Study of polymorphism of blood Albumin protein in Iraqi Buffalo

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Abstract

The study included the collection of blood samples from 80 Iraqi buffaloes in Basra Governorate. To study the genetic polymorphism of the protein albumin in the blood and the distribution of these genes by using the electrophoresis technique with polyacrylamide gel.Six genotypes were found for the blood protein albumin: AA, AC, AB, BB, BC and CC. Three alleles A, B and C are responsible for them. They are inherited according to Mendelian genetic laws and according to co-dominance. The frequency of the gene A, B and Cwere 0.43, 0.31, and 0.26 respectively. The frequency of the genotypes AA (0.255), AC (0.204), BB (0.173), AB (0.153), BC (0.112) and CC (0.102). The value of the observed and expected heterozygosity ratio was 0.469 and 0.6538, respectively. While the Fis for the A, B and C alleles was 0.229, 0.375 and 0.178, respectively. It is concluded from this study that there is a high genetic polymorphism of the albumin gene in Iraqi buffaloes.

Key words: Iraqi buffalo, electric migration, albumin protein.

Introduction

Albumin is characterized by its ability to bind with many compounds and this ability inhibits the action of some substances that cause poisoning of the body as well as its role in regulating the distribution of fluids outside cells through its effect on osmotic pressure. In regulating the acid-base balance of the blood (Melvin, 1970). It is one of the main proteins in the blood plasma and it is of special importance because of its high percentage, so it is the most studied blood protein in humans and animals, especially farm animals (Hrinca*et al*., 2008).The proportion of albumin in normal animals is about 40-60% of the total protein in the plasma and it works in the body as a storehouse of amino acids for tissues. Albumin is divided into three parts: pre-albumin, albumin, and post-albumin (Katusumato*et al*., 1998).

Genetic polymorphismsof albums were studied in Indian, Bulgarian and Italian river buffaloes, three genotypes were found responsible for alleles A and B (Kanna *et al*, 1968; Makavey, 1970; Masine *et al*, 1971).The study of Takashi et al (1980) found two alleles for this protein in the Malaysian and Philippine River and swamp buffaloes (A and B) (A and C), respectively, and the resulting of cross swamp and Philippine River buffaloes found three alleles (A, B, C).Tan et al (1993) found, during his study of river buffaloes, the presence of alleles (A and B) for the protein albumin, and Ahmed (1997) through his study of this protein in Egyptian buffaloes found the presence of the two alleles, (A andB). The albumin protein gene can be considered as a genetic marker for the reproductive state in buffaloes because there is a link between the state of the ovaries and the albumin protein, and animals with a high frequency of B allele have their ovaries more susceptible to infection (Wahid and

Magdy, 2017).Through several studies, the genetic sites of blood proteins were used as genetic markers to assess the reproductive efficiency of this animal (Zaabal and Ahmad, 2008, Alim *et al*; 2011, Wariah*et al*, 2015). Yuanita*et al* (2017) found three alleles (A, B, C) for albumin protein in Philippine buffalo in South Sumatra.Research has indicated that this protein is inherited according to Mendel's law of co-dominance. The genetic polymorphisms of blood proteins in river buffaloes were used to determine the types of breeds and the genetic distance between them, and was the first to use it was Giri and Pila (1956).In recent years, the genetic polymorphisms of DNA sequencing has been used to obtain genetic markers related to the economic traits of Friesian in Egypt (Zaabal and Ahmed, 2008, Elnahas *et al*., 2017), where it is possible to improve reproductive traits through the use of genotypes as markers for the selection process of healthy buffaloes.

The current study aims to reveal the genetic polymorphisms of the albumin gene and the genetic description of the buffalo breed in Iraq.

Materials and Methods

Animals

Eighty samples of buffalo blood of different ages were obtained, and 5 ml for each sample were collected from the jugular vein. Blood samples were placed in EDTA-free tubes to obtain blood serum and the possibility of detecting the genotypes of the albumin gene.

Electrophoresis

Electrophoresis was carried out using the PAGE method using a vertical electrophoresis device supplied by the British company Cleaver Scientific Ltd. Separation gel and staking gel solutions were prepared for the purpose of separating blood proteins.As shown in Table (1), adding the solutions is sequentially and sequentially with care to ensure that no air bubbles form and the solutions are mixed using a glass column so that the complete polymerization of the separation gel takes place after 30-40 minutes after addition.Then prepare the second gel and the mixture must be added and the modeling comb should be placed on top of the gel and after pouring is completed expose the gel to a fluorescent light 2-3 cm away from the source.When confirming the complete polymerization of the focus gel after 20-40 minutes, the comb is raised and the amount of water in the holes withdrawn using a small syringe (Micro Syringe).

Table (1) Electrophoresistechnique for albumin protein of Iraqi buffalo blood (pH 8.9)

Separation gel			Concentration gel			
Solution number	Amount of solution / 100 ml	The amount of solution in the mixture	solution number	Amount of solution / 100 ml	The amount of solution in the mixture	Amount of solution / 1000 ml
1	hydrochloric acid 48.0 ml	1 part	4	hydrated phosphate (25.6 ml)	1part	tris 6.0 g
	tris 36.6 g			tris 5.7 g		

	temed 0.5 ml			temed1.0 ml		
2	acrylamide 30.0 g	two parts	5	acrylamide 15.0 g	two parts	glycine28.8 g
	methylene bis acrylamide 1.0 g			methylene bis acrylamide 2.5 g		
3	ammonium per sulphate 0.28 g	four parts	6	riboflavin 4.0 mg	1 part	
	Urea 9 mol		7	sucrose 40.0 g	four parts	

Electrophoresis samples analysis

The electrophoresis samples were analyzed with a Gel Documentation device manufactured by Cleaver Scientific Ltd in the UK in 2009 and equipped with an application program (SoftWare) from the University of Cambridge, UK.

Statistical Analysis

The popgene program (version1.3, 1997) was used to analyze the results, estimate gene duplication, genotypes and some studied genetic parameters.

Results and Discussion

Allele and genotype frequencies

It appeared through electrophoresis assays that there are some bands of this protein, and this indicates that the protein is genetically formed into more than one genetic structure.There were six genotypes (AA, AB, BB, AC, CB and CC) responsible for three alleles (A, B). And C) these alleles are inherited according to Mendelian genetic laws in line with the phenomenon of co-dominance.The frequency of the A allele was (0.43), the B allele was (0.31) and the C allele was (0.26), while the value of chi-square was (17.5), this indicates that the animals are not subject to selection, which is the normal condition of the animal population (Table, 2).These results are in agreement with the study of Takashi et al, (1980), where they found six genotypes in the Philippine hybrid buffalo responsible for three alleles (X, B, A)) and the frequency of the A allele was higher than 0.532. They also found three genotypes in the Malaysian river buffalo and Philippine swamps are responsible for alleles (B, A)) and (C, A) respectively.

Table (2) The distribution of the genotypes of albumin protein in Iraqi buffaloes

Genotypes	Genotype Frequency	Chi-Squares	Allele Frequency		
			A	B	C
AA	0.255	17.5*	0.43	0.31	0.26
AC	0.204				

BB	0.173		
AB	0.153		
BC	0.112		
CC	0.102		

- **Significant at p< 0.05 level**

Our results agreed with the findings of Yuanita et al; (2017) in his study of the Philippine buffalo, and three alleles (C, B, A) were obtained, and it differed on the repetition level. The repetition level was 1 for each allele, where the heterozygosity is zero.The results did not agree with Ahmed, (1997) Wahid and Magdy, (2017), where they found the two alleles (B and A) and that the A allele is dominant. This difference is natural between these countries and Iraq, and may be due to the genetic distance between the Iraqi buffalo and the buffalo of those countries, where historical sources confirm the existence of the Iraqi buffalo thousands of years ago (Cockrill, 1974).

Number of observed and predicted alleles and Shannon's index

The number of observed and effective alleles was 3.00 and 2.86, respectively, Shannon's index reached 1.075 (Table, 3). The Shannon index has recently been assumed as a standard procedure for assessing diversity at those levels (Caggiotti et al., 2018; Sherwin, 2018).

Table (3) The number of observed and effective alleles for the protein albumin in the blood.

Gene	Number	Number of observed alleles	Number of effective alleles	Shannon index
albumin	196	3.0	2.86	1.075

Heterozygosity

The observed and expected heterozygosity was 0.4694 and 0.6538 (Table, 4). Most of the criteria that have been developed to measure genetic diversity between and within populations are the proportion of observed and expected heterozygosity (Ho, He), the number of alleles in one locus, the content of genetic polymorphisms, genetic distance or construct the phylogenetic tree.The goal is to contribute to the development of a permanent plan for genetic improvement for any species of agricultural animal.

Table (4) The observed and expected mixing ratio of albumin protein in the blood.

Gene	Observed heterozygosity	Expected heterozygosity	Nei **	Mean heterozygosity
albumin	0.4694	0.6538	0.6505	0.6505

It is clear from the general results of the current study and previous studies that there is a high genetic variance between individuals within the same clan with a low variance between different breeds and a high level of inbreeding coefficient (Sheriff and Alemayehu, 2018).The

observed ratios of observed and expected heterozygosity is the ratios resulting from balanced populations (Ojango et al., 2011),it is one of the most important criteria that is widely used to measure genetic diversity in breeds (Toro et al., 2009). Various studies show that the proportion of heterozygosity greater than 50% is appropriate for studies concerned with genetic diversity (Dorji et al., 2012; Davila et al., 2009).But in several related studies reviewed by Sheriff and Alemayehu (2018) showed that the values of heterozygosity in the Indian, Chinese, Chilean, Kenyan and Nigerian breeds are less than 50% during the genetic analysis of the data of these breeds.The severity of the decrease in heterozygosity may be due to small population size, high selection intensity, closed population, high inbreeding, and low or no migration of genotypes to the population under study (Canon et al., 2006).In the case of high rates of heterozygosity in populations reflects the presence of high genetic variance between members of one population. Most of the observed heterozygosity are genetically close to the expected ratios, but they are usually less valuable in most breeds, and the genetic loci are stable, alleles are repeated, and the populations are under Hardy equilibrium (Araujo et al., 2006).

Fixation index (Fis)

The Fis values for the A, B, and Calleles were 0.229, 0.375, and 0.178, respectively.

(Table, 5).The fixation index shows the decrease in the heterozygosity ratio. Since the decrease is clear in the proportion of heterozygosity, especially the B allele, most members of the population show a kind of inbreeding and an increase in the frequency of pure genotypes (wright, 1978).

Table (5). Fixation index of the albumin gene.

Allele	Fixation index (Fis)	Fit
A	0.229	0.2784
B	0.3755	
C	0.1784	

Neutrality

The observed neutrality value was 0.349, with an average of 0.711, which is close to the highest possible value of neutrality 0.969 (Table 6).Neutrality tests are used to test whether there is a balance in the populations. It is also used to test whether there is selection on a particular allele from a large number of alleles in a population,Statistical tests of neutrality are important tools in population genetics (Dogan and Dogan, 2017).Since the development of molecular genetics techniques has made it possible to obtain nucleotide sequences for the study of population genetics, a number of neutrality tests have been developed with the aim of facilitating interpretation of the increased volume of molecular data.Since Kimura (1968) first suggested that selectively neutral polymorphisms in neutral hypothesis testing have been

one of the main goals of the molecular genetics of populations.These tests are influenced by demographic factors. An important set of neutrality tests depends on the effect of repeating a single nucleotide polymorphism. It was suggested by Zenger (2006), Fay and Wu (2000), Fu and Li (1993), and Tajima (1989) the classic tests of neutrality, and they expressed the test with coefficients named after their names, Fay and Wu H, Fu and Li D, Tajima D and Zeng E.When these tests depend on the frequency of alleles, they are used to reveal the number of alleles on which natural selection worked (Komeliussen et al., 2013). Another benefit of the neutrality test is the knowledge of the correspondence between the observed polymorphisms of a particular gene in the DNA with the prediction of neutral evolution (Fu, 2001).

Table (6). Neutrality test for the blood albumin

Gene	No	K	Observed F	Min F	Max F	Mean	L 95	U 95
Albumin	196	3	0.3495	0.3333	0.9798	0.7115	0.3779	0.9698

It can be concluded that the Iraqi buffalo express high diversity reflected by high gene frequency of albumin genotypes, high heterozygosity.

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