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Electrophoretic pattern of blood serum proteins and enzymes activity of house sparrow (*Passer domesticus*) in Baghdad (Iraq)

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Abstract

The objective of this study was to determine the electrophoretic pattern of blood serum proteins and the activity of some blood serum enzymes included GOT, GPT and AP of house Sparrow (*Passer domesticus*) in Baghdad, Iraq. Seventeen (9 males and 8 females) individuals of house sparrow were collected from different regions of Baghdad. Samples of 1.0 ml of whole blood were taken from the wing vein from individuals to determined electrophoretic pattern of serum proteins and enzymes in three replicates for each sex within species. Results revealed that house sparrow blood serum proteins were separated into seven different regions, these bands were pre- albumen (3.68%), albumen (13.20%), post–albumen (8.37%), α–globulin (4.54%), β–globulin (27.49%), γ–globulin (30.15%) and transferrin (12.59%). Electrophoretic pattern of serum proteins were differed due to sex: females dominated males in pre–albumen, γ–globulin and total albumens. House sparrow blood serum GOT, GPT and AP enzymes activity were 38.4, 10.9 and 42.05 U/L respectively. Males had higher activity of GOT and GPT enzymes compared to females.

Keywords: House Sparrow, *Passer domesticus*, Serum Proteins, Electrophoresis, Serum Enzymes

Introduction

House Sparrow (*Passer domesticus*) is actually a member of the birds of Iraq (Allouse, 1962) belong to the weaver family, a large group of old world birds. House sparrows have spread from Eurasia, and can be found living with humankind around the globe. The house sparrow is a brown, chunky bird about 5 3/4 inches (15cm) long, and very common in human-made habitats (Joshi, 2009). The male has a distinctive black bib, white cheeks, a chestnut mantle around the gray crown, and chestnut-coloured feathers on the upper wings. The female and young are difficult to distinguish from some native sparrows. They have a plain, dingy-gray breast, a distinct, Buffy eye stripe, and a streaked back (Campbell et al., 2001; Moudhafer et al., 2006; BirdLife International, 2008). From 3 to 7 eggs are laid, 4 to 5 being the most typical (Baker, 1995). Incubation takes 10 to 14 days, and the young stay in the nest for about 15 days (Lowther and Cink, 1992).

Electrophoresis is being used with increasing frequency by avian taxonomists (Roman et al., 2009). This technique takes advantage of the different migration rates of protein molecules in an electric field. Electrophoresis is one of the most effective methods for the separation of ionic components of a mixture the resolving power of different electrophoretic methods is quite variable. To separate two component ions, it is necessary to permit migration to continue until one of the kinds of ions has travelled further than the other (Ordonneau et al., 2005).

Plasma protein electrophoresis is an invaluable diagnostic tool in avian taxonomy (Werner and Reavill, 1999). However, high inter-taxonomic variations have been observed in avian electrophoresis patterns (Sibley and Hendrickson, 1970; Zaias et al., 2000), which make their interpretation difficult for practitioners. For example, a previous study of avian albumin has shown that the same proteins can migrate over different distances, depending on species. For albumin, these differences have been attributed to variations in conformation and surface charge distribution (Archer and Battison, 1997; Roman et al., 2009).

The objective of this study was to determined electrophoretic pattern of house sparrow (*Passer domesticus*) blood serum proteins and the activity of some blood serum enzymes including GOT, GPT and AP as a species identification.

Materials and Methods

Seventeen (9 males and 8 females) individuals of house sparrow (*Passer domesticus*) were collected from different regions of Baghdad city, samples of 1.0 ml of

whole blood were taken from the wing vein on the inside of the elbow joint from individuals. The dove was held with its back downward and the wing laterally spread. Removal of a few feathers made the vein visible (Schermer, 1967).

Whole blood was drawn from each dove species by a BD insulin syringe needle and put in a 10 ml test tube until to clotting. The blood was centrifuged for 5 minutes. The serum was removed by a transfer pipette to clean test tube and frozen. A disc electrophoresis procedure was determined according to Davis (1964). The analysis were performed with a ten column electrophoresis apparatus utilizing stainless steel wires electrodes and two cubic reservoirs (17 cm deep and 11 × 16 cm in dimensions) constructed from 12 cm glass tubing. Acrylamide gels (0.5 cm diameter × 10 cm length) and Tris buffer at pH 8.3 were used throughout the study. Separation of 15 µl of serum samples was conducted at 25°C and at 3 mA per sample for 150 min, by EISCO power supply. After separation the staining was accomplished by using Coomassie brilliant blue R-250. The most distinct electrophoretic patterns were obtained with 15 µl of serum samples. The gels were then destained using 10% glacial acetic acid solution until separated band clearly appeared and the gels stored according to procedures outlined by Davis (1964). Protein bands and its percentages were determined according to the schematic diagrams obtained by Photo Capt Molecular Weight Software (2001).

The activities of GOT, GPT and AP enzymes in serum were determined photometrically using commercial Bio-test kit (RANDOX).

Statistical analysis was carried out using computerized statistical analysis program (SAS, 2001).

Results

House sparrow blood serum proteins were separated into seven different regions (Fig.1). These regions were pre–albumen, albumen, post–albumen, α –globulin, β-globulin, γ–globulin and transferrin respectively, from the cathode to the anode electrode. Schematic diagrams of electrophoretic pattern showed that nine bands protein were separated through acrylamide gels (Fig.2). The average values were 3.68, 13.20, 8.37, 4.54, 27.49, 30.15 and 12.59% for pre –albumen, albumen, post–albumen, α–globulin, β–globulin, γ–globulin and transferrin respectively. Significant sex differences were found in serum protein fractions ($P<0.05$). Females dominated males in the values of serum pre–albumin, γ–globulin and total albumins, whereas males dominated females in the values of serum β–globulin and total globulins. No sex differences were found in serum transferrin fractions (Table 1).

The average values of house sparrow serum GOT, GPT and AP enzymes activity were 38.4, 10.9 and 42.05 U/L respectively, males had higher activity of GOT and GPT enzymes compared to females (Table 2).

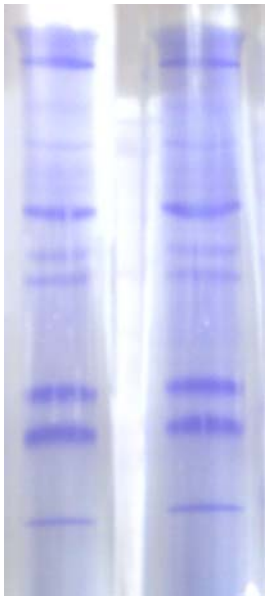

Fig. 1: Electrophoretic pattern of house sparrow (1: male, 2: female) serum proteins

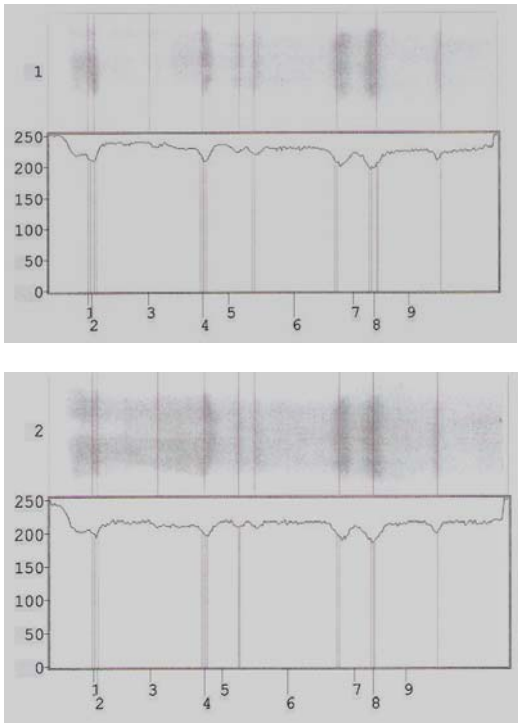

Fig. 2: Schematic diagrams of electrophoretic pattern of house sparrow (1: male, 2: female) blood serum proteins

Table 1: Blood serum proteins percentages of house sparrow

Serum proteins (%)	Sex		
	Male	female	Average
Pre–albumin	2.79±0.62[b]	4.56±0.33[a]	3.68±0.49
Albumin	13.47±1.15	12.93±1.11	13.20±1.13
Post–albumin	8.08±1.06	8.65±1.09	8.37±1.08
α–Globulin	4.40±0.76	4.68±0.80	4.54±0.77
β–Globulin	30.86±3.22[a]	24.11±3.09[b]	27.49±3.15
γ–Globulin	28.72±1.72[b]	31.57±1.66[a]	30.15±1.78
Total albumens	24.34±0.98[b]	26.14±0.96[a]	25.24±0.98
Total globulins	63.98±3.55[a]	60.36±3.57[b]	62.17±3.61
Transferrin	11.67±1.37	13.50±1.32	12.59±1.30

[a,b]different superscripts in a row differ significantly (P<0.05)

Table 2: Blood serum GOT, GPT and AP enzymes of house sparrow

Serum enzymes Activity	Sex		
	male	female	Average
GOT (U/l)	39.2±1.70[a]	37.6±1.68[b]	38.4±1.72
GPT (U/l)	11.7±0.85[a]	10.1±0.93[b]	10.9±0.97
AP (U/l)	42.3±1.31	41.8±1.34	42.05±1.27

[a,b] different superscripts in a row differ significantly (P<0.05)

Discussion

Since electrophoresis is one of the most effective methods for the separation of ionic components of a mixture, with this technique, over 20 serum protein bands are routinely separated from a sample of whole serum as small as one microliter (Ornstein, 1969). Avian total proteins consist of albumins, globulins and transferrin. All plasma proteins, except immunoglobulins, are manufactured in the liver (Lehninger, 1978).

Albumin is the largest single fraction in the healthy individuals. It serves as the major reservoir of protein. It is the main contributor of colloidal osmotic pressure. It is involved in acid-base balance, and it acts as a transport carrier for small molecules such as vitamins, minerals, hormones and fatty acids. Increase or decrease in albumin concentration is associated with diseases (Lehninger, 1978; Tohijo et al, 1995). Homeostasis of blood serum protein fractions, mainly the albumen are correlated with species genotype (Brandt et al., 1952; Rosa et al., 1993), and with the varieties or strains within a species (Zaias et al., 2000; Roman et al., 2009).

Pre–albumin is a separate and distinct fraction that precedes albumin in electrophoresis. The only known function of this fraction is the transportation of thyroid hormones. Pre–albumin has also been identified in the sera of female birds and positively correlated with high egg production laying hen strains (Al-Obaidi et al., 2007a; Al-Obaidi et al., 2009).

Post–albumin is also a separate and distinct fraction that retards albumin in electrophoresis, and it acts as a transport carrier for small molecules such as vitamins, minerals, hormones and fatty acids just like albumen fraction (Bell and Freeman, 1971). Increases or decreases in post–albumin concentration are associated with birds genotype and sex, female have high concentration than male in general (Al-Obaidi et al., 2007a), also post–albumin concentration increases are associated with heat stress (Al-Obaidi et al., 2009).

The globulins are composed of three fractions, designated alpha (α), beta (β) and gamma (γ). In birds, one or more sub-fractions of these globulins are identified (Ornstein, 1969; Bell and Freeman, 1971). Alpha and Beta globulins are groups of lipoproteins manufactured almost entirely by the liver. These proteins usually elevate during the acute phase of inflammatory liver disease, malnutrition and lipemia artefact (Schumaker and Adams, 1969), also some species differences were found in those protein fractions (Brandt et al., 1952; Rosa et al., 1993).

Unlike those found in mammals, in birds, the gamma fraction contains most of the immunoproteins, including IgM, IgA, IgE and IgG. Gamma globulins is usually elevated with ongoing antigenic stimulation, especially from infectious agents (Al-Obaidi et al., 2007b), but some species of birds have high gamma fraction concentration due to genotype, these possess and inherited genes for high immunity (Al-Obaidi et al., 2009).

Transferrin is a glycoprotein, and it acts as a transport carrier for cations in blood, this protein usually elevate during the acute phase of inflammatory infectious diseases, playing important role in non specific immunity (Tohijo et al., 1995), also some species differences were found in this protein fraction (Al-Obaidi et al., 2007b).

Serum transaminase enzymes of glutamicoxaloacetic acid transaminase (commonly abbreviated GOT), which also called aspartate aminotransferase (commonly abbreviated AST) and glutamic-pyruvic acid transaminase (commonly abbreviated GPT) are type of enzymes that help produce chemical reactions in the body. It is found mainly in the blood but also in certain body tissues, especially the heart and the liver. Transaminase enzymes help to form an acid known as oxaloacetic acid or pyruvic acid and an amino acid known as glutamic acid. Amino acids are groups of chemical substances that form proteins. Proteins are extremely complex, naturally occurring substances made of amino

acids that are essential to the body's structure and function. Alkaline phosphatase (AP) is present in nearly all tissues and organs, in particular liver and in bones, where it is associated with osteoblastic processes. In avian and poultry, males have consistently higher values for AP compared to females (Mauro et al., 1990; Niu et al., 2009). Al-Obaidi and Al-Shadeedi, (2011) found significant sex differences in serum enzymes activity, the males of two species of dove predominant females in the values of serum GOT, GPT and AP activity.

Serum protein electrophoresis and GOT and GPT enzymes activity are versatile and simple tests providing important information that can help the avian taxonomists in house sparrow identification.

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