

RELATIONSHIP BETWEEN IMMUNOGLOBULIN G (IgG) AND SOME BLOOD CONSTITUENTS IN CHICKENS

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ABSTRACT: The present study was carried out at the Department of Poultry and Fish Production, Faculty of Agriculture at Menoufia University, Shibin El-kom. The aim of the present study was to determine the concentration of immunoglobulin G (IgG) and investigate its relation to some blood constituents in chickens. Pullets were divided into three groups, control, high and low groups, based on IgG concentrations in blood serum.

The most important results can be summarized in the following points:-

1. The high immune response group (HR) had the highest IgG concentration (13.68 ± 0.339 mg/dl) compared to both of control (10.11 ± 0.362 mg/dl) and the low IgG (7.20 ± 0.339 mg/dl) groups. The statistical differences among groups were highly significant ($p \leq 0.01$)
2. There was a positive relationship between the immune response to SRBC antigen and hemoglobin concentration, hematocrit percentage, red blood cell counts, white blood cell counts, total plasma protein concentration, albumin and globulin concentration.
3. In general, the present results concluded that a complex relationship between immunoglobulin G and immunological and hematological traits of the chickens. The injection of SRBC as a natural antigen induced superior values of antibody response which resulted in good performance of some blood constituents in chickens.

key words: IgG, blood constituents ,chickens.

INTRODUCTION

1. The concentration of immunoglobulin G (IgG):

Three immunoglobulin classes, which are distinguishable in concentration structure, and immunochemical function, are found in birds. IgA, IgM, IgG. The birds of IgA and IgM are similar to mammalian IgA and IgM in molecular weight, structure and electrophoretic mobility (Carlander, 2002).

Also, IgG is the most abundant immunoglobulin found in chicken serum. It has been found at a concentration of 5.29 mg/ml, with a range of 3.7 to 8.0 mg/ml (Lestie and Martin, 1973).

2. Effect of IgG concentration on some blood constituents in chickens:

The constituents of blood reviewed were hemoglobin, hematocrit value, red blood cells, white blood cells, platelets, total plasma proteins, total plasma albumin, total plasma globulin and albumin to globulin ratio.

2.1. Hemoglobin concentration :

The amount of hemoglobin in avian blood is highly variable. Whittow (2000) demonstrated that much of this variations may be attributed to the methods of determination. Also, hemoglobin content of chickens blood depends on the breed and immune response.

In addition, Stromp Fova, et al., (2006) reported a significant increase in the concentration of hemoglobin measurement after application of probiotic. Although, it was reported that the high immune response chickens had higher counts of RBC which increased the hemoglobin content as compared to the low immune response chickens (Gebriel et al., 2010).

Also, **Khe** (2011) studied the interaction effect of different levels of primary immune response some blood constituents in Norfa chickens. She found that the high antibody titers to sheep red blood cells (SRBC) or bovine serum albumin (BSA) antigens increased some blood constituents including RBC, WBC, hematocrit and hemoglobin concentrations.

Also, **Nyaulingo (2013)** reported that increased hemoglobin concentration as chicks grew older, because of the increase in the number of RBC brought about by the increase of feed intake. The changes in hemoglobin concentration with age of chickens concurred with other reports (**Islam and Rahman, 2004 and Talebi et al., 2005**).

2.2. Hematocrit valume concentration (Packed cell volume concentration, PCV) :

Early studied by **Bakir et al., (1988)** who showed that there were significant differences in PCV values between four breeds (White Cornish, Sinai, White Plymouth Rock, and Dokki-4) at sexual maturity and at four months after laying.

Also, **Hanlon et al., (1997) and Kucharska et al., (1999)** reported that the high immune response line had higher values of most blood constituent parameters including hematocrit value, as compared to the low immune response line of chickens injected with BSA or SRBC antigens.

Also, **Saad, (2006) and El- Fiky, (2007)**. found that most of the blood constituent parameters had higher values in high immune response chickens to SRBC or BSA antigens as compared to the low immune response and control chickens.

2.3. Red Blood cells count: (Erythrocytes)

The count of red blood cells (RBC) varied greatly among breeds and within individuals of a breed, because the cells of RBC are not uniformly distributed in the blood vascular system (**Sturkie and Griminger, 1986**). In White Leghorn chickens at different ages, they found that the number of RBC in normal chickens increases from rather less than $2.00 \times 10^6/\text{mm}^3$ immediately after hatching to about $3.91 \times 10^6/\text{mm}^3$ in adult males and from $2.72 \times 10^6/\text{mm}^3$ to $3.00 \times 10^6/\text{mm}^3$ in adult females, which cleared that males had higher number of RBC than females.

Also, **Gebriel et al. (2010)** reported that the counts of red blood cells in Norfa chickens ranged from 1.12 to $4.81 \times 10^6/\text{mm}^3$ with total average of $3.18 \times 10^6/\text{mm}^3$.

In addition, **Khedr, (2011)** studied the interaction effect of different levels of primary antibody titers on some blood constituents in Norfa chickens. She found that the high immune response chickens to SRBC antigen had the highest counts of RBC as compared to low immune response or control chickens. The RBC count reached $3.53 \times 10^6/\text{mm}^3$ for high immune response group of chickens.

2.4. Red Blood cells indices:

Red blood cells indices including mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). There are some factors affecting on the RBC indices including immune response (**Gebriel et al., 1979**).

The high antibody response chickens to SRBC antigen had positive effect on mean cell volume (MCV) index and negative effect on both mean cell hemoglobin (MCH) and mean cell hemoglobin concentration in birds (**Campbell, 1988**). It was reported also that mean corpuscular volume is the expression of the average volume of individual RBC (**Talebi et al., 2005**).

3 Similar results were reported by Guo et al., (2004) who noticed that red blood cells play a vital role in white blood cells immune function modulation. They found positive association between antibody response and the mean cell volume (MCV).

66 Since the red blood cells and hemoglobin contents were increased in males as compared to females, which resulted in increasing of the mean cell volume of RBC index and decreasing the mean cell hemoglobin MCH, and mean cell hemoglobin concentration (MCHC) of the RBC indices in chickens (Campbell, 1988).

Similar results were reported by Whittow, (2000), who demonstrated that there were higher levels of hemoglobin in males than females of birds. This is correlated with the usual higher counts of RBC in the males, which increased of the MCV index and decreased the MCH and MCHC of the RBC indices in chickens.

2.5. White blood cells counts: (Leukocytes)

The number of WBCs varied greatly among breeds and within breeds. Ghany et al., (1961) found that the average numbers of WBCs in Fayoumi and Rhode Island Red Pullets were 67.0×10^3 and $73.0 \times 10^3/\text{mm}^3$ blood, respectively. Also, Brake and Baker (1982) found that the average number of WBCs was $35.0 \times 10^3/\text{mm}^3$ for females Rhode Island Red (RIR), which include 58.1% lymphocytes, 35.1% heterophils, 1.2% eosinophils, 3.1% basophils and 2.5% monocytes. They also reported that the number of WBCs in adult White Leghorn chickens was $28.9 \times 10^3/\text{mm}^3$.

Also, Gebriel et al., (2010) studied the WBC counts in control group in Norfa chickens. They found that the counts of WBC ranged from 21.34 to $39.10 \times 10^3/\text{mm}^3$ with total average of $29.89 \times 10^3/\text{mm}^3$.

2 In addition, Eid (2010) reported that the high immune response chickens had higher counts of WBCs as compared to the low immune response chickens to SRBC antigen, the differences were significant ($P \leq 0.05$).

2.6. Thrombocytes/ platelets in chickens:

16 Chicken thrombocytes are nucleated blood leukocytes homologous in function to mammalian platelets. They represent the most numerous white blood cell types in chickens blood and perform a hemostatic role similar to that of platelets (Chang and Hamilton, 1979). They also reported that avian thrombocytes are the most abundant white blood cell type in chicken blood.

2.7. Total plasma proteins concentration:

2 Kalamah (1995) found that the total plasma proteins in Norfa chickens was affected by the body weight. It was 5.39 and 5.44g/100ml in heavy and light body weight, respectively. The differences in total plasma proteins in relation to body weight statistically was significant ($P \leq 0.05$).

The functions of plasma proteins are (1) to help maintain normal blood pressure, (2) to influence the suspension stability of the erythrocytes, (3) to help regulate the acid – base balance of the blood, (4) to provide antibodies (immunoglobulins), (5) to affect the solubility of carbohydrates, lipids, and other substances held in solution in the plasma and (6) to transport substances bound by plasma proteins (Sturkie and Griminger, 1986).

8 Early studied by Ghany et al. (1961) who showed that the total plasma proteins in immature Fayoumi and Rhode. Island Red were 7.11 and 6.36g/100ml plasma, respectively, while, it reached to 7.45 and 9.38 g/100ml in adult females of Fayoumi and Rhode Island Red, respectively.

On the other hand, **Parmentier et al., (1994)**, **Hanlon et al., (1997)**, and **Kuckarska et al., (1999)** reported that the high immune response chickens line (HR) had higher values of most of hematological parameters including the total plasma proteins as compared to the low immune response chickens line (LR).

However, **Abd El-Rahman (2006 and 2012)** studied the relationship between immune response to sheep red blood cells and reproductive performance in chickens. She found that the statistical differences between strains of chickens were not significant. The total plasma proteins were 7.64 and 7.65 in both Norfa and WL strains.

2.8. Total plasma albumin concentration:

Plasma albumin is one of the most important proteins present in animal blood. It use as store for proteins in body and it also keeps the osmotic pressure stable in the blood. It was found that the albumin concentration in Norfa chicken hens was 2.26.g/100ml plasma, while the albumin concentration in cocks at 30.wk of age was 1.36g/ 100ml plasma (**Soliman et al., 2007**).

Also, **Gebriel et al. (2010)** found that the total plasma albumin concentration in Norfa chickens ranged from 1.07 to 6.04 g/100ml, with total average of 2.68 g/100ml.

In addition, **Eid (2010)** found that the high levels of antibody titers chickens had the highest concentration of total plasma albumin (1.50 g/100ml), followed by control chickens (1.44g/100ml) and the low antibody titers chickens. (1.41 g/100ml).

2.9. Total plasma globulin concentration:

Early studies stated that the average concentration of plasma globulin in White Leghorn hens was 2.94g/100ml plasma, while the concentration of plasma globulin in new Hampshire chickens were 2.14 to 3.50 g/100ml plasma at 8 and 16-wk of age, respectively. (**Sturkie and Griminger, 1986**)

In addition **Soliman et al., (2007)** found that the total plasma globulin concentration in Norfa adult hens was 3.08 g/100ml plasma, where the corresponding value in cocks was 3.10 g/100ml plasma. Also, **Gebriel et al., (2010)** showed that the concentration of plasma globulin in Norfa chickens ranged from 1.97 to 5.02 g/100ml with total average of 2.91g/100ml.

2.10. Albumin to globulin ratio:

Shim et al., (1979) reported that the mean value of albumin, globulin ratio (A/ G) ratio in immature females of White Leghorn was 1.40%. While, the mean value of A/G was decreased to 0.85% in adults females of White Leghorn chickens.

Also, **Ibraheem, (1987)** indicated that the values of A/G ratio of Fayoumi chickens were 0.70% and 0.61% at 131 and 219 days of age, respectively. However, **Saleh, (1997)** stated significant difference in A/G ratio between Lohman selected Leghorn and Baladi hens.

In addition , **Eid, (2010)** found that the values of the A/G ratio were 0.84% for males and 0.88% for females. He also reported that the antibody titers did not effect on the values of A/G ratio, being 0.87, 0.87 and 0.84% for high immune response, low immune response and control chickens, respectively.

MATERIALS AND METHODS

The present study was carried out at the Department of Poultry and Fish production, Faculty of Agriculture , Menoufia University, Shibin El- Kom . The experiments were

extended from Nov, 2018 to Feb. 2020, in order to determine the immunoglobulin G and investigate its relation to some blood constituents in chickens.

1. Chickens stock

Norfa strain was used in the present study as a synthetic local breed of chickens. It was developed at the Poultry Research Farm, Department of Poultry and Fish Production, Faculty of Agriculture, Menoufia university, Shibin El- Kom (Abdou, 1996)

2. Mating system and reproduction:

A total of 24 sires and 72 dams were used as parents for reproducing the next generation. The artificial insemination was used as a mating system for reproducing the chickens stock of the next generation. Each family contained one sire and three dams. Dams were assigned at random to each sire for reproducing the next generation.

Fertile eggs were collected two times a day and pedigreed according to their dams. Crooked, dirty and misshapen eggs were removed. Then, eggs were stored in egg storage room at 15-17°C for 7 days, with 80% relative humidity.

For hatching, all fertile eggs were moved to the incubation room and left for at least 12 hours at room temperature. Then, all eggs were set with wide end up in the setting trays according to their dams and incubated in a forced draft incubator at 99.5°F (37.8°C) with a relative humidity of 60.0% during the incubation period. On the day of hatching, chicks were wing banded, weighed and moved to brooding house.

3. Experimental stock managements:

Chicks identified according to their dams were weighed and moved from hatcher to brooding house. All chicks were brooded on floor brooder with wood shaving litter. The starting brooder temperature was 34°C during the first week. Then, the brooder temperature was decreased gradually from 2-3°C every week to reach 22-24°C at six weeks of age. The chicks were moved to rearing house at eight weeks of age.

All chicks were exposed to continuous artificial light for 24 hours during the first week of age, and then the light was decreased gradually to reach the natural day light by about 8-weeks of age. All chickens were received only natural day light from 9 to 17 weeks of age. At 18 weeks of age, pullets were moved to individual cages in laying house, and kept until 42-weeks of age under 16-hours of light a day. While, the males were moved to individual cages in cock house.

All chicks were fed *ad libitum* diet (which contained 19.88% crude protein and 2889 kcal ME/ kg diet), during both the brooding and rearing periods. Whereas, chickens were fed *ad libitum* diet (which contained 17.15% crude protein and 2739 kcal ME/Kg) during the production period until the end of experiments (NRC, 1994).

4. Experimental design and chicken groups:

A total number of 110 individuals (80 females and 30 males) of Norfa chickens were taken at random to be used in the present experiment. At 18 weeks of age, the pullets were housed individually in wire individual cages in laying house, where the hens were kept until the end of the experiment (42-weeks of age), where males were kept in cock house.

At 20-weeks of age, the immunoglobulin G (IgG) was determined in blood serum of each individual. Pullets were divided into three groups based on the IgG concentration as follows.

4.1. Control group (CG):

Pullets of control group (CG) were taken at random from the stock (80 females). Control group contained 21 females. No significant difference was found between the mean of the control group and that of the base data.

4.2. High immunoglobulin G (IgG) group (HG):

Pullets reached IgG concentration in blood serum more than ($\bar{X}+1Sd$) were taken and considered as high group (HG). The HG contained 24 females .

4.3. Low immunoglobulin G (IgG) group (LG):

Pullets had IgG concentration in blood serum lower than ($\bar{X}-1Sd$) were selected and considered as low group (LG). The low group contained 24 females .

5. Determination of total immunoglobulin G (IgG) in Pullet blood serum:

The IgG antibody was determined in the following steps.

5.1 Preparation of SRBC antigen:

The SRBC was brought to 2.5% vo/vo solution in PBS and used for immunization, according to Kundu et al., (1999).

5.2 Antigen immunization:

The SRBCs were immunized according to the method of Siegel and Gross (1980). In the primary immunization, at 10-week of age, each chicken received an intravenous inoculation via the branchial vein with 0.1 ml of 2.5% SRBCs suspension to induce the primary antibody response.

5.3 Blood samples collection and IgG determination:

At 2 weeks of age, about 2ml of blood sample was collected in dry tube via the wing vein, The blood samples were centrifuged at 3000 rpm for 15min at 4°C. The liquid that remained after clotted blood was collected, placed in disposable tubes and frozen for subsequent laboratory determination (Siegel and Gross, 1980).

The immunoglobulin G (IgG) concentration was determined in Lab Top in Zagazig city, Sharkia Governorate, using mono reagents, specific for IgG determination, using ELISA method according to Tip (2010). The IgG concentrations were recorded as mg/ ml.

6. Hematological determination:

At 34 weeks of age, blood samples were collected into EDTA test tubes from wing vein of each layer of all groups. Plasma was separated by centrifugation at 3000 rpm for 15 min and stored frozen at -20°C until hematological and chemical determinations.

6.1 Determination of hemoglobin (Hb) concentration:

Hemoglobin concentration was determined in anticoagulated blood samples. It was measured photometrically. In this technique, the absorbance of hemoglobin in a blood sample was measured electronically using a filter colorimeter or a direct read- out hemoglobin meter, with set wave length at 540 nm and Drabkin's neutral diluting fluid at pH 7.00 to 7.40, according to the method described by Lewis (1995). Hemoglobin concentration was expressed as (g/100ml).

6.2. Determination of red blood cells count (RBC):

Erythrocytes counts (RBC) were determined using thoma haemocytometer in red blood diluting pipette according to the method described by Campbell (1988) and expressed as counts ($10^6/cm^3$).

6.3. Determination of hematocrit value:

Hematocrit value was determined and expressed as percentage of packed cell volume (PCV%) according to the method described by Hunsaker (1969).

6.4. Determination of red blood cells indices:

Red blood cells indices determined included mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). The red blood cell indices were determined according to the method of Campbell (1988).

6.4.1. Mean cell volume determination:

The mean cell volume (MCV) provides information on cell size. It is measured in femtoliters. A femtoliter (FL) is 10^{-15} of a liter. The MCV was determined from the PCV and electronically obtained RBC count. It can be calculated as follows:

$$MCV (FL) = \frac{PCV \times 10}{n. of RBC (Cu. mm blood) \times 10^6}$$

6.4.2. Mean cell hemoglobin determination:

The mean cell hemoglobin (MCH) gives amount of hemoglobin in picogram (Pg) in an average red cell. A picogram (Pg) is 10^{-12} of gram. It is calculated from the hemoglobin and electronically obtained RBC count. It can be calculated as follows:

$$MCH (Pg) = \frac{Hemoglobin (g/dl) \times 10}{n. of RBC (Cu. mm blood) \times 10^6}$$

6.4.3. Mean cell hemoglobin concentration:

The mean cell hemoglobin concentration (MCHC) gives the concentration of hemoglobin in g/L in one liter of packed red cells. It is calculated from the hemoglobin (Hb) and PCV as follows:

$$MCHC (g/100ml) = \frac{Hemoglobin (g/dl) \times 10}{PCV}$$

6.5. Counting of white blood cells (WBC):

Serum sample from each hen was collected and immediately examined for total leukocyte cells counts (LC) by using white blood pipette, according to the method of Campbell (1988), which monitored to count by using photomicroscope provide with a monitor screen and counter.

7. Chemical determinations:

Chemical determinations included the determinations of total plasma protein (TP), plasma albumin (A) and plasma globulin (G).

7.1. Total plasma proteins (TP):

The colorimetric determination of total plasma proteins (TP) concentration was carried out with the principle that in the presence of an alkaline cupric sulfate, the protein produced a violet, the intensity of which is proportional to protein concentration according to the methods of Merck (1974).

7.2. Plasma albumin:

One of the most important serum proteins produced in the liver is albumin, the plasma albumin was determined according to the method of **Merck (1974)**.

7.3. Plasma globulin:

Plasma globulin (G) concentration was obtained by subtracting the value of albumin from the corresponding value of total plasma proteins.

8. Studied traits:

The following traits were studied during the experimental period:

8.1. The IgG concentrations in blood serum of chickens:

The concentrations of IgG were determined in blood sample at 20 wk of age as mg/ml.

8.2. Hemoglobin concentration:

Hemoglobin (Hb) concentration was individually determined at 34 wk of age for the different groups of layers, and expressed as Hb g/100ml.

8.3. Hematocrit value:

Hematocrit value was determined at 34 wk of age for the different groups of layers and expressed as percentage of packed cell volume (PCV%).

8.4. Red blood cells count:

Red blood cells counts (RBC) were determined for each layer at 34 wk of age for the different groups of layers and expressed as RBC counts ($10^6 \times \text{cm}^3$).

8.5. Red blood cells indices:

The RBC indices includes the following three indices.

A - Mean cell volume:

Mean cell volume (MCV) was calculated in femtoliters and expressed as MCV (fl).

B - Mean cell hemoglobin:

Mean cell hemoglobin (MCH) was determined from the hemoglobin and expressed as picogram MCH (Pg).

C - Mean cell hemoglobin concentration:

Mean cell hemoglobin concentration (MCHC) was determined from the hemoglobin and expressed as MCHC (g/100ml).

8.6. White blood cells count:

White blood cells (total leukocytes counts) were determined individually for each layer of different groups, and expressed as WBC ($10^3/\text{cm}^3$).

8.7. Total plasma proteins:

Total plasma proteins (TP) were determined individually for each layer of the different groups, and expressed as TP (g/dl).

8.8. Plasma albumin:

Plasma albumin (A) was determined individually for each layer of the different groups, and expressed as A (g/dl)

8.9. Plasma globulin:

Plasma globulin (G) concentration was determined individually for each layer of the different groups, and expressed as G (g/dl).

8.10. Albumin to globulin ratio:

Albumin to globulin ratio was calculated individually and expressed as A: G (%).

9. Statistical analysis:

Least square means and their standard errors ($\bar{X} \pm SE$) for each studied trait were calculated for the different groups. Data obtained were statistically analyzed using SPSS program (2004). Probability value, ($P \leq 0.05$) was considered for significant.

All percentages were converted to the corresponding arcsine, if necessary, prior statistical analysis as given by SAS (1988). Also, Duncan's the multiple Range (DMR) test was used for multiple comparisons of means (Duncan, 1955).

One way classification statistical fixed model was used for statistical analysis as the following:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where :

Y_{ij} = the i th observation of the individual over all means.

μ = the common mean.

G_i = The fixed effect of IgG response.

e_{ij} = Experimental error.

RESULTS AND DISCUSSION

1. The concentration of immunoglobulin G (IgG) in chickens:

The obtained results in Table (1) showed the concentrations of immunoglobulin G of different groups of Norfa chickens. The statistical analysis of the obtained data revealed that there were highly significant differences in the values of immunoglobulin G among control group and other two high and low Norfa chicken groups ($P \leq 0.01$). The highest mean value was recorded for high Norfa group (13.68 ± 0.339 mg/dl), followed by control group (10.11 ± 0.362 mg/dl) while low Norfa group registered the least value (7.20 ± 0.339 mg/dl).

Results, also indicated that the immunoglobulin G concentration of the high group of Norfa chickens was increased by 35.3% compared to control group, while it was decreased by 28.8% in the low group.

The present results are almost similar to the results reported by Carlander (2002). He found highly significant differences among lines of chickens in IgG concentrations in blood serum of chickens.

Similar results were reported recently by Sebea, (2021). She found that the IgG concentrations were 13.500 , 7.576 and 10.195 mg/ml blood serum in high, low and control chickens groups, respectively.

Table (1): The concentrations of immunoglobulin(G) IgG ($\bar{x} \pm SE$) of control , high and low groups of Norfa chickens , (mg/ dl) .

| Chicken groups | n. | Concentrations of | % change of control |
|----------------|----|-------------------|---------------------|
|----------------|----|-------------------|---------------------|

| | | IgG (mg/dl)* | |
|------------|----|----------------------------|-------|
| Control | 21 | 10.11 ± 0.362 ^b | 100.0 |
| High IgG | 24 | 13.68 ± 0.339 ^a | 135.3 |
| Low IgG | 24 | 7.20 ± 0.339 ^c | 71.2 |
| Total Ave. | 69 | 10.33 | - |
| P. value | - | 0.01 | |

abc : Means in the same column bearing different super scripts are significantly different .

2. The relationship between IgG concentrations and some blood constituents in chickens:

2.1. Hemoglobin concentrations:

Hemoglobin concentrations of Norfa chicken groups as affected by immunoglobulin G concentration are presented in Table (2). The statistical analysis of the obtained data revealed that the high immunity groups of Norfa chickens was recorded the highest significant hemoglobin concentration (12.82 ± 0.19 g/dl). when compared with control group (10.23 ± 0.21 g/dl), while low group recorded the least one (8.06 ± 0.19 g/dl).The statistical differences among groups were highly significant ($P < 0.01$).

Results, also indicated that hemoglobin concentration of the high group of Norfa chickens was increased by 25.3% compared to control group, while it was decreased by 21.2% in the low group.

The present results are in good agreement with the results reported by Kucharska et al. (1999). They reported that the high immune response thickeners had higher concentrations of hemoglobin as compared to low or control immune response thickeners.

Similar results were reported by Khedr (2011). She found that the high immunity group to SRBC or BSA antigens increased some blood constituents including RBC, WBC, hematocrit and hemoglobin concentrations.

Table (2): Effect of IgG antibody concentrations on hemoglobin concentrations (g/dl) of control and selected groups of chickens.

| Chicken groups | n. | Hb (g/dl) ($\bar{x} \pm SE$) | % change of control |
|----------------|----|-----------------------------------|---------------------|
| Control | 21 | 10.23 ± 0.21 ^b | 100.0 |
| High IgG | 24 | 12.82 ± 0.19 ^a | 125.3 |
| Low IgG | 24 | 8.06 ± 0.19 ^c | 78.8 |
| Total Ave. | 69 | 10.37 | - |
| P. value | - | 0.01 | - |

abc = Means in the same column bearing different superscripts are significantly different.

2.2. Hematocrit value (packed cell value):

Hematocrit values of Norfa chicken groups as affected by immunoglobulin G concentrations are presented in Table (3). The statistical analysis of the obtained data revealed that the high immunity group of Norfa chickens was recorded the highest significant hematocrit value ($28.75 \pm 1.64\%$) when compared with control group ($19.16 \pm 1.59\%$), while low group recorded the least one ($13.51 \pm 1.64\%$).

Results, also indicated that hematocrit value of the high group of Norfa chickens was increased by 50.1% compared to control group, while it was decreased by 29.5% in the low group.

Similar results were reported by, Hanlon et al., (1997) and Kucharska et al., (1999) They reported that the high immune response line had higher values of most blood constituent parameters including hematocrit value, as compared to the low immune response line of chickens injected with SRBC antigens.

Also, the present results are in good agreement with the results reported by Saad (2006) and El-Fiky (2007). They found that most of the blood constituents had higher values in high immune response chickens to SRBC of BSA antigen as compared to low immune response and control chickens.

In addition, Khedr , (2011) studied the effect of antibody titers on PCV% in Norfa chickens. She found that high antibody titers had significantly higher hematocrit value than low or control chickens.

Table (3): Effect of IgG antibody concentrations on hematocrit valume (PCV%) of the different groups of chickens .

| Chicken groups | n. | PCV (%) ($\bar{x} \pm SE$) | % change of control |
|----------------|----|---------------------------------|---------------------|
| Control | 21 | 19.16 \pm 1.59 ^b | 100.0 |
| High IgG | 24 | 28.75 \pm 1.64 ^a | 150.1 |
| Low IgG | 24 | 13.51 \pm 1.64 ^C | 70.5 |
| Total Ave. | 69 | 20.47 | - |
| P. value | - | 0.01 | - |

abc : Means in the same column bearing different superscripts are significantly different .

2.3 Red blood cells (Erythrocytes) counts:

The counts of red blood cell (RBC) of Norfa chicken groups affected by immunoglobulin G to SRBC are given in Table (4).The statistical analysis of the obtained data revealed that the high immunity group of Norfa chickens was recorded the highest significant counts of red blood cells (3.43 \pm 0.87) (10⁶/ cm³), while low group recorded the least one (1.54 \pm 0.87) (10⁶/cm³) and the control group was in between(2.45 \pm 0.93) (10⁶/cm³).

Results, also indicated that counts of red blood cells of the high immunity group of Norfa chickens were increased by 40% compared to control group, while it was decreased by 37.1% of the low group.

The present results are in good agreement with the results reported by Gebriel et al. (2010). They found that the counts of red blood cells (RBC) in Norfa chickens ranged from 1.12 to 4.80 X 10⁶ mm³with total average of 3.18 X10⁵ mm³.

Also, Khedr, (2011), studied the interaction effect of different levels of primary antibody titers on some blood constituents in Norfa chickens. She found that the high immune response chickens to SRBC antigen had the highest counts of RBC as compared to low immune response and control chickens. The RBC count reached 3.53 X 10⁶/ cm³ for high immune response group of chickens.

In addition, the present results were in good agreement with the results reported by Abd El-Rahman,(2012). She found that high immune response chickens had higher counts

of RBC than low immune response and control chickens. The counts were $3.52 \times 10^6/\text{cm}^3$, $2.10 \times 10^6/\text{cm}^3$ and $2.37 \times 10^6/\text{cm}^3$ for high, low immune response and control chickens, respectively. The statistical differences among immune response groups were highly ($P \leq 0.01$) significant.

Table (4): Effect of IgG antibody concentrations on red blood cells (RBC) count ($10^6/\text{cm}^3$) of the different groups of chickens.

| Chicken groups | n. | RBC ($10^6/\text{cm}^3$) ($\bar{x} \pm SE$) | % change of control |
|----------------|----|--|---------------------|
| Control | 21 | 2.45 ± 0.93^b | 100.0 |
| High IgG | 24 | 3.43 ± 0.87^a | 140.0 |
| Low IgG | 24 | 1.54 ± 0.87^c | 62.9 |
| Total Ave. | 69 | 2.47 | - |
| P. value | - | 0.01 | - |

abc : Means in the same column bearing different superscripts are significantly different.

2.5. Red blood cells indices :

Red blood cells indices including mean cell volume (MCV), Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). The effect of immunoglobulin G on red blood cells indices is given in Table (5). The present results showed that high immune response chickens to SRBC antigen had the highest value of MCV of RBC index, and the lowest values of both MCH and MCHC of RBC indices as compared to the low immunoglobulin G and control chickens. Whereas, the low immunoglobulin G and control chickens had the lowest value of MCH index and the highest values of both MCV and MCHC indices in Nofra chickens.

Since the red blood cells and hemoglobin contents were lower in low immune response and control lines of chickens, which resulted in increasing of the MCV of RBC index and decreasing the MCH and MCHC of the RBC indices in chickens, the statistical differences among immune response groups of chickens in RBC indices were significant ($P \leq 0.05$) or highly ($P \leq 0.01$) significant.

The present results were in close agreement with the result reported by Campbell (1988) and Guo et al., (2004). They found that the high immune response chickens to SRBC antigen had positive effect on MCV of the RBC index and negative effect on both MCH and MCHC of RBC indices in chickens.

Also, Abd El-Rahman, (2012), concluded that the high immune response to SRBC antigens had Positive association with MCV and negative association with both MCH and MCHC of the RBC indices with significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$) differences among immune response groups of chickens.

Table (5): Effect of IgG antibody concentrations on red blood cells indices of the different groups of chickens.

| Chicken groups | n. | $(\bar{x} \pm SE)$ | | MCHC (g/dl) |
|----------------|----|--------------------|--------------------|--------------------|
| | | MCV (fl) | MCH (Pg) | |
| Control | 21 | 42.87 ± 1.46^b | 17.42 ± 0.64^b | 46.68 ± 0.49^b |

| | | | | |
|------------|----|---------------------------|---------------------------|---------------------------|
| High IgG | 24 | 46.09 ± 1.36 ^a | 15.38 ± 0.60 ^c | 33.40 ± 0.46 ^c |
| Low IgG | 24 | 39.95 ± 1.36 ^c | 21.29 ± 0.60 ^a | 54.90 ± 0.46 ^a |
| Total Ave. | 69 | 42.97 | 18.03 | 44.99 |
| P. value | - | 0.05 | 0.01 | - |

30 : Means in the same column bearing different superscripts are significantly different.

MCV = Mean cell volume.

MCH = Mean cell hemoglobin

MCHC = Mean cell hemoglobin concentration.

2.6. White blood cells (leukocytes) counts:

White blood cells counts (WBC) of Norfa chicken groups as affected by immunoglobulin G to SRBC are presented in Table (6). The statistical analysis of the obtained data revealed that the high immunity groups of Norfa chickens was recorded the highest significant counts of white blood cells (31.27 ± 0.46) ($10^3/ \text{cm}^3$) when compared with control group (24.49 ± 0.48) ($10^3/ \text{cm}^3$), while low group recorded the least one (18.86 ± 0.46) ($10^3/ \text{cm}^3$).

Results also indicated that counts of red blood cells of the high immunity group of Norfa chickens were increased by 39.3% compared to control group, while it was decreased by 23.7% in the low group.

The present results were similar to the results reported by El- Fiky, (2007) and Eid (2010). They reported that the counts of WBC varied greatly due to the effect of antibody response. The high immune response chickens had higher counts of WBC as compared to the low immune response to SRBC antigen and control chickens.

Also, Gebriel et al. (2010) found that the counts of WBC ranged from 21.34 to 39.10 $\times 10^3 / \text{mm}^3$ with total average of $29.89 \times 10^3 \text{ mm}^3$.

Table (6): Effect of IgG antibody concentrations ($\bar{x} \pm SE$) on white blood cells (WBC) of the different groups of chickens.

| Chicken groups | n. | WBC ($10^3/ \text{cm}^3$) | % Change Of Control |
|----------------|----|-----------------------------|---------------------|
| Control | 21 | 24.49 ± 0.48 ^b | 100.0 |
| High IgG | 24 | 31.27 ± 0.46 ^a | 139.3 |
| Low IgG | 24 | 18.86 ± 0.46 ^c | 77.0 |
| Total Ave. | 69 | 24.87 | - |
| P. value | - | 0.01 | - |

abc = Means in the same column bearing different superscripts are significantly different.

2.7. Platelets

Platelets of Norfa chicken groups as affected by immunoglobulin G to SRBC are presented in Table (7). The statistical analysis of the obtained data revealed that the high immunity group of Norfa chickens was recorded the highest significant value of platelets (197.37 ± 12.89) ($10^3/ \text{ml}$) when compared with control group (153.33 ± 13.78) ($10^3/ \text{ml}$), while low group recorded the least one (110.83 ± 12.89) ($10^3/ \text{ml}$).

Result also indicated that value of platelets of the high group of Norfa chickens was increased by 28.7% compared to control group, while it was decreased by 27.7% in the low group.

It was reported that the highest platelet counts in indigenous chickens have previously been reported (Islam and Rahman, 2004), as compared to foreign chickens. Also Afolabi,

(2010) concluded that the platelets responsible for hemostasis, trephocytosis and phagocytosis in birds. It was lower in the blood of local grower chicken except for birds on d²³ that contained 10% PKC which recorded $17.35 \pm 0.55 \times 10^6/\text{mm}^3$ platelet, which was also similar ($p>0.05$) to those of birds on other diets.

In addition, El-Fiky (2007) found that the most of the blood constituents had higher values in high immune response chickens to SRBC antigen including platelets as compared to control or low immune response chickens.

Table (7): Effect of IgG antibody concentrations on platelets (PLT) of control and selected groups of chickens.

| Chicken groups | n. | PLT ($10^3/\text{ml}$) ($\bar{x} \pm SE$) | % change of control |
|----------------|----|--|---------------------|
| Control | 21 | 153.33 ± 13.78^b | 100.0 |
| High IgG | 24 | 197.37 ± 12.89^a | 128.7 |
| Low IgG | 24 | 110.83 ± 12.89^c | 72.3 |
| Total Ave. | 69 | 153.67 | - |
| P. value | - | 0.01 | - |

abc : Means in the same column bearing different superscripts are significantly different .

3. Chemical blood constituents :

The chemical blood constituents studied were total plasma protein (TB), Plasma albumin (A) plasma globulin (G) and A/G ratio in relation to the effect of immune response to SRBC antigen in Norfa Chickens.

3.1. Total plasma protein :

Total plasma protein of Norfa chickens groups as affected by immunoglobulin G to SRBC antigen are presented in Table (8). It is clear that high immunoglobulin G chickens had higher total plasma protein (5.95 ± 0.11) g/dl as compared to control chickens (5.05 ± 0.12) g/dl and low immunoglobulin G chickens (4.17 ± 0.11) g/dl. The statistical differences among immune response groups of Norfa chickens were highly ($P \leq 0.01$) significant.

Similar results were reported early by Edward and Peters, (1972). They found that the total plasma protein increased due to the immunization of chickens with bovine serum albumin antigen, or SRBC antigen.

Also, the present results are in good agreement with the results reported early by Parmentier et al., (1994) and Hanlon et al., (1997). They found that the high immune response chickens line (HR) had higher values of most of hematological parameters including the total plasma protein as compared to the low immune response line.

In addition, Abd El-Rahman, (2012), found that high immune response chickens had higher total plasma proteins (7.52 g/dl) as compared to control chickens (5.23 g/dl) and low immune response chickens (4.05 g/dl). The statistical differences among immune response groups of chickens were highly ($P \leq 0.01$) significant.

3.2. Total plasma albumin:

Total plasma albumin of Norfa chicken groups as affected by immunoglobulin G to SRBC antigen are presented in Table (8). It is clear that high immunoglobulin G chickens had higher total plasma albumin (2.42 ± 0.95) g/dl as compared to control chickens (1.83 ± 0.99) g/dl and low immunoglobulin G chickens (1.63 ± 0.95) g/dl.

Similar results were reported by **Eid, (2010)** who studied the association between immune response to SRBC and the plasma albumin concentration in broiler chickens. He found that the high levels of antibody titers chickens had the highest concentration of plasma albumin (1.50g/100ml), followed by control chickens (1.44g/100ml) and low level of antibody titers (1.41g / 100ml).

Moreover, **Abd El-Rahman, (2012)**, found that the high immune response chickens had significant higher concentration of plasma albumin (2.628 g/dl as compared to the low immune response (1.73) g/dl or control (2.38) g/dl chickens. The statistical differences were highly ($P \leq 0.01$) significant .

3.3. Total plasma globulin:

Total plasma globulin of Norfa chickens groups as affected by immunoglobulin G to SRBC antigen is given in Table (8). It is clear that high immunoglobulin G chickens had the highest plasma globulin concentration (3.53 ± 0.89) g/dl as compared to the low immunoglobulin G (2.62 ± 0.98) g/dl or control (3.22 ± 0.99) g/dl. The statistical differences among immunoglobulin G chickens groups were highly ($P \leq 0.01$) significant.

The present results did not agree with the results reported by, **Eid, (2010)**. They studied the relation between immune response and the plasma globulin in broiler chickens. He found that the plasma globulin of high and low immune response chickens had lower concentrations than the control chickens, but the statistical differences were not significant.

But The present results are in good agreement with, **Gebriel et al., (2010)**. They found that the high immune response chicken, had the highest plasma globulin concentration (5.26 g/dl), followed by control chickens (2.86 g/dl), then, the low immune response chickens (2.32 g/dl). The statistical differences among immune response chickens groups were highly significant ($P \leq 0.01$).

3.4. Albumin to globulin ratio:

Albumin to globulin ratio (A/G ratio) of Norfa chicken groups as affected by immunoglobulin G to SRBC antigen is given in Table (8). It is clear that the high immunoglobulin G chickens had the highest albumin to globulin ratio (0.69%) as compared to the control chickens group (0.57%) or low chickens group (0.62%). The statistical differences among immunoglobulin G groups of chickens were significant ($P \leq 0.05$).

On the other hand , **Eid , (2010)** , reported that the antibody titers did not affect the values of the A/G ratio, being 0.87, 0.87 , and 0.84% for high immune response, low immune response and control chickens, respectively.

In this respect, **Abd El-Rahman, (2012)**, found that high immune response chickens had significantly the highest concentration of total plasma proteins, albumin and globulin, followed by control chickens, while, the low immune response chickens had the lowest concentration, the control chickens had significantly the highest A/G ratio chickens (0.83%), followed by the low immune response chickens (0.75%), While the high immune response chickens had the lowest value of A/G ratio (0.50%).

Table (8): Effect of IgG antibody concentrations ($\bar{x} \pm SE$) on total plasma protein (TP), albumin (A), globulin (G) and A: G ratio (%) of control and selected groups of chickens.

| Chicken groups | n. | TP (g/dl) | A (g/dl) | G (g/dl) | A : G ratio % |
|----------------|----|-------------------|-------------------|-------------------|---------------|
| Control | 21 | 5.05 ± 0.12^b | 1.83 ± 0.99^b | 3.22 ± 0.99^b | 0.57 |

| | | | | | |
|------------|----|--------------------------|--------------------------|--------------------------|------|
| High IgG | 24 | 5.95 ± 0.11 ^a | 2.42 ± 0.95 ^a | 3.53 ± 0.89 ^a | 0.69 |
| Low IgG | 24 | 4.27 ± 0.11 ^c | 1.63 ± 0.95 ^c | 2.64 ± 0.89 ^c | 0.62 |
| Total Ave. | 69 | 5.09 | 1.96 | 3.13 | 0.63 |
| P. value | - | 0.01 | 0.01 | 0.01 | 0.05 |

abc = Means in the same column bearing different superscripts are significantly different.

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العلاقة بين الجلوبيولين المناعي (G) وبعض مكونات الدم في الدجاج

الملخص العربي

اجريت هذه الدراسة بقسم إنتاج الدواجن والأسماك ، كلية الزراعة بشيبي الكوم ، جامعة المنوفية. الهدف من الدراسة تقدير الجلوبيولين المناعي G وعلاقته ببعض مكونات الدم، تم تقسيم بدارى الدجاج إلى ثلاث مجاميع هي مجموعة المقارنة، مجموعة المناعة العالية ومجموعة المناعة المنخفضة، بناء على تركيز الجلوبيولين المناعي (G) في سيرم الدم.

ويمكن تخيص أهم النتائج في النقاط التالية :

- 1- حققت مجموعة عالية الإستجابة المناعية أعلى تركيز لـ IgG ($0,339 \pm 13,68$) ملجم /100مل مقارنة بكل من مجموعة المقارنة ($0,362 \pm 10,11$) ملجم /100مل ومجموعة منخفضة الاستجابة ($0,339 \pm 7,20$) ملجم /100مل .
- 2- وجدت علاقة معنوية موجبة بين كل من تركيز الأجسام المضادة والجلوبيولين المناعي G للمستضدات كرات الدم الحمراء للأغنام مع تركيز الهيموجلوبين والنسبة المئوية للهيماتوكريت وعدد كرات الدم الحمراء وعدد كرات الدم البيضاء وتركيز بروتينات بلازم الدم وتركيز الألبومين والجلوبيولين .
- 3- تلخص النتائج الحالية وجود علاقة قوية بين تركيز الجلوبيولين المناعي IgG وكل من الصفات المناعية لمكونات الدم في الدجاج وقد وجد أن حقن كرات الدم الحمراء للأغنام كمستضد طبيعي بسبب حدوث تفوق كبير في قيم الاستجابة لتكوين الأجسام المضادة حيث تؤدي إلى ظهور دجاج يتميز بالمظهر الجيد لمكونات الدم في الدجاج .

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