



## **Genetic polymorphism of transferrin gene in Najdi sheep in Iraq**

**Talib Ahmed Jaayid**

Genetic Engineering Lab., College of Agriculture, Basrah University (UoB), Basrah 320, Iraq

### **Abstract**

The study investigated the existence of polymorphism at transferrin (Tf) locus in Najdi sheep breed. A polyacrylamide gel electrophoresis under alkaline condition was used to distinguish Najdi Tf alleles. Analysis of 35 animals revealed that all animals were polymorphic, showing many genotypes with clear biodiversity in the Tf gene. Six Tf genotypes consisting of three homozygote types (AA, BB and CC) and three heterozygote types (AB, AC and BC) were detected. These genotypes are controlled by codominant autosomal gene according to the Mendelian laws of inheritance. The highest gene frequencies were calculated as 0.47 for Tf B. Differences between expected and observed number of Tf genotypes were significant. Therefore, polymorphism of sheep tfs can be used for the identification of its offspring and its possible benefit in genetic improvement programs of domestic animals and the conservation of bio-diversity. This will also be useful in genetic improvement process by enhancing selection and breeding programs.

**Keywords:** Polymorphism; transferrin gene; Najdi sheep breed; polyacrylamide gel electrophoresis

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### **Introduction**

The polymorphism genetically determined polymorphous systems in sheep blood and the opportunities for using them as genetic markers made it possible to conduct studies relating to the breed structure, as well as the changes that had occurred in them in the processes of introduction and selection. A number of scientists have conducted research in this area (Valenta and Stratil, 1978; Csizmadia et al., 1995; Guney et al., 2003; Jaayid et al., 2011a). Transferrin (tf) belongs to the group of beta-globulins, is not only found in blood serum, but also in milk and semen. The main function of tf in an organism is its participation in iron metabolism and in immune responses (Jurecka et al., 2009). Conservation of genetic variety of strains maintained in live gene banks is a high-priority task. The term genetic polymorphism defines the fact that each protein presents two or more genetically determined phenotypes by autosomal and codominant alleles. The study of polymorphism has many uses in medicine, biological research, and law enforcement. Over the last 10-20 years, considerable interest has

developed in blood protein polymorphism as well as increasing basic knowledge on protein fractions. Tf is the most heterogeneous polymorphic blood protein in goat (Guney et al., 2003), fish (Csizmadia et al., 1995; Jaayid and Aziz, 2009; Jaayid et al., 2011b), goose (Valenta and Stratil, 1978), chicken (Vyshinsky and Muravjev, 1970) and sheep (Jaayid et al., 2011c). A total of 4 co-dominant alleles have been found in Angora goats (Guney et al., 2003). Transferrin polymorphism has been demonstrated in different breeds of goats, such as Angora goat (Elmaci, 1998) and Damascus goats (Guney et al., 2003). Since then, several reports have been published concerning the gene frequencies in this system and the possible influence of polymorphism on disease resistance (Jurecka et al., 2009), weight at birth and daily gain in sheep (Bildik and Yur, 1999; Dellal, 2002; Rahman and Konuk, 1977), wool production (Dellal, 2001), milk production in goat (Yuce and Bilgen, 2004) and genetic performance (Khaertdinov and Gataulin, 2000; Lay et al., 1971). This great group of researches studies on tf has encouraged many countries especially neighbouring countries (Lay et al., 1971; Shahrabak et al., 2010) and

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**\*Corresponding author:** Talib Ahmed Jaayid, Genetic Engineering Lab., College of Agriculture, Basrah University (UoB), Basrah 320, Iraq; E-mail: taleb1968@yahoo.com

Turkey (Bildik and Yur, 1999; Elmaci, 1998; Dellal, 2002; Kargin et al., 2003; Mert, 2003). However, studies are yet to be carried out in Iraq, thus making this study important. This study investigates the current genetic structure of sheep population in Iraq according to the transferring polymorphous genetic systems.

## Materials and Methods

### Animals

Blood samples were collected from 35 Najdi sheep breed at the Animal Farm, Hartha Research Station, College of Agriculture, Basrah University and several farms in Basrah province. Samples of whole blood were taken into 10 ml heparinized vials.

### Electrophoresis

A polyacrylamide gel electrophoresis (PAGE) of tf protein fractions was carried out on 13 cm x 22 cm x 4 mm with 24 wells according to the method developed by Khaertdinov and Gataulin (2000). After applying an output voltage of 200 volts for 10 min, the inserts were removed and the same voltage continued for a further 15 min. The output voltage was then increased to 250 volts and continued until the brown line had migrated 9 cm beyond the insert line. The gel was then removed, sliced and stained for 10 min. with 0.1% (w/v) amido black in methanol-glacial acetic acid-water (50/7/43 by vol.). The gel was destained with a solution containing methanol- glacial acetic acid-water (40/10/50 by vol.). One or two changes of this solution were made when necessary until the background gel was light blue and the stained protein components remained darkly stained. Tf content was measured by using a UVband software (UVITec limited, Cambridge, UK).

### Statistical analysis

The allele frequencies of the Tf were estimated by direct counting of the genotypes. To ascertain the differences between observed and expected genotypes frequencies, popgene, version 1.31 (Yeh et al., 1999) and Chi-square ( $\chi^2$ ) on the basis of Hardy-Weinberg equilibrium was used.

## Results and Discussion

Figures 1 and 2 showed the electrophoretal patterns of some individual Najdi protein samples. Two bands were detected when tf was run. Figure 2 showed clear heterozygous genotypes composed of two bands (lanes no. 3 and 6-8). Clear homozygous genotypes composed of only one band was observed (lanes 1, 2, 4, 5 and 9-11). This confirms the accuracy of the electrophoresis procedure. The Najdi tf types were named according to the nomenclature suggested by Irnazarow and Bialowas (1994) and Jurecka et al. (2009). The results of tf obtained showing variations in the samples of Najdi sheep are presented in Table 1. Gene frequencies were calculated by the method of gene counting as described in Khaertdinov and Gataulin (2000).

The author found little agreement between the observed and expected numbers of genotypes on the basis of the law of Hardy Weinberg ( $\chi^2 = 3.03$ ). Thus there was a balance in the animals' populations, including samples taken and this is evidence of the absence of selection programs or any genetic improvement programs. The study showed the existence of genetic polymorphism of tf in Najdi sheep breed. In Najdi sheep, more than one genotypes was discovered; AA, BB, CC, AB, AC and BC in the order of decreasing mobility concerning three alleles (A, B and C), showing Mendel's law of inheritance and co-dominance.

Transferrin generally is highly polymorphic in sheep. Csizmadia et al. (1995) discovered 20 tf genotypes (AA, BB, DD, EE, FF, GG, AB, AD, AF, AG, BD, BE, BG, DE, DF, DG, EF, EG, FG, and FH) caused by 7 alleles (A, B, D, E, F, H and G) while Jaayid et al. (2011a) discovered 6 tf genotypes (AA, DD, EE, AD, AE and DE) in native cow. Mariana et al. (2005) found 5 genotypes in cattle. However, only a small number of studies have reported the relationship of genetic variation of blood proteins with production traits of goats (Bhat, 1987; Yuce and Bilgen, 2004). The frequencies results of tf in alleles obtained showed that B allele was predominant than allele A and C (0.47, 0.31 and 0.21), respectively. Therefore, the majority of

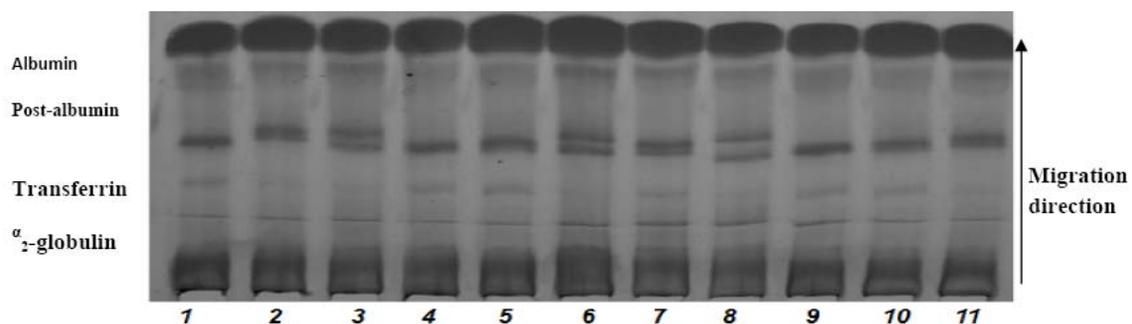


Fig. 1: Different transferrin genotypes as detected by PAGE technique in Najdi sheep breed

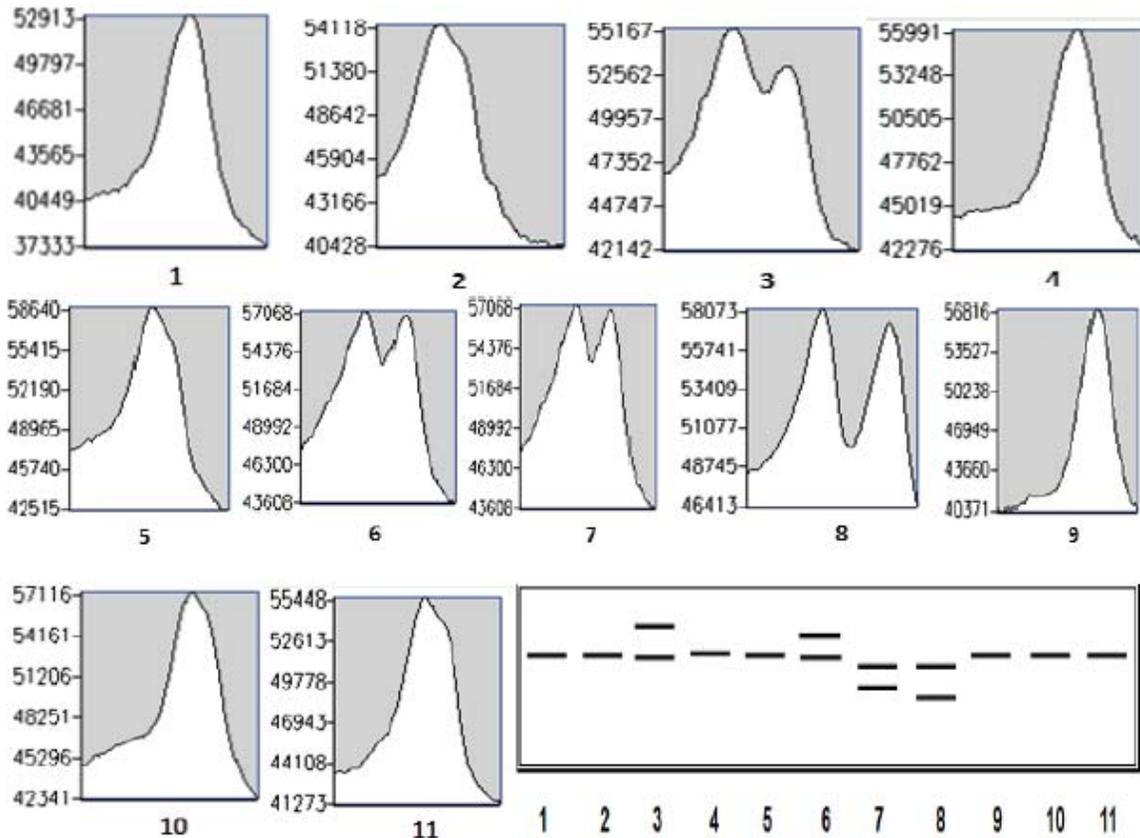


Fig. 2: Picture representation of the density of transferrin for lanes no. 1-11 in Najdi sheep breed

Table 1: Distribution of Tf genotypes and gene frequency of the Najdi sheep breed

|          | Transferrin genotypes, n= 35 |       |       |       |      |       | X <sup>2</sup><br>3.03 | Gene frequency |      |      |
|----------|------------------------------|-------|-------|-------|------|-------|------------------------|----------------|------|------|
|          | AA                           | BB    | CC    | AB    | AC   | BC    |                        | A              | B    | C    |
| Observed | 6                            | 11    | 4     | 7     | 3    | 4     |                        |                |      |      |
| Expected | 3.46                         | 7.78  | 1.61  | 10.37 | 4.71 | 7.07  |                        | 0.31           | 0.47 | 0.21 |
| %        | 17.34                        | 31.43 | 11.43 | 20.00 | 8.57 | 11.43 |                        |                |      |      |

Tf genotypes represented only AA and BB variants. These results had effects on only five genotypes. Homozygous genotypes BB and AB were predominant (31.43 and 20.00) % respectively, followed by AA, BC and AC (17.34, 11.43 and 8.57%), respectively.

Differences between expected and observed number for transferrin genotypes were not significant on the basis of the Hardy-Weinberg laws. Hence, the establishment of the fact that polymorphism of tf is genetically caused and the Najdi sheep breed populations of animals taken from the samples is in a genetic balance on that locus. This is evidence by the absence of any selection programs or genetic improvement programs.

### Conclusion

Six Tf genotypes consisting of three homozygous (AA, BB and CC) and three heterozygous genotypes (AB, AC and BC) composed of 3 alleles (A, B and C)

were detected. It may potentially help in genetic improvement programs of domestic animals and the conservation of bio-diversity. This may also be useful in genetic improvement through selection and breeding programs.

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