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Screening for the Genetic Diversity among Local and Pure Breeds of Asiatic Chickens Using Blood Protein Polymorphisms by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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A B S T R A C T

In this study, blood samples for protein analysis were collected from chicken of pure (boiler and layer) and local breeds for genotyping through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoregrams showed that there were similarities and differences in the molecular weight (MW) of the serum proteins among the accessions. A total of 14 bands, 9 were polymorphic and 6 were monomorphic. About 64.28 percent polymorphism was observed among the 12 accession during resolution of the proteins. Dendrogram developed using Jaccard's similarity coefficient by using UPGMA based Shan cluster revealed that the accession were clearly separated from one another and range of genetic similarity among the four strains was 69 percent to 100 percent. Dendrogram classified the 12 chicken accessions into three groups. First group was comprised of 4 accessions from Aseel (AS), White Leghorn (WL), Red Rhode Island-2 (RDD) and Red Rhode Island-1 (RCC). Second group had five accessions: White Plymouth Rock-2 (BDD), White Plymouth Rock-1 (BCC), White Cornish-1(BAA), Barred Plymouth Rock (RBB) and White Cornish-2 (BBB). Third group is consisting of three accessions with two closely related to White Cornish (SK) and Kadaknath (KD) and one distantly Red Cornish (RAA). It can be concluded that blood serum protein found useful in taxonomic study.

Keywords: Protein; Serum; SDS-PAGE; Polymorphisms; Banding Pattern; Chicken

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INTRODUCTION

In the past, the identification of chicken species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes often have been used as an aid in the species identification (Verimli *et al*., 2000a&b; Yigit *et al*., 2001) through electrophoresis in different species (Focant *et al*., 1981; Miyazaki *et al*., 1998; Brand and Ryckman., 1969; Khan and Gadru, 1988; Komagata *et al*., 1991; Li, 1991; Theophilus and Rao, 1998; Shahin, 1999; Mohamed *et al*., 2001). Recent studies indicated that serum proteins can present taxonomic values. In this study our emphasis was to establish the taxonomic value of serum protein for chicken Species using SDS-Page protein profile.

MATERIALS AND METHODS

Local Indian chickens were collected from different villages of India. Pure boiler and pure layers were collected from INDUS Poultry breeders Hyderabad India. Whole blood samples were taken to the laboratory for analysis. Serum was separated from the whole blood using centrifugation. The supernatant serum protein was carefully transferred into a clean 2 ml eppendorf micro tube and stored at −20°C. The sera were mixed with a sample buffer as described by Laemmli (1970). The final concentration of sera was adjusted to 5%. Samples were boiled for 3 min and stored at -70˚C until electrophoresis. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) analysis was carried out using the Bio-Rad Mini Protean II (10 ml capacity) for proteins. SDS-polyacrylamide denaturing gels (separating gels 7.5% and stacking gels 4%) were prepared as described by Sambrook *et al.* (1989). In all the samples, total protein was estimated according to Lowry *et al*. (1951). SDS-PAGE was performed according to Laemmli *et al.* (1970). Proteins were fractionated in all the samples by SDS-PAGE using 10% acrylamide gel and Tris-glycine (pH 8.3) as tank buffer. Gels were run at a current of 3 mA/sample till the dye reached the other end. Gels were fixed in 10% TCA and stained with 0.5% commassiie brilliant blue stain for 1 h and de-stained with 7% acetic acid and 10% methanol to remove the background stain and photographed when distaining was completed.

This method of analysis is a discontinuous buffer system, which means that the buffer in the reservoir is of a different pH and ionic strength from the buffer used to cast the gel. The stacking gel contained distilled water (6.1 ml), 0.5 M Tris-HCl at pH of 6.8 (2.5 ml), 10 percent SDS (100 µl), acrylamide/bisacrylamide solution (1.3 µl), 10 percent ammonium tetraoxosulphate (vi) (50 µl) and Initiator or N'N'N'N-Tetramethylene diamine (TEMED) (C6H16N2) (10 µl).

Statistical analysis

The presence of bands was designated as 1 and band absent was coded as 0. The computer program NTsys 2.0e designed for protein electrophoresis data analysis was used to develop the dendrogram produced by the unweighted pair group method with arithmetic mean UPGMA (Sneath and Sokal, 1973) using Shan clustering.

RESULTS AND DISCUSSION

In general, taxonomic studies are based on morphometric measurements, anatomical characteristics and DNA based molecular marker. Electrophoresis of serum proteins have been used in the taxonomic classification of chicken. These kinds of studies brought about a new look into taxonomical evaluation. Discrimination of related taxonomy can be easily made according to the electrophoretic results. The blood protein electrophoresis banding pattern of each accession of chicken (Local, Pure boilers and Pure layer) were developed. Out of 14 bands generated, 6 protein bands were monomorphic in nature and 8 protein bands were found to be polymorphic in nature. Protein band 2 differs in only one genotype with characteristics of absence band in KD accession. Protein band 5 have present on 8 genotype and absence in 4 genotype (BBB, RDD (L), RCC (L) and RAA (B). Protein band 6 is present on 7 accessions and absent on 5 accessions (AS, SK, WL, KD and RAA (B). Protein band 8 is present on 6 accession and 6 absent in 6 accessions (AS, SK, WL, KD, RDD (L) and RCC (L). Protein Band 9 has 10 presence of band and 2 absence of band that is RBB (B) and RAA (B). Protein band 10 had 9 presences and 3 absences (BBB, BAA and RAA (B). Protein band 12 presents in 10 accessions and absent in 2 accessions (SK and KD). Protein band 13 was present exclusively in SK genotype only. Rehan *et al.* (2002) studied genus Meriones sub groups of bloods serum proteins and reported that globulin, albumin, post albumin and pre albumin have varied number of protein bands while globulin is highly variable in species followed by pre albumin. Similar results were reported by Verimli *et al.* (2000a) in *Mesocricetus brandti*, Verimli *et al.* (2000a) in *Mesocricetus auratus* and Yigit *et al.* (2000) in *Rattus rattus*.

Blood protein pattern

Protein pattern A is reference pattern for accession AS and WL, with absence of two reference band (band 6 and band 8). Pattern A is characteristics pattern of two absence band and 12 presences of bands that were similar with Reference to SK and WL. Band 6 was found to be also similar with SK, KD and RAA (B). Band 8 was similar with SK, KD, RDD (L) and RCC (L). **Pattern B** was reference pattern for accession SK with absence of four reference band (protein band 6, protein band 8, protein band 12 and protein band 13). Pattern B is characteristics pattern of 4 absence protein band and 10 presences of

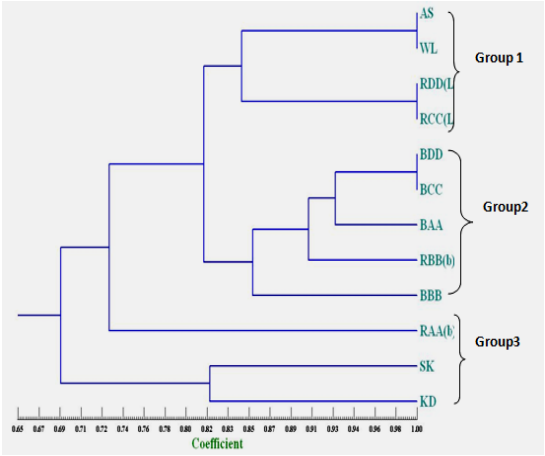

Fig. 1: Dendrogram generated using blood serum protein of 12 chicken accessions through SDS-PAGE profile

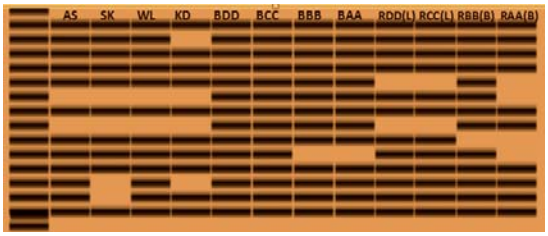

Fig. 2: Barcode presentation of 0, 1 binary matrix developed from SDS -PAGE profile for 12 chicken accession

	AS	SK	WL	KD	BDD	BCC	BBB	BAA	RDD(L)	RCC(L)	RBB(B)	RAA(B)
AS	1.00											
SK	0.83	1.00										
WL	1.00	0.83	1.00									
KD	0.83	0.81	0.83	1.00								
BDD	0.85	0.71	0.85	0.71	1.00							
BCC	0.85	0.71	0.85	0.71	1.00	1.00						
BBB	0.71	0.57	0.71	0.57	0.85	0.85	1.00					
BAA	0.78	0.64	0.78	0.64	0.92	0.92	0.92	1.00				
RDD(L)	0.84	0.69	0.84	0.69	0.85	0.85	0.84	0.78	1.00			
RCC(L)	0.84	0.69	0.84	0.69	0.85	0.85	0.84	0.78	1.00	1.00		
RBB(B)	0.78	0.64	0.78	0.64	0.92	0.92	0.78	0.85	0.78	0.78	1.00	
RAA(B)	0.69	0.53	0.69	0.53	0.71	0.71	0.83	0.76	0.69	0.69	0.76	1.00

Fig. 3: Jaccard`s similarity coefficient developed using blood serum protein of 12 chicken accession through SDS-PAGE profile

protein bands. Protein and 6 were found to be also similar with AS, KD, and RAAB (L). Protein band 8 was similar with AS, KD, RDD (L), and RCC (L). Protein Band 12 have similar with one accession KD. Protein band 13 is found to be unique among all. **Pattern C** is reference pattern for accession KD with absence of four reference band (protein band 2, protein band 6, protein band 8 and protein band 12). Pattern B is characteristics pattern of 4 absence protein band and 10 presences of protein bands. Protein band 2 was found to be unique among all. Protein band 6 was similar with AS, SK, WL, and RAA (B). Protein band 8 has similarity with AS, SK, WL, RDD (L) and RCC (L). Protein band 12 is found to be similar to SK. **Pattern D** is reference pattern for accession BDD and BCC with presence of 14 reference band. **Pattern E** is

reference pattern for accession BBB with presence of 12 reference protein band and two absence protein band (protein band 5, protein band 10,). Protein band 5 was found to be similar among RDD (L), RCC (L) and RAA (B). Protein band 10 was similar with BBA and RAA (B). Rest of protein Band has similar with presence of protein bands. **Pattern F** is reference pattern for accession BAA with presence of 13 reference protein band and 1 absence of protein band (protein band 10,). Protein band 10 was similar with BBB and RAA (B). Rest of protein band has similar presence of protein bands in respect of band. **Pattern G** is reference pattern for accession RDD (L) and RCC (L) with presence of 12 reference protein band and 2 absence protein bands (protein band 5, protein band 8). Protein band 5 was similar with BBB and RAA (B). Protein Band 8 has similiarity with AS, SK, WL and KD. **Pattern H** is reference pattern for accession RBB (B) with presence of 13 reference protein band and absence of band 9. Protein band 9 was similar with RAA (B). **Pattern I** is reference pattern for accession RAA (B) with presence of 10 reference protein band and absence of 4 protein bands (protein band 5, protein band 6, protein band 9 and protein band10).

Scoring band pattern and statistical analysis

Protein profiles of 12 chicken accessions were scored as matrix comparison pot 0 and 1 for missing and appearing protein bands to analyze for the distance similarity using Jaccard method, 1908 and UPGMA (Unweight Pair-Groups Method using Arithmetic Average). Then the data were used to make a dendrogram. The analysis revealed 3 groups classified by this method (Fig 3). The analysis showed high degree of similarity coefficient from all tested accession with a coefficient of 1.00 to 0.69. Investigating blood protein polymorphisms of local chicken in Laos, Okamoto *et al.* (1999) reported small genetic differentiation among the chicken populations.

Identification of genotype of local breeds

The dendrogram classified 12 chicken accessions into 3 groups (Fig. 1). Group I comprised the most similar accessions containing 4 accessions from AS, WL, RDD (L), and RCC (L) Group second has five accessions (BDD, BCC, BAA, RBB and BBB). Group third is consisting of 3 accessions with SK and KD, and RAA (B). Blood serum proteins of different groups was investigated by Verimli *et al.* (2000a&b, 2001), Yigit *et al*. (2001), Colak (2002a&b), and Colak and Ozkurt (2002). These authors reported variation in size, number and intensities of different protein bands.

Conclusion

Present study was successfully used in identification of different coherence of protein banding pattern with four chicken species. Blood protein varied with one breed to another hence it is advised that blood serum protein can be utilized for taxonomic identification.

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