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## STUDY OF THE RELATIONSHIP BETWEEN SOME GENETIC MARKERS AND MILK PRODUCTION AND ITS COMPONENTS IN IRAQI BUFFALOES

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### ABSTRACT

The study was conducted in private sector civil projects, at Al-Mishkhab district, Najaf governorate, from 1/10/2019 to 1/15/2020 for one productive season. A total of 30 buffaloes with their 30 offspring, data of the buffaloes used in the experiment were collected by knowing the ages and types of animals by the project owners. Milk yield was measured based on daily production and the entire production season, milk samples were taken every two weeks for each buffalo, during the trial period, by manual milking method, at six in the morning. The milk sample was taken after mixing the milk produced from the buffalo well from the morning milking, to make the sample more homogeneous and in an amount of about 50 ml, after that, transferred directly to check the components of the milk to preserve the samples, not to be exposed to sunlight or high temperatures, the milk components of fat and protein were calculated every two weeks for the length of the experiment, by using the EKO Milk laboratory milk analyzer after collecting samples during the milking process. The results indicated that there were no significant differences between the genetic markers BM1706, ETH02, ETH003 with average daily milk production, milk fat and protein in most weeks.

**Keywords:** genetic markers, milk production, milk components, Iraqi buffaloes.

### Introduction

Livestock resources in Iraq are one of the pillars of the national economy, contribution to attribution of added values to the national economic, meet the citizen's need of animal protein, Buffalo is an important source of red meat, as well as milk, characterized by a high proportion of fat, the importance of other products and its tolerance to different environmental conditions. The domestication of the buffalo was in the middle of the third millennium BC in the Mesopotamian valley at the ancient Sumerian city of Ur, so is the Minhjo-Daro in the Valley of India (Borghese, 2005). The population of buffaloes in Iraq is 285,000 (Ministry of Agriculture, 2008). Animals are the product of prolonged selection and improvement, since domestication by humans, the process has continued to find the best genotypes that produce high quantities of products (Giacomini *et al.*, 2008). Improving the production and reproductive performance of farm animals, including buffalo, especially under traditional breeding conditions, face multiple difficulties, led to a decrease in its productive and reproductive performance, therefore, needs special attention in order to improve and maintain its productive characteristics, the economy relies heavily on livestock, it is necessary to rely on some scientific strategies, for the purpose of obtaining more adapted and productive animals, led to the creation of alternative methods of traditional franchise, has been the case throughout the past decades, which requires considerable time and effort (Saleem *et al.*, 2015). The genetic improvement also has a positive effect, reflects on improved livestock production, especially reproductive performance and the quality of meat and milk,

in addition to resistance to internal and external diseases and parasites (El-Nahas *et al.*, 1998). Recent studies have indicated that improving the productive characteristics of buffaloes, requires new methods of genetic improvement, study the genotype of animals and choose the best, by studying the genes that affect production traits, to compare its genotype with the global strains to find out the genetic mutations occurring, and linking it to the phenotypic structure using the PCR (Polymerase Chain Reaction). The relationship of these genes to the traits of milk production, growth and fattening, characteristics of carcasses and milk production, include BM1706, ETH02, ETH003 genes (Hossam *et al.*, 2017; Talib and Maytham, 2013; Emel *et al.*, 2014). The lack of studies on these genes in buffalo in Iraq, this study aimed to find out the relationship between genetic markers (BM1706, ETH02 and ETH003), by using microsatellite technology, to determine the relationship of the genetic features of each marker to the rate of milk production and some of its components such as fat and protein ratios.

### Materials and Methods

#### Experiment animals

The study was conducted in private sector civil projects, at Al-Mishkhab district in the Najaf governorate, on a group of buffaloes from 1/10/2019 to 1/15/2020 for one productive season, as the experiment consisted of 30 buffaloes with their 30 offspring, data of the buffaloes used in the experiment were collected by knowing the ages and types of animals by the project owners.

### Calculating daily milk production and measuring milk components

Milk yield was measured based on daily production during the production season, milk samples were taken every two weeks for each buffalo by manual milking method, at six in the morning. The milk sample was taken after mixing the milk produced from the buffalo well from the morning milking, to make the sample more homogeneous and the amount of about 50 ml. It was transferred directly after that to check the components of the milk to preserve the samples, not to be exposed to sunlight or high temperatures. Then, the fat and protein components of the milk were calculated every two weeks, by using the EKO Milk laboratory milk analyzer after the samples were collected during the milking process.

DNA was extracted from buffalo blood samples using a measuring kit (Kit) provided by the Korean company Geneaid. Agaros gel preparation was prepared (Sambrook *et al.*, 2001). Then, the Microsatellites technique was used. Three markers were selected (BM1706, ETH003 and ETH02), to determine their relationship to the characteristic of producing milk and its components of buffalo, the annealing degree was determined by the complement sequence in the template DNA for each marker, using a grading process specific to each marker, to determine the success of the process of multiplying or amplifying the DNA segment to be determined by the markers used, during electrophoresis on an aqueous gel, as 5 µl of the PCR product was taken, it is placed in the pits with the use of a 25bp Lader tag (DNAMarker-25bp).

### Statistical analyses

Data were analyzed statistically using the Statistical Analysis System-SAS (2012), to study the effect of the three genetic markers (BM1706, ETH02, ETH003), on the studied traits on the Iraqi buffalo, significant differences between the means were compared using the polynomial Duncan (1955) test, by applying the General Linear Model-GLM method.

### Results and Discussions

Table 1. show that no significant differences between the genotypes: 230/211, 250/231 and 271/251, respectively, BM1706 in average daily milk production, there were no significant differences for these genotypes mentioned for BM1706 in the ratio of milk fat and milk protein in the different weeks. The mean milk production rate for BM1706 with its genotypes was 230/211, 250/231 and 271/251 were 7.20, 7.87 and 8.09 for the genotypes, respectively. The percentages for the first week of milk fat for the marker BM1706 with its genotypes were 230/211, 250/231 and 271/251 were 3.87, 5.63 and 5.02 for the mentioned genotypes, respectively. As for the eleventh week, the ratios ranged between 2.83, 4.15, and 4.12 for the genotypes: 230/211, 250/231 and 271/251, respectively. The percentages for the first week of milk protein for BM1706 with its genotypes were 230/211, 250/231 and 271/251 were 3.26, 3.47 and 3.43 for the mentioned genotypes, respectively. As for the eleventh week, the ratios ranged 2.99, 2.92 and 3.25 for the genotypes, 230/211, 250/231 and 271/251 base pairs, respectively. Agreed with Rushdi *et al.* (2017) that BM1706 did not have a significant effect on milk production and fat and protein ratio when studying Egyptian buffalo.

**Table 1 :** The relationship of genotypes for the BM1706 genotype to the percentage of milk fat and milk protein and the rate of daily milk production.

Milk components	Genotypes	Durations (weeks)			Milk production
		1	5	11	
Milk fat	230/211	0.76±3.87 a	0.90±2.45 a	1.03±2.83 a	230/211
	250/231	1.23±5.63 a	1.16±4.06 a	0.80±4.15 a	0.73±7.20 a
	271/251	0.82±5.02 a	1.03±4.35 a	0.81±4.12 a	250/231
Sig.		N.S	N.S	N.S	0.54±7.87 a
Milk protein	230/211	0.16±3.26 a	0.20±3.42 a	0.28±2.99 a	271/251
	250/231	0.17±3.47 a	0.21±3.35 a	0.21±2.92 a	0.39±8.09 a
	271/251	0.10±3.43 a	0.21±3.14 a	0.08±3.25 a	N.S
Sig.		N.S	N.S	N.S	

Table (2) showed that there were no significant differences between the genotypes: 215/200 and 230/216 base pairs, respectively, for marker ETH02 in average daily milk production. There were no significant differences for these genotypes mentioned for the marker ETH02 in the percentage of milk protein in the different weeks. The mean milk production rate for ETH02 with its genotypes was 215/200 and 230/216 base pairs, 7.50 and 8.00 for the mentioned genotypes, respectively. The percentages for the first week of milk protein for the marker ETH02 with its genotypes were 215/200 and 230/216 base pairs, 3.35 and 3.51 for the mentioned genotypes, respectively. As for the eleventh week, the percentages ranged from 3.08 to 3.19 for the genotypes: 215/200 and 230/216 base pairs, respectively. As for the proportions of milk fat, there were no significant

differences in the ratios of milk fat between the genotypes 215/200 and 230/216 for the marker ETH02 in the first week, the ratios of milk fat in the first week were 4.90 and 5.13 for the mentioned genotypes respectively. The presence of significant differences ( $P \leq 0.05$ ) between the genotypes for the marker ETH02 in the eleventh week, as there was an superiority in the percentage of milk fat for the genotypes 230/216 base pair over animals carrying the genotype 215/200 base pair. The ratios of milk fat for the eleventh week were 4.98 and 2.99 for the genotypes 230/216 and 215/200, respectively. Agreed with Rushdi *et al.* (2017) when studying the Egyptian buffalo, that they found a significant association ( $p \leq 0.01$ ) between the ETH02 genotype and the production of milk and its components.

**Table 2 :** The relationship of genotypes for the ETH02 genotype to the percentage of milk fat and milk protein and the rate of daily milk production.

Milk components	Genotypes	Durations (weeks)			Milk production
		1	5	11	
Milk fat	215-200	0.76±4.90 a	0.65±3.11 a	0.49±2.99 b	215-200
	230-216	0.63±5.13 a	1.14±4.65 a	0.91±4.98 a	0.31±7.50 a
Sig.		N.S	N.S	*	
Milk protein	215-200	0.08±3.35 a	0.15±3.30 a	0.12±3.08 a	230-216
	230-216	0.15±3.51 a	0.20±3.32 a	0.19±3.19 a	0.56±8.00 a
Sig.		N.S	N.S	N.S	N.S

Table (3) showed that there were no significant differences between the genotypes 125/110 and 145/126 base pairs respectively for marker ETH003 in average daily milk production. As the proportions were 7.76 and 7.44 for the mentioned genotypes, respectively. There were no significant differences between the genotypes 125/110 and 145/126 base pairs respectively for the marker ETH003 in the percentage of milk fat in the first and eleventh weeks, as the proportions of milk fat in the first week were 5.37 and 4.17 for the mentioned genotypes, respectively. The ratios of milk fat in the eleventh week, 4.58 and 3.03 for genotypes 125/110 and 145/126, respectively. There were significant differences ( $P \leq 0.05$ ) between the genotypes for the marker ETH003 in the percentage of milk fat in the fifth week. There was a superiority in the ratio of milk fat to the genotype 125/110 over the animals that carry the genotype 126/145, the ratios

were 5.14 and 2.23 for the mentioned combinations, respectively. No significant differences between genotypes 125/110 and 145/126 base pairs respectively for marker ETH003 in the percentage of milk protein at the fifth and eleventh weeks. As the ratios for milk protein in the eleventh week were 3.03 and 3.17 for the mentioned genotypes, respectively. There were significant differences ( $P \leq 0.05$ ) between the genotypes for the marker ETH003 in the percentage of milk protein in the first week. There was a superiority in the ratio of milk protein to the genotype 125/110 over the animals that carry the genotype 126/145, the ratios were 3.63 and 3.34 for the mentioned combinations, respectively. Agreed with Aminafshar *et al.* (2008) their study of Iranian buffalo in the south and southwestern Iranian Sea.

**Table 3 :** The relationship of genotypes for the ETH003 genotype to the percentage of milk fat and milk protein and the rate of daily milk production.

Milk components	Genotypes	Durations (weeks)			Milk production
		1	5	11	
Milk fat	125/110	0.64±5.37 a	0.87±5.14 a	0.73±4.58 a	125/110
	145/126	0.79±4.17 a	0.52±2.23 b	0.77±3.03 a	0.42±7.76 a
Sig.		N.S	*	N.S	
Milk protein	125/110	0.08±3.63 a	0.18±3.30 a	0.13±3.03 a	145/126
	145/126	0.08±3.34 b	0.20±3.34 a	0.19±3.17 a	0.47±7.44 a
Sig.		*	N.S	N.S	N.S

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