PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

POLIMORFISMO DO GENE *DGAT1* E SUAS RELAÇÕES COM RENDIMENTO DO LEITE DE GADO E SUA COMPOSIÇÃO QUÍMICA

DGAT1 GENE POLYMORPHISM AND ITS RELATIONSHIPS WITH CATTLE MILK YIELD AND CHEMICAL COMPOSITION

تعدد المظاهر الوراثية لجين DGAT1 وعلاقتها بأنتاج الحليب ومكوناته الكيميائية في الماشية

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RESUMO

Vários polimorfismos em diferentes lócus gênicos afetam características de produção, como rendimento e composição do leite. O presente estudo teve como objetivo determinar a frequência alélica e genotípica do gene DGAT1 e avaliar as associações entre variantes genéticas do DGAT1 e produção de leite e sua composição química de bovinos iraquianos locais. Amostras de sangue de 100 vacas foram obtidas para isolamento do DNA. O iniciador usado neste estudo amplificou fragmentos de 411-pb no exon 8 do gene DGAT1. Métodos de sequenciamento de DNA foram aplicados para detectar polimorfismo de nucleotídeo único do gene DGAT1 em 100 vacas. As sequências nucleotídicas do exon 8 do gene DGAT1 foram registradas para bovinos iraquianos locais no Centro Nacional de Informações de Biotecnologia (NCBI), Banco de Dados de DNA do Japão (DDBJ) e Arquivo Europeu de Nucleotídeos (ENA) sob os seguintes números de acesso (LC492073 e LC492074). Os resultados mostraram a presença de dois sítios polimórficos, levando à construção de 2 haplótipos diferentes na vaca. A diversidade de haplótipos foi de 0,536, enquanto a diversidade de nucleotídeos foi de 0,0031. Foram detectados dois locais de polimorfismo de nucleotídeo único (SNP) do gene DGAT1, a saber A10433G (A / G) e A10434C (A / C). O resultado dessa mutação altera a substituição da lisina por alanina na posição 232 (mutação A232K) da sequência de aminoácidos. O software genético V. 2020.0.4 foi utilizado para detectar genótipos do gene DGAT1, pois o alinhamento da sequência mostrou a presença de três genótipos. As frequências genotípicas de KK, KA e AA foram de 0,40, 0,30 e 0,30, respectivamente. As frequências dos alelos K e A foram de 0,60 e 0,40, respectivamente. O genótipo KK foi significativamente (P <0,05) associado ao maior rendimento de gordura. Portanto, o gene DGAT1 poderia servir como um marcador genético para a seleção do rendimento de gordura em vacas.

Palavras-chave: *Gene DGAT1, gado iraquiano local, produção de leite, polimorfismo de nucleotídeo único.*

ABSTRACT

Several polymorphisms in different gene loci have been noted to affect production traits such as milk yield and milk composition. The present study aimed to determine the allelic and genotypic frequency of the DGAT1 gene and evaluate the associations between DGAT1 genetic variants and milk yield and its chemical composition of local Iraqi cattle. Blood samples from 100 cows were obtained for DNA isolation. The primer used in this study amplified 411-bp fragments at exon 8 of the DGAT1 gene. DNA sequencing methods were applied to detect single nucleotide polymorphism of the DGAT1 gene in 100 cows. The nucleotide sequences of exon 8 of the DGAT1 gene were registered for local Iraqi cattle in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ), and the European Nucleotide Archive (ENA) under the following accession numbers (LC492073 and LC492074). The results showed the presence of two polymorphic sites leading to the construction of 2 different haplotypes in the cow. Haplotype diversity was 0.536, while nucleotide diversity was 0.0031. Two single-nucleotide polymorphism (SNP) loci of the DGAT1 gene were detected, namely A10433G (A/G) and A10434C (A/C). The resulting of this mutation changes lysine to alanine substitution at position 232 (A232K mutation) of amino acid sequence. Geneious software V. 2020.0.4 was used to detect genotypes of the DGAT1

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gene, as the sequence alignment showed the presence of three genotypes. The genotypic frequencies of KK, KA, and AA were 0.40, 0.30, and 0.30, respectively. Frequencies of K and A alleles were 0.60 and 0.40, respectively. The KK genotype was significantly (P<0.05) associated with higher fat yield. Therefore, the DGAT1 gene could serve as a genetic marker for the selection of fat yield in cows.

Keywords: *DGAT1 gene, local Iraqi cattle, Milk yield, single-nucleotide polymorphism.*

الخلاصة

العديد من التشكلات الوراثية في عدة مواقع جينية تم ملاحظة تأثيراتها في الصفات الانتاجية مثل انتاج الحليب ومكوناته. تهدف الدراسة الحالية قياس التباين الوراثي وتحديد تكرار الاليلات والتراكيب الوراثية لجين DGAT1 وعلاقتها بأنتاج الحليب ومكوناته الكيميائية في الماشية المحلية العراقية. تم الحصول على عينات الدم من 100 بقرة لغرض استخلاص الحامض النووي منقوص الاوكسجين. البادئات المستخدمة في هذه الدراسة لتضخيم قطعة بطول 411 زوج قاعدي في الاكسون الثامن من جين DGAT1. استعملت تقنية تتابعات الحامض النووي منقوص الاوكسجين لغرض تحديد التشكلات الوراثية للنيوكليوتيدات المنفردة. تم تسجيل تسلسل النوكليوتيدات exon 8 من جين DGAT1 للماشية المحلية العراقية في المركز الوطني لمعلومات التكنولوجيا الحيوية (NCBI) وبنك بيانات الحامض النووي الياباني (DDBJ) وأرشيف النيوكليوتيد الأوروبي (ENA) تحت أرقام الانضمام التالية (LC492073 و LC492074). أظهرت النتائج وجود نمطين فرديين(H) نتج عنهما تشكلين وراثيين (NH) في الابقار المدروسة. كانت قيمة تنوع النمط الفردي 0.536 بينما كانت قيمة تنوع النيوكليوتيدات 0.0031. تم تحديد تشكلين وراثيين للنيوكليوتيدات المنفردة (SNP) في جين DGAT1 هما A10433G و A10434C نتج عنها تغير الحامض الاميني اللابسين الى الحامض الاميني الالنين في الموقع 232 (A232K) من تسلسل الاحماض الامينية. استخدم برنامج Geneious (V. 2020.0.4) للكشف عن التراكيب الوراثية في الجين المدروس، اذ أظهرت محاذاة التتابعات عن وجود ثلاثة تراكيب وراثية. بلغ تكرار التراكيب الوراثية KK وKA وAA 0.40 و0.30 و0.30 على التوالي اما تكرار الاليلات K وA كانت 0.60 و0.40 على التوالي. اظهر التركيب الوراثي KK فرق معنوي (P<0.05) بزيادة نسبة الدهن مقارنة بالتراكيب الوراثية الاخرى لذلك يمكن استخدام هذا الجين كواسم وراثي لعلاقته بدهن الحليب في الابقار.

الكلمات المفتاحية: *جين DGAT1، الابقار المحلية العراقية، انتاج الحليب، التشكلات الوراثية للنيوكلوتيدة المنفردة.*

1. INTRODUCTION:

Iraqi local cattle are scattered on most areas and differ in appearance from each other and are believed to be due mostly to the origins of Indian cows (Zebu) where they have similar characteristics of the species (*Bos Indicus*), which occupy the hot areas, it differs from European cattle belonging to the *Bos Taurus* (Paulson and Thompson, 2015). Among these, Jenoubi is found in the southern, more humid part of Iraq. Alshawi *et al*., (2019) showed that a significant level of genetic diversity in indigenous Iraqi cattle in line with their history and genome-wide analysis releases the genes that play an important role in immunity (parasitic, bacterial disease) and other environmental adaptive traits (heat tolerance).

The synthesis of milk components has to be increased to improve the efficiency of milk production. This may be carried out by way of combining genetic enhancements and true management, which include improving the availability of the vital nutrients that the udder uses to produce milk. After advances in molecular biology, it became clear that DNA transcription in a laboratory and identifying the form of the genome that made up it became a compelling manner to persuade the phenotypic systems of individuals. In addition to transcription, many post-translation events could significantly affect the phenotype, along with protein phosphorylation constitutes a primary one (Osorio *et al.*, 2016).

The amount of milk, milk fat, and proteins are important traits in the dairy cattle breeding. Milk production depends on the ability of the mammary gland to metabolize fat. Milk fat consists of approximately 98% triglycerides, and the acyl-CoA: diacylglycerol acyltransferase 1 (DGAT1) enzyme has an essential function in milk fat synthesis because it catalyzes the final step in the formation of triglycerides (Lu, *et al*., 2015). Evidence has pointed to the role of DGAT1 enzyme on milk yield and composition (Cole *et al*., 2011).

The Diacylglycerol Acyltransferase-1 (DGAT1) gene is one of the functional candidate genes affecting milk composition traits (Juhlin *et al.*, 2012). The cattle DGAT1 gene is located on chromosome 14 and contains 17 exons ((Lešková *et al*., 2013). The dinucleotide change (AA/GC) at positions 10433 and 10434 (rs AJ318490.1) in exon 8 leads to a non-conservative substitution of Lysine by Alanine at position 232 and has been shown to affect milk yield strongly and milk composition in Italian Holsteins (Bobbo, *et al*., 2018), White Fulani and Borgou cattle breeds (Houaga *et al*., 2017) and Holstein, Simmental and Brown Swiss cattle breeds in Croatia (Dokso *et al*., 2015). In a study, demonstrated that the lysine variant, which represents the “wild type” and is defined by K allele, is characterized by a higher velocity rate in producing triacylglycerols than the A allele (alanine variant) and thus increasing the fat content in animal milk (Grisart *et al*., 2004). In animals with KK genotype, DGAT1 activity of KK

genotype was reported to be five times higher than AK and AA individuals (Lacorte *et al*., 2006)

This study aimed to provide an overview of the association between DGAT1 polymorphisms and milk yield and its chemical composition of local Iraqi cattle.

2. MATERIALS AND METHODS:

The present study was undertaken in the Genetic Engineering Laboratory, Department of Animal Production, College of Agriculture, University of Basrah, Iraq.

2.1. Animals and genomic DNA isolation

The study included the use of 100 Iraqi local cattle. Farmers own the animals used in this study. Before sampling, the objectives of the study were explained to them in their local languages so that they could make an informed decision regarding giving consent to sample their animals. Government veterinary, animal welfare, and health regulations were observed during sampling of the populations analyzed here. The procedures involving animal sample collection also followed the recommendation of directive 2010/63/EU. Collection of blood samples was permitted by the Iraqi Ministry of Agriculture.

The blood samples (5ml/cow) from the jugular vein were collected and immediately transported to the laboratory in a cool box containing ice and stored at −20°C until further analysis. A 50 ml tubes were used to collect milk samples and sent to the physiology laboratory (College of Agriculture, University of Babylon) for the analysis of milk components by Funke Gerber, Germany.

Genomic DNA was extracted from the whole blood using gSYNC™ DNA Extraction Kit manufactured by the Taiwanese Geneaid company. The DNA concentration was determined using Nanodrop Thermo scientific spectrophotometer (260/280) and then diluted to the final working concentration of a 50 ng/µl. A fragment (411bp) of the DGAT1 gene in cattle by using the primer F: 5′-GCACCATCCTCTTCCTCAAG-3′ and R: 5′-GGAAGCGCTTTCGGATG-3′ (Kaupe *et al*., 2004). The PCR amplifications were conducted in a 50 µl volume containing 6 µl genomic DNA, 25 µl of Master Mix, 2 µl each primer, 15 µl free water. The amplification conditions included one cycle of denaturation at 94° C for 2 min and 35 cycles for 30 min, 30 sec of annealing at 59°C, and extension at 72°C for 45 sec, as well as the final extension at 72°C for 10 min. The PCR results were extracted using apparatus at 2% agarose gel with the visualized by contact with ultraviolet light. The PCR product was sequenced by Yang ling Tianrun aoka biotechnology company.

2.2. Data analysis

The sequencing results of the DGAT1 gene were compared with accession No. MF351623 at the NCBI by BioEdit 7.0 software (Hall, 1999). Haplotype diversity (HD) and nucleotide diversity (π) were analyzed using DnaSP V5. 10 software (Librado and Rozas, 2009). The Geneious prime (version 2020.0.4) program was used to detect genotypes.

2.3. Statistical analysis

The Completely Randomized Design (CRD) was used to analyze the production data studied within the SPSS (2016) Statistical program Version 24. Least Significant Test within the program was performed to compare different means.

3. RESULTS AND DISCUSSION:

The nucleotide sequences of exon 8 of the DGAT1 gene were registered for Iraqi local cattle in the National Center for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ), and the European Nucleotide Archive (ENA) under the following accession numbers (LC492073 and LC492074).

3.1. Genetic Diversity

The results of the genetic diversity of the DGAT1 gene showed that their total number of sequences (N) was 100, and the number of haplotypes (H) was 2 haplotypes resulting in 2 genetic polymorphisms (NH). The values of haplotype diversity (HD) and nucleotide diversity (π) were 0.536 and 0.0031, respectively (Table 1).

The results in Figure 1 and Table 2 showed the analysis of nucleotides and protein of exon 8. They recorded two SNPs; adenine (A) to guanine (G) and adenine (A) to cytosine (C) in position 10433 and 10434, respectively. Thus, the amino acids changed to A232K.

The sequencing analysis of the *DGAT1* gene at exon 8 revealed three genotypes namely, KK, KA and AA in this fragment (Figure 2). The genotypic frequencies are shown in Table 3 (0.40, 0.30 and 0.30 respectively). Two different alleles K and A were identified. All alleles were present in

the studied animals but at different frequencies (0.60 and 0.40, respectively).

In this study, the higher frequency of the K allele (lysine variant, 0.60) at the DGAT1 locus was in line with reported frequencies in Kenya (Houaga *et al*., 2017) alleles were 0.91 and 0.77 in White Fulani and Borgou breeds respectively, Croatia (Dokso *et al*., 2015), New Zealand *(Spelman et al*., 2002) and Greece (Oikonomou *et al*., 2009), with values of 0.77, 0.60 and 0.62, respectively. But slightly different from other regions in German (Kaupe *et al*., 2007), UK (Banos *et al*., 2008), Polish (Nowacka-Woszuk *et al.*, 2008) and France (Vanbergue *et al*., 2016) Holsteins showed very similarly K allele frequency, ranging from 0.53 to 0.55. The differences in DGAT1 gene allele frequencies in different countries and regions may be due to differences in inbreeding and selection programs.

3.2. DGAT1 Polymorphisms and Milk Chemical Content

The present study supports the hypothesis that genotypes located within the DGAT1 gene may be associated with milk production traits in dairy cattle. The results were consistent with work in dairy cattle showing that the allele responsible for lysine (K) is usually associated with increased milk fat (Table 4). As in the following studies in Denmark (Bovenhuis *et al*., 2015), Croatia (Dokso *et al*., 2015) and Kenya (Houaga *et al*., 2017). However, the effect of diallelic DGAT1 on milk production traits also can be partly explained by the presence of multiple alleles in the locus of DGAT1 or other mutations in genes closely related (Kühn *et al*., 2004). The K allele is associated with a high-fat yield in milk (Argov-Argaman *et al*., 2013), the A allele is associated with high milk yield (Marchitelli *et al*., 2013). Previous studies on *Bos Taurus* and *Bos indicus* had determined the K allele index as a wild type and inferred that the replacement of A allele had occurred after the separation of the *Bos Taurus* and *Bos indicus* lineages (Kaupe *et al*., 2007). It was well documented that DGAT1 encoded an enzyme play a major role in the synthesis of triglycerides (Ali, 2015). Triglycerides are the major components of fat are formed by binding of diacylglycerol to long-chain fatty acyl-CoAs. These reactions controlled by some enzymes, one of them encoded by the DGAT1 gene (Klimov, *et al*., 2018). After these findings, DGAT1 was recommended as a functional candidate gene for milk production traits (Houaga *et al*., 2018).

4. CONCLUSIONS:

A significant association between the KK genotype of the DGAT1 gene with higher fat yield in Iraqi local cattle was detected. These results implied that the DGAT1 gene could be great candidate genes or linked to significant genes that affect milk production traits in cattle.

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Table 1. *Genetic Diversity of Iraqi Local Cattle Breed in the sample study*

Gene	Number of Sequences (N)	Haplotype Number (H)	Number of Polymorphisms (NH)	Haplotype Diversity (HD)	Nucleotide Diversity (π)
DGAT1	100	*2*	*2*	0.536	0.0031

Table 2. *Type of amino acid change in the DGAT1 gene in Iraqi local cattle.*

Location of mutation	Nucleotide change	Amino acid change	Type of mutation
232	AAG>GCG	Ala > Lys	Transition

Ala: Alanine, Lys: Lysine

Table 3. *Genotype and Allele frequency of the DGAT1 gene in the sample study.*

Breed	Number of animals	Genotype	Frequency	Allele	frequency	H.W.E (χ2-value)
Iraqi local cattle	40	KK	0.40	K	0.6	0.02
	30	KA	0.30	A	0.4	
	30	AA	0.30			

Table 4. *Association of DGAT1 K232A polymorphisms on milk composition in Iraqi local cattle.*

Genotype	Fat (%)	Protein (%)	Lactose (%)	SNF (%)
KK	4.35*	3.00	4.31	7.68
KA	3.59	3.25	4.37	7.81
AA	3.50	3.08	3.89	7.96
Total	3.86	3.10	4.20	7.80

*Significant at (P>0.05)

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MF351623  10274  ACCTCTGGTGCCGAGAGCGCAGGGCTGGGGCCAAGGCCAAGGCTGGTGAGGGCTGCCTCG  10333
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LC492074  1      ACCTCTGGTGCCGAGAGCGCAGGGCTGGGGCCAAGGCCAAGGCTGGTGAGGGCTGCCTCG  60

MF351623  10334  GGCTGGGGCCACTGGGCTGCCACTTGCCTCGGGACCGGCAGGGGCTCGGCTCACCCCCGA  10393
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LC492074  61     GGCTGGGGCCACTGGGCTGCCACTTGCCTCGGGACCGGCAGGGGCTCGGCTCACCCCCGA  120

MF351623  10394  CCCGCCCCCTGCCGCTTGCTCGTAGCTTTGGCAGGTAAGAAGGCCAACGGGGGAGCTGCC  10453
                 ||||||||||||||||||||||||||||||||||||||||||  ||||||||||||||||||||
LC492074  121    CCCGCCCCCTGCCGCTTGCTCGTAGCTTTGGCAGGTAAGGCGGCCAACGGGGGAGCTGCC  180

MF351623  10454  CAGCGCACCGTGAGCTACCCCGACAACCTGACCTACCGCGGTGAGGATCCTGCCGGGGGC  10513
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LC492074  181    CAGCGCACCGTGAGCTACCCCGACAACCTGACCTACCGCGGTGAGGATCCTGCCGGGGGC  240

MF351623  10514  TGGGGGGACTGCCCGGCGGCCTGGCCTGCTAGCCCCGCCCTCCCTTCCAGATCTCTACTA  10573
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LC492074  241    TGGGGGGACTGCCCGGCGGCCTGGCCTGCTAGCCCCGCCCTCCCTTCCAGATCTCTACTA  300

MF351623  10574  CTTCCTCTTCGCCCCCACCCTGTGCTACGAGCTCAACTTCCCCCGCTCCCCCCGCATCC  10632
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
LC492074  301    CTTCCTCTTCGCCCCCACCCTGTGCTACGAGCTCAACTTCCCCCGCTCCCCCCGCATCC  359
```

Figure 1. *Sequencing of DGAT1 gene in Iraqi Local Cattle in gene bank (LC492074) vs. reference Sequencing (MF351623).*

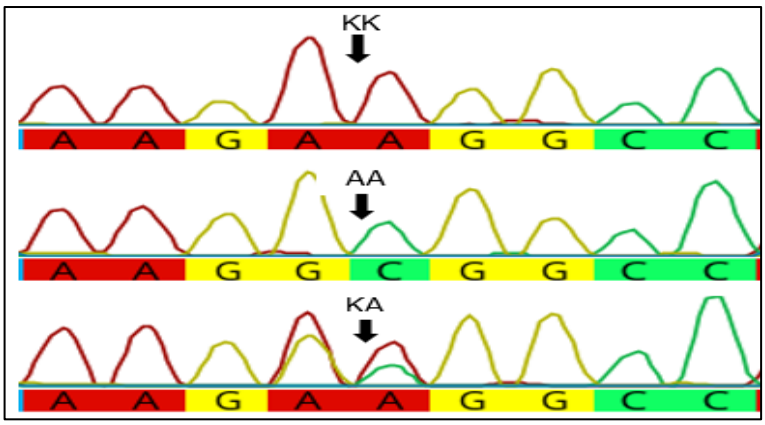

Figure 2. *The sequencing of genotypes namely, KK, KA and AA in exon 8 of the DGAT1 gene of Iraqi local cattle*