

Faculty of Veterinary Medicine  
Moshthor, Zagazig University  
Benha Branch



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

By

**HUSSEIN, S.A.**

*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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Department of Biochemistry; Faculty of Veterinary Medicine, Moshtohor Zagazig University, (Benha branch), Egypt.

which act in the hemostatic mechanism, in addition to immunoglobulins there by 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 immunoproteins.

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 emburgo cack 25%, fish meal 6%, bone meal 1.5%, calcium carbonate 1%, sodium

chloride 0.5% and magnesium sulphate 200 gm, vitamin AD<sub>3</sub> 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 (Weichselbaun, 1946), separation of protein fraction electrophoretically by (Koh 1968), serum IgG using immunoprecipitin analysis by (Hellsing, 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 comperatively 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 ablumin 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  $\alpha$  - 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 **Balash 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 cellulose 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 50 days of age. **Sturkie (1976)**, also stated that, five main fractions of plasma proteins can be descend 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. 2) showed a non significant increase in alpha-1 globulin which was (0.140 gm/dl) at 8 weeks, this increase became significant (0.195 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 hypoalbuminemia 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 wer 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).

Age	Six weeks ( $\bar{x} \pm S.E.$ )	Eight weeks ( $\bar{x} \pm S.E.$ )	Twelve weeks ( $\bar{x} \pm S.E.$ )
Total Proteins.	3.2 $\pm$ 0.06	3.35 $\pm$ 0.06	3.98 $\pm$ 0.04**
Albumin.	1.77 $\pm$ 0.06	1.54 $\pm$ 0.12	1.79 $\pm$ 0.11
Globulin.	1.43 $\pm$ 0.08	1.81 $\pm$ 0.14*	2.19 $\pm$ 0.13*
A/G ratio.	1.29 $\pm$ 0.11	0.92 $\pm$ 0.15	0.83 $\pm$ 0.05

\* Significant (P &lt; 0.05).

\*\* : High significant (P &lt; 0.01).

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

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

Age	Total proteins	Albumin	Total Globulins	Globulin fractions			
				Alpha -1	Alpha - 2	Beta	Gamma
Six weeks.	3.20 $\pm$ 0.06	1.77 $\pm$ 0.06	1.43 $\pm$ 0.08	0.119 $\pm$ 0.01	0.439 $\pm$ 0.03	0.552 $\pm$ 0.01	0.320 $\pm$ 0.02
Eight weeks.	3.35 $\pm$ 0.06	1.54 $\pm$ 0.12	1.81 $\pm$ 0.14*	0.140 $\pm$ 0.01	0.582 $\pm$ 0.04*	0.594 $\pm$ 0.02	0.470 $\pm$ 0.03*
Twelve weeks.	3.98 $\pm$ 0.04**	1.79 $\pm$ 0.11	2.19 $\pm$ 0.13*	0.195 $\pm$ 0.01**	0.801 $\pm$ 0.06**	0.620 $\pm$ 0.04	0.580 $\pm$ 0.05**

\* Significant (P &lt; 0.05).

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

\*\* : High significant (P &lt; 0.01).

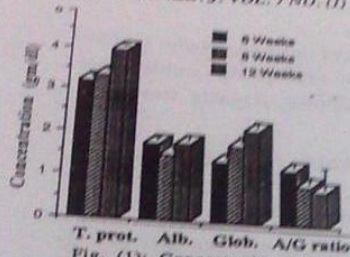
Table (3): Mean values of IgG concentrations and hemagglutination inhibition titres in serum from chickens at different age.

Age	Six weeks ( $\bar{x} \pm S.E.$ )	Eight weeks ( $\bar{x} \pm S.E.$ )	Twelve weeks ( $\bar{x} \pm S.E.$ )
IgG (mg/dl).	216.3 $\pm$ 8.81	346.8 $\pm$ 21.19**	409.2 $\pm$ 34.69**
Hemagglutination inhibition titre	2.4 $\pm$ 1.22	1.6 $\pm$ 1.06	3.2 $\pm$ 1.30

\*\* : High significant (P &lt; 0.01).

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



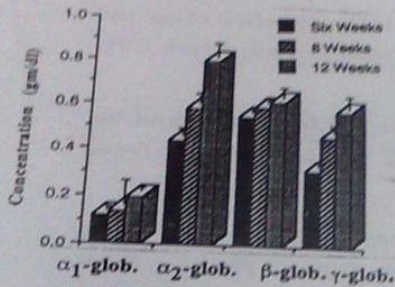


T. prot. Alb. Glob. A/G ratio

Fig. (1): Concentration of total proteins, albumin, globulin and A/G ratio in serum from chickens at different age groups.

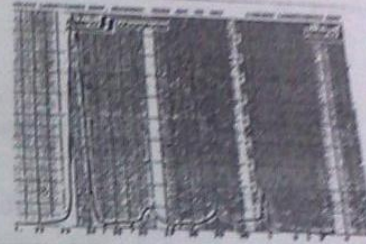


Graph (1): Scanning curve of serum protein fractions of chickens at six weeks of age.



$\alpha_1$ -glob.  $\alpha_2$ -glob.  $\beta$ -glob.  $\gamma$ -glob.

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



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

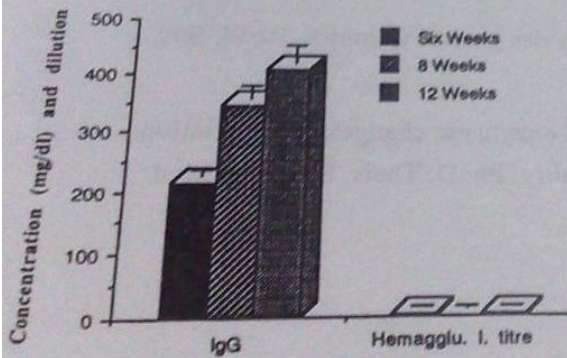
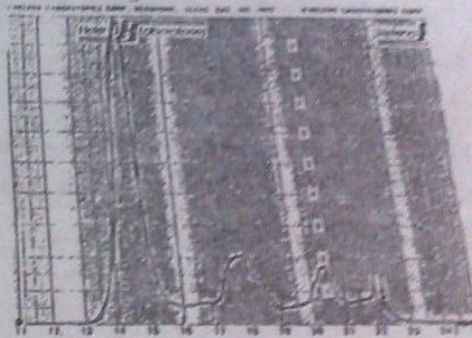


Fig. (3): Concentration of IgG and hemagglutination inhibition titres in serum from chickens at different age groups.



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

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نموذج الهجرة الكهربائية لمصل البروتينات ومستوى بروتينات المناعة (أى . جى . جى) فى الدجاج وعلاقتها بالعمر  
د. سامى على حسين

قسم الكيمياء الحيوية - كلية الطب البيطرى بمشهر - جامعة الزقازيق - فرع بنها - مصر  
إستخدم لإجراء هذا البحث عدد ٣٠ ككتوت عمر يوم تم تربيتها تحت ظروف  
صحية جيدة وذلك لتوضيح تأثير الأعمار المختلفة على محتوى البروتين الكلى ومشتقاته  
بالإضافة إلى بروتينات المناعة (أى . جى . جى) فى الدجاج الطبيعى.  
تم أخذ عينات الدم اللازمة لفصل المصل ثلاث مرات من عدد خمس كتاكيت  
وعلى فترات مختلفة من العمر وذلك عند عمر (٦، ٨، ١٢ أسبوع) من بداية التجربة  
(من عمر يوم) حتى نهاية فترة التجربة التى إمتدت إلى ١٢ أسبوع وذلك لإجراء التحاليل  
الآتية (تقدير مستوى البروتين الكلى، التحليل الكهربائى للبروتين ومشتقاته، وأيضاً تركيز  
بروتينات المناعة «أى . جى . جى» بالإضافة إلى إختبار مانع التلازن فى المصل) وقد أسفر  
هذا البحث عن النتائج التالية :-

- \* زادت نسب الجلوبيولين الكلى، الألبا - ٢ - جلوبيولين، الجاما-جلوبيولين زيادة  
معنوية عند الأسبوع الثامن من العمر بينما لوحظ وجود نقص معنوى فى نسبة الذلال  
إلى الجلوبيولين عند هذا العمر وذلك بمقارنة النتائج عند عمر ٦ أسابيع.
  - \* أظهر مستوى البروتين الكلى، الألبا-٢- جلوبيولين، الجاما-جلوبيولين زيادة عالية  
المعنوية عند عمر ١٢ أسبوع بينما لوحظ وجود زيادة معنوية فقط فى تركيز الجلوبيولين  
الكلى، والألبا - ١ - جلوبيولين عند هذا العمر .
  - \* لوحظ عدم وجود أى تغيرات معنوية فى مستوى الألبومين، البيتا جلوبيولين وأيضاً التتر  
فى إختبار مانع التلازن عند الأعمار المختلفة من التجربة .
  - \* بينما سجل مستوى بروتينات المناعة (أى - جى - جى) زيادة عالية المعنوية عند عمر  
٨، ١٢ أسبوع وذلك بمقارنة النتائج عند عمر ٦ أسابيع.
- من النتائج التى تم الحصول عليها يمكننا القول بأن هناك إختلافات كبيرة خاصة فى  
مستوى البروتين الكلى ومشتقاته ونسبة الذلال إلى الجلوبيولين بالإضافة إلى بروتينات المناعة  
(أى . جى . جى) أثناء الفترات المختلفة من العمر . لذلك من الضرورى إجراء تحليل لهذه  
المكونات على فترات منتظمة وذلك لإكتشاف أى تغيرات تعكس الحالة المرضية التى ربما  
تحدث داخل الجسم أثناء الفترات المختلفة من العمر .