Arylesterase, Ceruloplasmin and Amylase Types in Turkish Sheep Breeds

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Abstract: Polymorphisms of three biochemical systems (arylesterase, ceruloplasmin and amylase) were examined in the blood of five Turkish sheep breeds using starch gel electrophoresis. This is the first study on ceruloplasmin and amylase types on sheep breeds in Turkey. Totally 216 blood samples were analyzed. Both ceruloplasmin and amylase loci were found to be monomorphic while Arylesterase system was polymorphic among all analyzed samples. Differences between expected and observed number of arylesterase genotypes were nonsignificant.

Key words: Sheep, arylesterase, ceruloplasmin, amylase

Türkiye Koyun Irklarında Arilesteraz, Seruloplazmin ve Amilaz Tipleri

Özet: Bu çalışmada beş koyun ırkına ait toplam 216 örnekte arilesteraz, seruloplazmin ve amilaz tipleri yatay nişasta jel elektroforezi kullanılarak araştırılmıştır. Elde edilen bulgulara göre Türkiye koyun ırklarında ilk kez çalışılan seruloplazmin ve amilaz sistemleri monomorf olarak saptanırken, arilesteraz sisteminde polimorfizm gözlenmiştir. Arilesteraz lokusu bakımından beklenen ve gözlenen değerler arasındaki farklılık ise önemsiz bulunmuştur.

Anahtar sözcükler: Koyun, arilesteraz, seruloplazmin, amilaz

Introduction

Blood proteins have been used widely to characterize animal population because of their polymorphism and simple mode inheritance. Thus, blood protein polymorphisms have been studied in different species by using starch gel electrophoresis. They are useful in studies of basic genetics, population dynamics, clinical diagnosis and in gene mapping (Balakrishnan and Goswami, 1991). In addition, they can also be used for kinship analysis and as breed markers, a function of great importance, especially for the preservation of breeds (Igarashi et al., 2000).

There are great numbers of the studies in which blood protein polymorphism was demonstrated among different sheep breeds in all over the world. Although ceruloplasmin and amylase polymorphisms have been investigated on several species (Mazumder and Spooner, 1970; Tunon et al., 1989; Wussow and Plischke, 1990; Chung et al., 1990; Annunziata and Iorio, 1990; Wu et al., 1999; Elmaci and Asal, 2000; Elmaci, 2001), there are few studies on these loci in sheep breeds. The objective of the present study was to determine the arylesterase, ceruloplasmin and amylase types among five Turkish sheep breed populations.

Materials and methods

Samples

A total 216 sheep blood samples obtained by jugular venipuncture was collected from five different sheep breeds of Turkey; Dağlıç (20), Gökçeada (35), Sakız (54), Kıvırcık (56) and Merinos (51). The blood samples were collected in test tubes that contain heparin as anticoagulant and they were separated into plasma and red cells by centrifugation. Plasma samples were stored at –20 ºC until further the electrophoretic studies.

Electrophoresis and Staining Procedure

Plasma Arylesterase (EsA) phenotypes have been determined using the quick tube test developed by Tucker et. al. (1967). Using the quick tube test, after the addition of the substrate, within seconds the EsA-positive samples develop a deep yellow-brown coloration, whereas the EsA-negative turned dark green after standing for thirty or more seconds.

Horizontal starch gel electrophoresis in discontinuous buffer system and staining for ceruloplasmin and amylase were carried out as described previously (Annunziata and Iorio, 1990). The content of gel buffer solution was 15.2 mM Tris , 4.379 mM citric acid (pH:7.2); and the content of electrode buffer was 0.191 M boric acid and 0.039 M lithium hydroxide (pH: 8.0). The gel was prepared using 11% hydrolysed starch for electrophoresis. After electrophoresis gel was stained at 37 ºC for 1hr in a solution that contains 0.5 g p-phenylenediamine dihydrochloride and 2.46 g sodium acetate dissolved in 300 ml of distilled water (pH was adjusted to 5.7 with acetic acid). The ceruloplasmin zones appeared as dark blue bands on the gel. After Cp bands were recorded, the gel was left to overnight at 37 ºC and then immersed into destaining solution (5:5:1, distilled water: ethanol: acetic acid) at 4 ºC for approximately 4 hr. The gel was left in distilled water at room temperature for 2 hr, and then, on a glass plate for 1 hr at 37 ºC. The amylase variants were then read against light as distinct transparent bands.

Allele Frequency Estimation

Gene frequencies at locus EsA was calculated by using square-root method. Genetic equilibrium was checked using χ^2 (chi-square) method.

Results and discussion

Arylesterase

Arylesterase polymorphism has been demonstrated in most sheep breeds (Table 1). Table 1 shows that common arylesterase allele is EsA^- in all sheep breeds examined. In this study, the EsA locus was found to be polymorphic in five Turkish breeds studied (Table 2). All population was Hardy-Weinberg equilibrium. The frequencies EsA alleles observed in the present study are in agreement to the frequencies of different sheep breeds (Table 1).

Table 1. Arylesterase allele frequencies of some sheep breeds.

Breed	Country	n[a]	Allele frequencies		References
			EsA^+	EsA^-	
Kwale	Kenya	61	0.098	0.902	Mwacharo et. al., 2002
Makueni	Kenya	65	0.185	0.815	Mwacharo et. al., 2002
Siaya	Kenya	60	0.067	0.933	Mwacharo et. al., 2002
Kakamega	Kenya	77	0.364	0.636	Mwacharo et. al., 2002
Kajiado	Kenya	88	0.466	0.534	Mwacharo et. al., 2002
Merino	Kenya	40	0.450	0.550	Mwacharo et. al., 2002
Akkaraman	Turkey	96	0.330	0.670	Asal et. al. 1996
And. Merino	Turkey	123	0.470	0.530	Asal et. al. 1996
Ghezel	Iran	200	0.320	0.680	Osfoori and Fesus, 1996
Shahl	Iran	165	0.673	0.327	Osfoori and Fesus, 1996
Makoei	Iran	130	0.432	0.508	Osfoori and Fesus, 1996
Moghani	Iran	146	0.589	0.411	Osfoori and Fesus, 1996
Zel	Iran	192	0.641	0.359	Osfoori and Fesus, 1996
Karakul	Iran	124	0.500	0.500	Osfoori and Fesus, 1996
Baluchi	Iran	115	0.687	0.313	Osfoori and Fesus, 1996
Lori	Iran	175	0.360	0.640	Osfoori and Fesus, 1996
Shiraz Gray	Iran	177	0.458	0.542	Osfoori and Fesus, 1996
Sarda	Italy	414	0.058	0.942	Casati et al., 1990
Comisana	Italy	310	0.055	0.945	Casati et al., 1990
Bergamasca	Italy	199	0.042	0.958	Casati et al., 1990
Gentile di Puglia	Italy	283	0.083	0.917	Casati et al., 1990
Massese	Italy	375	0.433	0.567	Casati et al., 1990
Barbados	Texas	20	0.408	0.592	Wang et.al., 1990
Booroola	Nebraska	40	0.038	0.962	Wang et.al., 1990
Finnsheep	Nebraska	38	0.415	0.585	Wang et.al., 1990
Hampshire	Utah	41	0.037	0.963	Wang et.al., 1990
Karakul	Texas	20	0.553	0.447	Wang et.al., 1990
Rambouillet	Utah	30	0.034	0.966	Wang et.al., 1990
Rambouillet	Idaho	34	0.358	0.642	Wang et.al., 1990
Romanov	Nebraska	30	0.106	0.894	Wang et.al., 1990
St. Croix	Utah	35	0.044	0.956	Wang et.al., 1990
St. Croix	Virgin Isl.	30	0.051	0.949	Wang et.al., 1990
Columbia	Idaho	35	0.225	0.775	Wang et.al., 1990
Suffolk	Idaho	30	0.051	0.949	Wang et.al., 1990
Suffolk	Utah	31	0.139	0.861	Wang et.al., 1990
Churra	Spain	458	0.100	0.900	Ordas and Primitivo, 1986
Lacha	Spain	349	0.100	0.900	Ordas and Primitivo, 1986
Manchega	Spain	336	0.120	0.880	Ordas and Primitivo, 1986
Merino	Spain	859	0.179	0.821	Morera et al.,1983

a: sample size

Ceruloplasmin

The result of this study demonstrated the fact that ceruloplasmin was monomorphic among studied Turkish sheep breeds, because of all sample showed only one band. Although, heterogeneity in the ceruloplasmin has been reported in different species,

ceruloplasmin locus reported to be monomorphic in different sheep breeds (Manwell and Baker, 1977; Tsunoda et al., 1990; Wang et al., 1990)

Table 2. Distrubition of Arylesterase phenotypes and allele frequencies in five Turkish sheep breeds.

Breeds	Arylesterase Phenotype	Number of sheep obs.	exp.	Allelic frequency	χ^2
Dağlıç	EsA^+	15	15	EsA^+ =0.50	0.0000 n.s
(20)	EsA^-	5	5	EsA^- =0.50	
Gökçeada	EsA^+	24	24.03	EsA^+ =0.44	0.0001 n.s
(35)	EsA^-	11	10.97	EsA^- =0.56	
Sakız	EsA^+	39	38.83	EsA^+ =0.47	0.0026 n.s
(54)	EsA^-	15	15.17	EsA^- =0.53	
Kıvırcık	EsA^+	47	47.04	EsA^+ =0.60	0.0002 n.s
(56)	EsA^-	9	8.96	EsA^- =0.40	
Merinos	EsA^+	39	38.75	EsA^+ =0.51	0.0067 n.s
(51)	EsA^-	12	12.25	EsA^- =0.49	

Number in brackets indicate the sample size, n.s: not significant

Amylase

In this present study we did not observe any polymorphism for amylase since of the 216 samples showed only one zone. There are only few studies about amylase polymorphism in different sheep breeds. So far, no variation in the electrophoretic band pattern was revealed. Only single serum amylase band was observed in different sheep breeds (Ashton, 1965; Morera et al., 1983; Archibald, 1987).

Conclusion

Analysis of ceruloplasmin and amylase types among Turkish sheep breeds showed no polymorphism. However, our sample size does not represent the total population of Turkey. Thus, it is recommended that this work needs to be continued a greater number of animals and breeds.

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