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

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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). Agrawal 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 e9,t11+ C20:3n-6+ C20:4+ C20:5+ C22:5. USFA= MUFA+ PUF. 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'-AAGGCCAAGGCTTGTTAG-3'; reverse 5'-GGCAAGAGGAGTGTAG-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: \( Y_{ij} = \mu + T_i + e_{ij} \), where, \( Y_{ij} \) is the value of j observation that belongs to I treatment, \( \mu \) is the common mean, \( T_i \) is the genotype effect, and \( e_{ij} \) is the error associated with each observation, which is randomly and normally distributed with a mean of zero and variance of \( \sigma^2_e \).

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).

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

<table>
<thead>
<tr>
<th>Breed</th>
<th>H1 Frequency</th>
<th>H2 Frequency</th>
<th>H3 Frequency</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross</td>
<td>9</td>
<td>0.43</td>
<td>12</td>
<td>0.57</td>
</tr>
<tr>
<td>Local</td>
<td>3</td>
<td>0.50</td>
<td>3</td>
<td>0.50</td>
</tr>
<tr>
<td>Holstein</td>
<td>3</td>
<td>0.21</td>
<td>9</td>
<td>0.64</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>0.37</td>
<td>24</td>
<td>0.59</td>
</tr>
</tbody>
</table>

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 (%)

<table>
<thead>
<tr>
<th>Treats</th>
<th>Holstein (14)</th>
<th>CROSS (21)</th>
<th>LOCAL (6)</th>
<th>P value (Breed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0 (lauric)</td>
<td>4.68±0.30</td>
<td>4.62±0.29</td>
<td>4.98±0.29</td>
<td>0.037</td>
</tr>
<tr>
<td>C14:0 (myristic)</td>
<td>10.19±0.35</td>
<td>10.16±0.31</td>
<td>10.74±0.53</td>
<td>0.04</td>
</tr>
<tr>
<td>C15:0 (pentadecanoic)</td>
<td>1.65±0.10</td>
<td>1.63±0.09</td>
<td>1.80±0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>C16:0 (palmitic)</td>
<td>23.59±0.86</td>
<td>23.41±0.91</td>
<td>24.61±0.80</td>
<td>0.019</td>
</tr>
<tr>
<td>C17:0 (margaric)</td>
<td>1.33±0.21</td>
<td>1.30±0.18</td>
<td>1.55±0.18</td>
<td>0.024</td>
</tr>
<tr>
<td>C18:0 (stearic)</td>
<td>11.07±0.25</td>
<td>10.97±0.27</td>
<td>11.38±0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>SFA</td>
<td>52.52±2.04</td>
<td>52.10±2.02</td>
<td>55.05±2.13</td>
<td>0.012</td>
</tr>
<tr>
<td>C14:1 c9 (myristoleic)</td>
<td>1.91±0.27</td>
<td>1.97±0.26</td>
<td>1.63±0.24</td>
<td>0.024</td>
</tr>
<tr>
<td>C16:1 c9 (palmitoleic)</td>
<td>2.56±0.42</td>
<td>2.59±0.32</td>
<td>2.24±0.18</td>
<td>0.094</td>
</tr>
<tr>
<td>C18:1 c9 (oleic)</td>
<td>24.03±0.37</td>
<td>24.13±0.35</td>
<td>23.53±0.48</td>
<td>0.005</td>
</tr>
<tr>
<td>MUSFA</td>
<td>28.50±1.05</td>
<td>28.69±1.92</td>
<td>27.39±0.88</td>
<td>0.02</td>
</tr>
<tr>
<td>18:2 c9,c12 (linoleic)</td>
<td>4.26±0.38</td>
<td>4.30±0.29</td>
<td>3.65±0.47</td>
<td>0.001</td>
</tr>
<tr>
<td>18:3n-3 (linolenic)</td>
<td>0.57±0.09</td>
<td>0.58±0.09</td>
<td>0.49±0.05</td>
<td>0.064</td>
</tr>
<tr>
<td>18:2 c9,t11 (GLA)</td>
<td>0.59±0.05</td>
<td>0.61±0.06</td>
<td>0.54±0.02</td>
<td>0.038</td>
</tr>
<tr>
<td>C20:3n-6 (eicosatrienoic)</td>
<td>0.15±0.01</td>
<td>0.15±0.01</td>
<td>0.13±0.01</td>
<td>0.005</td>
</tr>
<tr>
<td>C20:4 (arachadonic)</td>
<td>0.49±0.06</td>
<td>0.52±0.06</td>
<td>0.44±0.02</td>
<td>0.017</td>
</tr>
<tr>
<td>C20:5 (timnodonic)</td>
<td>0.25±0.01</td>
<td>0.25±0.01</td>
<td>0.24±0.02</td>
<td>0.038</td>
</tr>
<tr>
<td>C22:5 (docosapentaenoic)</td>
<td>0.42±0.04</td>
<td>0.44±0.04</td>
<td>0.38±0.03</td>
<td>0.006</td>
</tr>
<tr>
<td>PUSFA</td>
<td>6.74±0.64</td>
<td>6.85±0.55</td>
<td>5.86±0.62</td>
<td>0.003</td>
</tr>
<tr>
<td>USFA</td>
<td>35.24±1.68</td>
<td>35.54±1.46</td>
<td>33.25±1.49</td>
<td>0.01</td>
</tr>
<tr>
<td>MUSFA/SFA</td>
<td>0.54±0.04</td>
<td>0.55±0.04</td>
<td>0.50±0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>USFA/SFA</td>
<td>0.67±0.06</td>
<td>0.68±0.05</td>
<td>0.61±0.05</td>
<td>0.015</td>
</tr>
<tr>
<td>PUSFA/SFA</td>
<td>0.13±0.02</td>
<td>0.13±0.02</td>
<td>0.11±0.02</td>
<td>0.007</td>
</tr>
<tr>
<td>IC14</td>
<td>15.82±2.34</td>
<td>16.28±2.23</td>
<td>13.19±2.21</td>
<td>0.019</td>
</tr>
<tr>
<td>IC16</td>
<td>60.40±4.86</td>
<td>61.11±4.08</td>
<td>55.40±3.71</td>
<td>0.023</td>
</tr>
<tr>
<td>IC18</td>
<td>68.47±0.81</td>
<td>68.74±0.18</td>
<td>67.39±0.91</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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).
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. Ardıcil 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 cholesterol 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 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

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Conflict of interest

There is no conflict of interest.

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علاقة تعدد الأشكال الوراثية لجين DGAT1 الأحماض الدهنية لحليب الأبقار المرأة في العراق (المحلية والمضربة والهولشتاين فريزيان)

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الخلاصة
أجريت هذه الدراسة على عينة من 41 بقرة (3 محلية، 21 هجين، 14 هولشتاين فريزيان) تم تربيتها في العراق تتراوح أعمارهم بين 4-3 سنوات. اشتملت الدراسة على عينات الدم وحليب. تم استخدام تقنية التسلسل لتحديد الطفرات في جين DGAT1 عن طريق قياس مستويات الأحماض الدهنية في حليب الأبقار. تم استخدام تقنية GC-MS لقياس مستويات الأحماض الدهنية في الحليب. أظهرت الدراسة وجود طفرة دينية، حيث كان التغيير في القاعدتين 148 و 149 في منطقة الترميز بأكملها لجين DGAT1 حيث تغيرت القاعدتان AA في منطقة الترميز بأكملها لجين DGAT1 من GU (Allele 2 في GC) إلى GA (Allele 1 في GC) أدت هذه الطفرة إلى تغيير حمض اللبنيسين (K) في الأليل 1 إلى الأليل 2 في الأليلات في الأليلات الثلاثة 2 و 3 على التوالي. تفوقت السلاسل المحلية على نسبة الأحماض الدهنية المشبعة على المضرب والهولشتاين فريزيان بينما تفوقت السلاسل المحلية غير المشبعة الأحادية وغير المشبعة تجاوز الأليل الأول معنويًا الأليلات الأخرى لجميع السلاسل في نسبة الأحماض الدهنية المشبعة. بالمقارنة مع الأليل الأول، أظهر الأليل الثاني للإفرادcrawler غير المشبعة مستويات أعلى من الأحماض الدهنية المشبعة غير المشبعة غير المشبعة. فيما يتعلق بالأحماض الدهنية غير المشبعة، فقد تفوق الأليل الثلاثة من سلالة فريزيان على الأليلات الأخرى. لذلك، يمكن الاعتماد على الأمانة الفردية لجين DGAT1 لتحديد الأليلات من سلالة فريزيان على الأليلات الأخرى. لذلك، يمكن الاعتماد على الأمانة الفردية لجين DGAT1 لتحديد الأليلات من سلالة فريزيان على الأليلات الأخرى.