ELECTROPHORETIC PATTERN OF SERUM PROTEINS AND IMMUNOGLOBULIN "IgG" LEVELS IN CHICKENS IN RELATION TO AGE

By 

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A total number of thirty, one day old Hubbard chicks were used in this study. Blood samples were collected three times from each five chickens and periodically at 6, 8 and 12 weeks of age from the onset of the experiment, which extended from one day up to 12 weeks of age. The determination of serum total proteins, protein fractions, IgG concentration and hemagglutination inhibition titre were estimated. The results revealed that, total globulins, Alpha-2 globulin and gamma globulin levels were significantly increased at 8 weeks of age, whereas the A/G ratio showed a significant decrease only as compared with the findings recorded at the 6 weeks of age. However, the level of total proteins, alpha-2 globulin and gamma globulin were highly significant increase at 12 weeks of age, whereas the concentration of total globulins and alpha-1 globulin, showed a significant increase. Moreover, the level of serum albumin, beta-globulins and hemagglutination inhibition titre showed a non-significant change. Serum immunoglobulin IgG levels showed a highly significant increase at 8 and 12 weeks of age.

Introduction

Chickens are considered as one of the most important sources of protein for human nutrients. Most plasma proteins are synthesized in the liver from amino acids derived either from the dietary proteins or tissue protein catabolism. The plasma also contain specialized protein, such as prothrombin and fibrinogen.

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which act in the hemostatic mechanism, in addition to immunoglobulins thereby, provide antibodies to resist infections. Moreover, transport protein bound hormones and other compounds, are the major source of cellular metabolic protein (Griminger, 1976).

Miller et al, (1951) stated that, the liver played a major role in the biosynthesis of the majority of plasma proteins, so the clinical determination of plasma proteins was important in diagnosis of cases in which there were hepatic disorders.

Sturkie (1976) stated that, immunoglobulin are synthesized by the cells of the reticuloendothelial system in response to a variety of antigenic stimuli that are ever present in the interior and exterior environment of all living organisms. In the electrophoretic pattern of fractionation, part of the beta 2 and essentially all of the gamma globulins are immunoglobulins.

Polonis (1982) indicated that, serum total protein level was increased from 3.56 gm/dl at the 5th weeks of age to 3.91 gm/dl at 8th weeks of age. Also, Morgan and Glick (1972) reported that, total serum protein in chickens increased from 2.68 gm/dl at one week to 4.63 gm/dl at 12 weeks of age.

Moreover, Heim and Schechtman (1954) studied the serum protein level of normal chicks from one to 27 days old at intervals of one week. They found that, serum total protein was increased from 2.15 gm/dl to 3.69 gm/dl.

So, the aim of the present study was planned to elucidate the influence of various ages on the level of total protein and its components as well as the immunoglobulin IgG concentration in the blood of normal Hubbard chickens.

Materials and Methods

Thirty one day old Hubbard chicks (obtained from general poultry Company, Cairo, Egypt), were used in this study. All chicks were kept throughout the experiment under good hygienic control. They were fed ad-libitum on a commercial ration which contains all food essential for optimum growth. This ration was composed of ground yellow corn 55%, ground seed cack 11%, rice embrgo cack 25%, fish meal 6%, bone meal 1.5%, calcium carbonate 1%, sodium
chloride 0.5% and magnesium sulphate 200 gm, vitamin AD3 E and Vit. B complex, were added. Blood samples were collected three times from each five chickens using wing vein technique and periodically at 6,8 and 12 weeks of age from the onset of the experiment which extended from one day up to 12 weeks of age. The clear serum was separated by centrifugation at 3000 r.p.m. for 15 minutes, and kept frozen at-20°C until analysis. All sera were analyzed for the following parameters total proteins by the Biuret method (Weichselbaum, 1946), separation of protein fraction electrophoretically by (Koh 1968), serum IgG using immunoprecipitin analysis by (Helling, 1973) and haemagglutination inhibition test by (Meszaros, 1964). Statistical, analysis of the obtained results were carried out using the method of Snedecor and Cochran (1967).

Results

The results obtained concerning the concentrations of immunoglobulin IgG and Electrophoretic pattern of serum proteins from chickens at different ages have been statistically summarized in three tables and three figures.

The obtained data Table (1) and Fig. (1) indicated that, there was a non significant increase in serum total protein level at 8th. weeks of age. This increase became highly significant at 12 weeks in comparison with the value of two weeks old. The value of serum albumin level revealed a non significant change. Whereas, the serum total globulin level showed a significant increase at 8 and 12 weeks of age. A/G ratio revealed a significant decrease at 8 weeks of age. This decrease became non significant at 12 weeks of age.

The obtained results table (2) and Fig. (2) revealed that there was a significant increase in serum alpha-2- and gamma- globulin fractions at 8 weeks of age. This increase became highly significant at 12 weeks. However, the serum alpha-1-globulin fraction showed a non significant increase at 8 weeks of age. This increase became significant at 12 weeks. A non significant increase in serum Beta-globulin fractions was noticed at 8 and 12 weeks of age when compared with the control values at 6 weeks of age.

The obtained data table (3) and Fig. (3) revealed that, there was a highly
significant increase in serum immunoglobulin IgG in chickens at 8 and 12 weeks of age. Whereas, serum Hemagglutination inhibition titre showed a non significant changes.

**Discussion**

The obtained data (table 1 and 2 and Fig. 1) indicate a non significant increase in serum total proteins (3.35 gm/dl) at 8 weeks of age. This increase became highly significant (3.98 gm/dl) at 12 weeks old in comparatively with the value at 6 weeks (3.20 gm/dl) of age. Our results were in agreement with those reported by Morgan and Glick (1972) who reported that, total serum proteins of normal chickens increased from 2.68 gm/dl at one week to 4.63 gm/dl at 12 weeks of age. Also these results agreed well with that of Polonis (1982) who indicated that, serum total protein level was increased from 3.56 gm/dl at the 5th. weeks of age to 3.91 gm/dl at 8th. weeks of age. Moreover, Brandt et al. (1951) showed that, serum total protein level of normal chickens was 3.36 gm/dl from 4 to 7 waks of age and then increased after 16 weeks to 4.49 gm/dl. The results also agreed well with that of Farahat et al. (1980) who reported that, serum total proteins of normal chickens was 3.27gm/dl at 6 weeks of age, while the value at 8 weeks was 5.58 gm/dl. The increase in serum total proteins level could be attributed to the increase in the level of immunoglobulin IgG and the total globulins concentrations especially the gamma-fractions which contains most of the antibody activity.

The obtained data (table 1 and 2 and fig. 1) in dicated that, there was no significant changes in the level of serum albumin fraction with age in the normal chickens. In contrary the value obtained at 6 weeks of age was 1.77 gm/dl which was agreed with the value obtained by Hoe (1925) who found that, serum albumin level of normal chickens was 1.82 gm/dl and with Chorine (1938) who recorded that, the level of serum albumin in normal chickens was 1.95 gm/dl. On the other hand, high values were recorded by Sturkie and Newman (1951) who stated that, the albumin level in males chickens was 2.33 gm/dl while in females was 3.27 gm/dl. The data showed a non significant decrease of serum albumin level at 8 weeks of age. Similar results were recorded by Meluzzi et al. (1992)
who observed that, albumin level did not appear to be influenced by the age. Such
non significant decrease in albumin fractions at 8 weeks of age although was non
significant might be due to reduced food intake, also albumin is believed to acts as
a protein reserve and a protein source for amino acids at times of subnormal
intake of food. These explanations agreed will with those of Strukie (1976) and
Shank et al. (1968) Moreover, Bertil-Laurell (1985) Who attributed such
decrease in serum albumin level to the shift of albumin to increased synthesis of
protective proteins particularly acute phase proteins.

The values of globulin fractions obtained in (table 1 and 2 and fig. 1) showed
that there was a significant increase in serum total globulins level at 8 and 12
weeks of age. These results were near to the values recorded by Farahat et al.
(1980) and Brandt et al. (1951). This increase in serum total globulins level
might be due to stimulation of immune system and consequently antibodies
produced and/or due to increased synthesis of protective proteins as a result of
general reaction of the body to infection. Moreover, the elevation of serum
globulin level was due to an enhanced synthesis of immunoglobulins and a large
increase in the level of α - glycoproteins, following tissue injury or during the
early stages of infection , probably due to its release from tissues as a part of the
inflammatory response (Butler, 1971).

The obtained data revealed that A/G ratio in the serum of normal chickens
decreased with age. 1.28 at 6 weeks of age, while at 12 week of age the A/G ratio
decreased to 0.83. This reduction may be attributed to the increase in globulin
fractions. Our results were agreed well with those of El-Shabiny (1984) who
stated that, the A/G ratio decreased from 0.97 to 0.58. On the other hand Balasch
et al. (1984) recorded a higher value of A/G ratio which was 1.98.

In our study, the electrophoretic technique revealed five fractions: albumin,
alpha 1- globulin, alpha 2-globulin, beta-globulin and gamma-globulin. These
results were nearly similar to that reported by Brandt (1951), Nasr El-Din
(1982) and El-Shabiny (1984) who reported that, serum protein fractionation
carried out using collulose acetate electrophoresis were separated into five
fractions which correspond to albumin, alpha-1, alpha-2 beta, and gamma
globulins fractions in normal Dokki-4 chickens of 35, 45 and 56 days of age. 
Strukie (1976), also stated that, five main fractions of plasma proteins can be
described in all system of protein fraction. The fractions correspond to albumin, and
four globulin fractions of mammalian plasma, alpha-1, alpha-2, beta and gamma
globulins.

The obtained data (table 2 and fig. 3) showed a non significant increase in
alpha-1 globulin which was (0.140 gm/dl) at 6 weeks, this increase became
significant (0.193 gm/dl) at 12 week of age. On the other hand serum alpha
2-globulin level exhibited significant and high significant increase which were
(0.582 gm/dl) and (0.801 gm/dl) at 8 and 12 weeks of age, respectively. Regarding
serum beta-globulin fraction, the results revealed a non significant increase during
the period of the experiment. Whereas, serum gamma- globulin level showed a
significant and highly significant increase at 8 and 12 weeks of age. These results
were nearly similar to the values obtained by Brandt et al. (1951) and Farahat et
al. (1980) who found that, alpha globulin of domestic fowl was increased from
0.74 gm/dl for the 4 to 7 weeks chicks to 1.3 gm/dl for the laying hens. The
increase of alpha-globulin may be attributed to increased synthesis of beta and
gamma globulins, Abdel Messih (1995). Moreover, Burtin and Grabar (1967)
demonstrated that, Alpha-globulin is linked with mucoproteins and glycoproteins
of plasma, and they attributed the increase in this fraction to hypalbuminemia
injury.

With regard to Beta-globulin fraction there was no significant increase at 8 and
12 weeks of age. The increase in the level of beta-globulin although was non
significant may resulted from the alteration in lipoprotein metabolism due to
hepatic disorder and/or antibody response by the infected host (El-Shabiny, 1984
and Bertil Laurell, 1985). Beta-globulin is the carrier of lipids and particularly
cholesterol, which is also associated with the antibody activity (Polyakove 1965).

The recorded increase of gamma-globulin fraction in serum of normal chickens
at 8 and 12 weeks of age was may be due to stimulation of reticulo endothelial
system (Immune system), Sturkie (1976) . Moreover, the increase of globulin
fraction, in particular gamma fraction was at its maximum at 12 weeks, since at
this week immunoglobulin IgG (antibodies) must be at their maximum level of peak and revealed a highly significant increased value. Moreover, the increase in gamma-globulin fraction may also be due to its enhanced synthesis by the defense system of the body against the infection (Abdel Messih, 1995).

Regarding serum IgG level the present data (table 3 and fig 3) cleared that, the mean value of IgG increased from 216.3 mg/dl at 6 weeks of age to 346.8 mg/dl at 8 weeks of age and reach to 409.2 mg/dl at 12 weeks of age. Our results were agreed well with those of Chhabra and Goel (1980) who stated that, the mean value of IgG, IgM and IgA concentrations in serum of normal chickens were 5.09, 1.35 and 0.31 mg/ml, respectively. Also with Higgins (1975), who recorded that the IgG level was ranged from 340 mg/dl to 570 mg/dl with a mean value of 500 mg/dl. However, Higgins and Calnek (1975) recorded lower values of IgG in normal serum of chickens. The increase in the IgG level in the present study may be due to the general reaction of the body to synthesis antibodies against any infectious agent.

Regarding the hemagglutination inhibition titres the obtained data revealed a non significant increase at 12 week of age in normal chickens. This test has been reported to be the most reliable test for detecting serum antibodies (Roberts et al., 1967). The present study corroborates this observation and leads to the inference that hemagglutination inhibition antibody activity was due to the increase in IgG concentration.

It is worthy to note that, there are wide variations concerning the total proteins, their fraction and A/G ratio as well as immunoglobulin IgG during the different stages of age. So, it could be valuable to estimate these parameters regularly to detect any change which may reflect a pathological condition which may occur in animal body during different stages of age.
Table (1): Mean values of total proteins, albumin, globulin concentrations and A/G ratio in serum from chickens at different age (gm/dl).

<table>
<thead>
<tr>
<th>Age</th>
<th>Six weeks (x ± S.E.)</th>
<th>Eight weeks (x ± S.E.)</th>
<th>Twelve weeks (x ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Proteins</td>
<td>3.2 ± 0.06</td>
<td>3.35 ± 0.06</td>
<td>3.98 ± 0.64**</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.77 ± 0.06</td>
<td>1.54 ± 0.12</td>
<td>1.79 ± 0.11</td>
</tr>
<tr>
<td>Globulin</td>
<td>1.43 ± 0.08</td>
<td>1.81 ± 0.14*</td>
<td>2.19 ± 0.13*</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>1.29 ± 0.11</td>
<td>0.92 ± 0.15</td>
<td>0.83 ± 0.05</td>
</tr>
</tbody>
</table>

* Significant (P < 0.05)  ** High significant (P < 0.01)

(\(\bar{X} \pm S.E\)) : Mean + Standard error.

Table (2): Electrophoretic pattern of serum proteins from chickens at different age (gm/dl).

<table>
<thead>
<tr>
<th>Age</th>
<th>Total proteins</th>
<th>Albumin</th>
<th>Total Globulins</th>
<th>Alpha-1</th>
<th>Alpha-2</th>
<th>Beta</th>
<th>Gammas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six weeks</td>
<td>3.20 ± 0.06</td>
<td>1.77 ± 0.06</td>
<td>1.43 ± 0.06</td>
<td>0.119 ± 0.01</td>
<td>0.439 ± 0.03</td>
<td>0.552 ± 0.01</td>
<td>0.320 ± 0.02</td>
</tr>
<tr>
<td>Eight weeks</td>
<td>3.35 ± 0.06</td>
<td>1.54 ± 0.12</td>
<td>1.81 ± 0.14*</td>
<td>0.140 ± 0.01</td>
<td>0.582 ± 0.04*</td>
<td>0.594 ± 0.02</td>
<td>0.470 ± 0.03*</td>
</tr>
<tr>
<td>Twelve weeks</td>
<td>3.98 ± 0.04**</td>
<td>1.79 ± 0.11</td>
<td>2.19 ± 0.13*</td>
<td>0.195 ± 0.01**</td>
<td>0.801 ± 0.06**</td>
<td>0.620 ± 0.04</td>
<td>0.580 ± 0.05**</td>
</tr>
</tbody>
</table>

* Significant (P < 0.05)  ** High significant (P < 0.01)

(\(\bar{X} \pm S.E\)) : Mean + Standard error.

Table (3): Mean values of IgG concentrations and hemagglutination inhibition titre in serum from chickens at different age.

<table>
<thead>
<tr>
<th>Age</th>
<th>Six weeks (x ± S.E.)</th>
<th>Eight weeks (x ± S.E.)</th>
<th>Twelve weeks (x ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/dl)</td>
<td>216.3 ± 8.81</td>
<td>346.8 ± 21.19**</td>
<td>409.2 ± 34.69**</td>
</tr>
<tr>
<td>Hemagglutination inhibition titre</td>
<td>2.4 ± 1.22</td>
<td>1.6 ± 1.06</td>
<td>3.2 ± 1.30</td>
</tr>
</tbody>
</table>

** : High significant (P < 0.01)  \((\overline{X} \pm S.E.)\) : Mean + Standard error.
Fig. (1): Concentration of total protein, albumin, globulin, and A/G ratio in sera from chickens at different age groups.

Graph (1): Scanning curve of serum protein fractions of chickens at six weeks of age.

Fig. (2): Concentration of globulin fractions in serum from chickens at different age groups.

Graph (2): Scanning curve of globulin fractions of chickens at eight weeks of age.

Fig. (3): Concentration of IgG and hemagglutination inhibition titre in sera from chickens at different age groups.

Graph (3): Scanning curve of serum protein fractions of chickens at twelve weeks of age.
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الملخص العربي

نصيوج الهجرة الكهربائية لمصل البروتينات وتراخيص البروتينات المناعية (أي. جي. جي.) في النجاح والتأثيرات على العمر

قسم الكيمياء الجزيئية - كلية الطب البيطري - جامعة القناة - الفرع بها - مصر

استخدمت هذه الدراسة عدد 30 كتكيرًا من عمر بوم تم تربيتها تحت ظروف صحية جيدة ومن ثم توصيل تأثير الأعمار المختلفة على محتوى البروتينات الكلية. وضعتها في الدراسة العلمية: 

الإدراج في بروتينات المناعة (أي. جي. جي.) في الدم الطبيعي.

تم أخذ عينات الدم للازاحة لفصل المصل ثلاث مرات من عدد خمس كتكيرات ، وعلى فترات مختلفة من العمر وذلك عند عمر (6، 8، 12 أسبوع) من بداية التجربة (6 عمر يوم) جهًا تفادى فترات التناحول التي تبدلت إلى 12 أسبوع وذلك لإجهاد النباتات.

النتيجة (تعدب مستوى البروتينات الكلية، التحليل الكهربائي للبروتينات والمستقبلات، وأيضًا تركز بالبروتينات المناعة (أي. جي. جي.) بالإضافة إلى إجراءات متابعة الثلاز في المصل). وقد أُسفر عن النتائج النبالية:

- زادت نسبة الجلوبولين الكلي والأنفأ-2- جلوبولين، الجاماك- جلوبولين زيادة ملحوظة عند الأسبوع الثامن من العمر بينما لوحظ وجود نقص معنوي في نسبة الذال.

- عينة عينة عند الأسبوع الثامن من العمر.

- نتائج عينة عند عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينة عينات

- في النتائج التي تم الحصول عليها يمكننا القول بأن هناك اختلافات كبيرة خاصة في مستوى البروتينات الكلية والمستقبلات وتيرة الذال إلى الجلوبولين بالإضافة إلى بروتينات المناعة (أي. جي. جي.) أثناء الفترات المختلفة من العمر. لذلك من الضروري إجراء تحليل لهذه المكونات على فترات متقطعة وذلك للكشف عن تغيرات تتعلق الحالة المرضية التي ربما تختلف داخل الجسم أثناء الفترات المختلفة من العمر.