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ESTIMATION OF GENETIC VARIANCE USING DATA OF DGAT1 GENE IN HOLSTEIN CATTLE

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ABSTRACT

This study was carried out in Iraq at the Taj Al-Nahrain Animal Production located in Diwaniyah province, one of the governorates of the Euphrates Middle, located south of the Iraqi capital Baghdad, about 180 kilometers away. This study analyzed the single nucleotide polymorphism (SNP) of 50 Holstein Friesian (HF). It was aimed to estimate the breeding value, additive, dominant variance and allele substitution of DGAT1 gene and their effect on milk production traits. The analysis revealed a heterogeneity site in the DGAT1 gene within the Exon8 region, in two locations. The first location in (7807) and carrying the symbol rs109234250 G/A changes the nitrogenous base from G to A, including three Genotype GG (Wild) GA (Heterozygous) and AA(Recessive) using (SNP) technique. The second location of heterogeneity (C7808A) carrying the symbol rs109326954 C/A changes the nitrogenous base from C to A, including three Genotype too, CC (Wild), CA (Heterozygous) and AA(Recessive) changes the nitrogenous base from C to A .The results showed no significant differences between the genotype in the heterogeneity site rs109234250 G/ A of DGAT1 gene for total milk yield trait (TMY) within breeding values for genotype (GG,GA and AA) were (72.4, -48.26, -168.92) respectively, dominance deviation (42.60, 99.40, 231.95) respectively and Additive variance (6114.71), Dominance variance (9881.47) and Genetic variance (15996.18), while recorded lactation milk yield trait (LMY) significant differences between of these genotypes GG , GA and AA within breeding values (16.08, -10.72, -37.52) respectively, dominance deviation (3.36, 7.83, 18.29) respectively, Additive variance (48.27), Dominance variance, (16.42) and Genetic variance (64.70). While the results showed in second the heterogeneity (C7808A) of DGAT1 gene for protein 2% and lactose 2% significant differences between the genotype within breeding values of these genotypes CC, CA and AA for protein 2% were (- 0.08, 0.06 and 0.20) respectively and dominance deviation (0.04, 0.09, 0.22) respectively and Additive variance (7.99) Dominance variance 8.54, and Genetic variance (16.3) also results showed significant differences between the genotype for lactose 2% percentage within breeding values of these genotypes CC, CA and AA (**-0.08, 0.06, 0.20**) respectively and dominance deviation (0.08, 0.18, 0.41) respectively and the Additive variance **(8.23)** Dominance variance **(0.03)** and Genetic variance (8.26).

Keywords: Breeding value, Dominance variance, alleles substitute, DGAT1

Introduction

Increased milk production has emerged as one of the main dairy breeding goals (Meredith *et al*., 2012) throughout the world. The genetic improvement in milk performance traits of native breeds could increase their potential values and improve production levels. The effective genetic improvement requires genetic information about the genetic variability and their effects on milk production. The potential value of indigenous livestock breeds for milk production traits must be analyzed and conserved to maximize the milk production so that they can become a self-sustainable resource. Livestock selection for improved production of milk has also influenced the evolution of animal breeds (Beja-Pereira *et al*., 2003) on global basis. Genetic improvement using selection by genetic markers and genome information is primarily based on the additive effect, which plays an important role in influencing the physiology and evolution of farm animals (Cloete *et al*, 2004). As that, the dominant effect has added value to the additive variation in the studied character variation, so many studies have begun to shed Highlight this effect (of the dominant variation) role in the total variation of complex productivity traits (Devendra and Owen, 1983; Eila *et al.*, 2005; Falconer, 1992). In order to understand the genetic content and genetic characteristics of the population/herd, a genetic analysis of the variation components, particularly the additive (VA), which represents the breeding value and dominance deviation (VD) and Interaction Deviation (VI) must be done (Falconer, 1996). Although the study of genetic maps has largely focused on additive genetic variation, many studies have stressed the importance of non-negligible contribution of non-additive effects in QTL (Hermiz, 2005). The different aspects of the different genotypes are mainly due to the pure or hybrid image of the alleles, in another meaning, the amount of the contribution of each allele negatively or positively in the phenotype of the character and the genetic selection in general, but the redistribution of the proportions of these alleles among individuals in order to increase replication at the level of the individual or herd, therefore, its consider an attempt to replace the unwanted allele with another one in the same locus has the most effect, known as the effect of substitution of alleles. The candidate gene, Diacylglycerol aminotransferase (*DGAT1*) located on the centromeric end of the bovine chromosome 14 in the QTL region (Farnir *et al*., 2002) is considered to be directly responsible for 50% variation in milk fat content and milk yield in dairy cattle (Banos *et al*., 2008) and has strong functional and positional role in milk traits (Furbass *et al*., 2006). The knockout trial

for *DGAT* gene has shown reduced or inhibited milk secretion in mouse lines (Smith *et al.,* 2000). A di-nucleotide polymorphism (GC/AA) in exon eight of *DGAT1* gene harbors a non-conservative lysine to alanine substitution (*K232A*) with profound effect on milk fat and milk yield (Winter *et al*., 2002) in different bovine breeds (Sorensen *et al.*, 2006). The polymorphic status of *DGAT1* gene and their association with milk yield and milk fat has been amply reported in exotic cattle, however, there have been scant studies in Holstein cattle. The identification of specific pattern of allele and genotypic frequencies in indigenous cattle breeds and their association with lactation performance may result in detection of causal factors responsible for variation in performance for milk production. Detects single nucleotide polymorphisms (SNPs) located at restriction site, easy to conduct and less cumbersome. The aim of this research is to estimate the breeding values, calculate the total genetic variance and determine the additive and dominant variability as well as the effect of the effect of substitution of alleles and the average effect of DGAT1gene in Holstein cattle and their association with milk performance traits.

Material and Methods

Experimental animals

The study was carried out in southern Iraq in the Taj Al-Nahrain tributaries belonging to Taj Al-Nahrain, located within the administrative boundaries of Diwaniyah Governorate for the period from 1/6/2019 to 1/3/2020, on a sample of 50 cows from the Holstein-Friesian cattle imported from Germany. The birth sequences were heterogeneous.

Laboratory side

Laboratory side: While genetic tests (laboratory part) of blood samples were carried out in order to separate the genetic material and determine the genetic structure of the animals Genotype, For gen DGAT1 .And the relationship of this to the performance of production and physiological, as well as to study the proportions of the distribution of structures in the herd and repeat the resulting alleles.

DNA extraction

DNA was extracted from the blood samples of the cows for the purpose of conducting the molecular examination of the studied gene (DGAT1) in the scientific progress laboratory located in Baghdad/ Harthiya for the period from 1/11/2019 to 1/2/2020 in order to separate the genetic material and determine the genetics of the Diacylglycerol Acyl transferase gene (DGAT1).

Molecular characterization and selection of the initiator of the studied gene

The Diacylglycerol Acyl Transferase gene (DGAT1) determined Exon8. The studied gene was made up of 411 Pb and analyzed by analyzing the polymorphism of the single nucleotide SNP in the Exon8 region of the DGAT1 gene located on chromosome 14. The first to discover the polymorphisms of single-nucleotide SNP (Winter *et al*., 2002).

Table 1 : Primers sequence used in the study.

Primer sequence	Primer Name	Amplicon size (pb)	Name gene	Annealing temp C°
GCACCATCCTCTTCCTCAAG F-	Exon8	411 pb	DGAT1	52
GGAAGCGCTTTCGGATG R-	Exon8			
Based on gene sequences available in the gene bank database Gene bank) Sequence number				

Statistical analysis

Data were analyzed statistically using a program SAS-Statistical Analysis System to study the effect of a gene structure Diacylglycerol Acyl Trans fereace using the mathematical model:

First Mathematical Model:

$$Yijklm = \mu + G1i + + eijklm$$

Yijklm: The value of viewing.

μ: Average.

G1i: Effect of multiple genetic manifestations of Gen DGAT1 G/A rs109234250 (GG, GA, AA)

Eijklm: Random error, which is naturally distributed with an average of zero and a variation of its magnitude σ2e.

Second Mathematical Model

$$Yijklm\ = \mu + G2i + + eijklm$$

Yijklm: The value of viewing.

μ: Average

G2i: Effect of multiple genetic manifestations of gen DGAT1 C/A rs109326954 (CC, CA, AA).

Eijklm: Random error, which is naturally distributed with an average of zero and a variation of its magnitude σ2e.

I also used the Kay square test (Chi-square- χ2) to compare the percentages of the distribution of genotypes for each mutation in the studied cattle sample. The following law was also applied to calculate night repetition according to the Hardy Weinberg's equilibrium rule.

$$PA = \frac{2\times \text{No. of Homozygous} + 1\times \text{No. of Heterozygous}}{2\times \text{Total number of sample}}$$

The equations for each calculated value were applied as follows (Falconer and Mackay, 1996):

1- The average effect of allele G = q [a+d (q – p) = αG
2- The effect of substitution of alleles = αG- αA
3- The breeding values : GG= 2 α1, GA = 2pqd, AA = 2 α2
4- The dominant deviations : GG = $2q^2$ d, GA = 2pqd, AA = $2p^2$ d
5- The average effect of allele A =- q [a+d (q – p) = αA
6- The different variances : VA = 2pq α^2 , VD = 4 $p^2q^2d^2$, VG = VA + VD

Results and Discussion

DNA was extracted and its concentration was between 50 to 75ng/ µl as the first step to extract the DGAT1 bovine gene within PCR technology and using the diagnostic kit supplied by Promega USA and the method of work referred to in the separation of materials and methods of work. The product was then migrated from each sample.

Single nucleotides polymorphism Exon8 of DGAT1 Gen

Huge piece of Diacylglycerol Acyl transfereace gene DGAT1 with PCR technology and using several US company Promega PCR The initiator of DGAT1 gene Exon8(Primers), DNA samples and control of the thermal cycle device As mentioned above, and then A 5 µL sample of each sample was carried out in 2% agarose gel with 100 volts voltage difference and at 50 mA for 70 min and paging output to ensure successful extraction and acquisition of 411bp required DNA fragments(100-1500 bp DNA Ladder Marker) As shown in Figure(1).

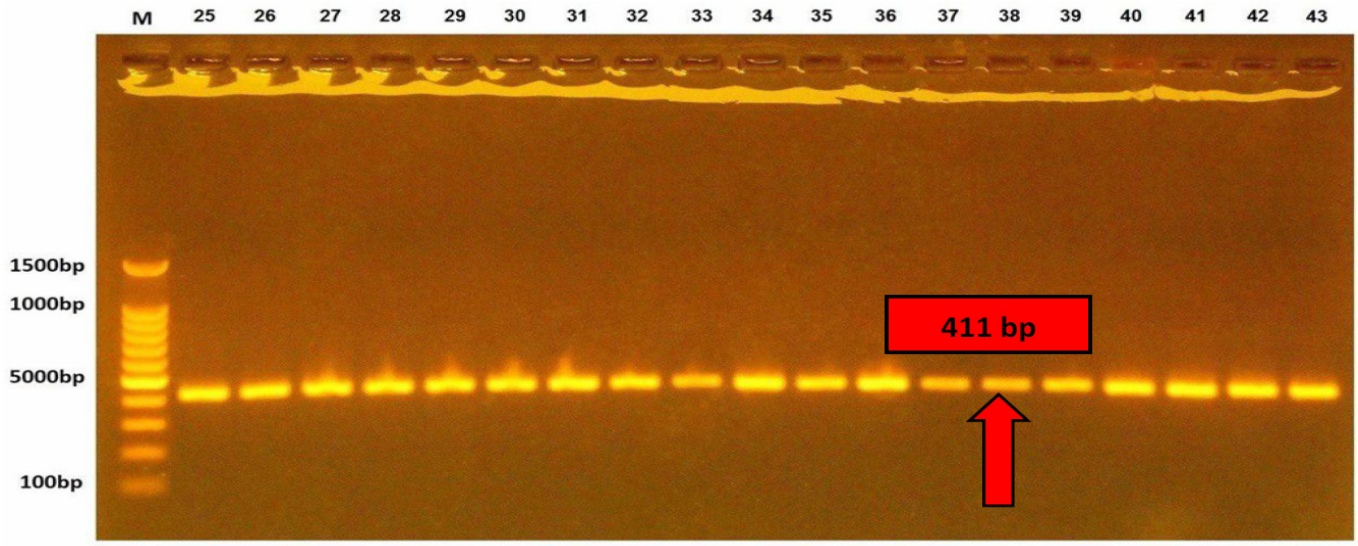

Fig. 1 : The electrical transfer of the Exon8 PCR product to the DGAT1 Gen in the size of 411 bp in the Holstein-Friesian cows, M (Marker).

The location of heterogeneity in the sequence of nitrogen bases, percentages of genotypes and allelic replication of DGAT1 in the Holstein Frisian cows:

The analysis revealed a heterogeneity site in the DGAT1 gene within the Exon8 region, in tow locations. The first location in 7807 and carrying the symbol G/A rs109234250 and the second location in 7808 and carrying the symbol C/A rs109326954, the genotype of experimental animals was determined in the studied DGAT1 gene using SNP technology as shown in **Figure (2).** Genotype was recorded for heterogeneity site G/A rs109234250 where it contained three genotypes: GG (Wild), GA (Heterozygous) and AA (Recessive) and heterogeneity sit C/A rs109326954 it included three genotypes too: GG (Wild), GA (Heterozygous) and AA (Recessive).

Results showed that the nitrogen base was changed from G to A in the first location and from C to A for the second location.

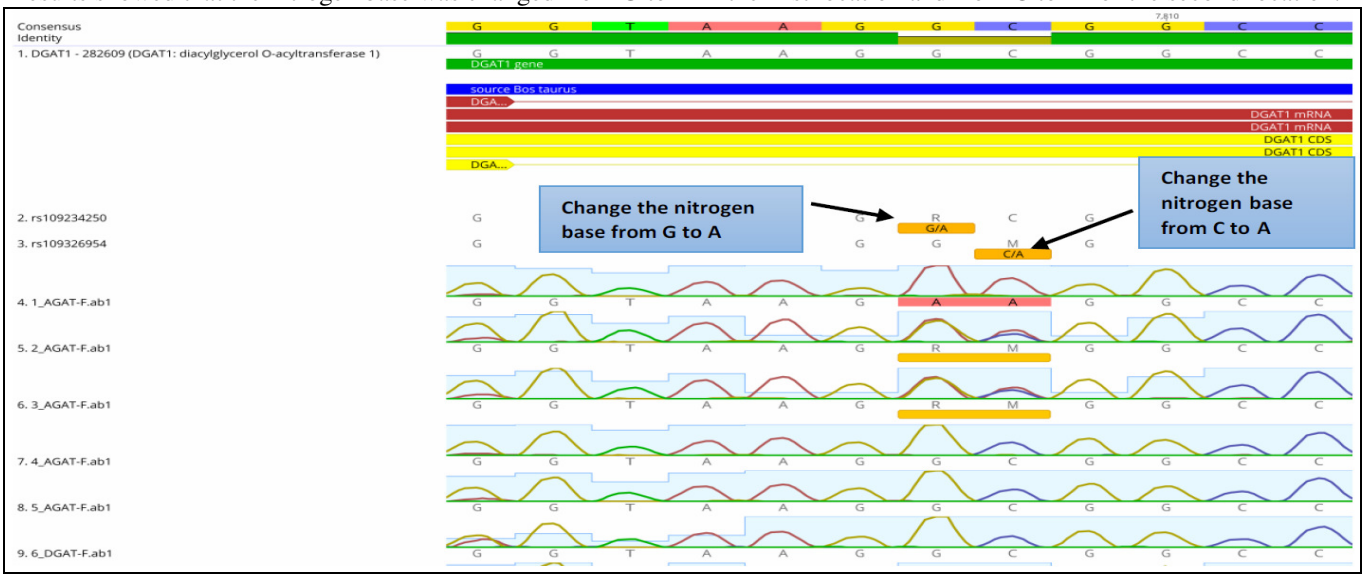

Fig. 2 : The heterogeneity of the nitrogen bases at the first heterogeneity site of rs109234250 G/A and the second heterogeneity site of the exon8 component of the DGAT1 gene is determined for a sample of Holstein-Friesian cows.

The genetic ratios of the heterogeneity site are rs109234250 Were 52.00, 36.00 and 12.00 % for genotypes, GG (n = 26), GA (n = 18) and AA (n=6) respectively, with a total frequency of 0.70 for G and 0.30 for allele A.

Table 2 : Number and percentage of genotypes of the DGAT1 gen rs109234250 G / A in the studied Holstein cows sample.

Genotype	Number	Percentage%
GG	26	52.00
GA		36.00
AA	6	12.00
% 100	50	**SUM**
** 19.920	---	**The value of the Chi square χ^2**
		Allelic Frequency
0.70		**G**
0.30		**A**
**** P<0.01**		

Explain (table 3) the genetic ratios of the heterogeneity site are rs109326954Were 52.00, 36.00 and 12.00 % for genotypes, CC (n = 26), CA (n = 18) and AA (n=12) respectively, with a total frequency of 0.70 for C and 0.30 for allele A.

Table 3 : Number and percentage of genotypes of the DGAT1 gene: rs109236954 C / A in the studied Holstein cows sample.

Genotype	Number	Percentage(%)
CC	26	52.00
CA	18	36.00
AA	6	12.00
% 100	50	**SUM**
** 19.920	---	**The value of the Chi square χ^2**
		Allelic Frequency
0.70		**C**
0.30		**A**
****P<0.01**.		

The difference in the results of the genetic composition of imported Holstein cattle from some studies and the differences between the other studies may be due to differences in the variety and suitability of environmental conditions as well as to the difference in the size of the studied sample. This difference may also be due to the fact that the current study population is not in the Hardy-Weinberg.

Through analysis of the results of my heterogeneities in the segment studied none of the variations were observed, which led to the alteration of the amino acid and was injected into the same amino acid, as these mutations were mutated mutations and were not encoded into a different amino acid.

Table 4 : The average gene effect and the Average effect of gene substitution of alleles for Total milk yield (TMY), Lactation milk yield (LMY) for G/A rs109234250 and Protein 2%, Lactose 2% for C/A rs109326954, for DGAT1 gene.

Trait	Alleles	Average gene effect	Average effect of gene substitution
TMY	G	36.20	120.66
	A	- 84.46	- 120.66
LMY	G	8.04	26.8
	A	- 18.76	- 26.8
Protein 2%	C	- 0.042	- 0.138
	A	0.098	0.138
Lactose 2%	C	- 0.04	- 0.14
	A	0.098	0.14

Effect of genotype in the segment studied of rs109234250 G/ A heterogeneity on breeding values of total milk yield (TMY) and lactation milk yield (LMY) traits of the Holstein cattle:

Table (5) After SNP - PCR conducting, three genotypes of the studied DGAT1 gene Holstein cattle, were found to be the GG, GA and AA with P = 0.70 and q = 0.30, respectively, with 26 cows carrying the GG genotype, while the cows carrying the GA gene were 18 cows and the AA genotype were 6 cows of the total experimental animals.

Table (5) shows the effect of breeding value, dominance deviation and genetic variations genotypes of DGAT1 gen G/A rs109234250 for (TMY) trait (LMY) of the Holstein-Freizian cows. The GG was superior in terms of the breeding value of the next generation of (TMY) and (LMY) traits and the dominant deviations (DD) were less than the breeding values of the GG genotype for (LMY) and (TMY) traits.

The additive variance (VA) “as a part of total genetic variance was less than the dominant variance (VD) in (TMY) trait, which reflecting the level of allelic interaction between alleles in DGAT1 gene and its high contribution to total genetic variation. In the case of the lactation milk yield trait (LMY) characteristic, the VA value (48.27) was higher than VD value (16.42) .The results from Table(5) showed no significant differences between the GG, GA and the AA genotype in the number of vaccinations for heterogeneity site rs109234250 G/ A from the DGAT1 gene for (TMY) trait.

The averages were respectively for each of the three genotypes GG, GA and AA (4585.67, 4607.02, and 4155.01). However, differences may be considered to be of economic benefit Livestock breeding cattle milk. In addition, Table (5) shows statistically significant differences between the genotypes of (LMY) trait. This study agreement with study (Mohamed *et al*, 2015).

Results show in (table 5) that GG genotype can be selected to improve both (TMY) and (LMY) traits in Holstein cows because breeding values are the true value inherited by off spring, which can be used to select parents to the next generation (Warmington and Kirton , 1990). The average effect of G allele was positive for all studied traits, In contrast to mutant allele (A) which was negative Table (4). The average effect of gene substitution for the studied traits was positive for G allele and negative for A allele Table (4).

Table 5 : Breeding value, dominance deviation and genetic variations genotypes in DGAT1 gen G/A rs109234250 for trait total milk yield (TMY) and Lactation milk yield (LMY).

Total milk yield (kg)							
Genotype	**No**	**Mean**	**BV**	**DD**	**VA**	**VD**	**VG**
GG	26	4585.67	72.4	42.60	6114.71	9881.47	15996.18
GA	18	4607.02	- 48.26	99.40			
AA	6	4155.01	- 168.92	231.95			
Lactation milk yield (days)							
Genotype	**No**	**Mean**	**BV**	**DD**	**VA**	**VD**	**VG**
GG	26	282.64	16.08	3.36	48.27	16.42	64.70
GA	18	267.03	- 10.72	7.83			
AA	6	214.11	- 37.52	18.29			

BV: Breeding value, DD: dominance deviation, VA: Additive variance, VD: Dominance variance, VG: Genetic variance.

Effect of genotype in the segment studied of rs109234250 C/A heterogeneity on breeding values of percentages Protein 2%, lactose 2 of the Holstein cattle

Table (6)The analysis shows that there are three genotypes within rs109234250 C/ A site and both tow percentage (Protein 2% and Lactose 2%) , with 26 cows carrying the CC genotype and 18 cows for CA genotype while the cows carrying the AA genotype were 6 cows of the total experimental animals. The results showed statistically significant differences between the genotypes within this site within the Protein 2% Percentage. The averages for the CC, CA and AA were measured respectively (2.52, 2.79, and 2.61) also the results in table(6) showed statistically significant differences between the genotypes within this site within the lactose 2% Percentage and the averages for the CC, CA and AA were measured respectively (3.91, 4.30, and 3.85).

Shows the effect of breeding value, dominance deviation and genetic variations genotypes in DGAT1 gen C/A rs109326954 for Protein 2% and lactose 2% percentage of the Holstein-Freizian cows. The AA was superior in terms of the breeding value of the next generation of Protein 2% Percentage and lactose 2% Percentage and the dominant deviations (DD) were higher than the breeding values of the CC and CA genotype for both Protein 2% and lactose 2%. Table (6) showed the additive variance (VA) "as a part of total genetic variance "was less than the dominant variance (VD) in Protein 2%, which reflecting the level of allelic interaction between alleles in DGAT1 gene and its. High contribution to total genetic variation. While the lactose 2%, the VA value (8.23) was higher than VD value (0.03).

Results show in (table 6) that AA genotype can be selected to improve both Protein 2% and Lactose 2% in Holstein cows because breeding values are the true value inherited by offspring, which can be used to select parents to the next generation (Warmington and Kirton (1990). The average effect of A allele was positive for all studied traits, In contrast to mutant allele (C) which was negative Table (4). The average effect of gene substitution for the studied traits was positive for A allele and negative for C allele Table (4).

Table 6 : Breeding value, dominance deviation and genetic variations genotypes in DGAT1 gen C/A rs109326954 for percentage protein 2% and lactose 2%.

Protein 2%							
Genotype	**No**	**Mean**	**BV**	**DD**	**VA**	**VD**	**VG**
CC	26	2.52	- 0.08	0.04	7.99	8.54	16.53
CA	18	2.79	0.06	0.09			
AA	6	2.61	0.20	0.22			
Lactose 2%							
Genotype	**No**	**Mean**	**BV**	**DD**	**VA**	**VD**	**VG**
CC	26	3.91	- 0.08	0.08	8.23	0.03	8.26
CA	18	4.30	0.06	0.18			
AA	6	3.85	0.20	0.41			

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