

## Variation of egg proteins between bird varieties by using SDS-PAGE

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### Abstract

Proteins are essential constituents of all organisms; both egg white proteins and egg yolk are source of protein. The aim of this study was conducted to perform preliminary studies to analyses and compare egg white proteins and yolk proteins from different avian species (guineafowl, dwarf hens, local hen, Shami, turkey, duck, geese, partridge and quail) via or with SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis ). 18 Fresh eggs of different poultry species (guineafowl, dwarf hens, local hen, Shami, turkey, duck, geese, partridge and quail) were collected from various farms in the Sulaimani province. Data on egg proteins were analyzed using Statistical Xlstate used for dendrogram construction and PCA. The main egg white proteins were Ovomicin, Ovotransferrin, Ovalbumin, Flavoprotein,  $\alpha$ -chymotrypsinogen, and Trypsin inhibitor. The main lipoproteins were Apovitellenin VI, Apovitellenin Vb, Apovitellenin V, Apovitellenin IIIa, Apovitellenin III, Apovitellin 7, B-Livetin, Apovitellenin IIa, Apovitellenin II, and Apovitellenin I. All these lipoproteins were observed in the nine birds species. The egg white proteins and yolk lipoproteins for nine species were examined. It can be concluded the large differences were found in a mount of egg white proteins and yolk lipoproteins of the nine species of birds.

### التباين في بروتينات البيض بين أصناف الطيور باستخدام SDS-PAGE

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### الخلاصة

البروتينات هي المكونات الأساسية لجميع الكائنات الحية، كصفار البيض و بياض البيض. كان الهدف من هذه الدراسة هو إجراء دراسات أولية لتحليل و مقارنة بروتينات بياض و صفار البيض في أنواع مختلفة من الطيور مثل دجاج غينيا، الدجاج القزم، الدجاج المحلي، الدجاج الشامي، الديك الرومي، البط، الأوز، و طائر السمان الياباني باستخدام تقنية SDS-PAGE . تم جمع البيض من مزارع مختلفة في محافظة السليمانية. و قد تم تحليل البيانات باستخدام Xlstate الاحصائية المستخدمة لبناء Dendrogram و PCA . كانت البروتينات الرئيسية هي Ovomicin , Ovotransferrin, Ovalbumin, Flavoprotein,  $\alpha$ -chymotrypsinogen , و chymotrypsinogen و مثبط التربسية. اما البروتينات الدهنية هي: Apovitellenin VI و Apovitellenin Vb و Apovitellenin V و Apovitellenin IIIa و Apovitellin 7 و Apovitellin III و Apovitellenin II و B-Livetin و Apovitellenin II و Apovitellenin I و Apovitellenin II . يمكن ان نستنتج ان هنالك اختلافات كبيرة في بروتينات بياض البيض و البروتينات الدهنية لصفار البيض لمختلف الطيور.

**Keyword: albumin, yolk, SDS-PAGE, protein.**

## Introduction

Proteins are essential constituents of all organisms; both egg white proteins and egg yolk are source of protein (Desert, 2001). Egg white proteins is the common name for the clear liquid contained within an egg Albumin, which have been investigated by many workers for existence of genetic variants detected by electrophoresis (Obeidah et al., 1977). Other components are present in very low amount (Mine, 1995). The main protein fractions of egg white proteins are like: Ovalbumin, Ovotransferrin, Ovomuroid, Ovomucin, Ovogobulin G1, Ovogobulin G2, Ovogobulin G3, Flavoprotein, Ovoglycoprotein, Ovomacroglobulin, Ovoinhibitor, Avidin, and Cystatin (Wetter, et al., 1953).

Yolk is fat-in-water emulsion with about 50% dry weight. Protein and lipoproteins identified in egg yolk Apovitellenin VIa,  $\gamma$ -Livetin + Apovitellenin VI, Apovitellenin Va, Apovitellin 3 + 4, Apovitellenin Vb, Apovitellenin V, Apovitellin 5+6,  $\alpha$ -Livetin, Apovitellenin IV, Apovitellenin IIIa, Phosvitin,  $\alpha$ -Livetin/Apovitellenin III, Apovitellin 7,  $\beta$ -Livetin, Apovitellin, Apovitellenin IIa, Apovitellenin II, Apovitellenin I, Apolipoprotein II.

Three main techniques were used to study and characterize the proteins like electrophoresis, chromatography (Galyean & Cotterill, 1979; Gorg et al., 2000; Herbert et al., 2001; Valuev et al., 2003), and SDS-PAGE (Holen & Elsayed, 1990). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a powerful technique used in many fields concerning biotechnology (Piatigorsky, 1987), biochemistry (Tu, et al., 2000), molecular biology (Kasahara, et al., 2010), and forensic science (Allen & Goldberg, 1995). It is used to characterize chicken by separating molecular weight of egg proteins (Hamazume, et al., 1984).

In animal breeding and genetics, we often deal with large number of possibly

correlated traits, which makes data presumably complex to handle and interpret. Such difficulty in data handling and interpretation can be quelled using principal component analysis. Principal component analysis (PCA) is a multivariate technique that analyzes a data table in which observations are described by several inter-correlated quantitative dependent variables (Abdi & Williams, 2010).

A dendrogram is a branching diagram used to visualize the arrangement of the clusters based on the degree of similarity of certain characteristics( Wu et al.,2010) As protein dendrogram construction is used in other areas, such as multiple protein sequence alignments, it is very important that the most related protein sequences be identified and align first (Chrysostomou & Seker.,2013).

The aim of this work was to perform preliminary studies to analyses and compare egg white proteins and yolk proteins from different avian species (guineafowl, dwarf hens, local hen, Shami, turkey, duck, geese, partridge and quail) by Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE).

## Materials and methods

Current study was done in the animal production laboratories in the college of agricultural sciences, University of Sulaimani during March 2018 to July 2018. 18 Fresh eggs of different birds species (guineafowl, dwarf hens, local hen, brahma, turkey, duck, geese, partridge and quail) were collected from various farms in the Sulaimani province in maturation age. When the egg was broken Yolk and white (albumin) were carefully separated from the eggshell. The protein samples (yolk and egg white proteins) were taken from the chosen poultry species, after that the samples was distilled water at rate of 1:10 folds (one volume egg sample:nine volume H<sub>2</sub>O), the samples were liquated in the test tubes and

stored at -20 °C till it applied. SDS (Polyacrylamide gel electrophoresis) were done as described via Laemmli (1970). Egg samples (yolk and white) were cleared for electrophoresing with an environment of reduction when diluted with three parts of buffer to one parts of diluted samples (yolk and egg white) (10% v/v). After that Samples has been heated at 95°C for five minutes before keeping into wells of Polyacrylamide gel using long tips of micropipette. The sample dye reaches the end of the gel. When gels walked at constant energy (150 voltage) .Removal of gel occur after electrophoresis completed and the proteins stained Coomassie brilliant blue R 250. A broad range (200: 14.4 kDa) marker were applied for differentiation, scanning of the gel were done and stored in 1% acetic acid then kept in room temperature or fridge

### Result

SDS-PAGE was used to analysis egg white proteins of nine birds species (Figure 1: a, b). The main egg white proteins were Ovomicin, Ovotransferrin, Ovalbumin, Flavoprotein,  $\alpha$ - chymotrypsinogen, and Trypsin inhibitor. Ovomicin was observed just in turkey and partridge, and both Ovotransferrin and Ovalbumin was observed in all the nine species with some differences. Flavoprotein was observed in guinea fowl, dwarf fowl, local hen, and shami.  $\alpha$ - chymotrypsinogen was observed just in partridge. Finally Trypsin inhibitor was observed in each of turkey, duck, geese, partridge, and quail.

Yolk lipoproteins were presented a wide range of relative molecular masses (Figure 2: a, b). The main lipoproteins were Apovitellenin VI, Apovitellenin Vb, Apovitellenin V, Apovitellenin IIIa, Apovitellenin III, Apovitellin 7, B-Livetin, Apovitellenin IIa, Apovitellenin II, and Apovitellenin I. All these lipoproteins were observed in the nine birds species. The cluster of white proteins of egg analysis base of dendrogram of the nine birds

species are given in figure 3. The dendrogram of the egg white proteins grouped the nine birds species into four clades. The first was composed of quail, turkey, and partridge; the second, duck and geese; the third, dwarf hen and local hen; and the fourth, guineafowl and shami. Figure 4 was shown the dendrogram of the yolk lipoproteins for the nine bird species. The dendrogram of the yolk lipoproteins of grouped the nine bird species into three clades. The first clade was composed of geese, duck, and quail; the second, local hen, guineafowl, dwarf hen, and shami; the third, turkey and partridge.

The Principal Component biplot figure 5, explains the four principal components had Eigen values of 4.887 (PC1), 1.767 (PC2), 1.032 (PC3) and 0.313 (PC4). The Eigen values showed the amount of variance explained by each of the principal components out of the total variance. The result explain in turkey the major protein that observed albumin was ovalbumin, Flavoprotein, lysozyme, carbonic and anhydrase but in partridge shows trypsin inhibitor was major protein in guineafowl, dwarf hen, shami, duck, geese, quail all protein have same value, ovotransferrin is the major protein was observed in local hen. The Principal Component biplot for egg yolk in figure 6, explain An Eigen values against their principal components are (1.919) for F1, (0.724) for F2 and (0.357) for F3. The Eigen values showed the amount of variance explained by each of the principal components out of the total variance. The all factor of PCA explain in turkey, partridge and quail the major protein in yolk protein was trypsin inhibitor, duck and geese quail all protein have same affect on total yolk protein, ovalbumin, lysozyme, Flavoprotein, ovotransferrin, have large protein was observed in local hen and dwarf hen but carbonic anhydrase, ganalbumin, x-lactoalbumin, Avidin have major albumin protein was observed in shami and guineafowl.

**Discussion**

As a result for the SDS-PAGE, Ovalbumin appeared as the largest band on the gel for all the nine varieties, as expected, because it is the most abundant protein in egg white (54%) (Li-Chan, et al., 1995; Handa & Kuroda, 1999). The other five protein bands detected in the white fraction was Ovotransferrin, Flavoprotein, Ovomicin,  $\alpha$ -chymotrypsinogen, and Trypsin inhibitor. Furthermore the variation of egg white proteins and yolk lipoproteins between the nine species may be because the different egg sources, and the health of the hens (Miguel, et al., 2005).

**Conclusion**

The egg white proteins and yolk lipoproteins for nine varieties were examined. It can be concluded the large differences were found in a mount of egg white proteins and yolk lipoproteins of the nine birds. Furthermore the types also differ between the species depending on the genetic and environments surrounding the flock.

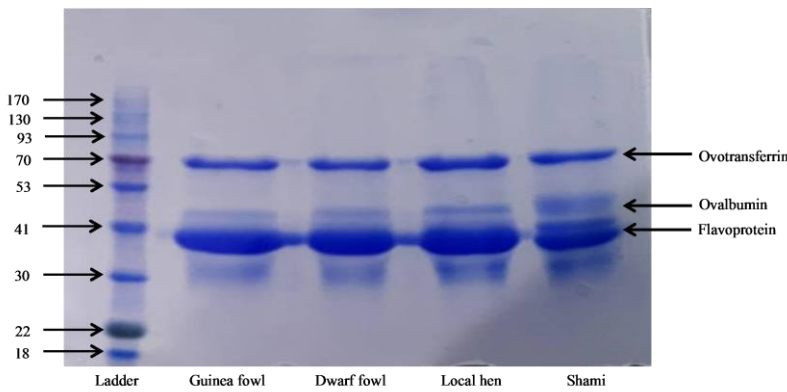


Figure 1 a: SDS-PAGE analysis of four bird species white proteins

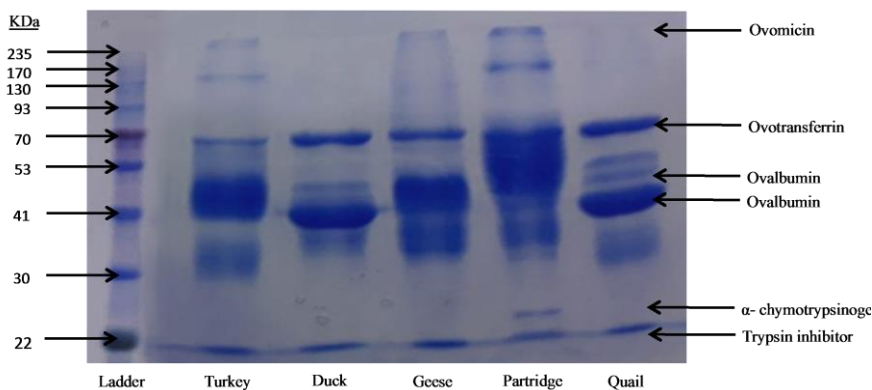


Figure 1 b: SDS-PAGE analysis of five bird species white proteins

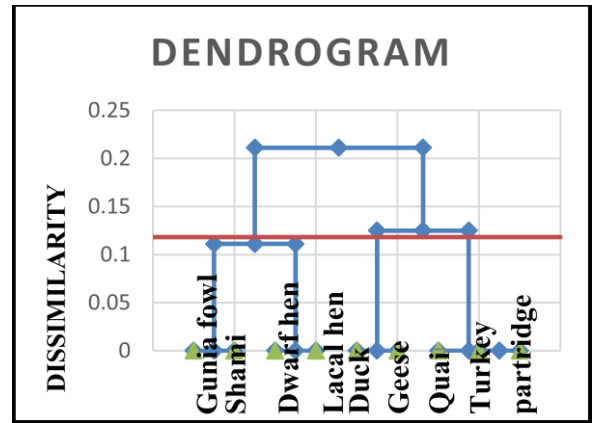


Figure 3: Cluster analysis of egg white protein base of dendrogram of the nine birds species

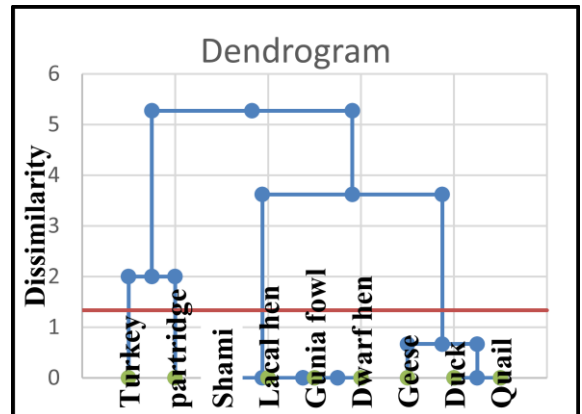


Figure 4: The dendrogram of the yolk lipoproteins grouped the nine birds species

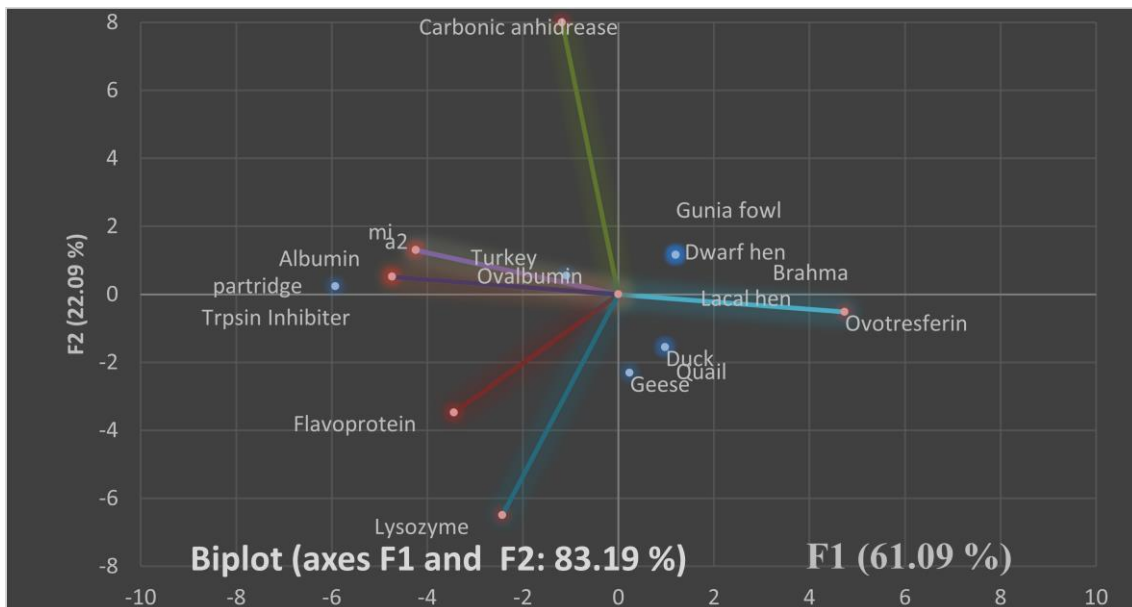


Figure 5: The Principal Component biplot for egg white protein

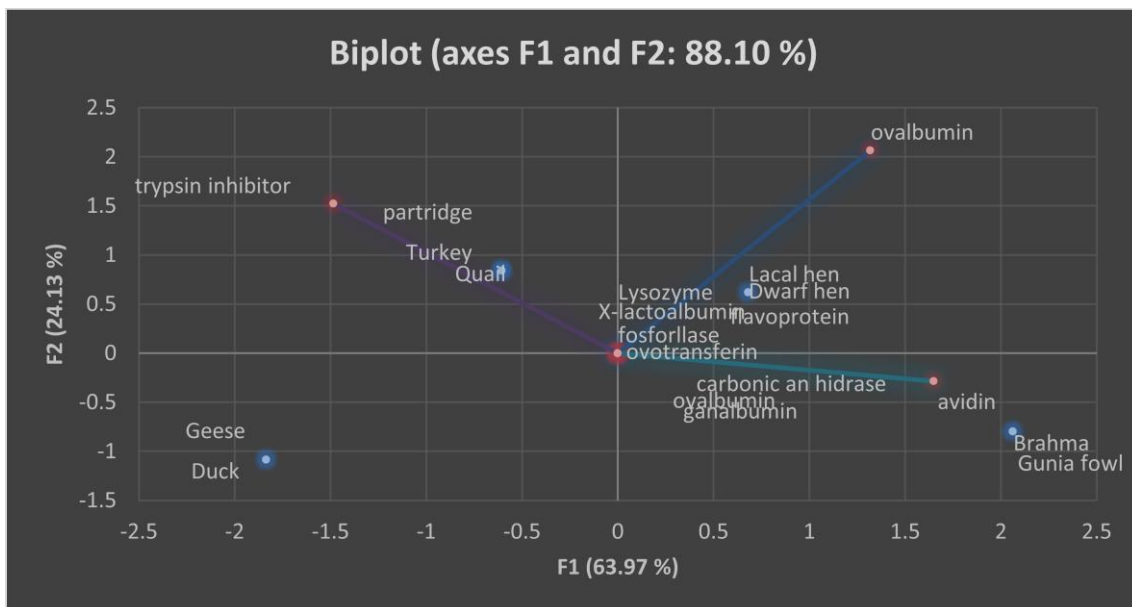


Figure 6: The Principal Component biplot for the yolk lipoproteins

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