استخدام نمط الترحيل الكهربائي لبروتينات مصل الدم لتصنيف نوعي الحمامة الفاختة (*Streptopelia decaocto*) والحمامة الضاحكة (*Streptopelia senegalensis*)

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الخلاصة

استهدف البحث فصل بروتينات مصل دم كل من الحمامة الفاختة (*Streptopelia decaocto*) والحمامة المطوقة (*Streptopelia senegalensis*) بطريقة الترحيل الكهربائي كطريقة تصنيفية مابين النوعين . تم جمع ٢٣ حمامة فاختة (١٣ ذكر و١٠ إناث) و ١٥ حمامة ضاحكة (٨ ذكر و٥ إناث) من مناطق مختلفة من مدينة بغداد ، وتم سحب٠,١ مل من الدم من الوريد الموجود في الجناح لدراسة نمط الترحيل الكهربائي لبروتينات مصل الدم وبثلاثة مكررات لكل جنس ضمن كل نوع . بينت النتائج إن بروتينات مصل دم كل من الحمامة الفاختة والحمامة المطوقة قد انفصلت إلى سبعة مناطق بروتينية وهي pre – albumen و albumen و post – albumen و α-globulin و β-globulin و γ- globulin و transferrin بالتتابع من القطب الموجب إلى السالب. اختلف نمط الترحيل الكهربائي لبروتينات مصل الدم بين النوعين من الحمامة وكذلك بين الجنسين ضمن كل نوع ، إذ تفوقت الحمامة الفاختة على الضاحكة في المعدل العام لقيم نسب بروتينات pre – albumen و post – albumen و Total albumens في حين تفوقت الحمامة الضاحكة على الفاختة في المعدل العام لقيم نسب بروتينات albumen و-β globulin و γ- globulin و total globulin و transferrin مصل الدم.

ELECTROPHORETIC PATTERN OF COLLARED DOVE (*Streptopelia decaocto*) AND LAUGHING DOVE (*Streptopelia senegalensis*) BLOOD SERUM PROTEINS AS A SPEICES CLASSIFICATION

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Abstract

The objective of this study was to separate blood serum proteins by using electrophoresis of Collared Dove (*Streptopelia decaocto*) and

Laughing dove (*Streptopelia senegalensis*) as a species classification. Twenty three (13 males and 10 females) individuals of Collared dove and fifteen (8 males and 7 females) of Laughing dove were collected from different regions of Baghdad city, samples of 1.0 ml of whole blood were taken from the wing vein from individuals to determined electrophoretic pattern of serum proteins in three replicates for each sex within species. Results revealed that Collared Dove and Laughing dove blood serum proteins were separated into seven different regions, these bands were pre – albumen, albumen, post – albumen, α-globulin, β-globulin, γ- globulin and transferrin respectively, from the cathode to the anode electrode. Electrophoretic pattern of serum proteins were differed due to species of the dove and sex within species, Collared Dove predominant Laughing dove in blood serum pre - albumen, post - albumen and total albumen, when as Laughing dove predominant Collared Dove in blood serum albumen, β-globulin, γ- globulin, total globulin and transferrin.

Introduction

Collared Dove (*Streptopelia decaocto*) and Laughing doves (*Streptopelia senegalensis*) are actually members of the birds of Iraq, they have well adapted in Baghdad areas, nesting on the top of buildings, window sills and any other place they can build a stable nest (Allouse, 1962 ; Moudhafer *et.al*., 2006). The Collared Dove (*Streptopelia decaocto*), also spelled Eurasian Collared-Dove is one of the great colonisers of the avian world. Its original range was warmer temperate regions from southeastern Europe to Japan. Laughing doves (*Streptopelia senegalensis*), also called Palm dove are found throughout Africa, the Middle East, some parts of Asia and Australia. The Laughing dove earned its name because of the distinctive coo that sounds just like a human laughing (BirdLife International, 2004).

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 traveled further than the other (Ordonneau *et.al.*, 2005).

Plasma protein electrophoresis is an invaluable diagnostic tool in avian taxonomists (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 Collared dove (*Streptopelia d.decaocto*) and Laughing dove (*Streptopelia senegalensis*) blood serum proteins as a species classification.

Material and Methods

Twenty three (13 males and 10 females) individuals of Collared dove (*Streptopelia d.decaocto*) and fifteen (8 males and 7 females) of Laughing dove (*Streptopelia senegalensis*) were used in this study, 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).

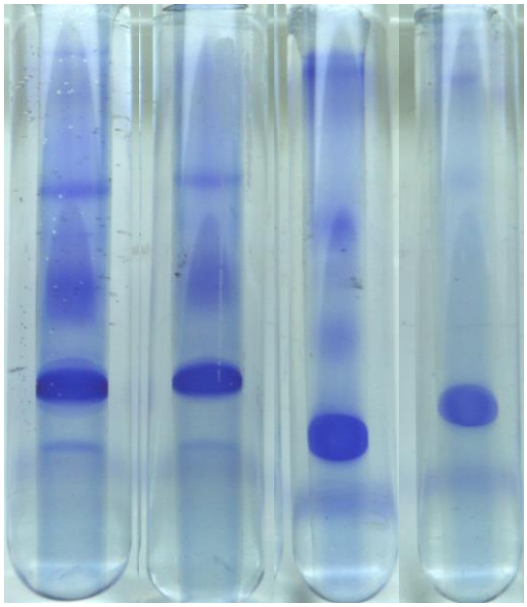
Whole blood was drawn from each dove species by a B-D insulin syringe needle and put in a 10 ml test tube until to clotting. The blood was centrifuged for 5 minutes. The serum was removed by a transfer pipette to clean test tube and frozen. Disc electrophoresis procedures determined according to Davis (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 X 16 cm in dimensions) constructed from 12 cm glass tubing. Acrylamide gels (0.5 cm diameter x 10 cm length) and TRIS buffer at pH 8.3 were used throughout. Separation of 15 ul 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 ul 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).

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

Results and Discussion

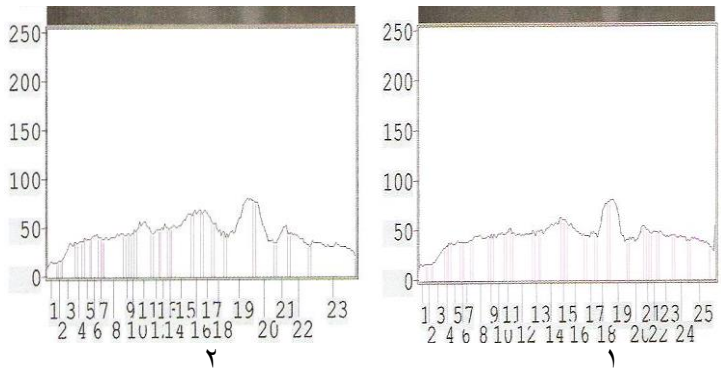
Collared Dove and Laughing doves blood serum proteins were separated into seven different regions (Fig.1), these regions were pre – albumen,

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 over twenty band proteins were separated through acrylamide gels (Fig.2). Since electrophoresis is one of the most effective methods for the separation of



1 2 3 4

Fig. (1): electrophoretic pattern of Collared dove (1: male, 2 : female) and Laughing dove (3: male, 4 : female) blood serum proteins.



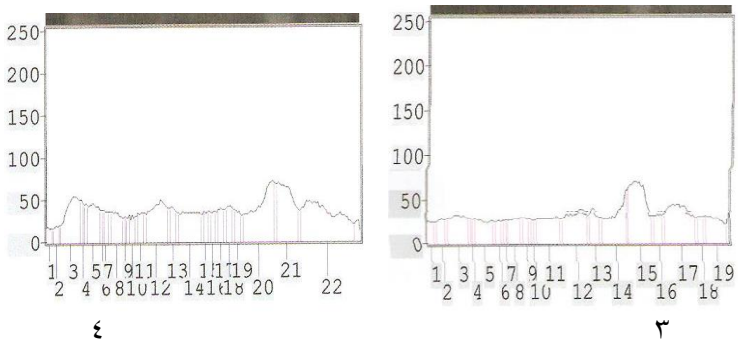


Fig. (2): Schematic diagrams of electrophoretic pattern of Collared dove (1: male, 2 : female) and Laughing dove (3: male, 4 : female) blood serum proteins.

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). Significant species differences were found in serum protein fractions ($P<0.05$), Collared dove predominant Laughing dove in the average values of serum pre-albumin which were 3.15 and 2.69 %, post-albumin were 17.64 and 12.32 % respectively, whenas Laughing dove predominant Collared dove in the average values of serum albumin which were 22.95 and 20.54 % respectively (Table 1). Also, significant sex differences were found in serum protein fractions ($P<0.05$), females of the two species predominant males in the values of serum pre-albumin and post-albumin, whenas Laughing dove males predominant females in the values of serum albumin.

Avian total proteins consist of albumins and globulins. 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. Increases or decreases in albumin concentration are associated with diseases (Lehninger, 1978 ; Tohjo *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).

Table (2) showed that significant species differences were found in serum globulin fractions ($P<0.05$), Collared dove predominant Laughing dove in the average values of β – Globulin and γ - Globulin which were 6.63 , 4.83 % and 13.77 , 11.08 % respectively , whenas no significant

Table (1): Blood serum albumens percentages of Collared dove and Laughing dove.

Species	Pre-albumin	Albumin	Post- albumin
Collared dove (males)	2.40 b	٢٠,٦٨ a	.٣١ b١٧
Collared dove (females)	3.89 a	20.39 a	.٩٧ a١٧
Average	3.15 A	20.54 B	.٦٤ A١٧
Laughing dove (males)	2.02 b	24.30 a	.٣٩ b١١
Laughing dove (females)	2.45 a	21.57 b	.٢٤ a١٣
Average	2.24 B	22.94 A	.٣٢ B١٢

Different letters among columns revealed significant differences ($P<0.05$) : * large letters between species. ** small letters between sex.

Table (2): Table (1): Blood serum globulins percentages of Collared dove and Laughing dove.

Species	α- Globulin	β - Globulin	γ - Globulin
Collared dove (males)	8.18 b	٥,٢٩ a	.٠١ a١٢
Collared dove (females)	9.23 a	4.37 b	.١٥ b١١
Average	8.71 A	4.83 B	.٠٨ B١١
Laughing dove (males)	7.43 b	5.45 b	.٣٥ b١٣
Laughing dove (females)	8.82 a	7.80 a	.٢٠ a١٤
Average	8.13 A	6.63 A	.٧٧ A١٣

Different letters among columns revealed significant differences (P<0.05) : * large letters between species. small letters between sex.**

species differences were found in α-globulin fraction. Also, significant sex differences were found in serum globulin fractions (P<0.05), female predominant male in the values of serum α-globulin fraction of the two species, whenas male predominant female in the values of β – Globulin and γ - Globulin fractions of Collared dove, female predominant male in those values of Laughing dove.

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 artifact (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 usually elevate with ongoing antigenic stimulation, usually 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).

Table (3) showed that significant species differences were found in serum total albumens, total globulin and transferrin fractions (P<0.05), Collared dove predominant Laughing dove in the average values of total albumens which were 41.32, 37.49 % respectively, whenas Laughing dove predominant Collared dove in the average values of total globulin and transferrin fractions which were 28.53, 24.62 % and 10.35, 9.43 % respectively. Also, significant sex differences were found in serum total albumens and total globulin (P<0.05), male predominant female in serum total albumens of Collared dove, whenas female predominant male in serum total globulin of Laughing dove. No sex differences were found in serum transferrin fractions of the two dove species.

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 (Tohjo *et.al.*, 1995), also some species differences were found in this protein fraction (Al-Obaidi *et.al.*, 2007b).

Serum protein electrophoresis is a versatile and simple test providing important information that can help the avian taxonomists in dove species classification.

Table (3): Table (1): Blood serum Total albumens, Total globulins and Transferrin percentages of Collared dove and Laughing dove.

Species	Total albumens	Total globulins	Transferrin
Collared dove (males)	40.39 b	٢٥,٤٨ a	.١٤ a٩
Collared dove (females)	41.35 a	24.75 a	.٧١ a٩
Average	41.32 A	24.62 B	.٤٣ B٩
Laughing dove (males)	37.71 a	26.23 b	.٤١ a١٠
Laughing dove (females)	37.26 a	30.82 a	.٣٠ a١٠
Average	37.49 B	28.53 A	.٣٥ A١٠

Different letters among columns revealed significant differences (P<0.05) : * large letters between species. ** small letters between sex.

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