POLYMORPHIC OF DGAT1 GENE (INTRON 1) AND THEIR RELATIONSHIP TO PERFORMANCE IN IRAQI BUFFALO

Ali Fadhel kadhim Wafaa ismail ibrahim
Animal production Department- college of Agricultural Engineering Sciences- Baghdad University

ABSTRACT

This study was conducted in two locations (al-Fadelian and Althahab al'abaith) in Baghdad governorate on a sample of 60 Iraqi buffalo females (*Bubalus bubalis*) for the period from 1/11/2017 to 1/11/2018. This study was conducted to: identifying the genotypes, the proportions of their distribution of fragment (Intron1-1146 bp) of DGAT1 gene and the relationship of those genotypes to performance of the Buffalo were the main goals of this study.The genotypes of the target non-coding area of the specific Fragment (Intron1-1146 bp) differed from DGAT1 gene depending on the different genetic packets resulting from enzymatic digestion, which showed three genotypes represented by CC, CT and TT and their distribution ratios were 60.00, 33.33 and 6.67% Respectively, the discrepancy between these three ratios was highly significant ($P < 0.01$),while the frequency of allele was 0.77 and 0.23 for both allele's C and T respectively. The results showed that the production of daily milk was not significantly affected by the different in the genotypes of this Fragment of DGAT1 gene, while the age of the first birth was significant affected ($P < 0.05$) by the variation of the genotypes for the studied fragment by DGAT1 gene of buffalo with genotype CT and CC in comparison with its peers that had the genotype TT. The fat ratio had been significantly influenced where it had been at maximum in buffalo milk that had the genotype TT (8.00 ± 0.52 %) and CT (7.43 ± 0.71 %), while other milk component ratio had not been significantly affected by the genotype variation . The body dimensions also had not been significantly affected at mother except chest circumference ($P<0.05$), the highest production was in buffalo with TT genotype (213.00± 0.00cm).

Keywords: *Bubalus bubalis*, DGAT1 gene, (Intron1-1146 bp), Sequencing.

المظاهر الوراثية للقطعة(intron1)من جينDGAT1 وعلاقتها بالاداء في الجاموس العراقي

الخلاصة

أجريت هذه الدراسة في موقعين (الفضيلية و الذهب الابيض) في محافظة بغداد على عينة مكونة من 60 من اناث الجاموس العراقي *(Bubalus bubalis)*، فضلا عن مختبر التقدم العلمي للتقانات الاحيائية وتحاليل الوراثة الجزيئية للمدة من 2017/11/1 حتى 2018/11/1، بهدف فصل المادة الوراثية وتحديد التراكيب الوراثية (Genotype) ونسب توزيعها للقطعة (Intron1-1146bp – Sequencing) من جين DGAT1 وعلاقة تلك التراكيب بالاداء الانتاجي والتناسلي، وفي ما يأتي أهم النتائج المتحصل عليها. تباينت نسب توزيع التراكيب الوراثية CC و CT و TT للقطعة (Intron1-1146bp - Sequencing)من جين DGAT1 معنويا ($P<0.01$) في العينة المدروسة، إذ بلغت نسبها 60.00 و 33.33 و 6.67 % بالتتابع، وبتكرار أليلي بلغ 0.77 و 0.23 لكل من الأليلين C و T على التوالي. أظهرت نتائج الدراسة الحالية أن انتاج الحليب اليومي لم يتأثر معنويا باختلاف التراكيب الوراثية لهذه القطعة من جين DGAT1 . اتضح ان تأثير التراكيب الوراثية لهذه القطعة من جين DGAT1 في العمر عند اول ولادة كان معنويا ($P<0.05$) وبتفوق للأمهات ذات التركيب CT و CC موازنة بمثيلاتها ذات التركيب TT . تأثرت نسبة الدهن معنويا باختلاف التراكيب الوراثية لهذه القطعة من جين DGAT1 ،اذ بلغت النسبة أقصاها في حليب الجاموس ذات التركيب الوراثي TT (8.00 ± 0.52%) و CT (7.43 ± 0.71%), بينما لم تتأثر معنويا نسب باقي مكونات الحليب الاخرى باختلاف التراكيب الوراثية. كما لم تتأثر أبعاد الجسم التي تمت دراستها لدى الأمهات باستثناء محيط الصدر($P<0.05$) ولصالح الافراد ذات التركيب الوراثي TT (213.00± 0.00 سم) باختلاف التراكيب الوراثية لهذه القطعة من جين DGAT1 .

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1- INTRODUCTION

The Iraqi buffalo belongs to the bovine family (Bovidae) bubalis genus, which spreads from northern Iraq to its south near marshes, river and streams. The great adaptability in severe environmental conditions and the high biological efficiency in the use of aquatic plants such as reed, papyrus and fodder that available in its areas of existence, for these reasons, it is protected from extinction risk (1). Generally, the traditional genetic improvement of farm animals, including buffalo based on the statistical methods and the individual selection of best appearance composition resulted in a significant gains in genetic improvement. However the tremendous development in scientific research and the huge knowledge of genome work lead to develop more accurate, less time-and cost selection programs. The biological development , molecular genetics, the discovery of genetic maps has led to the identification of ways and programs to improving animal performance (15).The latest discovery of the polymerase change reaction PCR did a qualitative leap that changes the way of thinking in the biological sciences, which its influenced to several sub-disciplines in biology (8). Scientists have invested this technology to study and trace genetic mutations that occur on genetic material in organisms such as Single nucleotide polymorphism SNP and used it as a genetic markers. These markers used for selecting the production traits with a low genetic equivalent, which are controlled by a number of genetic sites that known as Quantitative Traits loci QTL (14). Among these markers that can be used in the selection process and genetic improvement are diacylglycerol acyltransferase gene, which known as DGAT1 gene that detected individually in 2002 to had refer studies performed on this gene in Iraq. This gene is related to milk production and the percentage of milk fat(6). Since the marker assisted selection being an effective tool in improving the production performance of the buffalo and the lack of studies at the local level on this aspect, this study was carried out in order to identify the polymorphism of the specific fragment(Intron1-1146bp) of DGAT1 gene and its relationship to milk production and composition, age at the first birth and the body dimensions of mothers in the buffalo samples.

2- Materials and Methods

This study was carried out in two locations (al-Fadelia and Athahab al abaith) in Baghdad Governorate on a sample of 60 buffalos for the period from 1/11/2017 to 1/11/ 2018. Daily milk production for mothers was recorded for the production season (2017 – 2018), The daily milk production was calculated every two weeks after birth for 6 times during (three month) for each Buffalo. As well as , sample of milk produced was taken from milking female at morning from the milking pot directly and twice (for the first and second months after birth) for each female buffalo, these samples were analyzed at the research and development unit at ABI Gharib dairy factories and using electrical device called ultrasonic milk analyzer (Master LM2) in order to estimate milk composition. Notes were recorded on buffaloes health, physiological and reproductive condition by veterinarians with the help of Breeders. Furthermore, body dimension was recorded and blood samples were taken. Whereas genetic analyses (laboratory part) of blood samples were performed in the laboratory for scientific progress of molecular genetics and biotechnologies in Al-Harithiy, Baghdad governorate, with the aim of isolating the genetic material and identifying the genotypes of the specific fragment (Intron1-1146bp) of DGAT1gene by the use of (sequencing) and its relationship to performance of the Buffalo, as well as the study of the distribution ratios of their genotypes in the herd and alleles frequency of the obtained.

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Statistical analysis system (11) was used to study the effect of genotypes for the specific fragment(Intron1- 1146bp) of the DGAT1 gene in the studied traits. The significant differences between the means were compared using Duncan test (1955) by applying least square means. The factors and traits considered above have been analyzed according to the following linear model:

$$Yijk = \mu + Gi + eijk$$

Where:-

Y_{ijk}: The K-View value of genotypes i and birth sequence j.

μ: General means of the trait.

G_i: Effect of genotypes of the specific fragment (Intron1- 1146bp) of DGAT1 gene (CC,CT and TT).

e_{ijk}: Random error that is distributed naturally with an means equal to zero and a variation of $\sigma^2 e$.

2-1: Choosing the primer of the studied gene fragment

The primers were selected as listed in Table 1 in order to perform the molecular detection and identify the phenotypic diversity of the specific fragment resulting from the existence of the SNP for DGAT1 gene (12) and (5).

Table 1: The primers sequence that used in the study

Gene name	Amplification area	Sequence
DGAT1 Genbank:102390126	specific fragment (Intron1 -1146bp) sequencing	Forward = 5´- TGGATTTGGGGTCACTTT -3´
		Reverse =5´- GTCCCTCTACCAGCCTTCC -3´

3- Result and Discussion

3-1: DNA isolating and DGAT1 gene extraction

Figures 1 and 2 represents PCR amplified for the specific fragment (Intron1-1146bp) of the DGAT1 gene within sequencing technology. The precise output was photographed to Confirm the success of the extraction process and to obtain the required fragment (Intron 1),which was in the size of 1146 base pairs(bp), the results of the polymerase reaction were then both ends(forward and Revers) in volume 25 microliter to company Macrogen Corporation-Korea, after obtaining the results have been used a program genious software on the World Gene Bank website www. Ncbi.nih.go and informatics program BioEdit, the nucleotide sequence file was used to determine the presence or absence of the mutation and the curve file to determine the phenotypic diversity of the DGAT1 gene.

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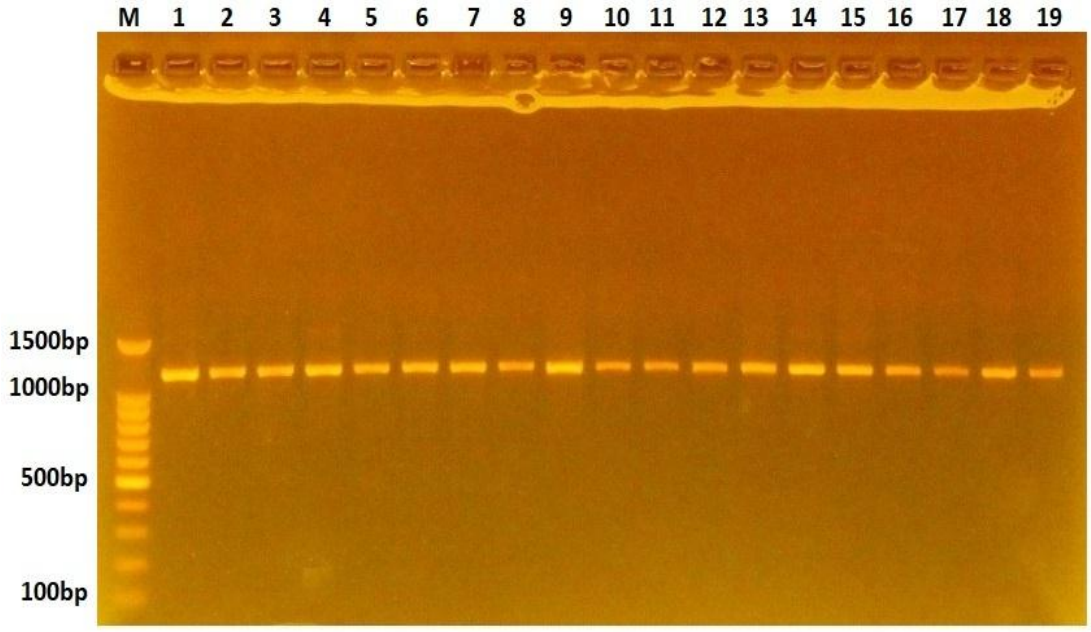

Figure 1: The specific PCR fragment (Intron1-1146bp) of DGAT1 gene using agarose gel electurphaesis (1.5%).
M : refers to ladder maker and lanes 1-19 refer to amplified samples.

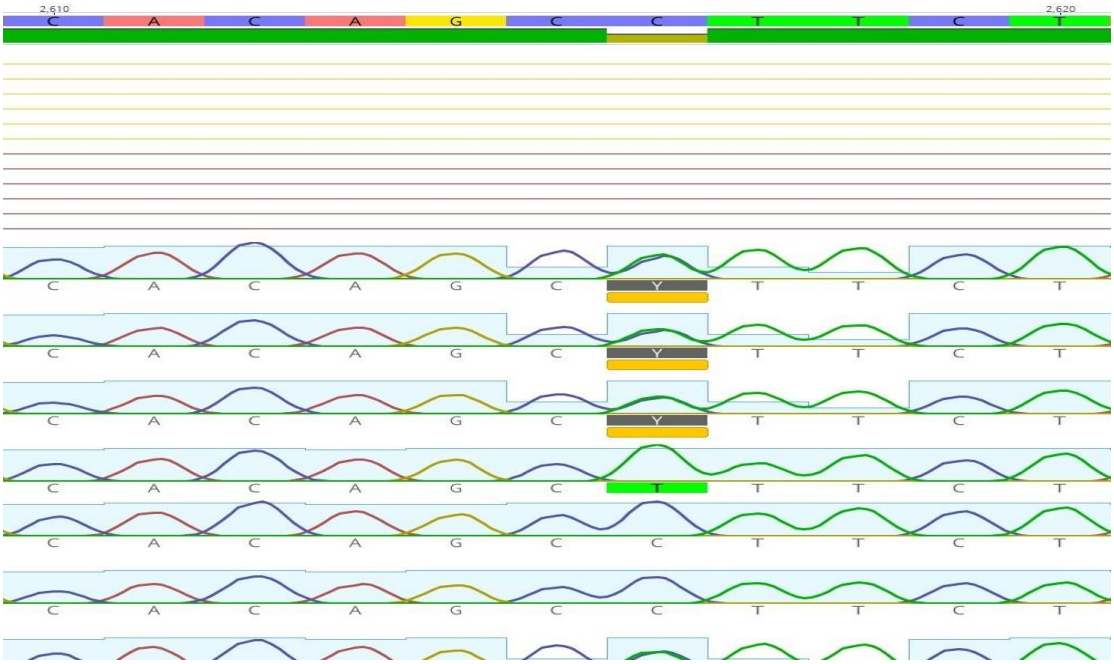

Fig. 2: The products of sequencing technology for fragment (Intron1-1146bp) of DGAT1 gene

The genotype of CC =Wild
The genotype of CT =heterozygous
The genotype of TT =mutant

3-2: The Percentage of genotypes distribution and the allele's frequency for the specific fragment of Intron1-1146 bp for DGAT1 gene.

Table 2 shows the number of genotypes, their percentages and the allele's frequency of the studied specific fragment (Intron1-1146 bp) for DGAT1 gene. There were three genetic genotypes of this

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fragment CC, CT and TT, while numbers that carried these three genotypes were 36, 20 and 4 and by a percentage of 60. 00 ,33.33 and 6. 67 % respectively, that mean of the allele's frequency C and T was 0.77 and 0.23 respectively. The findings show that there was a high significant difference ($P < 0.01$) between the distribution ratios of the sample's genotypes. The appearance of CC genotype with a higher percentage of the TT genotype in this study was similar to what (9) and (10), they pointed out the prevalence of allele's C and by up to 0.89 and 100% respectively at They study on Indian Buffalo.

Table 2: Number and percentages of genotypes and the allele's frequency of the fragment (Intron1-1146bp) for DGAT1 gene in the sample of Buffalo studied

Genotype	Number	Percentage (%)
CC	36	60.00
CT	20	33.33
TT	4	6.67
Total	60	% 100
Value ($\chi 2$)	---	** 20.40
Allele's frequency		
C	0.77	
T	0.23	
.(P<0.01) **		

3-3: Relationship of genotypes of the specific fragment (Intron1-1146 bp) of DGAT1 gene to the production of daily milk for Buffalo

The results showed that there was not significant effect for the observed genotypes of DGAT1 fragment on the daily milk production as shown in Table 3. This result was similar with (10) in relation to the carrying individuals genotypes CC which amounted to 6.15 kg, when his study on Indian Murrah Buffalo, while the result was different with respect to genotypes CT and TT which amounted to each 4.31 and 7.34 kg respectively, there were also significant differences ($P < 0.05$) between them.

Table 3. Relationship of genotypes for the specific fragment (Intron1-1146bp) of DGAT1 gene on the daily milk production for Buffalo

Genotype	Number of buffalo (Number of samples)	Average ± standard error Daily milk Production (kg)
CC	36 (216 samples)	6.02 ± 0.50 a
CT	20 (120 samples)	6.40 ± 0.70 a
TT	4 (24 samples)	4.00 ± 1.00 a
Significant level	Total number 60	NS
Averages with similar characters within a single column are no Significant different. Non Significant different.		

3-4: Relationship of genotypes of the specific fragment (Intron1- 1146bp) of DGAT1 gene with Buffalo milk composition

The results showed that there was a significant effect ($P < 0.05$) for the different genotypes to the specific fragment (Intron1- 1146bp) of DGAT1 gene with regard to milk fat percentage. As individuals that carry the mutant TT genotype have the superiority on individuals that carry the heterozygous

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genotype of CT , whereas it was in below individuals that carry the wild genotype of CC and the amount of milk produced was 8.00 ± 0.52 , 7.43 ± 0.71 and 5.33 ± 0.62 % respectively, while the differences were not significant in terms of percentages of each protein, lactose, non-faty solids and ash (Table 4). This result presented a good agreement with that obtained by (9) and (10) for more than individuals that carry the mutant TT genotype for the percentage fat in milk when studying on Murrah Buffalo which of the values of TT of both 7.39 and 7.32 % respectively. Note that the percentage fat in milk is one of the most important characteristics structure milk which determines the quality of milk buffalo its price and type of product which makes it so that the adoption of genotype of this fragment of the gene in improving this attribute seems feasible through the results of this study, as there are inverse relationship between the amount of milk producers.

Table 4. Relationship of genotypes of the specific fragment (Intron1-1146bp) of DGAT1 gene to the milk competition of Buffalo

Genotype	**Number of Buffalo (Number of samples)**	**Mean ± standard error**				
		Protein (%)	**Fat (%)**	**Lactose (%)**	**Non-fatty solids (%)**	**Ash (%)**
CC	36 (72 samples)	3.96 ± 0.26 a	5.33 ± 0.62 b	4.45 ± 0.19 a	9.18 ± 0.50 a	0.36 ± 0.14 a
CT	20 (40 samples)	4.02 ± 0.17 a	7.43 ± 0.71 a	4.38 ± 0.23 a	9.95 ± 0.66 a	0.50 ± 0.04 a
TT	4 (8 samples)	3.88 ± 0.09 a	8.00 ± 0.52 a	4.46 ± 0.16 a	9.02 ± 0.62 a	0.52 ± 0.07 a
Significant level	Total number 60	NS	*	NS	NS	NS

Averages with different characters within a single column are Significant different.
NS: Non- Significant ,(P<0.05) *

3-5: Relationship of genotypes of the specific fragment (Intron1-1146bp) of DGAT1 gene in age at the first birth of female buffalo

The results showed that there was a significant effect (P < 0.05) for the different genotypes to the specific fragment (Intron1-1146bp) of DGAT1 gene on age at first birth. As individuals that carry the heterozygous genotype CT have the superiority on individuals that carry the wild genotype of CC , whereas it was in below individuals that carry the mutant genotype of TT and their values of was 2.37 ± 0.03 , 2.39 ± 0.02 and 2.55 ± 0.05 year respectively, as shown in Table 5. This result was less than from what(13) and (3) reached, when they studied the two Buffalo breeds the Pakistani Nili-ravi and the Indian Murrah breed, as this result was less than from what (7) reached and both of the three genotypes where the result was 2.61 years when studying on the Iraqi Buffalo in al-Fadelian.

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Table 5. Relationship of genotypes of the specific fragment (Intron1-1146 bp) of DGAT1 Gene in age at the first birth of female buffalo

Genotype	Number of Buffalo	Mean ± standard error
		Age at first birth(year)
CC	36	2.39 ± 0.02 ab
CT	20	2.37 ± 0.03 b
TT	4	2.55 ± 0.05 a
Significant level	Total Number . 60	*
Averages with different characters within a single column are Significant different. (P<0.05) *		

3-6: Relationship of genotypes of the specific fragment (Intron1-1146 bp) of DGAT1 gene to the mother's body dimensions

The results showed that there was a significant effect (P < 0.05) for the different genotypes of mothers Buffalo for this fragment of the gene in the circumference of the chest for the individuals that carry the mutant genotypes TT compared to the individuals that carry the genotypes CT and CC, which were their rates) (213.00 ±0.00,(200.60 ±6.76) and (198.86 ±3.02)cm respectively, while were not any significant effect between the different genotypes for the rest of attributes that are abdominal circumference and the length of the body and height at the front and height at the rear(table 6). A number of researchers reported that the body's shape and dimensions were different breed, age and sex of the buffalo, and that the body's dimensions in the buffalo are the most important evidence that reflects the consistency of the body that can be exploited in breeding and selection programs (2).

Table 6. Relationship of genotypes of the specific fragment (Intron1-1146bp) of DGAT1 gene to the mother's body dimensions

Genotype	Number of Buffalo	Mean ± standard error				
		Chest circumference (cm)	Abdominal circumference (cm)	Body length (cm)	Height at the front (cm)	Height at the rear (cm)
CC	36	198.86 ± 3.02 b	244.86 ± 2.46 a	153.46 ± 1.78 a	138.73 ± 1.60 a	135.60 ± 1.79 a
CT	20	200.60 ± 6.76 ab	250.40 ± 4.57 a	155.80 ± 3.56 a	138.00 ± 2.54 a	134.60 ± 2.31 a
TT	4	213.00 ± 0.00 a	250.00 ± 0.00 a	163.00 ± 0.00 a	140.00 ± 0.00 a	135.00 ± 0.00 a
Significant level	Total number 60	*	NS	NS	NS	NS
Mean with different characters within a single column are significant different. NS: Non- Significant ,(P<0.05) *						

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4- Conclusion

1- which showed three genotypes represented by CC, CT and TT and their distribution ratios were 60.00, 33.33 and 6.67% Respectively, the discrepancy between these three ratios was highly significant ($P < 0.01$),while the frequency of allele was 0.77 and 0.23 for both allele's C and T respectively.

2- The results showed that the production of daily milk was not significantly affected by the different in the genotypes of this Fragment of DGAT1 gene,

3- while the age of the first birth was significant affected ($P < 0.05$) by the variation of the genotypes for the studied fragment by DGAT1 gene of buffalo with genotype CT and CC in comparison with its peers that had the genotype TT.

4- The fat ratio had been significantly influenced where it had been at maximum in buffalo milk that had the genotype TT (8.00 ± 0.52 %) and CT (7.43 ± 0.71 %), while other milk component ratio had not been significantly affected by the genotype variation .

5- The body dimensions also had not been significantly affected at mother except chest circumference ($P<0.05$), the highest production was in buffalo with TT genotype (213.00± 0.00cm).

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