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Genetic polymorphisms in Iranian native poultry breeds Part II. Isoenzyme polymorphisms with special reference to alkaline phosphatase and esterase

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ABSTRACT

Esterase and alkaline phosphatase polymorphisms were studied in five Iranian native chicken breeds maintained in genetic reserve: Naked Neck, Dashtyary, Lary, Marandy and Common breed. The frequency of esterase allele A is ranging from 0.50 to 0.19, that of allele B from 0.79 to 0.49 and C from 0.14 to 0.02 in the breeds studied. The genotypic frequencies of alkaline phosphatase AA ranged from 0.481 to 0.315 that of genotype aa from 0.648 till 0.519 for the breeds respectively. No electrophoretic band was found in the Dashtyary and Common breed. The gene frequencies of allele A ranged from 0.279-0.176 that of gene a ranged from 0.823-0.720. The heterozygosity index range for esterase was 0.579-0.344 and for alkaline phosphatase 0.599-0.480. The accuracy to distinguish chickens from one breed from the other taking random samples is low both for esterase loci PD 0.75 to 0.52 and alkaline phosphatase loci PD 0.67 to 0.62, for more accurate separation more polymorphic loci are needed to be typed.
(Keywords: alkaline phosphatase, esterase, genetic polymorphism, native chicken)

ÖSSZEFOGLALÁS

Irán helyi baromfi fajtáinak genetikai polimorfizmusa
II. rész: Izoenzim polimorfizmus különös tekintettel az alkalikus foszfatázra és észterázra

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Az alkalikus foszfatáz és észteráz polimorfizmusát vizsgálták a következő 5 iráni helyi tyúk fajtában melyet genetikai tartalékként tenyésztettek: kopasznyakú, Dashtyary, Lary, Marandy és parlagi. Az észteráz A allél frekvenciája 0.50-0.19, a B-é 0.79-0.49, a C-é pedig 0.14-0.02 között változott a vizsgált állományokban. Az alkalikus foszfatáz AA genotípusos frekvenciája 0.481-0.315, az aa genotípusé pedig 0.648-0.519 a különböző fajtákban. Nem találtak elektroforézises sávot a Dashtyary és a parlagi állományokban. Az A allél génfrekvenciája 0.79-0.176, az a allélé 0.823-0.720 között alakult. Az észteráz heterozigozitás indexe 0.579-0.344, az alkalikus foszfatázé pedig 0.599-0.480. A véletlenszerűen kiválasztott mintákban az egyik fajtájú csirke másik fajtájútól történő megkülönböztethetőségének pontossága mind az észteráz locus PD (0.75-0.52), mind az

alkalikus foszfatáz locus PD (0.67-0.62) esetében alacsony, ezért több polimorfizmus locust kell beazonosítani.
(Kulcsszavak: alkalikus foszfatáz, észteráz, genetikai polimorfizmus, helyi tyúk fajták)

INTRODUCTION

There is lack of information regarding isoenzyme polymorphisms relevant to native indigenous breeds of great economic importance in developing countries except the paper published by *Okada et al.* (1988) referring to isoenzyme polymorphisms of the native chicken breeds of Bangladesh. In this paper we present data regarding alkaline phosphatase and esterase polymorphisms in five native chicken breeds of Iran constituting the majority of the 34 million indigenous chicken population of the country.

MATERIALS AND METHODS

Breeds used: 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. These breeds were maintained as a gene pool in this institute. Determination of the isoenzyme: Blood was taken in citrated vacuum tubes directly from the 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 -20°C until electrophoresis were performed.

Phenotyping of Esterase: plasma samples were analysed by horizontal polyacrylamide gel electrophoresis according to *Gahne et al*. (1977), *Kuryl et al*. (1986). The gel consisted of two parts. The concentration of acrylamid for the first part (staking gel) was 8% and for the second part (resolution gel) was 12% with the buffer of Tris-HCl and citric acid and boric acid to adjusted for pH=9. Staining solution contained 50 mg of α-naphtyle butyrate added with 50 mg Fast blue RR salt in 0.2 M Phosphate buffer (pH=7.4). To destain the gel, it was immersed in the 5% acetic acid solution.

Phenotyping of alkaline phosphatase: Horizontal polyacrylamid gel electrophoresis was used for separation of variant in alkaline phosphatase was according to *Washburn et al.* (1980), *Tamaki and Tanabe* (1970). Preparation of gel was the same as mentioned before with 5% concentration of acrylamide gel buffer consisted of Tris-HCl , 0.188M with pH=8.8. Electrode buffer contains 8 g NaOH added to 75 g boric acid diluted up to 3 lit. distilled water pH=8.2. Staining solution was solution A (β-naphtyle sodium phosphate 0.05%) and solution B (0.097 barbital buffer with pH=9.4 containing 0.02M $MgCl_2$ with naphthanyl diazo blue B 0.05%). Barbital buffer was made by mixing 974 cm^3 of 0.1 M barbital sodium salt solution with 26 cm^3 of 0.1 N HCl. The plasma was incubated for 60 minutes at 37°C with the substrate (solution A) and then with solution B as an inhibitor and dye coupler. For destaining the gel it was washed with distilled water and immersed in solution of 50% ethanol.

RESULTS AND DISCUSSION

Esterase

Data on esterase in all five breeds studied are summarised in *Table 1* and *2*. As shown in the tables frequency of allele A is ranging from 0.5 to 0.19, allele B from 0.79 to 0.45 and allele C from 0.14 to 0.02 in all the breeds. Therefore the most frequent allele is B

and the rarer allele is C. In Dashtyary breed the BB genotype was the most frequent with frequency of 0.65 while CC genotype was absent among the tested animals. In the Lary breed the maximum (0.39) and the minimum (0.02) genotypic frequencies were observed for AB and CC respectively. In the Marandy breed BB genotype had the maximum frequency while CC and AC had the minimum. In Naked neck the frequency of BB and BC genotypes were 0.42 and 0.015 respectively. In the Common breed BC and CC Genotype was not observed but AA with frequency of 0.34 was the most frequent genotype.

The gene production of esterase is controlled by three co-dominant alleles in an autosomal locus (*Hashigushi et al.*, 1968; *Okada and Sasaki*, 1970; *Okada*, 1973; *Grunder*, 1968). *Table 1* showed the frequency of different alleles in esterase system in the breeds under study. Allele B is predominant among breeds except Common breed in which allele A is predominant. These data are not in agreement with the data of *Grunder* (1971), who reported the B allele is infrequent among the egg strains. However in meat type strains the frequency of B allele was high as stated by *Kuryle et al*. (1986). Our observation for C allele that had the lowest frequency among breeds is in agreement with the result of *Grunder* (1971). *Okada* (1988) reported that C allele exists in only two out of ten native breeds in Bangladesh and the most frequent allele was B and its frequency ranged from 0.5 to 0.85.

Number of AA genotype in samples of Dashtyary and Marandy breeds were only 3 and 7 respectively, while it was more frequent in the other breeds tested. The CC genotype was not found in Dashtyary and Common breeds. Genotype BB was most frequent among Naked-neck and Marandy breeds. The frequency of AB genotype was also high in these two breeds. As these breeds are rather egg type, then our data are in agreement with those published by *Grunder and Merrit* (1977) who performed tests on Ottawa Meat Control Stains and their crosses.

The BC genotype in Marandy and Lary has high frequency. *Grunder et al.* (1970) and *Kuryl et al.* (1986), showed that the esterase activity was highest in the BB genotype, intermediate in AA genotype and lowest in CC genotype. In our data the genotype BB was the most frequent. It can be said that in average the activity of esterase is high among the breeds tested.

Alkaline Phosphatase

Alkaline Phosphatase sources are in bacteria, fungi, green algae, plants protozoa, invertebrates and vertebrates. Alkaline phosphates act on orthophosphoric monoester and change it to alcohol and orthophosphate (*Gennady and Mancheko*, 1994). Inheritance of alkaline phosphates isozymes determined by a single autosomal dominant gene named as A which is allelic to a recessive gene responsible for the slow type named as a by *Tamaki and Tanabe*, (1970); *Law and Munro*, (1965) and *Wilcox*, (1966). Two different alleles which are controlling the variation of alkaline phosphatase in chicken breeds tested in this study are alkaline phosphatase A and a. Genotypic and allelic frequencies are presented in *Table 3*.

Allele frequencies as well as the frequencies of heterozygote and dominant homozygote types have been estimated on the basis of the Hardy-Weinberg formulae assuming the breeds are in equilibrium for the genes controlling this enzyme. *Table 3* shows that frequencies of a allele is higher than A. In some birds also no banding has been observed except in the Dashtyary breed. The genotypic frequency of aa is relatively high in all breeds.

Table 1

Frequency of Esterase genotype in Iranian chicken breeds

Breeds(1)	n	Genotype frequency(2)					
		AA	BB	CC	AB	AC	BC
Dashtyary	49	0.061	0.653	0.000	0.245	0.020	0.020
Lary	54	0.241	0.204	0.019	0.389	0.037	0.111
Marandy	62	0.113	0.323	0.032	0.306	0.032	0.194
Naked-neck	66	0.167	0.424	0.030	0.318	0.045	0.015
Common breed	67	0.343	0.328	0.000	0.269	0.060	0.000

1. táblázat: Iráni csirke fajták észteráz genotípusos frekvenciái

Fajták(1), Genotípusos frekvencia(2)

Table 2

Frequency of Esterase alleles in different breeds

Breeds(1)	n	Gene frequency(2)		
		A	B	C
Dashtyary	49	0.194	0.786	0.020
Lary	54	0.450	0.450	0.093
Marandy	62	0.282	0.573	0.145
Naked-neck	66	0.348	0.591	0.061
Common breed	67	0.507	0.463	0.030

2. táblázat: Az észteráz allélek frekvenciái különböző fajtákban

Fajták(1), Génfrekvencia(2)

Table 3

Genotype and gene frequencies of Alkaline Phosphates system in Iranian native breeds

Breeds(1)	n	Genotype frequency(2)			Gene frequency(3)	
		AA	aa	no band	A	a
Dashtyary	49	0.429	0.571	0.000	0.244	0.755
Lary	54	0.315	0.648	0.037	0.176	0.823
Marandy	62	0.387	0.597	0.016	0.227	0.772
Naked-neck	66	0.333	0.621	0.045	0.211	0.788
Common breed	77	0.481	0.519	0.000	0.279	0.720

Genotypic and gene frequencies were estimated on the basis of Hardy-Weinberg formula. (*A genotípusos és génfrekvenciákat a Hardy-Weinberg képlet alapján számolták.)*

3. táblázat: Az alkalikus foszfatáz rendszer genotípusos és génfrekvenciái az iráni fajtákban

Fajták(1), Genotípusos frekvencia(2) Génfrekvencia(3)

From the *Table 3* it can be seen that gene frequency for A allele is less than a allele among all the breeds. Allele A is ranging from 0.176 in Lary to 0.279 in Common breed. These ranges are similar to those reported by *Wilcox* (1966), who reported that the low frequency for A allele 0.3 for random bred control line of White Leghorn which was developed in Cornell University and, *Okada et al*. (1988) reported 0.072 to 0.225 for A allele in indigenous breeds of Bangladesh, but is not in agreement with observation of *Tamaki* and *Tanabe* (1970) according to whom allele frequency for A allele was 0.64 and for a allele is 0.36 for White Plymouth Rocks maintained at the National Institute of Animal Industry of Japan *Wilcox* (1966) found some birds in White Leghorns with no electrophoretic band. We also found some birds that showed no electrophoretic band.

Genotypic frequency of aa was ranging from 0.519 in Common breed to 0.648 in Lary to 0.52 in Common breed. The mean frequency was in agreement with the result of *Tamaki and Tanabe* (1970) who reported the frequency of 49.3 for aa genotype in White Plymouth Rock. Heterozygosity indexes based on alkaline phosphatase and esterase are shown in *Table 4*. This index for esterase locus is ranging from 0.579 in Lary to 0.344 in Dashtyary and for alkaline phosphatase 0.599 in Common breed to 0.480 in the Lary breed.

Table 4

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

Breeds(3)	Heterozygosity index(1)		PD(2)	
	ESTERASE(4)	ALKALINE PHOSPHATASE(5)	ESTERASE	ALKALINE PHOSPHATASE
Dashtyary	0.3446	0.4898	0.5256	0.6197
Lary	0.5797	0.4808	0.7315	0.6472
Marandy	0.5714	0.4940	0.7469	0.6373
Naked-neck	0.5257	0.5030	0.6867	0.6672
Common breed	0.5275	0.5992	0.6656	0.6246

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

Heterozigozitás index(1), Valószínűség(2), Fajták(3), Észteráz(4), Alkali foszfatáz(5)

The accuracy of distinguishing chickens of one breed from the other taking random samples is low for alkaline phosphatase and esterase (ranging from 0.7469 to 0.5256 in Esterase loci and 0.6672 to 0.6197 for alkaline phosphatase) respectively. For a more accurate separation additional polymorphic loci are needed to be typed.

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