two alleles. The Holstein breed (9 and 3, respectively) has a frequency of 0.64 and 0.21 for the two alleles. The number of animals for the third allele was 2 with a frequency of 0.14, which was unique to the Holstein-Friesian breed. While the number of animals bearing the first and second alleles of the local strain was 3, with a frequency of 0.5 for each (Table 1).

Table 1: The number of animals from different breeds with the polymorphisms belongs to alleles 1, 2, and 3

Breed	H1	Frequency	H2	Frequency	H3	Frequency	Total
Cross	9	0.43	12	0.57	-	-	21
Local	3	0.50	3	0.50	-	-	6
Holstein	3	0.21	9	0.64	2	0.14	14
Total	15	0.37	24	0.59	2	0.05	41

Figure 2 shows the network of alleles of cows bred in Iraq. The Holstein-Friesian breed was unique to the H3 allele, while it shared with the two local and crossed cattle the H1 and H2 alleles. The H3 allele differed from the H1 allele with the nitrogen base 148 and the H2 allele with the nitrogen base 149.

Effect of DGAT1 gene on fatty acid levels in milk of cows raised in Iraq

Table 2 shows the breeds' effect on milk's fatty acid content. The local breed showed its superiority in all saturated fatty acids (SFA), as it recorded 55.5%, compared to both the Holstein 52.52% and the cross cows 52.12%.

Also, the local breed was superior to the other breeds with the saturated acids C12:0, C14:0, C15:0, C16:0, and C17:0. Still, it showed an arithmetic increase in the percent of C18:0. The local breed reported the lowest percentages of monounsaturated, polyunsaturated, and total fatty acids, with values of 27.39%, 5.86%, and 33.25%, respectively. At the same time, the local breed showed a significant decrease in the ratios of monounsaturated fatty acids, polyunsaturated fatty acids, and total/saturated fatty acids compared to the cross and Holstein cows 0.50, 0.61, and 0.11%, respectively. When calculating the acid indices IC14, IC16, and IC18, the local breed also recorded a significant decrease with values of 13.19, 55.40, and 67.39, respectively.

Figure 2: alleles network of locales, Holstein and their crosses.

The alleles of the DGAT1 gene were significantly associated with fatty acids. Both palmitic and oleic fatty acids had higher proportions than the rest of the other fatty acids in the milk fat of cows raised in Iraq local, frisian, and cross breeds. The first allele was significantly superior to the second allele in saturated palmitic fatty acid for all breeds. The second allele was superior in the concentration of oleic fatty acid (C18:1) of the cross and local breed, while the third allele was superior in the Holstein-Friesen breed (Table 3).

Table 2: Effect of different breeds (Holstein, cross, and local cattle) on milk fatty acids contents (%)

Treats	Number (Breed)			P value (Breed)
	Holstein (14)	CROSS (21)	LOCAL (6)	
C12:0 (lauric)	4.68b±0.30	4.62b±0.29	4.98a±0.29	0.037
C14:0 (myristic)	10.19b±0.35	10.16b±0.31	10.74a±0.53	0.04
C15:0 (pentadecanoic)	1.65b±0.10	1.63b±0.09	1.80a±0.13	0.03
C16:0(palmitic)	23.59ab±0.86	23.41b±0.91	24.61a±0.80	0.019
C17:0 (margaric)	1.33b±0.21	1.30b±0.18	1.55a±0.18	0.024
C18:0 (stearic)	11.07a±0.25	10.97a±0.27	11.38a±0.24	0.06
SFA	52.52b±2.04	52.10b±2.02	55.05a±2.13	0.012
C14:1 c9 (myristoleic)	1.91a±0.27	1.97a±0.26	1.63b±0.24	0.024
C16:1 c9 (palmitoleic)	2.56a±0.42	2.59a±0.32	2.24a±0.18	0.094
C18:1 c9 (oleic)	24.03ab±0.37	24.13a±0.35	23.53b±0.48	0.005
MUSFA	28.50a±1.05	28.69a±0.92	27.39b±0.88	0.02
18:2 c9,c12 (linoleic)	4.26a±0.38	4.30a±0.29	3.65b±0.47	0.001
18:3n-3 (linolenic)	0.57a±0.09	0.58a±0.09	0.49a±0.05	0.064
18:2 c9,t11 (GLA)	0.59ab±0.05	0.61b±0.06	0.54b±0.02	0.038
C20:3n-6 (eicosatrienoic)	0.15a±0.01	0.15a±0.01	0.13b±0.01	0.005
C20:4 (arachadonic)	0.49ab±0.06	0.52a±0.06	0.44b±0.02	0.017
C20:5 (timnodonic)	0.25a±0.01	0.25a±0.01	0.24b±0.02	0.038
C22:5 (docosapentaenoic)	0.42b±0.04	0.44a±0.04	0.38c±0.03	0.006
PUSFA	6.74a±0.64	6.85a±0.55	5.86b±0.62	0.003
USFA	35.24a±1.68	35.54a±1.46	33.25b±1.49	0.01
MUSFA/SFA	0.54a±0.04	0.55a±0.04	0.50b±0.03	0.02
USFA/SFA	0.67a±0.06	0.68a±0.05	0.61b±0.05	0.015
PUSFA/SFA	0.13a±0.02	0.13a±0.02	0.11b±0.02	0.007
IC14	15.82a±2.34	16.28a±2.23	13.19b±2.21	0.019
IC16	60.40a±4.86	61.11a±4.08	55.40b±3.71	0.023
IC18	68.47a±0.81	68.74a±0.18	67.39b±0.91	0.005

SFA: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid. USFA: unsaturated fatty acid.

This was reflected in each of the saturated fatty acids (SFA), polyunsaturated fatty acids (PUSFA), and mono (MUSFA). The first allele showed a significant superiority of saturated fatty acids for all breeds. On the other hand, the second allele showed a significant superiority for the monounsaturated and polyunsaturated fatty acids of the local and cross-breed, while the third allele showed a significant superiority for fatty acids, saturated, mono- and poly saturated of Holstein-Friesen bred in Iraq. Figure 3 shows the presence of an expansion in the population size that includes the three breeds after passing through the bottleneck of the DGTA1 gene due to the presence of a cross between the local and the Holstein breeds (Figure 3).

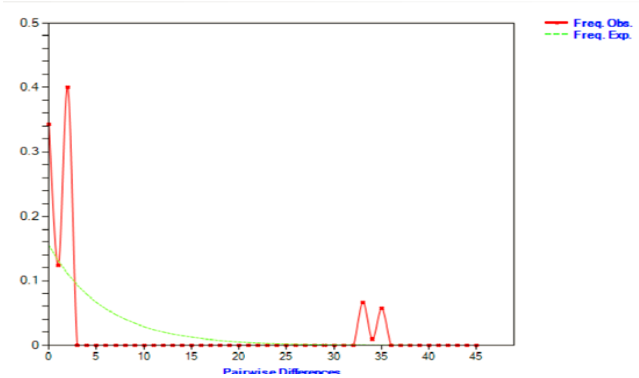

Figure 3: Matching of observed and expected genotypes frequencies of DGAt1 gene of local, Holstein and their crosses.

Table 3: Effect of DGAT1 alleles on milk fatty acids contents

TRIATS	Cross*		Local*		Hol*		
	Allele 1/9	Allele 2/12	Allele 1/3	Allele 2/3	Allele 1/3	Allele 2/3	Allele 3/3
C12:0	4.93a±0.11	4.39b±0.02	5.24a±0.02	4.72b±0.01	5.15a±0.02	4.63a±0.07	4.22b±0.07
C14:0	10.49a±0.09	9.91b±0.04	11.20a±0.12	10.27b±0.01	10.69a±0.08	10.16b±0.04	9.57c±0.09
C15:0	1.72a±0.03	1.56b±0.01	1.91a±0.01	1.68b±0.01	1.80a±0.04	1.64b±0.02	1.47c±0.01
C16:0	24.36a±0.06	22.70b±0.13	25.31a±0.17	23.91b±0.13	24.76a±0.34	23.56b±0.07	21.99c±0.22
C17:0	1.48a±0.04	1.16b±0.02	1.70a±0.06	1.40b±0.00	1.59a±0.02	1.34b±0.02	0.91c±0.07
C18:0	11.26a±0.03	10.76b±0.04	11.56a±0.13	11.20b±0.01	11.33a±0.01	11.09b±0.08	10.55c±0.00
C14:1	1.69b±0.07	2.19a±0.02	1.41b±0.05	1.84a±0.01	1.56c±0.01	1.91b±0.04	2.45a±0.04
C16:1	2.30b±0.08	2.80a±0.08	2.08b±0.02	2.40a±0.03	2.14c±0.01	2.49b±0.03	3.48a±0.01
C18:1 c9	23.76b±0.09	24.40a±0.04	23.15b±0.22	23.90a±0.01	23.53c±0.05	24.05b±0.10	24.71a±0.05
18:2 c9,c12	4.01b±0.01	4.52a±0.05	3.26b±0.17	4.04a±0.01	3.98c±0.01	4.17b±0.13	5.10a±0.16
18:3n-3	0.48b±0.01	0.65a±0.01	0.44b±0.01	0.53a±0.01	0.45c±0.01	0.57b±0.03	0.75a±0.04
18:2 c9,t11	0.54b±0.02	0.66a±0.01	0.52b±0.00	0.56a±0.00	0.52c±0.01	0.59b±0.02	0.69a±0.01
C20:3n-6	0.14b±0.01	0.16a±0.00	0.13a±0.00	0.14a±0.00	0.14c±0.01	0.15b±0.00	0.18a±0.01
C20:4	0.45b±0.01	0.57a±0.01	0.42b±0.00	0.46a±0.00	0.42c±0.01	0.48b±0.02	0.62a±0.04
C20:5	0.24b±0.01	0.27a±0.00	0.22b±0.01	0.25a±0.00	0.23c±0.01	0.25b±0.01	0.28a±0.00
C22:5	0.40b±0.01	0.47a±0.00	0.35b±0.00	0.41a±0.00	0.36c±0.02	0.43b±0.02	0.49a±0.01
SFA	54.23a±0.35	50.49b±0.26	56.92a±0.51	53.18b±0.16	55.31a±0.51	52.43b±0.30	48.71c±0.46
MUSFA	27.75b±0.23	29.39a±0.14	26.64b±0.28	28.14a±0.05	27.23c±0.06	28.45b±0.16	30.64a±0.09
PUSFA	6.27b±0.05	7.29a±0.07	5.33b±0.19	6.39a±0.01	6.12c±0.05	6.64b±0.21	8.10a±0.25
USFA	34.02b±0.28	36.68a±0.21	31.97b±0.47	34.53a±0.06	33.35c±0.11	35.09b±0.37	38.73a±0.34
MUSFA/SFA	0.51b±0.01	0.58a±0.01	0.47b±0.01	0.53a±0.00	0.49c±0.01	0.54b±0.01	0.63a±0.01
USFA/SFA	0.63b±0.01	0.73a±0.01	0.56b±0.01	0.65a±0.00	0.60c±0.01	0.67b±0.01	0.80a±0.01
PUSFA/SFA	0.12b±0.01	0.14a±0.00	0.09b±0.00	0.12a±0.00	0.11c±0.00	0.13b±0.00	0.17a±0.01
IC14	13.86b±0.57	18.10a±0.19	11.21b±0.42	15.16a±0.07	12.74c±0.17	15.83b±0.34	20.38a±0.39
IC16	57.20b±1.21	64.04a±0.78	52.05b±0.36	58.75a±0.36	54.31c±0.63	60.24b±0.53	70.21a±0.19
IC18	67.86b±0.14	69.40a±0.11	66.70b±0.45	68.09a±0.02	67.51b±0.07	68.43b±0.25	70.08a±0.05

This means that different letters horizontally differ significantly at P<0.05.

Discussion

Concerning the prevalence and dominance of genotypes, Agrawal *et al.* (30), in their study of the eighth coding segment of the DGAT1 gene of Indian Sahiwal cows, there was no genetic conformation of the DGAT1 gene, as the study showed that all experimental animals had one genetic form, which is the dominant one. Studies on genetic polymorphisms of DGAT1 and their relationship to milk traits were mentioned. Lešková *et al.* (31) indicated that the genotypes of the DGAT1 gene had significant effects on fat milk content, as the dominant genotype had higher breeding values for milk fat content. The results of Dakhil (32) indicated three genotypes of the DGAT1 gene, dominant KK, heterozygote KA, and recessive AA. The results showed a high correlation of the dominant genotype KK with fat content and increased fat production 4.09%, compared to the AA genotype, which was associated with low fat 3.30%, while the KA homozygote recorded 3.68 % and high milk productivity. The reason is attributed to the K232A mutation that caused the substitution of the K allele encoding lysine, which was associated with high fat, to the allele A, encoding alanine, which was associated with increased milk production and decreased fat, about the characteristic of the production peak. Ardicli *et al.* (33) pointed out the significant role of the DGAT1 gene in triglyceride synthesis. In general, the Holstein Friesian breed showed a significant increase in unsaturated fatty acids because this breed was selected and improved intensively to increase milk yield, and this led to a decrease in the fat percentage due to a negative correlation between them. Therefore, improved milk production improved milk quality through higher levels of different types of unsaturated fatty acids.

DGAT1 polymorphisms affect the fatty acid composition of milk in cattle. Carvajal *et al.* (34) found that the DGAT1 GC/GC allele was associated with lower milk fat and protein content, lower saturated fatty acid levels, and higher polyunsaturated FA (PUFA), n-3 and n-6 FA, and a-linolenic acid to cholesterolemic FA ratios, which implied a healthier FA profile. The DGAT1 K232A polymorphism influenced the fatty acid composition: milk from AA cows had a more favorable fatty acid composition due to lower total saturated fatty acids, saturated to unsaturated ratio, atherogenic index, and higher levels of oleic acid and total unsaturated fatty acids (35). However, the effects of DGAT1 polymorphisms on milk fatty acids may also depend on other factors such as feeding system, breed, and environment (35).

The lipid polymorphisms detected in this study are consistent with previous studies in other cattle breeds **(**36-38). Fatty acid profiles were generated individually and revealed that in most cases, C16:0 fatty acids were the most abundant, accounting for more than 25% of the total milk fat obtained from genotypes than total unsaturated fatty acids such as oleic acid (C18: 1cis9) accounts for more than 20% of the total fat in cattle milk. A study of the DGAT1 polymorphism revealed a high genetic variation of lipid profile (39). These results indicate increased fat selection in the milk of Romanian Holstein cows, the obvious effect of polymorphism on the fat content and composition of milk (40-44). These findings agree with the results of the current study. The mismatch test showed a major unimodal distribution with peaks of even differences. This reflects a correspondence between the observed and expected frequencies, an expansion in the population, and the studied gene not being affected by the influential forces that affect gene replication. In addition, the population size for these genotypes is large, and there is no risk of losing some genotypes or alleles. In addition to the free movement of animals within Iraq and between different provinces (45,46). Lastly, some breeding stations in different regions of Iraq import Holstein cows. Most neutrality tests compare several mutational parameter estimates derived from empirical data: Fay and Wu's FW-test compares Tajima's estimate to a different estimate weighted by the homozygosity of the derived variants. In contrast, Tajima's D compares Watterson's estimate based on the mean number of differences between pairs of sequences to Tajima's estimate based on the mean number of segregating sites in the sample (47).

Conclusions

These findings indicate that the DGAT1 gene alleles can significantly affect the fatty acid composition of milk in cows raised in Iraq, which can be important for the quality and nutritional value of milk products. Overall, these factors could have contributed to the observed differences in fatty acid composition between Iraq's local Holstein and crossbred cows.

Acknowledgment

The authors thank the University of Basrah, College of Agriculture for all the facilities to achieve this study.

Conflict of interest

There is no conflict of interest.

References

1. Rothschild MF. Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe Newslett Agric Genom. 1997;8:13-20. [available at]
2. Mohammed SA, Rahamtalla SA, Ahmed SS, Elhafiz A, Dousa BM, Elamin KM, Ahmed MK. DGAT1 gene in dairy cattle. Glob J Anim Sci Res. 2015;3(1):191-198. [available at]
3. Tung HT, Chau LT, Van Hanh N, Long LT, Son HN. The diversity of dgat1 and scd1 gene in Vietnamese native, Sindhi and Brahman crossbred cattle. J Entomol Zool Stud. 2019:7(2):1253-1255. [available at]
4. Winter A, Krämer W, Werner FA, Kollers S, Kata S, Durstewitz G, Buitkamp J, Womack JE, Thaller G, Fries R. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA: Diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. Proc Nat Acad Sci. 2002;99(14):9300-9305. DOI: 10.1073/pnas.142293799
5. Faraj SH, Ayied AY, Seger DK. DGAT1 gene polymorphism and its relationships with cattle milk yield and chemical composition. Period Tche Quimica. 2020;17(35):174-180. DOI: 10.52571/ptq.v17.n35.2020.16_faraj_pgs_174_180.pdf
6. Khan MZ, Ma Y, Ma J, Xiao J, Liu Y, Liu S, Khan A, Khan IM, Cao Z. Association of DGAT1 with cattle, buffalo, goat, and sheep milk and meat production traits. Front Vet Sci. 2021;8:712470. DOI: 10.3389/fvets.2021.712470
7. Samuel B, Mengistie D, Assefa E, Kang M, Park C, Dadi H, Dinka H. Genetic diversity of DGAT1 gene linked to milk production in cattle populations of Ethiopia. BMC Genom Data. 2022;23(1):64. DOI: 10.1111/1541-4337.12751
8. Magan JB, O′ Callaghan TF, Kelly AL, McCarthy NA. Compositional and functional properties of milk and dairy products derived from cows fed pasture or concentrate-based diets. Compr Rev Food Sci Food Saf. 2021;20(3):2769-800. DOI: 10.1111/1541-4337.12751
9. Riuzzi G, Davis H, Lanza I, Butler G, Contiero B, Gottardo F, Segato S. Multivariate modelling of milk fatty acid profile to discriminate the forages in dairy cows' ration. Sci Rep. 2021;11(1):23201. DOI: 10.1038/s41598-021-02600-9
10. Paszczyk B, Tońska E. Fatty acid content, lipid quality indices, and mineral composition of cow milk and yogurts produced with different starter cultures enriched with *Bifidobacterium bifidum*. Appl Sci. 2022;12(13):6558. DOI: 10.3390/app12136558
11. Al-Shuhaib MB, Al-Thuwaini TM, Al-Fihan RA, Al-Qutbi AA. Genotyping of diacylglycerol acyltransferase 2 gene in Holstein cattle population. Agric Conspec Sci. 2019;84(2):211-8. [available at]
12. Rahawy M. Study on the post-partum disorders and their relationship with the reproductive performance in Iraqi cow-buffaloes. Iraqi J Vet Sci. 2021;35(2):313-317. DOI: 10.33899/ijvs.2020.126771.1387
13. Elzaki S, Korkuć P, Arends D, Reissmann M, Brockmann GA. Effects of DGAT1 on milk performance in Sudanese Butana× Holstein crossbred cattle. Trop Anim Health Prod. 2022;54(2):142. DOI: 10.1007/s11250-022-03141-7
14. Świtoński M. Advances in domestic animal genomics. NAUKA. 2008;1:27-43. [available at]
15. Safdar M, Ozaslan M. The role of diglyceride acyltransferase (DGAT) genes polymorphisms on milk production in dairy goats. Ger J Vet Res. 2021;1(1):15-18. DOI: 10.51585/gjvr.2021.0003
16. Komisarek J, Michalak A, Walendowska A. The effects of polymorphisms in DGAT1, GH and GHR genes on reproduction and production traits in Jersey cows. Anim Sci Pap Rep. 2011;29(1):29-36. [available at]
17. Anton I, Kovács K, Holló G, Farkas V, Szabó F, Egerszegi I, Rátky J, Zsolnai A, Brüssow KP. Effect of DGAT1, leptin and TG gene polymorphisms on some milk production traits in different dairy cattle breeds in Hungary. Arch Anim Breed. 2012;55(4):307-14. DOI: 10.5194/aab-55-307-2012

18. Mahmoud KG, Sakr AM, Ibrahim SR, Sosa AS, Hasanain MH, Nawito MF. GnRHR gene polymorphism and its correlation with semen quality in Buffalo bulls (*Bubalus bubalis*). Iraqi J Vet Sci. 2021;35(2):381-386. DOI: 10.33899/ijvs.2020.126886.1407
19. DeVita RJ, Pinto S. Current status of the research and development of diacylglycerol O-acyltransferase 1 (DGAT1) inhibitors: Miniperspective. J Med Chem. 2013;56(24):9820-5. DOI: 10.1021/jm4007033
20. Chopade MM, Shrinivas J, Deshmukh RS, Katkade BS, Sawane MP. Study of Effect of various genetic and non-genetic factors on milk yield in Frieswal Cattle. Indian J Anim Sci. 2023;93(1):67–71. DOI: 10.56093/ijans.v93i1.128719
21. Banos G, Woolliams JA, Woodward BW, Forbes AB, Coffey MP. Impact of single nucleotide polymorphisms in leptin, leptin receptor, growth hormone receptor, and diacylglycerol acyltransferase (DGAT1) gene loci on milk production, feed, and body energy traits of UK dairy cows. J Dairy Sci. 2008;91(8):3190-200. DOI: 10.3168/jds.2007-0930
22. Purwin C, Pysera B, Minakowski D, Sederevičius A, Traidaraitė A. Composition of milk and blood metabolites in higth productivity dairy cows on pasture. Vet Ir Zootech. 2005;32(54):57-60. [available at]
23. Li Y, Zhou H, Cheng L, Edwards GR, Hickford JG. Effect of DGAT1 variant (K232A) on milk traits and milk fat compositionin outdoor pasture-grazed dairy cattle, New Zealand J Agric Res. 2019;64(1):101-113. DOI: 10.1080/00288233.2019.1589537
24. Habib HN, Al-Rishdy KA, AL-Hellou MF. Molecular description of melatonin receptor 1A gene in Iraqi buffalo. Iraqi J Vet Sci. 2022;36(4):905-912. DOI: 10.33899/ijvs.2022.132532.2103
25. Coakley M, Banni S, Johnson MC, Mills S, Devery R, Fitzgerald G, Paul Ross R, Stanton C. Inhibitory effect of conjugated α-linolenic acid from bifidobacteria of intestinal origin on SW480 cancer cells. Lipids. 2009;44:249-56. DOI: 10.1007/s11745-008-3269-z
26. Al-Rikabi AK, Al–Fekaiki DF, Zaalan AZ. Diagnosis of fatty acids in the goat's fat milk (three strains) using GC-MS. Iraqi J Agric Res. 2017;22(5):143-151. [available at]
27. Oh D, La B, Lee Y, Byun Y, Lee J, Yeo G, Yeo J. Identification of novel single nucleotide polymorphisms (SNPs) of the lipoprotein lipase (LPL) gene associated with fatty acid composition in Korean cattle. Mol Biol Rep. 2013;40:3155-63. DOI: 10.1007/s11033-012-2389-y
28. Samuel B, Dadi H, Dinka H. Effect of the DGAT1 K232A mutation and breed on milk traits in cattle populations of Ethiopia. Front Anim Sci. 2023;4:1096706. DOI: 10.3389/fanim.2023.1096706
29. Statistical Packages of Social Sciences (SPSS). IBM SPSS Statistics for Windows, Version 24.0. USA: IBM Corp; 2016. DOI: 10.1002/9781118901731.iecrm0237
30. Kęsek-Woźniak M, Wojtas E, Zielak-Steciwko AE. Impact of SNPs in ACACA, SCD1, and DGAT1 genes on fatty acid profile in bovine milk with regard to lactation phases. Anim. 2020;10(6):997. DOI: 10.3390/ani10060997
31. Lešková L, Bauer M, Chrenek P, Lacková Z, Soročinová J, Petrovič V, Kováč G. Detection of DGAT1 gene polymorphism and its effect on selected biochemical indicators in dairy cows after calving. Acta Vet Brno. 2013;82(3):265-9. DOI: 10.2754/avb201382030265
32. Dakhil MH. Genetic influence of the gene responsible for milk fat content in crossbred cows with the babil local strain. Al-Furat J Agric Sci. 2017;9(4):738-748. DOI: 10.1021/jm4007033
33. Ardicli S, Soyudal B, Samli H, Dincel D, Balci F. Effect of STAT1, OLR1, CSN1S1, CSN1S2, and DGAT1 genes on milk yield and composition traits of Holstein breed. Rev Bras Zootec. 2018;47. DOI: 10.1590/rbz4720170247
34. Carvajal AM, Huircan P, Dezamour JM, Subiabre I, Kerr B, Morales R, Ungerfeld EM. Milk fatty acid profile is modulated by DGAT1 and SCD1 genotypes in dairy cattle on pasture and strategic supplementation. Genet Mol Res. 2016;15(2). DOI: 10.4238/gmr.15027057
35. Tumino S, Criscione A, Moltisanti V, Marletta D, Bordonaro S, Avondo M, Valenti B. Feeding system resizes the effects of DGAT1 polymorphism on milk traits and fatty acids composition in Modicana cows. Anim. 2021;11:1616. DOI: 10.3390/ani11061616
36. Spelman RJ, Ford CA, McElhinney P, Gregory GC, Snell RG. Characterization of the DGAT1 gene in the New Zealand dairy population. J Dairy Sci. 2002;85(12):3514-3517. DOI: 10.3168/jds.S0022-0302(02)74440-8
37. Tăbăran A, Balteanu VA, Gal E, Pusta D, Mihaiu R, Dan SD, Tăbăran AF, Mihaiu M. Influence of DGAT1 K232A polymorphism on milk fat percentage and fatty acid profiles in Romanian Holstein cattle. Anim Biotechnol. 2015;26(2):105-111. DOI: 10.1080/10495398.2014.933740
38. MacGibbon AH. Composition and structure of bovine milk lipids. In: Fox PF, McSweeney PH, editors. Advanced dairy chemistry. USA: Springer; 2006. 1–42 p. DOI: 10.1007/0-387-28813-9_1
39. Sanjayaranj I, MacGibbon AH, Holroyd SE, Janssen PM, Blair HT, Lopez-Villalobos N. Association of single nucleotide polymorphism in the DGAT1 gene with the fatty acid composition of cows milked once and twice a day. Genes. 2023;14(3):767. DOI: 10.3390/genes14030767
40. Grisart B, Farnir F, Karim L, Cambisano N, Kim JJ, Kvasz A, Mni M, Simon P, Frère JM, Coppieters W, Georges M. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. Proc Nat Acad Sci. 2004;101(8):2398-2403. DOI: 10.1073/pnas.0308518100
41. Shorten PR, Pleasants TB, Upreti GC. A mathematical model for mammary fatty acid synthesis and triglyceride assembly: the role of stearoyl CoA desaturase (SCD). J Dairy Res. 2004;71(4):385-397. DOI: 10.1017/S0022029904000354
42. Dokso A, Ivankovic A, Zecevic E, Brka M. Effect of DGAT1 gene variants on milk quantity and quality. Mljekarstvo. 2015;65(4):238-242. DOI: 10.15567/mljekarstvo.2015.0403
43. Gurcan EK, Cobanoglu O, Kul E, Abaci HS, Cankaya S. Determination of the genetic polymorphism for DGAT1 gene in Holstein, Jersey and native cattle breeds of Turkey. Indian J Anim Res. 2018;53(2):146-150. DOI: 10.18805/ijar.B-873
44. Li F, Cai C, Qu K, Liu J, Jia Y, Hanif Q, Chen N, Zhang J, Chen H, Huang B, Lei C. DGAT1, K232A polymorphism is associated with milk production traits in Chinese cattle. Anim Biotechnol. 2020;32(4):427-431. DOI: 10.1080/10495398.2020.1711769
45. Li Y, Zhou H, Cheng L, Edwards GR, Hickford JG. Effect of DGAT1 variant (K232A) on milk traits and milk fat composition in outdoor pasture-grazed dairy cattle. New Zeeland J Agric Res. 202164(1):101–113. DOI: 10.1080/00288233.2019.1589537
46. Ali MH, Hassanin SD. Subclinical ketosis: Prevalence and some risk factors in cross breed and imported breed dairy cow. Iraqi J Vet Sci. 2022;36(2):273-277. DOI: 10.33899/ijvs.2021.129949.1707
47. Mousset S, Derome N, Veuille M. A test of neutrality and constant population size based on the mismatch distribution. Mol Biol Evol. 2004;21(4):724-31. DOI: 10.1093/molbev/msh066

علاقة تعدد الأشكال الوراثية لجين DGAT1 وإنتاج الأحماض الدهنية لحليب الأبقار المربأة في العراق (المحلية والمضربة والهوليشتاين فريزيان)

منتهى يعقوب يوسف[1]، هناء علي الغالبي[1] و حيدر عدنان البطاط[2]

[1]قسم الإنتاج الحيواني، كلية الزراعة، جامعة البصرة، [2]مديرية زراعة البصرة، البصرة، العراق

الخلاصة

أجريت هذه الدراسة على عينة من ٤١ بقرة (٦ محلية، ٢١ هجين، ١٤ هولشتاين فريزين) تم تربيتها في العراق تتراوح أعمارهم بين ٣-٤ سنوات. اشتملت الدراسة على عينات دم وحليب. تم استخدام تقنية التسلسل لتحديد الطفرات في جين DGAT1 لتحديد تأثير الأنماط الجينية على نوعية الأحماض الدهنية في حليب البقر لجميع السلالات. تم استخدام جهاز GC-MS لقياس مستويات الأحماض الدهنية في الحليب. أظهرت الدراسة وجود طفرة بديلة، حيث كان التغيير في القاعدتين ١٤٨ و ١٤٩ في منطقة الترميز بأكملها لجين DGAT1. حيث تغيرت القاعدتان AA في (Allele 1) إلى GC في (Allele 2) ثم إلى GA في (Allele 3). أدت هذه الطفرة إلى تغيير حامض اللايسين (K) في الأليل ١ إلى الألانين والجلوتامين في الأليلات ٢ و ٣ على التوالي. تفوقت السلالة المحلية على نسبة الأحماض الدهنية المشبعة على المضرب والهولشتاين فريزيان. بينما تفوقت المضرب و هولشتاين فريزيان وفريزيان بشكل ملحوظ على السلالة المحلية غير المشبعة الأحادية وغير المشبعة. تجاوز الأليل الأول معنويا الأليلات الأخرى لجميع السلالات في نسبة الأحماض الدهنية المشبعة. بالمقارنة مع الأليل الأول، أظهر الأليل الثاني للأبقار المضربة مستويات أعلى من الأحماض الدهنية الأحادية غير المشبعة والمتعددة غير المشبعة. فيما يتعلق بالأحماض الدهنية غير المشبعة، فقد تفوق الأليل الثالث من سلالة الفريزيان على الأليلات الأخرى. لذلك، يمكن الاعتماد على الأنماط الفردية لجين DGAT1 كعلامات في عملية الاختيار لأغراض جودة الحليب.