Relationship between Selection for Humoral Immune Response and Productive and Reproductive Traits in Fayoumi Male of Chicken Nahla A. M.; A. M. Rizk; H. A. Abdellatif and M. H. Abdelfatah Animal production Research Institute, ARC, Dokki, Giza, Egypt



# ABSTRACT

The present study was conducted to elucidate the relationship between humoral immune response selection and some productive traits in three lines of Favoumi cocks. A total of 300 male Favoumi chicks were divided into three lines, control, high and low according to their immune response against SRBC injection . They were fed the formulated diets in adlibitum . Results showed that the high line males ND,H9 had significantly higher immune response against NDV and H9 than the other lines . Thyroid hormones concentration in the control and low line were significantly higher than high line . males but non significant effect on T3/T4 ratio between all male lines . The control line had better testosterone hormone level than the high line, while. serum corticosterone hormone concentration was higher in the control and high lines than the low line males . Ejaculate volume ,abnormal sperm percentage and total abnormal sperm / ejaculate did not significantly different between lines studied, the low line in sperm motility (score) was significantly higher than that from the high line. non significantly difference between the low line and the control. Sperm concentration, total sperm /ejaculate and total live sperm / ejaculate were significantly higher in the low and control lines compared by the high line males . Both comb width, and length, wattle width and length did not significantly influenced by Favoumi - selected lines. Serum total protein, albumin and globulin were significantly improved for the high line followed by the control line while the low line recorded the lowest value . both total cholesterol, triglycerides , AST and ALT did not significantly influenced by Fayoumi males selected lines . Avrage of immunoglobulins of different lines of Fayoumi males after 10 days from injection of SRBC was significantly higher in the high line males than the other line. It is concluded that the high line males had significantly higher antibody titer against SRBC in terms of higher plasma IgG and IgM compared to males from the control and low line males .

# **INTRODUCTION**

In recent years, different strategies have been applied to improve poultry productivity and profitability . The most important one is directed towards maintaining health, reducing diseases outbreak and improving general immunity. The non-genetic factors mitigating against good performance from poultry in developing countries typically include disease challenge . So, immune selection in commercial genotypes for improving tolerance to prevailing conditions was recently applied by Robert (2011). There is an increasing demand for poultry meat that is nutritious and free of drug residues . Antibody titer against sheep red blood cells (SRBC's), a non -pathogenic multivalent antigen has been extensively used to measure immuno competence (Balemans et al., 2005). The immune response to challenge with SRBC's reflects antigen handling, antigen presentation of macrophages and the proliferation of B-lymphocytes (Sarker et al., 1999). Divergent selection for humoral immune response against SRBC's in poultry was positively related to the improvement of cell - mediated immune parameters . Yao-Dong et al .(2012) have observed that there were moderate to high genetic correlation between immune response to SRBC's and anti-AI and anti-NDV titers which were higher in high line than low line chickens . Van-der Most et al. (2011) showed that selection for growth compromises immune function, however selection for immune function did not necessarily affect growth . Results of Fayoumi chickens selected for antibody response against SRBC's antigen demonstrated that broiler strains are more immune responsive (Gharib, 2006).

Therefore, the main objectives of the present study were to further investigate the relationship between humoral immunity and both produdive and reproductive traits of Fayoumi males. Bonker and Beuving (1989) showed that corticosterone level had a negative effect on immune organs (thymus, bursa and spleen) development in birds.

# **MATERIALS AND METHODS**

The present study was carried out at the El-Azab Poultry Research Center , Animal Production Research Institute , Agricultural Research Center , Ministry of Agrculture , Egypt .

## **Experimental birds**

The pedigreed progenies of 2nd generation of Fayoumi chickens were used in this study. The selection criterion was based on primary antibody titer against SRBCs antigen that was injected intra – muscular (i.m) at 56 days of age and antibody titer was evaluated 7 days post – immunization . Then the highest females (10.32) and highest males (11.05) in the antibody titers were selected as parents to produce HR line in the progeny of second generation . On the other hand , the lowest females (5.09) and lowest males (5.90) in antibody titers were selected as parents to produce the low line (LR) in the progeny of the 2nd generation . While females (7.94) and males (8.17) were taken at random and mated to produce the control line progeny (CL) of the 2nd generation .

# **Experimental stock management**

At hatching , day all chicks were removed from hatchery , wing banded according to their families for indentification . The chicks of each line were housed on floor pens in semi-open system up to 18 weeks of age , then the birds were transferred to wire cage . The starting brooder temperature was 33 C during the first 3 days and then the temperature was reduced gradmally by 3 C to reach 24-25 C . The chicks were exposed to continuaus light during the first three days of age and then they were exposed to natural day light until 17 week of age . At 18 weeks of age they received 14 hours of light a day . The light period was increased 30 minutes every other week using artificial light at night , until it became fixed at 16 hours daily .

Birds were provided with clean fresh water and feed ad – libitum following the recommended standard diets for each age formulated (from 1 day to end of

experimental) according to feed composition tables for Animal and Poultry feedstuffs used in Egypt (2001). **Studied traits** 

Schedule of immunization and evaluation of humoral immune response.

All birds were vaccinated for Newcastle Disease Virus (NDV) at 7,18 and 21 days of age . Avian Influenza Virus (AIV) was given as follows:, (H5N1 at 12 and 37 days of age) ,and (H9N2 at 9 and 30 days of ages) . Then at 56 days of age , all brids were challenged intramuscular (i.m) by one ml of 25% SRBCs suspension that was prepared by washing of SRBCs in phosphate buffer saline (PBS) (Ghaffari laleh *et al* ,2008)

After 7 days post –immunization by SRBCs (at 63 days of age) a blood sample of 4 ml was collected from the brachial vein of each bird and serum samples were prepared by clotting at 37 C and centrifugation in the meantime . Antibody titers against NDV,AIV and SRBCs were evaluated using hemagglutination inhibition , test and expressed as log 2 of the reciprocal of the highest serial dilution where the hemaggulation occurred .

### **Reproductive performance traits**

Body weight at sexual maturity was recorded individually for each selected male to the nearest gram. Semen characteristics measurements

At 36 weeks of age, semen was collected from cocks of each line using abdominal massage method. Three successive ejaculates were collected from each male within 10 days. Semen samples were individually evaluated according to kalamah *et al*. (2000). The following characteristics were measured for each ejacutate :-

The ejaculate valume to the nearest 0.01 ml using 1.00ml tuberculin syringe , motility , pH , live sperm , abnormal sperm , sperm concentration , total sperm /ejaculate , total live sperm/ ejaculate and total abnormal sperm/ ejaculate .

Semen pH was measured by comparative pH paper , percentage of live and abnormal sperms were determined after staining with eosin and nigrosine and sperms concentration was determined by using themes – zies hem cytometer (El-wardany *et al*.,1995).

#### Hormonal profile

At 36 weeks of age , blood samples were collected from randomly selected five cocks from each line . Estrogen hormone was evaluated using RIA estradiol immunotech Beckman coulter company , Franc serum samples of cocks were evaluated for leve of testosterone hormone by using RIA( testosterone direct Beckman Coulter company) Moreover , tri – iodothyronine (T3) ,tetraiodothyronine (T4) and corticosterone hormone leve were measured in serum samples using (RIA kits purchased from Cambridge Medical Diagnostic Lab (Billerica MA).

#### Statistical analysis

Data were subjected to one-way analysis of variance using general linear models (GLM) procedure of SAS Institute (2000). Means were separated by using Duncan's multiple range test (Duncan, 1955). The percentage values were transferred to percentage angle using arcsine equation before subjected to statistical analysis, and then actual means are presented.

The following model was used:

#### $\mathbf{Y}_{ij} = \mathbf{U} + \mathbf{L}_i + \mathbf{e}_{ij}.$

Where:  $Y_{ij}$  = observation for each dependent variable; U = overall mean;  $L_i$  = Line effects (i = 1,2 and 3);  $e_{ij}$  = Random error.

### **RESULTS AND DISCUSSION**

 Table 1. The composition of the experimental basal diets.

Ingredients	Percentage (%)
Yellow corn	61.57
Soya bean 44%	17.00
Wheat bran	6.70
Corn gluten 60%	4.50
Di Ca P export	1.39
Lime stone	8.16
Salt	0.37
*Premix	0.30
L Methionine	0.01
Total	100.00
Calculated values (%)	
Crude Protein	16.5
Metabolizable energy (M.E.) kcal/kg	2699
Crude fiber (C. F.)	3.468
Ether extract	2.964
Calcium	3.399
Available Phosphorous	0.397
Total Phosphorous	0.610
Sodium	0.164
Arginine	1.28
Lysine	0.730
Methionine	0.335
Methionine & cysteine	0.619

\*Premix added to the 1 kg of diet including Vit.A 10000 I.U; vit. D3 2000 I.U; vit. E 15 mg; vit. K3 1 mg; vit B1 1mg; vit. B2 5 mg; vit. B12 10 μg; vit B6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; folic acid 1mg; Biotin 50 μg; choline 300 mg; zinc 50mg; copper 4mg; iodine 0.3 mg; iron 30mg; selenium 0.1mg; manganese 60mg; cobalt 0.1mg and carrier CaCo3 up to 1kg.

Relationship between selection to immune response to SRBC ,NDV and avian Influenza of Fayoumi males at 20 weeks of age is illustrated in Table-2 -Data clearly showed that the high line males had significantly higher immune response against NDV and H9 than the other lines .On the other hand ,data showed non significant effect on immune response to H5 between all fayoumi –selected lines .

 

 Table 2. Relationship between selection to immune response to SRBC, NDV and avian Influenza of Favoumi males at 36 weeks of age.

Téana		Line				
Item	High	Control	Low	SEM		
NDV	7.380 <sup>a</sup>	6.167 <sup>b</sup>	5.433 <sup>b</sup>	0.325		
H9	5.533 <sup>a</sup>	4.280 <sup>b</sup>	4.097 <sup>b</sup>	0.315		
H5	4.120	3.780	3.543	0.251		
<sup>a, b</sup> Means within each row have no similar letter(s) are significantly						

Means within each row have no similar letter(s) are significantly different (P  $\leq$  0.05)

The relationship between selection to immune response to SRBC and thyroid activity of Fayoumi males at 36 weeks of age is illustrated in table -3- Data clearly showed that T3 and t4 hormones concentration for the control and low line cocks were significantly higher than high line males. On the other hand , data showed non significant effect on T3/T4 ratio between all male lines.

Moreover ,the control line had better testosterone hormone level than the high line . It is observed from the

table that the significantly high an average of plasma corticosterone for males was recorded for the high line strain compared to males from the control and low line. In addition, the low line had better estradiol hormone than the control and high line. On the other hand, non - significantly difference between average progesterone hormone in control line and the high line and low line, respectively.

Table 3. Relationship between selection to immune<br/>response to SRBC, and plasma<br/>corticosterone, thyroid hormones level and<br/>sex hormones of Fayoumi males at 36<br/>weeks of age.

weeks of age.					
Itom		SEM			
Item	High	Control	Low	SEM	
T3 (ng/ml)	2.603 b	3.480 a	3.893 a	0.214	
T4 (ng/ml)	14.343 b	16.170 ab	17.923 a	0.779	
T3/T4 ratio	0.182	0.215	0.218	0.015	
corticosterone (ng/ml)	2.37a	1.92b	1.55c	0.46	
Testosterone (ng/ml)	2.477c	2.956 b	3.390 a	0.121	
Estradiol (ng/ml)	250.33c	288.67 b	331.00 a	9.308	
progesterone (ng/ml)	0.216 b	0.240 ab	0.280 a	0.012	
ab as the a			· · ·	101 (1	

<sup>····</sup> Means within each row have no similar letter(s) are significantly different ( $P \le 0.05$ )

It is clear from the table that , the low line had significantly higher serum testosterone ,estradiol and progesterone levels than the control and high line . More ever,the control line had better testosterone and estradiol hermones response than the high line . plasma corticosterone hormone level of the control line was higher than the low males ... Thyroid hormones was reported to has an obvious effect on immune organs and hence , immunity of living organisms . In this respect ERF and MARCH (1987),Scott and Glick(1989),Haddad and

Mashaly (1990)) and Johnson et al. (1992) have reported that thyroid hormones level had a stimulatory influence on immune organs development and consequently the immune response of birds . On the other hand , adrenal gland was known to exert its effect on immunity by producing corticosterone . Donker and Beuving (1989) showed that corticosterone level had a negative effect on immune organs (thymus, bursa and spleen) development in birds .Our results confirm those obtained by many others who bostulated that the avian immune system is under the control of genes and endocrine secretions (Biozzi, et al .,1982) and Van der Zjp (1983). Moreover Lassila et al (1979) stated that the genetic control of the cell - mediated immunity in chickens is not related to sex of the bird. On the other hand. EL-kaiaty (1993) reported that the immune response against SRBC was higher in Fayoumi chickens at 4,11 and 18 weeks of age compared to both LSL and Baladi chickens . A similar trend was observed by Atta et al (1996) who found that Fayoumi chicks responded better to SRBC in a dose dependent manner than LSL chicks . Also Davis, (1998) found that endocrine system is the main regulator of different environmental factors affecting immunity of birds, since, it help birds to successfully compete the viral challenges. This was also observed by many authors who claimed that hormones can modulate the immune system responses (Eglezos et al, 1993 and Trout and Mashaly, 1995).

Table -4 showed the relationship between selection to immune response to SRBC and semen quality traits of Fayoumi cocks at 36 weeks of age. Data revealed that ejaculate volume ,abnormal sperm percentage and total abnormal sperm / ejaculate did not significantly different between lines studied. However, semen pH of the control line was significantly higher compared to the high line.

Table 4. Relationship between selection to immune response to SRBC and semen quality traits of Fayoumi cocks at 36 weeks of age.

Item	Line			SEM
	High	Control	Low	SEM
Ejaculate volume (ml)	0.378	0.418	0.526	0.070
Semen pH	7.30 <sup>b</sup>	7.87 <sup>a</sup>	7.50 <sup>ab</sup>	0.143
Sperm motility (1-5)	2.70 <sup>b</sup>	3.40 <sup>ab</sup>	3.80 <sup>a</sup>	0.299
Live sperm %	78.90 <sup>b</sup>	86.20 <sup>a</sup>	87.00 <sup>a</sup>	2.168
Abnormal sperm %	15.50	14.60	13.70	0.941
Sperm concentration $(x10^9/ml)$	3.019 <sup>b</sup>	3.307 <sup>ab</sup>	3.655 <sup>a</sup>	0.165
Total sperm/ejaculate (x10 <sup>9</sup> /ml)	1.155 <sup>b</sup>	1.386 <sup>ab</sup>	1.900 <sup>a</sup>	0.234
Total live sperm/ejaculate $(x10^9 / ml)$	2.427 <sup>b</sup>	2.874 <sup>ab</sup>	3.191 <sup>a</sup>	0.192
Total abnormal sperm/ejaculate (x10 <sup>9</sup> )	0.477	0.485	0.497	0.042

<sup>a, b...</sup> Means within each row have no similar letter(s) are significantly different ( $P \le 0.05$ )

Data clearly showed also that in sperm motility (score) of the low line was significant higher than that from the high line. On the other hand, data showed non significantly difference between the low line and the control. Live sperm percent of both the low and control lines was significantly higher compared to the high line. Results clearly show that both sperm concentration, total sperm /ejaculate and total live sperm / ejaculate were significantly higher in the low and control lines compared by the high line strain. However, both low and control lines did not significantly differ in these traits. The improved semen quality traits in our study may be related to the genetic background of the selected lines although they are produced from the same fayoumi breed. It appears that selection for higher immunity is assocated with changes in the expression of genes responsible for semen production and quality. In this respect, El -Wardany, et al. (1995) examined semen quality traits in three local strains of chicken (originated from Favouni breed ) and found significant differences in semen quality traits which in close agreement with our study . Moreover, Kalamah et al .(2000) showed that semen volume of Norfa cocks was 0.33 ml which is nearly similar to the value of the high line strain in our study. The present study is in agreement with the findings reported by Khedr (2005) who studied the association between the immune response and semen volume, semen characteristics sperm motility(%) and semen pH in Turkey were not significant and found that all traits . These results are in agreement with the results reporded by

Hafez (1968). He reported that fresh semen of cocks is usually slightly alkaline. Also, Zaki *et al*.(1982) found that the statislical differences among strains of chicken in pH values were not significant. In addition, El-Hammady *et al*. (1995) showed that the semen pH in Dandarawi cocks was 7.25 and the corresponding value in fayoumi cocks was 7.29 which is nearly similar to our results.

Table-5 showed the relationship between selection to immune response to SRBC and comb and wattle measurment of Fayoumi cocks at 36 weeks of age . Results clearly showed that both comb width , and length , wattle width and length did not significantly influenced by Fayoumi – selected lines . The increased wattle length in response to SRBC may be mediated through cytokines release and / or as an endocrine regulation of antibody production by B-lymphocytes . This is in accordance with the reports by Tizard (1996) who found that antibodies display a high degree of interaction on antigens that may stimulate antibodies production and hence induce a response on the secondary sexual characters , i.e.increase of wattle and length .

Table 5. Relationship between selection to immune response to SRBC and comb and wattle measurement of Fayoumi cocks at 36 weeks of age.

	CEM		
High	Control	Low	SEM
12.39	12.58	13.04	0.378
6.96	6.80	7.34	0.255
5.31	5.65	5.33	0.641
5.27	5.22	5.02	0.392
	High 12.39 6.96 5.31 5.27	Line           High         Control           12.39         12.58           6.96         6.80           5.31         5.65           5.27         5.22	Line           High         Control         Low           12.39         12.58         13.04           6.96         6.80         7.34           5.31         5.65         5.33           5.27         5.22         5.02

<sup>k, b...</sup> Means within each row have no similar letter(s) are significantly different (P ≤ 0.05)

The relationship between selection to immune response to SRBC and blood plasma proteins and lipids concentrations and liver enzymes activity of Fayoumi males at 36 weeks of age is illustrated in Table-6 results clearly showed that both total protein and albumin were significantly improved for the high line followed by the control line while the low line recorded the lowest values.

Table 6. Relationship between selection to immune response to SRBC, blood plasma proteins, lipids concentrations and liver enzymes of Fayoumi males at 36 weeks of age.

Itom		SEM			
Item	High	Control	Low	SEIVI	
Plasma proteins:					
Total protein (g/dl)	5.822 <sup>a</sup>	5.224 <sup>b</sup>	5.172 <sup>b</sup>	0.146	
Albumin (A) (g/dl)	3.196 <sup>a</sup>	3.048 <sup>ab</sup>	2.934 <sup>b</sup>	0.079	
Globulin (G) (g/dl)	2.626 <sup>a</sup>	2.176 <sup>b</sup>	2.238 <sup>b</sup>	0.111	
A/G Ratio	1.222	1.414	1.328	0.071	
Plasma lipids:					
Total lipids (mg/dl)	384.69 <sup>a</sup>	387.16 <sup>a</sup>	341.54 <sup>b</sup>	12.999	
Total cholesterol (mg/dl)	158.89	140.14	144.92	10.619	
Triglycerides (mg/dl)	82.75	76.44	70.24	4.726	
Liver functions:					
AST (U/L)	50.43	49.34	49.24	1.785	
ALT (U/L)	23.98	24.71	23.62	1.820	
<sup>a, b</sup> Means within each row with no similar letter(s) are significantly					
different ( $P \le 0.05$ )					

On the other hand, A/G ratio was not significantly different for Fayoumi males selected lines. However, data showed significant reduction in total lipids for the control and the high lines ,while the low line recorded the lowest value. Data clearly showed that both total cholesterol, triglycerides , ASTand ALT did not significantly influenced by fayoumi males selected lines . The significant increases in both plasma protein and plasma lipids profiles may be due , in part to the high immune response as a result of SRBC injection . This was explained by several authors as related to the increase in immunoglobulin's secretion , where they are higher in the high antibody lines than the low ones (Okada and Yamamato, 1987,Martin *et al* . , 1990 and Elasyaid *et al* . , 2011).

Table-7- Showed avrage of immunoglobulins of different lines of Fayoumi males after 10 days from injection of SRBC. It is clear from the table that the high line males had significantly higher antibody titer against SRBC in terns of higher plasma IgG and IgM compared to males from the control and low line strain. The significant increase in IgG and IgM in the high line provides evidence that both played an important role in the activation of acquired immune response of chickens. This agreed with the results by Lee and Klassing , (2004) and Yao – Dong , *et al*, (2012) who reported that IgG and IgM provides a defense mechanism against various types of pathogen

Table 7. Avrage of immunoglobulins of different linesof Fayoumi malesafter 10 dayspost SRBC

injecuo	л.				
Item		SEM			
	High	Control	Low	SEN	
IgG(mg/dl)	3.383 <sup>a</sup>	2.570 <sup>b</sup>	2.113 <sup>b</sup>	0.139	
IgM (mgdl)	3.250 <sup>a</sup>	$2.520^{ab}$	1.960 <sup>b</sup>	0.219	
a, b, N			4(.)	C 41-	1

<sup>b...</sup> Means within each row with no similar letter(s) are significantly different ( $P \le 0.05$ )

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# العلاقة بين الانتخاب للاستجابه المناعية في الدم والصفات الإنتاجية والتناسلية في ذكور دجاج الفيومي نهله عبدالرازق محمد ، احمد محمد رزق ، هشام احمد عبداللطيف و محمد حامد عبدالفتاح معهد بحوث الانتاج الحيواني ، مركز البحوث الزراعية ،وزارة الزراعه ، مصر

أجريت الدر اسة الحالية لتوضيح العلاقة بين الانتخاب للاستجابة المناعية في الدم وبعض الصفات الإنتاجية في ثلاثة خطوط من سلاله الفيومي. تم تقسيم مجموعه من ذكور الفيومي إلى ثلاثة خطِّوط كنترول ومرتفع ومنخفض طبقا للاستجابه المناعيه للحقن بكرات الدم الحمراء للغنم ، و أظهرت النتَّائج أن الذكور ذوي الخط المرتفع لديهم استجابة مناعية أعلى بشكل ملحوظ ضد NDV و H9 أكثر من الخطوط الأخرى. توضح النتائج ايضا أن هرمونات الغده الدرقية (T3 لوي الحط العرائع ليهم السبب منابيه المنفض أعلى بشن متعوط صد ٧ (١٢) و ١٦٦ عن من المحطوط الحرى. توضع المنابع العلى المرموت العام المربي (٢٦ و T4) في مجموعه المقارنة والخط المنخفض أعلى بكثير من الذكور عالية المناعة بدون تأثير كبير على نسبة T4 / 73 بين جميع الذكور. كان مستوى هرمون التستوستيرون مرتقعا في مجموعه المقارنة عن المجموعه مرتقعه المناعة . ان تركيز هرمون كورتيكوستيرون في الدم وتبعا في المجموعه منخفضة المناعه وكذلك المرتقعه ولا توجد فروق معنويه في حجم السائل المنوي ، ونسبة الحيوانات المنوية الشائعه والحيوانات المنوية الشائعة / القذفه الواحده بين الخطوط التي تمت دراستها. كان الخط المنخفض في حركة الحيوانات المنوية (درجة) أعلى بكثير من ذلك من الخط المرتفع. لا فرق كبير بين الخط المنخفض التخطوط التي تمت دراستها. كان الخط المنخفض في حركة الحيوانات المنوية (درجة) أعلى بكثير من ذلك من الخط المرتفع. لا فرق كبير بين الخط المنخفض والكنترول. كان كل من تركيز الحيوانات المنوية وعدد والحيوانات المنوية / الفذفه ومجموع الحيوانات المنوية الحية / الفذفه أعلى بكثير في خطوط الكنترول والخط المنخفض مقارنة بالخط العالي . كان مستوى كل من البروتين الكلي والألبيومين والجلوبيولين مرتفعا بصورة معنويه للخط العالي يليه خط الكنترول بينما سجل الخط المنخفض أدنى قيمة. لم يتأثر كل من الكوليسترول الكلي والدهون الثلاثية ونشاط انزيم AST وانزيم ALT بشكل كبير بالخطوط المختارة من الذكور الفيومي. كان مستوى الجلوبيولينات المناعية مرتفعا في دم الديوك المنتخبه لارتفاع المناعة ضد كرات دم الغنم الحمراء مقارنه بالديوك الموجوده في الخطوط الاخري