Plant Archives Vol. 19 No. 2, 2019 pp. 3782-3784 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ASSOCIATION OF *FABP4* GENE POLYMORPHISMS WITH SOME MILK COMPONENTS IN HOLSTEIN COWS

Younis W. Younis[1], Emad G.H. Alabbasy[1] and Riyadh Hamad Senkal[2]

[1]Department of Animal Production, Collage of Agriculture, University of Tikrit, Iraq.
[2]Department of Animal Production, Collage of Agricultural Engineering Sciences, University of Baghdad, Iraq.

Abstract

This investigated was conducted in Taj Al-Nahrain station for Dairy cattle /private sector, and for the period from 1-8-2018 to 30-12-2018 on 49 Holstein cows, to determine the association between *FABP4* **(**Fatty acid binding protein 4) gene's polymorphisms with some milk components (pH, protein%, casein%, Fat%, milk density, water%, freezing point and nonfat solid) in Holstein cows. The results of *FABP4* gene analysis showed a highly significant different ($P<0.01$) between genotypes of *FABP4* gene's genotypes GG, GA & AA, the percentage were 57.14, 38.78 and 4.08% respectively, with an allele frequency of alleles G 0.76 and A 0.24% respectively. The results of this study showed that there was no significant differences between the *FABP4* gene polymorphisms and milk components. It was possible to conclude from this study the possibility of *FABP4* gene's polymorphisms in the development of genetic improvement strategies and in breeding programs in dairy cows.

Key words : *FABP4*, Polymorphism, Milk Components, Holstein, Sequencing.

Introduction

Improving the productive and reproductive performance in animals farm including cows under traditional breeding conditions faces many difficulties that researchers are attempt to crossing it so as to increase their productivity through genetic improvement programs, than stimulate researchers and breeder to find alternative means to the traditional selection that was followed over the past decades and which requires great time and effort (Saleem *et al.,* 2015). Molecular biology has undergone tremendous development during the past three decades, This development has led to the detection of genetic markers at the molecular level that have helped to build genetic improvement programs in less time, cost and speed is greater to improve the performance of productive animals (Yadav *et al*., 2017). There are many genes have a high genetic diversity and are therefore it's important in the application of selection programs and among these genes is *FABP* gene which is located on chromosome 14 in cows, it is involved in intracellular transport and is linked with the yield of milk, lipids and protein percentage and its association with fatty acids in milk, in the majority of previous studies three genotypes were found, FABP is found in several types, including FABP3 and FABP4, Some studies have also indicated to the relationship of this gene with the number of body cells in milk, infection with mastitis and a number of reproductive traits (Cho *et al*., 2008). Therefore, this study aims to investigate the effect of this gene polymorphisms and its association with the proportions of milk components.

Materials and Methods

Samples of blood were collected from 49 Holstein cows breeding at Taj Al-Nahrain station for Dairy cattle (Diwaniyah Governorate) 120 kilometers south of Baghdad. Blood collected by a medical syringe from the jugular vein in a 15 ml sterile polypropylene tubes containing 0.5 ml of EDTA (0.5 M) as an anticoagulant by the phenol chloroform extraction by the veterinarian at the station, The blood samples were then transferred by a cool box then stored in freezing at -20^{0}C temperature till transferred to the lab to extracting DNA, The genetic material DNA was extracted from the blood according to instructions of the Geneaid kit as a first step, the DNA samples were checked for their quality, purity and concentration, the quality of the genomic DNA was checked by using agarose gel electrophoresis, DNA

samples of good quality, purity and concentration were used for further analysis, the polymerase chain reaction (PCR) technique for *FABP4* typing is based upon the extensive polymorphism that is present an in region of gene (part of Intron 2, Whole Exon3) was amplified by using primers table 1 (Shin *et al.,* 2012).

Chain Polymerase Reaction (PCR)

The molecular detection was carried out using the polymerase chain reaction (PCR) of studied region and using Promega's kit with volume 25 µl and placed in the polymerase chain reaction device according to the reaction conditions that special of each piece gene studied, After the interaction table 2 was finished the interaction output was carried out (electrophoresis) to ensure that the required region was doubled.

Electrophoresis of DNA

7 µl DNA were mixed with 3µl from Loading dye, after that samples were loaded in agarose gel, the samples were carried (electrophoresis) over 70 volts and 45 mA for 70 minutes then extracted the submerged gel plot in TBE liquid and 1% bromide atheideum dye for the purpose of viewing the DNA bundle and detecting the size of the studied piece of the gene (Fig. 1).

Polymorphisms select of FABP4 gene by using sequencing technique

The PCR product was sent by Advanced Scientific Co/ Harithiya to the laboratories of the company Macrogen Corporation-Korea for procedure Sanger sequencing technique the results were analyzed by using genius software program.

Milk components analysis

Use a special device to measure the milk components (Julie z7), the measured components were milk content of fat, protein, casine, density, added water, freezing point and nonfat solids In addition to milk pH milk was measured by PH Meter.

Statistical analysis

The data were analyzed statistically using a program Statistical Analysis System SAS (2012), To study the effect of polymorphisms of the gene FABP4 in the proportions of milk components and after the comparison of the differences between the averages of the components through the use of the test of the mean squares (Least square means).

Mathematical model; $Y_{ijk} = \mu + G_i + P_j + e_{ijk}$

As each of the following:

Y_{ijk}: The value of the observation l belonging to the genotype i and the sequence of the production cycle j.

µ: average of trait.

G_i: effect multiple polymorphism of FABP4 gene.

P_j: effect productive cycle sequencing (2^{nd}, 3^{rd}).

e_{ijk}: Random error which is distributed naturally at an average of zero and a variation of $\sigma^2 e$

Also used Chi-square-x^2 test to compare between percentage of gene polymorphisms and alleles frequency.

Results and Discussion

Distribution of genotypes and allele frequency

After completion PCR and Sequencing technique, Shows the presence mutation of an SNP that recorded in the NCBI rs110652478 (G/A), as it is found is three polymorphisms GG, GA, AA. The table 3 is shown there are a highly significant difference ($P<0.01$) between genotype and allele frequency percentage, the percentage was 57.14, 38.78, 4.08 % and allele frequency of G, A 0.76, 0.24 % respectively.

Table 1: Primers used in duplication program.

Gene	Studied piece	Sequence
FABP4	Part Intron2	Forward = 5' ACCCCTATGATGCTATTCCACA 3
	Whole Exon3	Reverse = 5' ATACGGTTCACATTGAGAGGGA 3

(Shine *et al*., 2012).

Table 2: Using program in molecular detection (PCR Technique).

No.	Steps	Temperature	Time (minute)	Cycle number
1	First Denaturation	95	4	1
2	Denaturation	94	1	35
3	Annealing	60	1	
4	Extention	72	1.5	
5	Final extension	72	5	1
6	Incubation	4	.....	.....

(Ardicili *et al*., 2017).

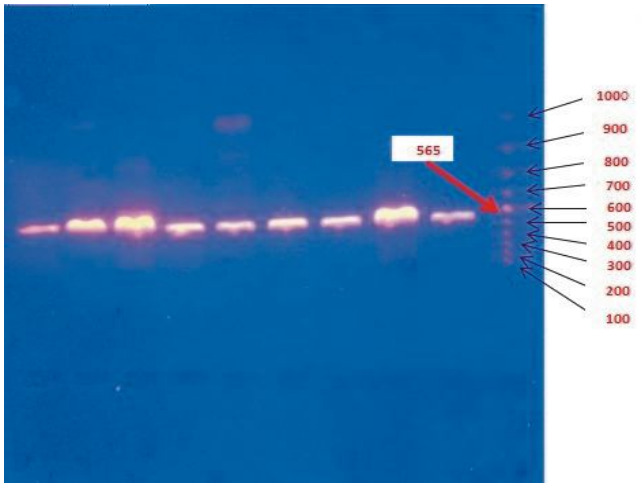

Fig. 1: Studied region of *FABP4* gene (565 bp).

Table 3: Genotype and allele frequency of *FABP4* gene.

Genotype	No.	Percentage %
GG	28	57.14
GA	19	38.78
AA	2	4.08
Total	49	100
Chi square x^2 value	.....	**30.061
Allele frequency		
G		0.76
A		0.24

**(P<0.01).

Table 4: Average percentage some milk components in Holstein cows.

Genotype	Freezing point	Added water	Density	Fat%	Casein%	Protien%	PH	Non-fat solids
AA (2)	53.30±0.80	4.20±1.44	25.15±0.65	5.37±0.13	2.47±0.04	3.09±0.05	6.92±0.05	8.12± 0.14
AG (18)	53.70±0.73	4.27±0.97	26.07±1.11	4.71± 0.45	2.54±0.04	3.17±0.05	7.02±0.04	8.23±0.17
GG (29)	55.07±2.09	5.10±1.17	26.39±0.71	4.15±0.36	2.46±0.04	3.08±0.05	6.97±0.01	8.13±0.14

Association between *FABP4* gene polymorphisms and percentage of some components in Holstein cow's milk

The results in table 4 showed that there was No significant differences between genotypes of *FABP4* gene in protein, fat, casein percentage average and also no significant differences in Density, added water, freezing point, non-fat solids in milk and pH milk. This is consistent with some studies in this matter, these results were agreed with the results of a study Kulig *et al*., 2010 who studies effect of single nucleotide polymorphism in FABP4 on milk components in jersey cows and Kaczor *et al*., 2017 who studies effect of FABP4 gene polymorphism on milk components in Holstein Frisian polish cows.

This may be due to the fact that this gene mainly affects the deposition of fat in the body (Ardicli *et al.,* 2017) and is considered as a genetic factor affecting the storage and isolation of fat between muscles and tissues (Fortes *et al.,* 2009), This gene showed its effect in many characteristics and traits such as the thickness of the back fat and the degree of marbling as well as the weight of the carcass (Maharani *et al.,* 2012, Shin *et al.,* 2012). Making him a candidate gene for use as a molecular genetic marker in the slection for meat-producing animals and calves breeding projects.

Conclusion

We conclude from the results of our present study that there is no association between the multiple polymorphisms of *FABP4* gene with the proportions of the main milk components such as milk pH, fat % protein %, casein %, density, water added, freezing point and non-fat solids in Holstein cow's milk with the possibility of using this gene as a molecular genetically marker in the genetic improvement programs for other traits such as calves breeding projects.

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