POLYMORPHIC GENOTYPES FOR FRAGMENT (INTRON1-661BP) FROM DGAT1 GENE AND THEIR RELATIONSHIP TO PERFORMANCE IN IRAQI BUFFALO USING RELP TECHNOLOGY

Ali Fadhel Kadhim and Wafaa Ismail Ibrahim

Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

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. The separating the genetic material, identifying the genotypes and the proportions of their distribution of Fragment (661bp-Intron1) 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 coding area of the specific Fragment (661bp-Intron1) differed from DGAT1 gene depending on the different genetic packets resulting from enzymatic digestion, which showed two genotypes represented by CT and TT and their distribution ratios were 23.33 and 76.67% Respectively, the discrepancy between these two ratios was highly significant ($P < 0.01$),while the frequency of allele was 0.88 and 0.12 for both allele's T and C respectively. The results showed that the production of daily milk was Significant affected ($P < 0.05$) due to the different in the genotypes of this Fragment of DGAT1 gene of buffalo with heterozygous CT genotype at a rate of 10.28 ± 0.69 kg, while the age the first birth was not affected by the variation of the genotypes for the Studied Fragment by DGAT1 gene. Finally, all percentage of milk component that studied and the body dimension for the mothers were not affected by the variation of the genotypes for the specific Fragment of DGAT1 gene. The results that obtained from this study can be used to adopt new genetic improvement methods in Buffalo to maximize the economic return of their breeding projects by selecting and increasing the genotypes that have achieved the best economic qualities.

Key words : *Bubalus bubalis*, DGAT1 gene, (Intron1-661bp), PCR-RFLP.

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 (Alhasnaoui, 2012). 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 (Yadav *et al.,* 2007). 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 (Lorenz, 2012). 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. (Williams, 2005). 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). This

gene is related to milk production and the percentage of milk fat (Grisart *et al.*, 2002). 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 (Intron1-661bp) of DGAT1 Gene and its relationship to milk production and its composition, age at the first birth and the body dimensions of mothers in the buffalo samples

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 was 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 some milk composition, notes was recorded on buffaloes health, physiological and reproductive condition by veterinarians with the help of Breeders, furthermore body dimension was recorded, as well as blood samples was 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 separating the genetic material and identifying the genotypes of the specific fragment (Intron1-661bp) of DGAT1gene by the use of Restriction fragment length polymorphism (RFLP) 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.

Statistical Analysis System-SAS (2012) was used to study the effect of genotypes for the specific fragment (Intron1- 661bp) 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:

$$Y_{ijk} = \mu + Gi + Pj + ei_{jk}$$

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- 661bp) of DGAT1 gene (CT and TT).

P_j: Birth sequence effect (from 1st to 8th).

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

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 (Selvaggi *et al.,* 2015: Gabor. *et al.,* 2014).

DNA separation and DGAT1 Gene extraction

Figures 1 and 2 represents the separation and extraction of DNA for the specific fragment (Intron1-661bp) of the DGAT1 gene within PCR-RFLP technology using 2 Microliter from loading dye with 2.5% concentration of Agarose gel, the voltages was set to 70 volts with a current of 40 milliamperes for an hour and a half. 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 661 base pairs (bp) with the use of DNA fragment information volumes (Lader 100-1500 bp). The packages resulting from the enzymatic digestion of the restriction enzyme *Smal* was differed according to the different genotypic structures of the encryption area of Intron1 for this Gene.

The genotype of CC (Wild) is not found in the studied Buffalo samples.

The genotype CT (heterozygous genotype) shows at columns 7 and 8.

The TT genotype (mutant) appears at columns 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14 and 15.

Result and Discussion

The Percentage of genotypes distribution and the allele's frequency for the specific fragment of Intron1-661bp for DGAT1 gene

(Table 2) shows the number of genotypes, their percentages and the allele's frequency of the studied specific fragment (Intron1-661bp) for DGAT1 gene, there were two genetic genotypes of this fragment TT and CT. The non-appearance of the members that carry the

Table 1: The primers sequence that used in the study.

| Gene name	Amplification Area	Sequence
DGAT1Genbank:102390126	specific fragment(661bp - Intron1) PCR-RFLP	Forward = 5´-GGCCTCTCCCCTTACAAAAC - 3´
		Reverse=5´-CACACACCAATTCAGGATGC - 3´
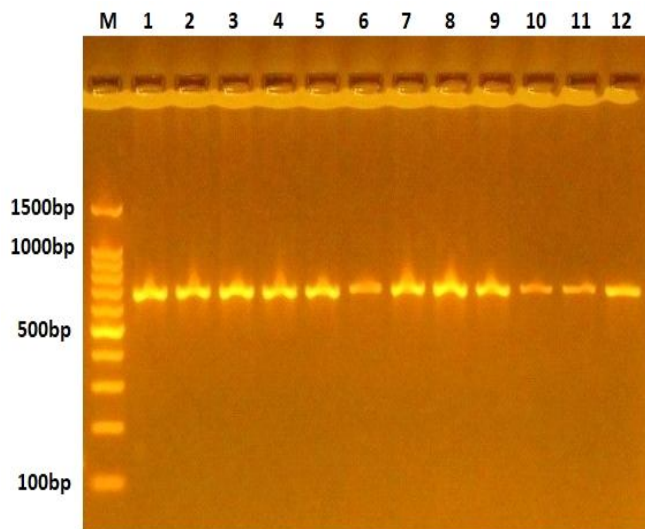
Fig. 1: The specific fragment (Intron1-661bp) of DGAT1 Gene using PCR-RFLP technology.

wild pure genetic genotypes (Wild-CC) within the studied sample, while the total numbers that carried those two genotypes were 46 and 14 and by a percentage of 76, 67% and 23, 33% respectively, i.e. that mean the allele's frequency T and C was 0, 88 and 0, 12 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 TT's genotype with a higher percentage of the CC's genotype in this study was similar to what Mishra reached and others. (2007), he pointed out the prevalence of allele's T and by up to 0.80 while the result was different from what Rahul reached at (2009) which indicated the allele's prevalence C by 0.85 at Their study on Indian Buffalo in a specific location (5545) of DGAT1 gene.

Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 gene to the production of daily Milk for Buffalo

The results showed that there was a significant difference (P < 0.05) in the daily milk production between the buffalos with the genotypes variation of this fragment of gene. The members that carry the heterozygous CT genotype have the superiorityon the members that carry the mutant genotype of TT and the amount of milk produced was 10.28 ± 0.69 and 8.64 ± 0.55 kg respectively as shown in table 3. This result was different with the
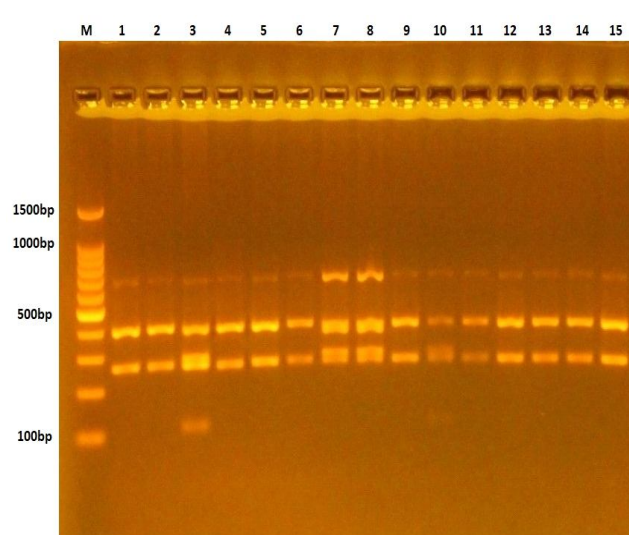
Fig. 2: The products of the digested fragment (Intron1-661bp) of DGAT1 gene using restriction enzyme *SmaI*.

Table 2: Number and percentages of genotypes and the allele's frequency of the fragment (Intron1-661bp) for DGAT1 Gene in the sample of Buffalo studied.

Genotype	Number	Percentage (%)
CT	14	23.33
TT	46	76.67
Total	60	100 %
Value (÷2)	.....	53.78 **
Allele's frequency		
C	0.12	
T	0.88	

** (P<0.01).

(Patel *et al.*, 2009) findings that there are no significant differences in the rate of daily milk production according to his study on Indian Mehsana Buffalo specifically in the locations (8087), (8259), (8426), (3674) and (3627) of DGAT1gene.

Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 gene with Buffalo milk composition

The results showed that there was no Significant effect for the different genotypes to the specific fragment (Intron1-661bp) of DGAT1 gene on some milk composition such as protein, fat, lactose, non-faty solids and ash as shown in table 4. This result presented a good

Table 3: Relationship of genotypes for the specific fragment (Intron1-661bp) of DGAT1 gene to the production of daily Milk for Buffalo.

Genotype	Number of buffalo (Number of samples)	Average ± standard error
		Daily milk Production (kg)
CT	14 (84 samples)	a 0.69 ± 10.28
TT	46 (276 samples)	b 0.55 ± 8.64
Significant level	Total number 60	*

Averages with different characters within a single column are Significant different.* (P<0.05).

Table 4: Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 gene to the milk competition of Buffalo.

Genotype	Number of Buffalo (Number of samples)	Average ± standard error (%)				
		Protein (%)	Fat (%)	Lactose (%)	Non-fatty solids (%)	Ash (%)
CT	14 (28 samples)	3.64 ± 0.64 a	5.77 ± 0.97 a	4.61 ± 0.39 a	10.78 ± 0.43 a	0.49 ± 0.27 a
TT	46 (92 samples)	3.89 ± 0.14 a	5.93 ± 0.33 a	4.50 ± 0.12 a	10.00 ± 0.49 a	0.38 ± 0.06 a
Significant level	Total number 60	NS	NS	NS	NS	NS

Averages with identical characters within a single column are not Significant different. NS: Non- Significantly.

Table 5: Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 Gene in age at the first birth of female buffalo.

Genotype	Number of buffalo	Average ± standard error (year) Age at first birth
CT	14	a 0.05 ± 2.38
TT	46	a 0.02 ± 2.41
Significant level	Total Number 60	NS

Averages with identical characters within a single column are not Significant different. NS: Non- Significantly.

Table 6: Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 gene to the mother's body dimensions.

Genotype	Number of Buffalo	Average ± standard error (cm)				
		Chest circumference	Abdominal circumference	Body length	Height at the front	Height at the rear
CT	14	207.60 ± 7.28 a	248.00 ± 5.00 a	157.60 ± 4.01 a	140.80 ± 3.30 a	137.80 ± 3.05 a
TT	46	203.84 ± 2.46 a	248.84 ± 1.87 a	155.96 ± 1.33 a	139.56 ± 1.05 a	136.60 ± 1.15 a
Significant level	Total number 60	NS	NS	NS	NS	NS

Averages with identical characters within a single column are not Significant different. NS: Non- Significant.

The results showed that there is no Significant effect for the different genotypes to the specific fragment (Intron1-661bp) of DGAT1 Gene on age at first birth as shown in (Table 5). It was noted that there were mathematical differences for the individuals that carry the CT heterozygous genotypes compared to the members that carry the pure genotypes TT.

This result was less than from what (Sethi, 2003) and (Borghese, 2010) 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 Idriss and his colleagues at (2009) reached and both of the two genotypes where the result was 2.61 years When studying on the Iraqi Buffalo in al-Fadelian.

Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 gene to the mother's body dimensions

agreement with that obtained by (Rahul *et al*., 2009) as followed, there was no significant differences in milk fat percentage when studying on Murrah Buffalo at the two locations (5545) and (6067) of DGAT1 gene. There was a lack in significant differences in the amount of milk and its composition among the individuals that carry two genotypes TT and CT may be attributed mainly to the nature of this gene's work, which is characterized by a strong competition between the wild allele and the mutent allele's genotype at work and thus the individuals are close in the processes of gene expression for this gene although its genotypes was different.

Relationship of genotypes of the specific fragment (Intron1-661bp) of DGAT1 Gene in age at the first birth of female buffalo

The results showed that there is no Significant effect for the different genotypes of mothers Buffalo for this fragment of the gene in all body's dimensions which is the circumference of the chest and abdominal circumference and the length of the body and height at the front and height at the rear, and the rates of the body dimensions of the individuals which carrying the pure genetics (TT) was 203.84, 248.84, 155.96, 139.56, 136.60 cm Sequentially as for heterozygous ratios (CT), it amounted to 207.60, 248.00, 157.60, 140.80 and 137.80 cm Sequentially (Table 6). A number of researchers have 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 (Baghdasar *et al.,* 2014).

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