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Immunocompetence of Two Broiler Strains Fed Marginal and High Protein Diets

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Abstract: Immunocompetence of broiler chicks fed marginal and high dietary protein levels was studied. Four hundred one-day-old broiler chicks (200 Cobb and 200 Hubbard) were divided into two equal groups within each strain. The marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP. The high protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively. Starter ration was provided for *ad libitum* consumption from hatching up to 3 weeks of age, while the finisher one was provided from 4 to 6 weeks of age. The average high and low ambient temperatures recorded during the experimental period were 21 and 18°C, respectively. At 3 weeks of age, 60 chicks (15 chicks/strain/treatment) were used for determined the cell-mediated immunity via Phytohemagglutinin-P (PHA-P) injection. Also, humoral immune responses were determined. On day 14, chicks were slaughtered and the bursa of Fabricius, spleen, thymus, liver and heart were removed and weighed to the nearest milligram. The present results showed that the body weights did not significantly affected by strain, dietary protein level and their interaction. However, the Hubbard broiler chicks significantly consumed more feed and had a better feed conversion ratio compared to Cobb ones. The broiler chicks fed a high protein diet significantly consumed less feed and had a better feed conversion ration compared to other that fed a marginal protein diet. With respect to immunocompetence, it could be noticed that the Hubbard broiler chicks had significantly hyper responder to PHA-P injection and significantly higher total anti-SRBCs antibody titer compared to Cobb ones. The high dietary protein level significantly increased the toe-web swelling and total anti-SRBCs antibody titer compared to marginal protein level. The relative lymphoid organs weight did not significantly affected by strain. Similar trend, except for bursa, was noticed for dietary protein level. The relationships between body weight and toe-web swelling measured at all times were very low in Cobb fed a marginal protein diet. Similar trend, but moderate, was noticed in Hubbard-H group. However, significantly positive relationships between body weight and toe-web swelling measured at all times were observed in Cobb fed a high protein diet and Hubbard fed a marginal protein diet. Negative relationship between body weight and total anti-SRBC antibody titer was observed in all groups. In conclusion, under winter season of Egypt, the Hubbard broiler chicks had lower mortality rate and better immune competence compared to Cobb ones. Also, the high protein level improved the immunity of the broiler chicks.

Key words: Broiler chicks, protein level, immunocompetence

Introduction

Over the last several decades, genetic selection for faster growth rate, better feed efficiency and higher disease resistance are intensively considered in commercial broiler production. Measures of immunity that have been commonly used and assessed in poultry are antibody response to foreign antigens (Patterson and Siegel, 1998), lymphoid organ weights (Gross and Siegel, 1983) and lymphocyte blastogenesis assays (Gogal *et al*., 1997). Lymphoid organs weights are easily measured and reflect the body's ability to provide lymphoid cells during an immune response. Primary and secondary lymphoid organs provide the site for maturation lymphocytes and for the interaction between lymphocytes and antigens. It has been documented that deficiency or excess of dietary protein (Glick *et al*., 1981, 1983; Payne *et al*., 1990) or amino acids (Bhargava *et al*., 1970; Tsiagbe *et al*., 1987) changes immune responses. The effect of nutrition on antibody response to Sheep Red Blood Cells (SRBCs) is variable. Tsiagbe *et al*. (1987) found a dose related increase in total and immunoglobulin-G (IgG) antibodies against SRBCs when the broiler chicken diet was supplemented with methionine. However, Rao *et al*. (1999) found no significant differences in humoral response to SRBCs among the chicks fed high, medium and low protein diets. Similarly, dietary protein and energy content had no significant influence on broiler chick responses to SRBCs (Praharaj *et al*., 1997). On the other hand, Carlomagno *et al*. (1980) reported that protein deficiency inhibited antibody production and the development of antibody production cells in response to T-dependent antigens. Glick *et al*. (1983) showed that diet deficient in protein (33% of requirement) could reduce numbers of lymphocytes in the thymus of chickens. Spleen is identified as the secondary lymphoid tissue (Glick,

2000). Whiles all plasma proteins, except immunoglobulins, are manufactured in the liver. Spleen size was not affected by dietary protein, this indicated that deprivation of protein level in diets from 18% to 14% did not give and harmful effect on the secondary lymphoid tissue (Bunchasak *et al.*, 2005), whereas higher protein intake may induce bigger liver size due to high protein synthesis (serum protein fractions) and fat synthesis (triglyceride). Conversely, Payne *et al.* (1990) reported that deprivation of protein in chicken's diet reduced numbers of lymphocytes in the circulation and the spleen. Moderate protein deficiency has been intensively examined for its effect on immune response and infectious disease resistance. An additional important role of nutrition is that poultry are not only fed for production or reproductive performances but must also be fed to minimize infectious disease and their concomitant stresses. Substantial information is available in literature to indicate that administration of certain vitamins, minerals, amino acids and their different combinations to chicken in excess of their supposed requirements enhance their disease resistance. This increased resistance has been attributed to significant stimulation of humoral, cellular immunity and phagocytosis. Therefore, this experiment was conducted to evaluate the effect of dietary protein level, strain and their interaction on immunocompetence of meat-type chickens under Egyptian environmental conditions.

Materials and Methods

Birds and husbandry: This experiment was carried out at the poultry farm of Poultry Production Department, Faculty of Agriculture, Ain Shams University. Two strains of broiler chicks (200 Cobb and 200 Hubbard) were used in this experiment. Upon arrival, the chicks were wing-banded and randomly divided into two equal groups within each strain. The first group fed a marginal diet, while the second group fed a high protein diet. The marginal protein starter diet had 3075 Kcal ME/kg and 20% Crude Protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP. The high protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively. The starter was provided from hatching to 3 weeks of age, while the finisher was provided for the last three weeks of age. The feed and water were offered for *ad libitum* consumption. All chicks were brooded and reared under the same environmental, managerial and hygienic conditions. They were brooded in electrical brooding batteries. At 3 weeks of age, 60 chicks (15/strain/treatment) were randomly taken and housed in individual cages and the remaining chicks were raised on a deep litter up to marketing age (6 weeks). The average high and low ambient temperatures recorded during the experimental period were 21 and 18°C, respectively.

Measurements and observations

Phenotypic parameters: Body weights were individually determined for each strain within protein level. Individual feed consumption was determined from 2 to 6 weeks of age and feed conversion ratio was calculated. Cumulative mortality rate was recorded from hatching time up to 6 weeks of age.

Immunocompetence parameters

Cell-mediated immunity: Response induced in vivo by mitogen was evaluated by injection of phytohemagglutinin-P (PHA-P) into the two webs between the second and the third digits of chicks. At 2 weeks of age, 15 chicks from each treatment within strain were used. Each chick was intradermally injected in the toe web of the left foot with 100 µg phytohemagglutinin-P (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml of sterile saline measured with a constant tension caliper before injection and at 24, 48 and 72 hr post PHA-P injection. The toe web swelling was calculated as the difference between the thickness of the toe web before and after injection.

Lymphoid organs and some organs weight: After completion of PHA-P, the chicks were weighed and slaughtered. The lymphoid organs weight including bursa of Fabricius, spleen and thymus (all lobes from left side of the neck) were removed and weighed. Additionally, some organs (liver and heart) were weighed to the nearest milligram.

Humoral immune response: At 3 weeks of age, 60 chicks (15/strain/treatment) were randomly assigned for assessing humoral immunity response. The Sheep Red Blood Cells (SRBCs) were collected and washed 3 times in phosphate-buffer saline (PBS). After that, the packed cells were brought to a 7% vol/vol solution in the PBS. Chicks were injected into thigh muscle with SRBC (3% suspension in PBS, 1 ml/chick) followed by a booster injection of SRBC suspension at 4 wks (at 14 days of the first injection). Blood samples were drawn at 7, 14 days after the first and second injections. Plasma was stared at-20°C until tested. The antibody levels against SRBC were measured by hemagglutination test using 2% SRBCs suspension. Plasma was heat inactivated at 56°C for 30 min and then analyzed for total, mercaptoethanol-sensitive (Presumably IgM) and mercaptoethanol-resistant (IgG) anti-SRBC antibodies as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994). Briefly, 50 µL of plasma was added in an equal amount of PBS in the first column of a 96-well V-shaped bottom plate and the solution was incubated for 30 min at 37°C. A serial dilution was then made and 50 µL of 2% SRBC suspension was added to each well. Total antibody titers were then read offer 30 min of incubation at 37°C.

Table 1: Body weight, feed consumption and feed conversion ratio of broiler chicks fed a marginal and high protein diet

	Body weight (g)		Body weight gain (g)	Feed consumption (g)	Feed conversion ratio
	2wk	6wk	2-6wk	2-6wk	2-6wk
Protein level (PL)					
Marginal	374.50	1606.8	1232.4	2735.70	2.22
High	411.10	1686.5	1275.4	2669.30	2.10
Prob.	0.05	NS	NS	0.02	0.01
Strain (S)					
Cobb	382.10	1607.5	1225.4	2689.70	2.20
Hubbard	403.50	1685.9	1283.3	2715.20	2.12
Prob.	0.05	NS	NS	0.04	0.01
PL*S					
Cobb-marginal	357.60	1582.4	1224.8	2743.60	2.24
Cobb-high	406.50	1632.5	1226.0	2635.90	2.15
Hubbard-marginal	391.30	1631.2	1239.9	2727.80	2.20
Hubbard-high	415.70	1740.5	1324.8	2702.60	2.04
Pooled SEM	8.52	26.7	15.4	21.16	0.03
Prob.	NS	NS	NS	0.02	NS

Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively

The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For MER (IgG) response, 50 µL of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the pervious mentioned procedure. The difference between the total and IgG response was considered to be equal to the IgM antibody level.

Blood constituents: After completion of SRBCs, the same chicks were weighed and slaughtered. A portion of blood was used for hematocrit level determination using capillary tubes and a microhematocrit centrifuge. The hematocrit figures were measured after spinning microhematocrit for 12 min. The resulting plasma was stored at -20°C for later analysis. The frozen plasma was thaw prior to analysis. Total protein and albumen levels were determined in plasma by enzymatic methods using available commercial kits SCLAVO INC., 5 Mansard count, Wayne NJ 07470, USA. The globulin level was calculated as the difference between the total protein and albumen levels.

Statistical analysis: Data were subjected to a two-way analysis of variance with strain and dietary protein level effects using the General Linear Model (GLM) procedure of SAS User's Guide (2001) according to the model of

$$Y_{ijk} = \mu + S_i + PLj + (S*PL)_{ij} + e_{ijk}$$

Where; μ = overall mean,
S_i = strain effect (i = 1,2),
PI_j = dietary protein effect (j = 1,2)
$(S*PL)_{ij}$ = interaction between strain and dietary protein level and
e_{ijk} = experimental error. Correlation coefficients (PROC CORR) were calculated to assess the relationship between some traits.

Results and Discussion

Phenotypic parameters: Body weight, feed consumption and feed conversion ratio of broiler chicks fed a marginal and high protein diets are presented in Table 1. It could be noticed that the Hubbard broiler chicks had significantly heavier body weight at 2 weeks of age compared to Cobb ones. Similar trend did not observed at marketing age (6wk), whereas there was no significant difference between strains for 6wk-body weight. With respect to dietary protein level, it could be speculated that the broiler chicks fed a high protein level had significantly heavier 2-wk-body weight compared to the other fed a marginal protein level. However, there was no significant difference between dietary protein levels for 6-wk-body weight. The body weights did not significantly affected by interaction between strain and dietary protein level. In accordance to feed consumption, the Hubbard chicks significantly consumed more feed compared to Cobb ones. Also, the broiler chicks fed a marginal protein level significantly consumed more feed compared to the other fed a high protein diet. The interaction between strain and protein level was significantly effect of feed consumption. Concerning feed conversion ratio, the Hubbard broiler chicks had a better feed conversion ration compared to Cobb ones. Also, the broiler chicks fed a high protein diet had a significantly better feed conversion ratio compared to the other which fed a marginal protein level. The interaction between strain and dietary protein level did not significantly effect on feed conversion ratio. Solangi *et al.* (2003) showed that the broiler growth, feed consumption, feed conversion ratio and carcass parameters were significantly affected by increasing level of dietary protein. With respect to mortality rate, data presented in Fig. 2 showed that the Hubbard broiler

Table 2: Toe web swelling of broiler chicks fed marginal and high dietary proteins

	Toe-web swelling (mm)		
	24h	48h	72h
Protein level (PL)			
Marginal	0.37	0.27	0.14
High	0.50	0.39	0.22
Prob.	0.01	0.01	0.01
Strain (S)			
Cobb	0.36	0.27	0.14
Hubbard	0.50	0.39	0.22
Prob.	0.01	0.01	0.01
S*PL			
Cobb-marginal	0.31	0.21	0.11
Cobb-high	0.41	0.32	0.17
Hubbard-marginal	0.42	0.32	0.17
Hubbard-high	0.59	0.45	0.27
Pooled SEM	0.02	0.03	0.01
Prob.	NS	NS	NS

Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively; 24h = after 24h post PHA-P injection 48h= after 48h post PHA-P injection; 72h = after 72h post PHA-P injection

chicks had a lower mortality rate compared to Cobb ones. Likewise, the broiler chicks fed a high protein diet had lower mortality rate compared to the other that fed a marginal protein level.

Cell-mediated immunity: Phytohemagglutinin is a lectin isolated from red kidney bean and stimulates T-cell proliferation with minimal effects on B cells. Therefore, lymphocyte proliferation response to intradermally injection of PHA-P is considered an *in vivo* measurement of T-cell function. Data presented in Table 2 showed that the Hubbard broiler chicks had significantly hyper responder to PHA-P injection at all times compared to Cobb ones. Cell-mediated immunity of chickens has been examined and demonstrated to be under the influence of genetic origin (Lamont and Smyth, 1984). With respect to dietary protein level, it could be noticed that the toe-web swelling measured at all times for broiler chicks fed a high protein diet were significantly higher than that of broiler chicks fed a marginal protein diet. The toe-web swelling measured at all times did not significantly affected by interaction between strain and dietary protein level.

Body weight, relative lymphoid organs weight and some organs: Data presented in Table 3 showed that the effect of strain, protein level and their interaction on body weight, lymphoid organ and some organs weights of broiler chicks. It could be speculated that the body weight of broiler chicks fed a high protein diet was heavier than those of other fed a marginal protein diet, but the difference did not statistically significant. The 3wk-body weight did not significantly affected by strain and interaction between strain and dietary protein level. The bursa of Fabricius is a key lymphoid organ that is responsible for the development and maturation of B-lymphocytes and the humoral antibody response is dependent on this central organ (Zhang *et al*., 2006; Cheema *et al*., 2007). For, example, a high antibody response to SRBC has been associated with a larger bursa size in White Leghorn chicken strains (Ubosi *et al*., 1985). Furthermore, Zhang *et al.* (2006) showed a clear association between non-MHC genes and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines. Our results indicated that the Hubbard broiler chicks had significantly higher relative bursa weight compared to Cobb ones. Similar trend, but not statistically significant, was noticed for dietary protein level, whereas the broiler chicks fed a high protein level had higher relative bursa weight compared to other fed a marginal protein diet. Moreover, the relative bursa weight was not significantly affected by interaction between them. Spleen is identified as the secondary lymphoid tissue (Glick, 2000). The current results showed that the relative spleen weight did not significantly affected by strain, dietary protein level and their interaction. Spleen size was not affected by dietary protein; this indicated that deprivation of protein level in diets from 18% to 14% did not give and harmful effect on the secondary lymphoid tissue (Bunchasak *et al*., 2005). The immunological function of thymus is to provide a specific environmental essential for T-cells differentiation, which essential for cell-mediated immunity and modulation of immune response (Owen, 1977). The present result indicated that there was no significant difference between strains for relative thymus weight. Similar result was observed for dietary protein level. However, the relative thymus weight was significantly affected by interaction between strain and dietary protein level; whereas the Cobb broiler chicks fed a marginal diet associated with higher relative thymus weight compared to other fed a high protein diet. Similar results did not observed in Hubbard ones. The relative liver weight did not significantly affected by strain. Concerning dietary protein level, it could be observed that the broiler chicks fed a high protein diet had significantly higher relative liver weight compared to other fed a marginal protein diet. Bunchasak *et al.* (2005) reported that higher protein intake may induce bigger liver size due to high protein synthesis (serum protein fractions) and fat synthesis (triglycerides). The relative heart weight was significantly affected by strain and dietary protein level. The Cobb broiler chicks had significantly higher relative heart weight compared to Hubbard ones. Also, the broiler chicks fed a high protein diet had significantly higher relative heart weight compared to other fed a marginal diet. There is evidence

Table 3: Body weight, lymphoid organs and some organs weights of broiler chicks fed a marginal and high dietary protein diets

Lymphoid organs	Body weight (g)	Bursa	Spleen	Thymus	Liver	Heart
				(%)		
Protein level (PL)						
Marginal	577.8	0.19	0.18	0.26	3.39	0.75
High	602.8	0.21	0.16	0.28	3.70	0.87
Prob.	NS	NS	NS	NS	0.01	0.01
Strain (S)						
Cobb	587.8	0.18	0.17	0.28	3.56	0.83
Hubbard	592.7	0.22	0.17	0.27	3.52	0.79
Prob.	NS	0.05	NS	NS	NS	0.05
S*PL						
Cobb-marginal	573.4	0.17	0.17	0.25	3.45	0.71
Cobb-high	602.2	0.19	0.16	0.30	3.67	0.94
Hubbard-marginal	582.1	0.20	0.18	0.27	3.32	0.78
Hubbard-high	603.3	0.23	0.15	0.26	3.72	0.80
Pooled SEM	10.22	0.01	0.04	0.04	0.18	0.02
Prob.	NS	NS	NS	0.02	NS	NS

N = 15 chicks/strain/protein level; Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively

Table 4: Total anti-SRBCs antibody, immunoglobulin-M and immunoglobulin-G of broiler chicks fed a marginal and high dietary protein diets

	Total anti-SRBCs antibody				Immunoglobulin-M (IgM)				Immunoglobulin-G (IgG)			
	7PPI	14PPI	7PSI	14PSI	7PPI	14PPI	7PSI	14PSI	7PPI	14PPI	7PSI	14PSI
Protein level (PL)												
Marginal	4.1	2.1	4.7	2.7	2.2	1.1	1.8	0.5	1.9	1.1	3.0	2.2
High	4.9	3.1	5.9	4.0	3.2	2.1	2.6	1.5	1.7	1.6	3.3	2.6
Prob.	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	NS	NS	NS	NS
Strain (S)												
Cobb	4.0	2.3	4.8	3.0	2.9	1.7	2.0	1.0	1.1	0.6	2.8	2.0
Hubbard	5.0	3.0	5.8	3.7	2.6	1.4	2.4	1.0	2.5	1.1	3.5	2.8
Prob.	0.05	0.02	0.01	0.02	NS	NS	NS	NS	0.01	0.02	0.01	0.05
PL*S												
Cobb-marginal	3.5	1.7	4.2	2.5	2.4	1.1	2.0	0.5	1.1	0.6	2.2	2.0
Cobb-high	4.4	2.8	5.3	3.4	3.3	2.3	1.9	1.5	1.1	0.5	3.4	1.9
Hubbard-marginal	4.7	2.5	5.2	2.8	2.0	1.0	1.5	0.5	2.7	1.5	3.7	2.3
Hubbard-high	5.3	3.4	6.4	4.6	3.1	1.8	3.2	1.4	2.2	1.6	3.2	3.2
Pooled SEM	0.04	0.02	0.06	0.04	0.02	0.02	0.04	0.03	0.01	0.04	0.02	0.02
Prob.	NS	0.01	NS	NS	NS	NS	0.01	NS	NS	NS	0.01	0.01

Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively; 7PPI = 7 days post primary SRBCs injection; 14PPI = 14 days post primary SRBCs injection; 7PSI = 7 days post; secondary SRBCs injection; 14 PSI = 14 days post secondary SRBCs injection; NS = not significant

in the literature suggesting improved lymphoid organs growth in low weight strains when the dietary protein level is increased from 18 to 23% (Rao *et al*., 1999). Conversely, Cheema *et al*. (2003) found that the relative lymphoid organs weight did not significantly affect by high dietary protein diet (22%) compared to marginal protein diet (20%). The conflicting results could be attributed to the difference conditions and strains.

Humoral immune response: Total anti-SRBCs antibody, immunoglobulin-M (IgM) and immunoglobulin-G (IgG) of broiler chicks fed a marginal and high dietary protein levels are summarized in Table 4. With respect to strain effect, it could be noticed that the Hubbard broiler chicks had significantly higher total anti-SRBC antibody titer by about 25 and 30.4% at 7 and 14 days post primary secondary SRBC-injection, respectively compared to Cobb ones. Similar trend was noticed post secondary SRBCs injection, whereas the Hubbard broiler chicks had significantly higher total anti-SRBCs antibody by about 20.8 and 23.3% at 7 and 14 days post secondary SRBCs-injection, respectively compared to Cobb ones. The last result could be indicated the Hubbard broiler chicks had a better immunological memory than that of Cobb ones and the two types of responses may be under different genetic control. Boa-Amponsem *et al*. (1999) concluded that immunological memory would to be influenced by genetic selection. The present result also may indicate that the Hubbard broiler chicks had more resistant to parasites and viruses diseases.

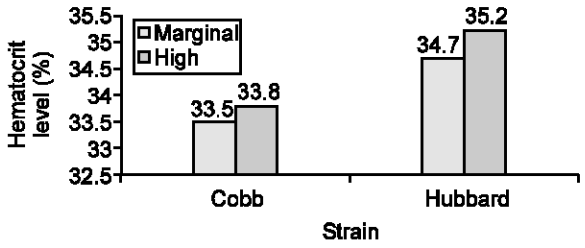

Fig. 1: Hematocrit level of broiler chicks fed a marginal and high protein diet. Marginal protein starter diet had 3075 Kcal ME/kg and 20% Crude Protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP. High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively

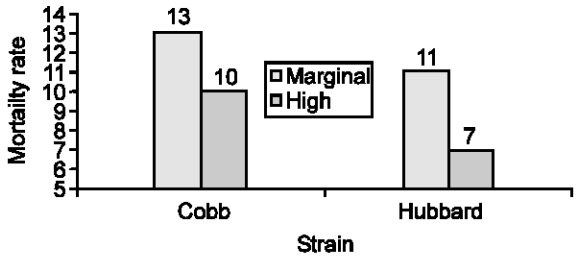

Fig. 2: Mortality rate (%) of broiler chicks fed a marginal and high protein diets Marginal protein starter diet had 3075 Kcal ME/kg and 20% Crude Protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP. High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively

Lines of chickens selected for their ability to produce high antibody to SRBC exhibited higher antibody to Newcastle disease virus, were more resist to Mycoplasma gallisepticum (van der Zijpp, 1983; van der Zijpp *et al*., 1983) and lower mortality rate when they were exposed to Marek's disease virus (Pinard *et al*., 1993) than the chicken lines that produced low antibody. Therefore, disease resistance may be indirectly improved by selection for immune parameters. The higher secondary response in Hubbard broiler chicks might positively affect the effectiveness of vaccination. Parmentier *et al*. (1996) found that a line of chicken selected for humoral response to SRBC antigen responded better to vaccination with viral antigens than a line selected in the opposite direction. Deficiency or excess of dietary protein (Glick *et al*., 1981, 1983; Payne *et al*., 1990) or amino acids (Bhargava *et al*., 1970; Tsiagbe *et al*., 1987) changes immune responses. Our results noted that the total anti-SRBCs antibody titers measured post primary and secondary SRBCs-injection for broiler chicks fed a high protein diet were significantly higher than those of other fed a marginal protein diet. Therefore, administration of protein to chicks in excess of their supposed enhances their disease resistance. The total anti-SRBCs antibody determined at all times did not significantly affected by interaction between strain and protein diet. In accordance to immunoglobulin-M, the present result showed that there was no significant difference between strains for immunoglobulin-M measured at all times. Inversely, the broiler chicks fed a high protein diet had significantly higher immunoglobulin-M compared to other fed a marginal protein diet. The immunoglobulin-M measured at 7 days post secondary SRBCs-injection was significantly affected by interaction between strain and dietary protein level. This result could be attributed to the Hubbard broiler chicks fed a high protein diet had higher immunoglobulin-M compared to other fed a marginal protein diet. Similar trend did not observed in Cobb broiler chicks. With respect to immunoglobulin-G, it could be noted that the immunoglobulin-G (IgG) anti-SRBC antibody titer measured at all times of Hubbard broiler chicks were significantly higher than that of Cobb ones. Okada and Yamamoto (1987) demonstrated that the high immunoglobulin-G (IgG) level was associated with high antibody response to SRBC and lipopolysaccharides. Also, Martin *et al*. (1989) reported that IgG level was higher for high antibody level than low antibody level. In accordance to dietary protein level, there was no significant difference between treatments for immunoglobulin-G measured at all times. The immunoglobulin-G measured at 7 and 14 days post secondary SRBCs-injection were significantly affected by interaction between strain and dietary protein level. The last result could be attributed to the Cobb broiler chicks fed a high protein diet had a higher IgG measured at 7 days post secondary SRBCs injection compared to other fed a marginal protein diet. Similar trend did not observed in Hubbard ones. Moreover, the IgG measured at 14 days post secondary SRBCs for Hubbard chicks fed a high protein diet was significantly higher than that of Hubbard chicks fed a marginal protein diet, but the same trend did not observed in Cobb ones.

Blood constituents: Data illustrated in Fig. 1 showed that the Hubbard broiler chicks had significantly higher hematocrit level compared to Cobb ones. Also, the broiler chicks fed a high protein diet had significantly higher hematocrit level compared to other fed a marginal protein diet. Furthermore, the hematocrit level was significantly affected by interaction between strain and dietary protein level. The last result could be attributed to the high protein level led to increase hematocrit level in Hubbard strain, but not in Cobb ones. The higher level of hematocrit may have enhanced oxygen delivery to the tissue. Also, this increment is supposed to be a factor

Table 5: Plasma total protein, albumen and globulin of broiler chicks fed a marginal and high dietary protein diets

	Plasma total protein (mg/dl)				Albumen (mg/dl)				Globulin (mg/dl)			
	7PPI	14PPI	7PSI	14PSI	7PPI	14PPI	7PSI	14PSI	7PPI	14PPI	7PSI	14PSI
Protein level (PL)												
Marginal	5.5	4.3	5.4	4.2	3.5	3.0	3.7	2.6	2.0	1.3	1.7	1.6
High	6.5	5.6	6.0	5.2	4.1	3.8	4.1	3.3	2.4	1.8	1.9	1.9
Prob.	0.01	0.01	0.05	0.01	0.01	0.02	0.05	0.01	NS	0.02	NS	NS
Strain (S)												
Cobb	5.9	4.4	5.6	4.3	3.8	3.4	4.0	3.1	2.1	1.0	1.6	1.2
Hubbard	6.2	5.2	5.9	5.1	3.9	3.3	3.8	2.9	2.3	1.9	2.1	2.2
Prob.	0.01	0.01	NS	0.01	NS	NS	NS	NS	NS	0.05	0.05	0.01
PL*S												
Cobb-marginal	5.4	4.2	5.2	3.9	3.6	3.1	3.7	2.8	1.8	1.1	1.5	1.1
Cobb-high	6.3	5.6	5.9	4.7	4.0	3.8	4.2	3.4	2.3	1.8	1.7	1.3
Hubbard-marginal	5.6	4.4	5.6	4.5	3.5	2.8	3.6	2.5	2.1	1.6	2.0	2.0
Hubbard-high	6.7	5.9	6.1	5.6	4.2	3.7	3.9	3.2	2.5	2.2	2.2	2.4
Pooled SEM	0.21	0.18	0.22	0.32	0.14	0.18	0.20	0.16	0.10	0.08	0.11	0.05
Prob.	NS	NS	NS	NS	NS	0.05	0.05	NS	0.02	NS	NS	NS

Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively; 7PPI = 7 days post primary SRBCs injection; 14PPI = 14 days post primary SRBCs injection; 7PSI = 7 days post; secondary SRBCs injection; 14 PSI = 14 days post secondary SRBCs injection; NS = not significant

for increased blood volume as a reaction to increase body oxygen requirement. In avian species, total proteins in plasma or serum are consisted with albumen and globulins and these parameters are commonly used in nutritional studies. Data illustrated in Table 5 showed that Hubbard broiler chicks had significantly higher plasma total protein measured at 7 and 14 days post primary SRBCs injection compared to Cobb ones. Inversely, there was no significant difference between strains for total plasma protein measured at 7 days post secondary SRBCs injection. However, total plasma protein measured at 14 days post secondary SRBCs injection of Hubbard broiler chicks was higher than that of Cobb ones. With respect to dietary protein level, it could be noticed that the broiler chicks fed a high protein diet had significantly higher plasma protein level measured at all times compared to other fed a marginal protein diet. Agbede and Aletor (2003) have reported that total serum protein, albumen and globulin syntheses were not affected by sources of dietary protein (quality of protein). Conversely, Eggum (1989) and Tewe (1985) stated that total serum protein, globulin and albumen were directly responsive to both protein quality and quantity. The interaction between strain and dietary protein level did not significantly effect on plasma total protein. Albumen is serves as the major rservoir of protein and involved in colloidal osmotic pressure, acid-base balance and it acts as a transport carrier for small molecules such as vitamins, minerals, hormones and fatty acids (Margaret, 2001). Our results noted that there was no significant difference between strains for plasma albumen. Conversely, the plasma albumen measured at all times of broiler chicks fed a high protein diet was significantly higher than that of broiler chicks fed a marginal diet. The plasma protein measured at 14 days post primary SRBCs injection and 7 days post secondary SRBCs injection was significantly affected by interaction between strain and dietary protein level. This result may be the high protein diet was increased plasma albumen measured at 14 days post primary SRBCs injection in the Hubbard broiler chicks, but not in Cobb ones. Opposite trend was noticed at 7 days post secondary SRBCs injection. The globulins are composed of three fractions, designated alpha, beta and gamma. Alpha-globulins are a group of proteins manufactured almost entirely by the liver. Normally, these proteins increase with acute nephritis, severe active hepatitis, active, usually systemic inflammation, malnutrition and in nephritic syndromes (Margaret, 2001). The gamma-globulin fraction contains most of the immuno-proteins, including IgM, IgA, IgE and IgG. These usually elevate with ongoing antigenic stimulation, usually from infectious agents (Margaret, 2001). Our results indicated that there was no significant difference between strains for plasma globulin measured at 7 and 14 days post primary SRBCs injection. Inversely, at 7 and 14 days post secondary SRBCs injection, the Hubbard broiler chicks had significantly higher plasma globulin compared to Cobb ones. Concerning dietary protein level, the plasma globulin measured at all time, except of 7 days post secondary SRBCs injection, did not significantly affected by dietary protein level. The plasma globulin measured at 7 days post primary SRBCs injection was significantly affected by interaction between strain and dietary protein level.

Correlations

Cell mediated immunity and phenotypic parameters: Correlation coefficients among body weight, relative

Table 6: Correlation coefficients among body weight, relative lymphoid organs weight and toe-web swelling of broiler chicks

	Bursa %	Spleen %	Thymus %	D24	D48	D72	Strain	Protein level
Body weight, g	-0.24	0.08	-0.57*	0.11	0.05	0.02	Cobb	Marginal
	-0.35	0.13	-0.26	0.67**	0.43*	0.49*		High
	-0.19	-0.61*	-0.50*	0.54*	0.60*	0.44*	Hubbard	Marginal
	-0.38	0.46	-0.14	0.23	0.38	0.31		High
Bursa,%		-0.24	0.54*	-0.08	-0.21	-0.13	Cobb	Marginal
		-0.43	0.33	-0.59*	-0.52*	-0.43		High
		-0.65*	-0.58*	-0.03	-0.09	-0.26	Hubbard	Marginal
		-0.16	-0.21	-0.32	-0.69*	-0.32		High
Spleen,%			-0.52*	0.01	0.08	0.09	Cobb	Marginal
			-0.69**	0.35	0.20	0.08		High
			0.82**	0.12	0.11	0.02	Hubbard	Marginal
			0.43	0.63*	0.26	0.27		High
Thymus				-0.28	-0.03	-0.01	Cobb	Marginal
				-0.15	-0.10	-0.13		High
				-0.16	-0.19	-0.37	Hubbard	Marginal
				-0.03	-0.29	-0.15		High
D24					0.89**	0.78**	Cobb	Marginal
					0.77**	0.64*		High
					0.89**	0.84**	Hubbard	Marginal
					0.68*	0.47		High
D48						0.90**	Cobb	Marginal
						0.81**		High
						0.87**	Hubbard	Marginal
						0.67*		High

D24 = toe-web swelling measured at 24h post PHA-P injection; D48 = toe-web swelling measured at 48h post PHA-P injection; D72 = toe-web swelling measured at 72h post PHA-P injection; Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively

Table 7: Phenotypic correlation coefficients between immunocompetence parameters and some productive traits of broiler chicks

	7PPI	14PPI	7PSI	14PSI	Strain	Protein level
Body weight, g	-0.61*	-0.66*	-0.42	-0.51*	Cobb	Marginal
	-0.72**	-0.71	-0.65*	-0.62*		High
	-0.67*	-0.58*	-0.54*	-0.48*	Hubbard	Marginal
	-0.68*	-0.60*	-0.66*	-0.55*		High
Bursa, %	-0.51*	-0.48*	-0.61*	-0.38	Cobb	Marginal
	-0.48*	-0.42	-0.32	-0.51*		High
	0.43	0.35	0.51*	0.46	Hubbard	Marginal
	0.65*	0.71**	0.68*	0.65*		High
Spleen,%	-0.20	-0.18	-0.37	-0.40	Cobb	Marginal
	-0.48*	-0.52	-0.36	-0.57*		High
	-0.32	-0.25	-0.19	-0.36	Hubbard	Marginal
	-0.22	-0.25	-0.18	-0.37		High
Thymus, %	-0.28	-0.35	-0.27	-0.42	Cobb	Marginal
	0.25	0.23	0.27	0.31		High
	-0.35	-0.48*	-0.30	-0.29	Hubbard	Marginal
	0.18	0.23	0.28	0.42		High
Globulin, g/dl	0.50*	0.66**	0.72**	0.50*	Cobb	Marginal
	0.61*	0.55*	0.54*	0.38		High
	0.47*	0.38	0.40	0.42	Hubbard	Marginal
	0.51*	0.54*	0.38	0.40		High

7PPI = at 7 days post primary SRBC-injection; 14PPI = at 14 days post primary SRBC-injection; 7PSI = at 7 days post secondary SRBC-injection; 14PSI = at 14 days post secondary SRBC-injection; Marginal protein starter diet had 3075 Kcal ME/kg and 20% crude protein (CP) while the marginal protein fishier diet had 3152 Kcal ME/kg and 18% CP; High protein starter diet and finisher diets contained 3086 Kcal ME/kg, 22% CP and 3130 Kcal ME/kg, 20% CP, respectively; * $p<0.05$** $p<0.01$

lymphoid organs weight and toe-web swelling of broiler chicks are presented in Table 6. It could be noticed that the body weight was moderately negative correlated with relative bursa weight in all groups. The relationship between body weight and relative spleen weight was very low in both marginal and high Cobb groups. However, significantly negative relationship (rp = -0.61) between body weight and relative spleen weight was observed in Hubbard-marginal group. Negative relationships, with statistically significant in both Cobb

and Hubbard fed a marginal and high protein diet, respectively, between body weight and relative thymus weight was observed in all groups. The relationships between body weight and toe-web swelling measured at all times were very low in Cobb fed a marginal protein diet. Similar trend, but moderate, was noticed in Hubbard-H group. However, significantly positive relationships between body weight and toe-web swelling measured at all times were observed in Cobb fed a high protein diet and Hubbard fed a marginal protein diet. Relative spleen weight was negatively correlated with relative bursa weight in all groups, with statistically significant (rp = -0.65) in Hubbard strain fed a marginal protein diet. Relative thymus weight was positively correlated with relative bursa weight in both marginal and high Cobb strain. Inversely, these relationships were negative in both marginal and high Hubbard strain. Opposite trend was noticed between relative thymus and spleen weight, whereas the relationships between relative thymus and spleen weight were negative in both marginal and high Cobb strain. However, these relationships were positive in both marginal and high Hubbard groups. Both relative bursa and thymus weight was negatively correlated with toe-web swelling measured at all times in all groups. Conversely, relative spleen weight was positively correlated with toe-web swelling measured at all times in all groups. There were highly significant positive correlations among toe-web swelling measured at all times in all groups. Finally, it could be noticed that there was negative relationship between live body and relative lymphoid organs weight In chicken, Muir and Jaap (1967) reported that bursa weight at hatching was negatively associated with post-hatching body weight. Similar relationship was observed in turkey (Li *et al*., 2001). Also, Rao *et al*. (1999) reported that the lowest body weight showed highest bursa weight. The modern commercial broiler strains selected for enhanced broiler performance exhibits reduced relative growth of both primary and secondary lymphoid organs. This may support the "resource allocation theory" (Rauw *et al*., 1998), which suggests that artificial selection for a particular that such as increasing body weight, in animals leads to a change in the allocation of resources to the different function of the animal that may affect its ability to maintain its immunocompetence and health.

Humoral immune response and phenotypic parameters: Phenotypic correlation coefficients between some phenotypic parameters and humoral immune response of Cobb and Hubbard broiler chicks fed a marginal and high protein deits are summarized in Table 7. Immunocompetence and growth are influenced by genetic and non-genetic factors. The present results speculated that there was a negative relationship between body weight and total anti-SRBC antibody titer in all groups. There is evidence in the literature regarding negatively correlation between growth and anti-SRBC antibody response in Leghorn (Siegel *et al*., 1982), broilers (Qureshi and Havenstein, 1994), brown egg-layers (Kreukniet *et al*., 1994) and Egyptian native breeds (Yakoub *et al*., 2005). Negative correlation between body weight and level of antibody response based on pleiotropic effects for genes associated with immunoresponsiveness (Martin *et al*., 1989). On the other hand, arguments for resource allocation for prioritization of resource use for various demands by chickens artificially selected for body growth (Dunnington and Siegel, 1996). Also, this inverse relationship could be because utilization of resources such as energy and protein might be diverted toward the support of the production of immune products and away from stimulated growth (Mashaly *et al*., 2000). Benson *et al*. (1993) reported that stimulation of immune response resulted in decreased chick growth. Positive relationship between relative bursa weight and total anti-SRBC antibody titer was observed in Hubbard strain. However, inverse relationship was observed in Cobb one. These results indicated that the bursa size may not necessarily be associated with antibody titer. Yamamoto and Glick (1982) found that a chicken line selected for small bursa size had higher total and 2-mercaptoethanol-resistant antibody titers in the primary response to SRBC and also had higher total antibody titers in the line selected for large bursa size. Ubosi *et al*. (1985) observed that a chicken line selected for high response to SRBC had a larger bursa size than the line selected for low response. There was inverse relationship between relative spleen weight and total anti-SRBC antibody titer in all groups. This suggests the size of spleen did not affect the antibody immune response. Relative spleen weight was negatively correlated with total anti-SRBC antibody titer in Cobb and Hubbard broiler chicks fed a marginal protein diet. Conversely, these relationships were positive in both Cobb and Hubbard broiler chicks fed a high protein diet.

In conclusion, under winter season of Egypt, the Hubbard broiler chicks had a lower mortality rate and better immune competence compared to Cobb ones. Also, the high protein level improved the immune competence of broiler chicks.

References

Agbede, J.O. and V.A. Aletor, 2003. Evaluation of fish meal replaced with leaf protein concentrate from gyricidia in diets for broiler chicks: Effect on performance, muscle growth, haematology and serum metabolites. Int. J. Poult. Sci., 2: 424-250.

Benson, B.N., C.C. Calvert, E. Roura and K.C. Klasing, 1993. Dietary energy sources and density modulate the expression of immunological stress in chicks. J. Nutr., 123: 1714-1723.

Bhargava, K.K., R.P. Hanson and M.L. Sunde, 1970. Effects of methionine and valine on antibody production in chickens infected with Newcastle disease virus. J. Nutr., 100: 241-248.

Boa-Amponsem, K., E.A. Dunnington, K.S. Baker and P.B. Siegel, 1999. Diet and immunological memory of lines of white leghorn chickens divergently selected for antibody response to sheep red blood cells. Poult. Sci., 78: 165-170.

Bunchasak, C., K. Poosuwan and R. Nukraew, 2005. Effect of dietary protein on egg production and immunity of laying hens during peak production period. Int. J. Poult. Sci., 4: 701-708.

Carlomagno, M.A., A.E. Alito, S.U. Rife and A.L. GImeno, 1980. B-cell immune response during total protein deprivation. Acta Physiol. Lat. Am., 30: 187-192.

Cheema, M.A., M.A. Qureshi and G.B. Havenstein, 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poult. Sci., 82: 1519-1529.

Cheema, M.A., M.A. Qureshi, G.B. Havenstein, P.R. Ferket and K.E. Nestor, 2007. A comparison of the immune response of 2003 commercial turkeys and a 1966 randombred strain when fed representative 2003 and 1966 turkey diets. Poult. Sci., 86: 241-248.

Dunnington, E.A. and P.B. Siegel, 1996. Long-term divergent selection for eight-week body weight in White Plymouth Rock chicken. Poult. Sci., 75: 1168-1179.

Eggum, B.O., 1989. Biochemical and methodological principles. In: H.D. Bock, B. Eggum, A.G. Low, O. Simon and T. Zebrowska (Eds.), Protein metabolism in farm animals. Evaluation, Digestion, Absorption and Metabolism (Oxford Science Publication, Deutscher Handwirtschafts Verlag, Berlin), pp: 1-52.

Glick, B., 2000. Immunophysiology. In: Sturkie's Avian Physilogy, Fifth Edition, Edition by G. Causey Whittow, Academic Press, Sandiego, California, USA, pp: 657-685.

Glick, B., E.J. Day and D. Thompson, 1981. Calorieprotein deficiencies and immune response of the chicken. I. Humoral immunity. Poult. Sci., 60: 2494-2500.

Glick, B., R.L. Taylor Jr., D.E. Martin, M. Watabe, E.J. Day, and D. Thompson, 1983. Calorie-protein deficiencies and immune response of the chicken. II. Cell mediated immunity. Poult. Sci., 62: 1889-1893.

Gogal, R.N.J., S.A. Ahmed and C.T. Larsen, 1997. Analysis of avian lymphocyte proliferation by a new, simple, nonradioactive assay (Lympho-Pro). Avian Dis., 41: 714-725.

Gross, W.B. and P.B. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis., 27: 972-979.

Kreukniet, M.B., M.G.B. Nieuwland and A.J. van der Zijpp, 1994. Phagocytic activity of two lines of chickens divergently selected for antibody production. Vet. Immunol. Immunopath., 44: 377-387.

Lamont, S.J. and J.R. Smyth, 1984. Effect of selection for delayed amelanosis on immune response in chickens. 2. Cell-mediated immunity. Poult. Sci., 63: 440-442.

Li, Z., K.E. Nestor, Y.M. Saif, J.W. Anderson and R.A. Patterson, 2001. Effect of selection for increased body weight in turkey on lymphoid organ weights, phagocytosis. and antibody responses to fowl cholera and Newcastle disease-inactivated vaccines. Poult. Sci., 80: 689-694.

Margaret, A.W., 2001. Avian Plasma Proteins. http://www.exoticpetvet.net.

Martin, A., W.B. Gross and P.B. Siegel, 1989. IgG and IgM responses in high and low antibody selected lines of chickens. J. Heredity, 80: 249-252.

Mashaly, M.M., M.J.W. Heetkamp, H.K. Parmentier and J.W. Schrama, 2000. Influence of genetic selection for antibody production against sheep red blood cells on energy metabolism in laying hens. Poult. Sci., 79: 519-524.

Muir, F.V. and R.G. Jaap, 1967. A negative genetic correlation between bursa weight at hatching and post hatching body growth of chickens. Poult. Sci., 46: 1483-1488.

Okada, I. and Y. Yamamoto, 1987. Immunocompetence and Marek's disease resistance in three pairs of chicken lines selected for different immunological characters. Poult. Sci., 66: 769-773.

Owen, J.J.T., 1977. Ontogenesis of Lymphocytes. In B and T all in immune recognition. John wile and Sons. New York. NY., pp: 21-34.

Parmentier, H.K., M.G.B. Nieuwland, E. Rijke, G. Devries Reilingh and J.W. Schrama, 1996. Divergent antibody response to vaccines and divergent body weights of chickens lines selected for high and low humoral responsiveness to sheep red blood cells. Avian Dis., 40: 634-644.

Patterson, P.H. and H.S. Siegel, 1998. Impact of cage density on pullet performance and blood parameters of stress. Poult. Sci., 77: 32-40.

Payne, C.J., T.R. Scott, J.W. Dick and B. Glick, 1990. Immunity to *Pasteurella multocida* in protein-deficient chickens. Poult. Sci., 69: 2134-2142.

Pinard, M.H., J.M.A. Van Arendonk, M.G.B. Nieuwland and A.J. van der Zijpp, 1993. Divergent selection for humoral responiveness in chickens: distribution and effect of major histocompatibility complex types. Genet. Sel. Evol., 25: 191-203.

Praharaj, N.K., E.A. Dunnington, Gross and P.B. Siegel, 1997. Dietary effects on immune responses of fast-growing chicks to inoculation of sheep eryrocytes and Eschirichia coli. Poult. Sci., 76: 244-247.

Qureshi, M.A. and G.B. Havenstein, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. Poult. Sci., 73: 1805-1812.

Rao, S.V., N.K. Praharj, M.R. Reddy and B. Sridevi, 1999. Immunocompetence, resistance to Eschirichia coli and growth in male broiler parent chicks fed different levels of crude protein. Vet. Res. Comm., 23: 323-326.

Rauw, W.M., E. Kanis, E.N. Noordhuizen and F.J. Grammers, 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Prod. Sci., 56: 15-33.

SAS Institute, 2001. SAS/STAT User's Guide Version 8.2 Ed: Statistics. SAS Institute Inc., Cary, NC.

Siegel, P.B., W.B. Gross and J.A. Cherry, 1982. Correlated response of chickens to selection for production of antibodies to sheep erythrocytes. Anim. Blood Groups Biochem. Gen., 13: 291-297.

Solangi, A.A., G.M. Baloch, P.K. Wagan, B. Chanchar and A. Memon, 2003. Effect of different levels of dietary protein on the growth of broiler. J. Anim. Vet. Adv., 5: 301-304.

Tewe, O.O., 1985. Protein metabolism in growing pigs fed corn or cassava peel based diets containing graded protein levels. Res. Vet. Sci., 29: 259-263.

Tsiagbe, V.K., M.E. Cook, A.E. Harper and M.L. Sunde, 1987. Enhanced immune responses in broiler chicks fed Methionine-supplemented diets. Poult. Sci., 66: 1147-1154.

Ubosi, C.O., W.B. Gross, P.B. Homilton, M. Ehrich and P.B. Siegel, 1985. Aflatoxin effects in White Leghorn Chickens Selected for response to sheep erythrocyte antigen. 2. Serological and organ Characteristics. Poult. Sci., 64: 1071-1076.

van der Zijpp, A.J., 1983. Breeding for immune responsiveness and disease resistance. World's Poult. Sci. J., 39: 118-131.

van der Zijpp, A.J., K. Frankena, J. Boneschanscher and M.G.B. Nieuwland, 1983. Genetic analysis of primary and secondary immune response in the chicken. Poult. Sci., 62: 565-572.

Yakoub, H.A., A. Galal, S.A. El-Fiky and M.M. Fathi, 2005. Genetic differences between Fayoumi and Dandarawi Egyptian chicken strains. 2. Antibody response against Sheep Red Blood Cells (SRBCs). Egypt. Poult. Sci., 25: 1069-1083.

Yamamoto, Y. and B. Glick, 1982. A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of Fabricius. Poult. Sci., 61: 2129-2132.

Zhang, H.M., H.D. Hunt, G.B. Kulkarni, D.E. Palmquist and L.D. Bacon, 2006. Lymphoid organ size varies among inbred lines 63 and 72 and their thirteen recombinant congenic strains of chickens with the same major histocompatibility complex. Poult. Sci., 85: 844-853.