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Genetic polymorphisms in Iranian native poultry breeds Part III. Albumin and transferrin polymorphisms

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ABSTRACT

Albumin and transferrin polymorphisms were studied in five Iranian native chicken breeds maintained in genetic reserve Dashtyary, Lary, Marandy, Naked neck and Common breed. The frequency of albumin allele A is very low or non-existent in the breeds ranging from 0.000 to 0.031, B allele is very frequent ranging 0.917 to 0.960 and the frequency of C allele ranges between 0.020 to 0.016. The frequencies of transferrin allele A vary between breeds from 0.016 to 0.075, B has a frequency range of 0.355 to 0.523 and C allele is characterised by frequencies from 0.522 to 0.629. The heterozygosity index for transferrin regarding all breeds lies within the range of 0.478 to 0.559 and for albumin 0,077 to 0.155. The accuracy to distinguish individual chickens taking random samples is very low for albumin PD<0.27 in all breeds the accuracy is higher for transferrin PD<0.71.
(Keywords: albumin, transferrin, genetic polymorphism, native chicken)

ÖSSZEFOGLALÁS

Irán helyi baromfi fajtáinak genetikai polimorfizmusa
III. rész: Albumin és transzferrin polimorfizmus
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Az albumin és transzferrin polimorfizmusát vizsgálták a következő 5 iráni helyi tyúk fajtában, melyet genetikai tartalékként tenyésztettek: kopasznyakú, Dashtyary, Lary, Marandy és parlagi. Az albumin A frekvenciája rendkívül alacsony vagy az allél egyáltalán nincs is jelen a vizsgált állományokban (0.000-0.031), a B allél nagyon gyakori (0.917-0.960), a C frekvenciája pedig 0.020-0.016 között van. A transzferrin A allél frekvenciája 0.016-0.075, a B-é 0.355-0.523, míg a C-é 0.522-0.629 a vizsgált fajtákban. A transzferrin heterozigozitás indexe az értékelt fajtákra vonatkoztatva 0.478-0.559, az albuminé pedig 0.077-0.155 között változott. A véletlenszerűen kiválasztott mintákban a csirkék megkülönböztethetőségének pontossága az albumin esetében rendkívül alacsony (PD<0.27), míg a transzferrinnél jóval magasabb (PD<0.71).
(Kulcsszavak: albumin, transzferrin, genetikai polimorfizmus, helyi tyúkfajták)

INTRODUCTION

With the development of techniques for the characterisation of individual, populations, breeds and strains become applicable there arose the necessity to screen also native breeds of great economic importance. Polymorphism of the largest protein fraction in plasma of chicken is controlled by at least five autosomal codominant alleles (*Crawford,* 1990; *Hashigushi et al.,* 1981). Albumin plays a major role in osmotic regulation and serves a transport function for fatty acids, trace elements, drugs and calcium, (*Stryer,* 1995). Iron is transported in the plasma by transferrin, a protein that binds two ferric ions, and stored in tissue molecules of ferritin (*Stryer,* 1995). Inheritance of serum transferrin are controlled by a single autosomal locus with three co-dominant alleles, (*Ogden et al.,* 1962; *Crawford,* 1990).

MATERIALS AND METHODS

Breeds

Blood samples were collected from five different indigenous Iranian chicken breeds Naked neck, Dashtyary, Lary, Marandy and Common breed reared in the Poultry Breeding department of Animal Science Research Institute of Iran. These breeds were maintained as a gene pool in this institute. Blood was taken in citrated vacuum tubes directly from wing vein and centrifuged within half an hour after sampling at 1500 rpm for ten minutes, to separate erythrocytes and serum. Separated serum samples were kept at -20oC until electrophoresis were performed.

Phenotyping of albumin

Horizontal starch gel electrophoresis was used for phenotyping the albumin in the serum samples of Iranian chicken breeds. For preparation of the gel buffer 52 cm^3 Stock solution A (10.5 g Citric acid diluted up to 1000 cm^3 distilled water) were added to 36 cm^3 stock solution B (230 g Tris-HCl diluted up to 1 lit distilled water). 180 cm^3 of the mentioned buffer were used with 20 g hydrolysed starch for gel preparation. Electrode buffer consist of 10.5 g citric acid added to 92.75 g boric acid and 20 g NaOH, pH=8.65. For the staining of the gel after electrophoreses it cut along its thickness by the help of fine nylon wire and the upper layer removed and lower part immersed in protein staining dye such as amino black and then placed in 8% acetic acid and 40% methanol and 52% distilled water for destaining and fixation of the protein.

Phenotyping of transferrin

Horizontal polyacrilamid gel electrophoresis was used for phenotyping the transferrin in the serum samples. Gel was prepared as proposed by *Gahne et al.* (1977). The working table is as follows (*Table 1*).

TEMED has been added in to the stock solutions B1 and B2 in the amount of 30 µ l per 10 ml of the buffers. Two gel buffers used were B1 and B2. The first buffer prepared with tris-HCl 0.75M and H_2SO_4 0.54M adjusted for pH=7.78. The second buffer, B2 which used for stacking gel consist of Tris-HCl 105M with H_2SO_4 0.54M adjusted for pH=8.98. Electrode buffer contains 40 g Tris-HCl 0.2M and 20 g NaOH 0.1N adjusted for pH=9.3.

Table 1

Working table of polyacrylamide gel preparation

No.	Acrylamide %(1)	A sol. ml	B_1 sol. ml	B_2 sol. ml	Distilled water ml(2)	C sol. ml	Gel height (cm)(3)
l	11.11	12.5	10	-	4	9.5	14
2	4	1.5	-	3	4.5	4	4
3	8	2	-	2	2	2	2

1. táblázat: A poliakrilamid gél előállítás munkamenete

Akrilamid(1), Desztillált víz(2), A gél hossza(3)

Staining procedures were performed when electrophoresis was completed after the albumin zone reached to 2-3 cm in the resolution gel. The gel was placed in solution containing 2 g of Commassie brillant blue for 30 minutes. Then the gel was immersed in solution containing 20% methanol, 7% acetic acid for destaining and fixation of the proteins.

RESULTS AND DISCUSSION

Albumin

Three alleles namely A, B and C have been found during phenotyping of the samples. *Table 2 and 3* show genotypic and allelic frequencies of albumin in different breeds. Six genotypes were found from the combination of three alleles in this system. As it can be seen from the *Table 3* that allele A has lowest frequency, allele B is the most frequent allele and allele C is being intermediate in all breeds except in Dashtyary which C allele is relatively more frequent than allele A. In Naked neck and Common breed the frequencies of A allele is nil. BB genotype has highest frequency in Dashtyary breed while AA, AB and BC were relatively rare and the frequencies of CC and AC are nil. In Lary and Marandy breed, AA, CC and AC genotypes were absent but BB genotype showed the highest frequency. Genotypes AA and CC were absent also in Naked neck and Common breeds. Where genotypes AB and AC had not been found in these two breeds. BB genotype is the most frequent and BC has the lowest frequency.

Most of the birds were homozygote for BB genotype followed by BC and AB heterozygote. These result is in agreement with those published by *Okada et al.* (1988) screening native populations in Bangladesh. It is of interest to note that similar results were found in Leghorns, Rhode Island and Sussex strains by *Mc Indoe* and *Ogden et al.* in 1962.

Transferrin

In transferrin system three alleles have been observed. Allelic and genotypic frequencies of different transferrin variants in serum sample of Iranian indigenous chicken breeds are presented in *Tables 4 and 5.* The allele C is the most frequent allele which is followed by B and A allele respectively in different breeds. Genotypic frequencies of AA, BB and AC in Dashtyary and Lary are nil, while BC genotype has the highest frequency, but the genotypes CC and AB have smaller frequencies respectively after BB.

In Marandy and Naked neck genotypes AA and BB are absent. The frequency of BC is high in Marandy, in which the frequencies of CC, AB and AC are very small. In Naked neck the frequencies of CC, AC and AB genotypes are low. In Common breed AA genotype has not been observed in this study, but BC has been observed with highest frequency. CC, AC, AB and BB genotypes have been observed with small frequencies in the serum samples of this breed.

The AA and BB genotypes were absent in all breeds except in Common breed that BB genotype was observed in 4 birds. The most frequent genotype was BC followed by CC, AB and AC respectively. Data show that the most frequent allele was C among all breeds and A allele had lowest frequency. These results were not in agreement with the results of *Okada et al.* (1988), who reported that the most frequent allele in their study was B. He also stated that B allele was ranging from 0.85 to 0.5 in native chicken in Bangladesh. *Stratil* (1968) and *Ogden et al.* (1962) stated the frequency of B allele was highest in strains under their study (in Stratil study the strain was Light Sussex, White Cornish, White Leghorn, Partridge Leghorn and some of their crosses and Ogden et al screened the strains of White and Black Leghorns and also Rhode Island Red and Light Sussex).

Table 2

Genotype frequency in Albumin system

Breeds(1)	n	Genotype frequency(2)					
		AA	AB	BB	BC	CC	AC
Dashtyary	49	0.020	0.020	0.920	0.040	0.000	0.000
Lary	54	0.000	0.040	0.830	0.130	0.000	0.000
Marandy	62	0.000	0.020	0.920	0.060	0.000	0.000
Naked-neck	66	0.000	0.000	0.850	0.150	0.000	0.000
Common breed	67	0.000	0.000	0.090	0.100	0.000	0.000

2. táblázat: Az albumin rendszer genotípusos frekvenciái

Fajták(1), Genotípusos frekvencia(2)

Table 3

Frequency of Albumin alleles in different breeds

Breeds(1)	n	Gene frequency(2)		
		A	B	C
Dashtyary	49	0.031	0.949	0.020
Lary	54	0.019	0.917	0.065
Marandy	62	0.008	0.960	0.032
Naked-neck	66	0.000	0.924	0.076
Common breed	67	0.000	0.948	0.052

3. táblázat: A különböző fajták albumin alléljeinek frekvenciái

Fajták(1), Genotípusos frekvencia(2)

Table 4

Frequency of genotypes of the Transferrin system

Breeds(1)	n	Genotype frequency(2)					
		AA	AB	BB	BC	CC	AC
Dashtyary	49	0.000	0.060	0.000	0.820	0.120	0.000
Lary	54	0.000	0.040	0.000	0.780	0.190	0.000
Marandy	62	0.000	0.020	0.000	0.690	0.270	0.020
Naked-neck	66	0.000	0.030	0.000	0.830	0.080	0.060
Common breed	67	0.000	0.060	0.030	0.690	0.130	0.090

4. táblázat: A transzferrin rendszer genotípusos frekvenciái

Fajták(1), Genotípusos frekvencia(2)

Table 5

Frequency of Transferrin alleles in different breeds

Breeds(1)	n	Gene frequency(2)		
		A	B	C
Dashtyary	49	0.031	0.439	0.531
Lary	54	0.019	0.407	0.574
Marandy	62	0.016	0.355	0.629
Naked-neck	66	0.045	0.432	0.523
Common breed	67	0.075	0.523	0.522

5. táblázat: A különböző fajták transzferrin alléljeinek frekvenciái

Fajták(1), Genotípusos frekvencia(2)

Table 6

Heterozygosity index and the probability of distinguishing one individual from the other (PD)

Breeds(3)	Heterozygosity index(1)		PD(2)	
	Albumin	Transferrin	Albumin	Transferrin
Dashtyary	0.095	0.525	0.184	0.665
Lary	0.155	0.504	0.278	0.644
Marandy	0.077	0.478	0.147	0.627
Naked-neck	0.150	0.538	0.250	0.683
Common breed	0.099	0.559	0.183	0.712

4. táblázat: Az egyed megkülönböztetésének (PD) heterozigozitás indexe és valószínűsége

Heterozigozitás index(1), Valószínűség(2), Fajták(3)

Heterozygosity index *Table 6* shows that in albumin system compare to transferrin the heterozygosity is much lower and it is ranging from 0.077 for Marandy to 0.155 in Lary. Transferrin system has more heterozygosity, ranging from 0.559 to 0.478. It shows that more or less in all breeds there is almost the same heterozygosity.

The accuracy of distinguishing chickens of one breed from other taking random samples is low for albumin and transferrin (ranging from 0.278 in Lary breed to 0.147 in Marandy breed and in transferrin it is ranging from 0.712 in the Common breed to 0.627 in Marandy breed) respectively. For a more accurate separation additional polymorphic loci are needed to be typed.

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