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EVALUATION OF LYMPHOCYTE ACTIVITY AND DELAYED HYPERSENSITIVITY OF LOCAL CHICKENS

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SUMMARY

The present work was carried out at the Poultry Breeding Farm, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

This study involved three local breeds: Fayoumi, Dandarawi and Sena obtained from El-Fayoum Takamoly Project.

A total number of fifteen immature males of each breed at fourteen weeks of age were used in this research to evaluate the lymphocytes activity and delayed hyperesensitivity of local breeds of chickens.

Obtained data indicated that Fayoumi had greater absolute and relative weight (mg/100 gm. body weight) of spleen than other two breeds. With regard to thymus, the differences between breeds were non-significant. Plasma total protein values of Fayoumi were higher than both Dandarawi and Sena, the differences were significant ($P < 0.05$). Data concerning albumin showed that the three breeds have the same level. Plasma cholesterol data showed that the young Fayoumi males has a high level, the difference was significant ($P < 0.05$) than Dandarawi and Sena.

Splenomegaly reaction (spleen Index) for Sena was induced at early age; but Fayoumi had a little spleen index, Dandarawi had a minor splenomegaly. The genetic differences between the three breed may be caused the differences in splenomegaly (Hala *et al.*, 1976).

Phytohemagglutinin responses (PHA reaction) or Wattle test at young males (12 weeks) were lower than those of adult males (20 weeks), the PHA response as humoral response matures during development (with advanced of age), Bacan and Lee (1981), Schauenstein *et al.*, (1985).

We can suggest that Fayoumi breed has a hyperactivity in cell-mediated immunity (T-lymphocytes).

Keywords: Local breed, Fayoumi, Dandarawy, lymphocytes, PHA, splenomegaly

INTRODUCTION

Control of the immune response is the normal embryonic development of thymus–derived lymphocytes (T-cells) and bursal or bone marrow derived lymphocytes (B-cells) (Glick, 1978). Because of the importance of T and B-cells lymphocytes in the immune response, much research has focused on determining mechanisms by which T and B-cells activities are regulated. Many workers examined the relationships between endocrine system and immune response (cell-mediated immunity) in chickens (Bachman and Mashaly, 1987).

Phytohemagglutinin (PHA) and concanavalin-A (Con-A) belong to a class of mitogens that activate normal peripheral T-lymphocytes to undergo blast transformation and proliferation. They have been used extensively in the study of immune responses in normal and diseased animals (Lee and Bacan, 1983). Miggiano *et al.* (1976) presented evidence of a single-gene control of difference in response to Con-A between two different inbred lines of chicken. Cell-mediated immunity, which is mainly influenced by the thymus and its lymphocytes, is one of the major parts of the immune response.

Most of Egyption local strains of chickens (highly inbred strains) has a high disease resistance, many workers suggest that the natural selection caused that. Limited information is known about the activity or capacity of the local (native) strains immune system. This study was designed using three strains "Fayoumi, Dandarawi and Sena" to investigate (evaluate) lymphocyte activity and capacity of immune system.

MATERIALS AND METHODS

(a) Birds

A number of 15 immature males (12 weeks of age) Single Comb from each Fayoumi, Dandarawi and Sena obtained from El-Fayoum Takamoly project were used in this study. Chicks were housed in the rearing pins with ground floor. Feed, according to NRC, and water were available *ad-libitum*. Chicks received 14 hr of light and 10 hr darkness. The chicks were housed under the natural local conditions of temperature and relative humidity during March to June.

(b) Blood analysis

For chemical constituents, a sample of 2 ml of blood were taken from each bird, samples were centrifuged at 3000 rpm for 15 minutes, plasma were separated and stored at -20°C until the time of analysis. A calourimetric method was used to determine plasma total protein and albumin according to (Doumas, 1975), (Weichsclboun, 1946), Glucose (Trinder, 1969), Cholesterol (Waston, 1960), GOT and GPT (Reitman and Frankel, 1957).

(c) Body and lymphoid organs weight

A number ranged from 12 – 15 birds (males) were weighted and sacrificed. Spleen and thymus were removed and weighted to determine their relative weights (mg/100g body weight) by El-Kaiaty (1993) and Bachman and Mashaly (1987).

(d) Immunocompetence measures (delayed hypersensitivity)

(1) Delayed Wattle reaction (DWR)

Cell-mediated immune response was measured by using wattle response to phytohemagglutinin (PHA-P)[5]. A volume of 0.1 ml of PHA solution in (100 µgPHA/0.1ml saline) was injected subcutaneously into a defined area on the wattle. Saline (0.1 ml) was injected into the other wattle and served as a control.

The thicknesses of both wattles were measured before and 24 hrs after PHA injection (El-Kaiaty, 1993), (Bachman and Mashaly, 1987).

PHA response, in millimeters, was calculated as:

PHA = [(Post PHA Inj.) – (Pre PHA Inj.)] – [Post PBS Inj. – Pre PBS Inj.].

Van der Zijpp, (1983), Lamont and Smyth (1984) and Clave *et al.*, (1985).

(2) Graft-vs-Host (GvH) or splenomegaly reactions

GvH was induced by injection of 0.1ml hepranized RBC's of our birds into the eggs at 13-day of embryonic development then killed by chilling on day 19. The ratio, sleen/embryo weight was used to measure splenomegaly according to Johnson and Edgar (1986). The control (approximately 35 embryos) were received saline solution as executed by Lamont and Smyth (1983).

Statistical analysis

The data were analyzed by analysis of variance and 5% probability level ($p<0.05$) or lower was accepted as significant (SAS, 1985). The comparison between groups means was made by using Duncan multiple range Test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Lymphoid organs

The absolute and relative weight (mg/100 g B.W.) of spleen and thymus for different breeds are shown in Table 1. Obtained data showed that Fayoumi had greater absolute and relative weight of spleen than Dandarawi and Sena. With regard to thymus, the differences between breeds were non-significant. These results agree with those of Nady Esa (1997) who reported that spleen values ranged from 2.5 to 4.8 g in Fayoumi and 2.4 to 4.3 g in Dandarawi breed.

Table 1. Means ± S.E. of spleen and thymus in some local breeds of chickens

	Spleen		Thymus	
Treatments	Relative* %	Absolute	Relative* %	Absolute
Fayoumi	359.42^{a} ±39.13	6.161^{a} ±0.616	138.490^{a} ±8.748	2.404^{a} ±0.132
Dandarawi	65.28^{ab} ±39.13	4.296^{b} ±0.616	121.010^{a} ±8.748	1.957^{b} ±0.132
Sena	243.93^{b} ±44.15	4.686^{ab}±0.695	143.073^{a}±9.869	2.763^{a} ±0.148

* mg/100 gms of BW

$^{a, b}$ Means within each column with no common superscripts are differ significantly ($P< 0.05$).

2. Plasma constituents

2.1. Total protein and albumin

Data in Table 2 showed some plasma components. Obtained data showed that plasma total protein values of Fayoumi were significantly ($P <0.05$) higher than that of both Dandarawi and Sena. These results agree with those of El-Kaiaty (1996), Nady Esa (1997), Abd El-Latif *et al.*, (1997) and Gharib (1998). Data concerning the albumin levels showed that the three breeds had the same levels and the differences between breeds were non-significant. These results agree with those reported by Nady Esa (1997) and Gharib (1998) who reported the same values, approximately, for Fayoumi and Dandarawy. Abo-Eita (1997) for White Baladi (local strain of chicken) Hamdy *et al.* (1998) pointed out similar in Japanese Quail.

2.2. Plasma cholesterol

The plasma cholesterol for Fayoumi, Dandarawi and Sena is presented in Table 2. The data show that the differences between Fayoumi and both Dandarawi and Sena was significant ($P < 0.05$). The present data for young males ranging from 340 to 456 mg/100 ml is more than double of that reported by El-Kaiaty and Hamdy (1997) and Nady Esa (1997) who reported values from 152 to 231 ml/100 ml in force molting Fayoumi hens. Nady Esa (1997) and Gillbert (1971) suggested that plasma cholesterol utilization is related to sexual hormones. Siegel (1971) indicated that physiological stress was accompanied with a significant increase in blood cholesterol, this could explain the increasing levels of cholesterol in male chicken.

2.3. Plasma glucose

The mean values of plasma glucose are shown in Table 2. At early age (12 weeks) Fayoumi had significantly higher mean than both Dandarawi and Sena, While, at 18 weeks of age, the three breeds had the nearly similar values. At late age (23 weeks) the three breeds had smaller values than early ages. Nady Esa (1977) reported smaller values for plasma glucose than us, 192, 183, 184, 176, 172 and 170 versus 226, 224, 193, 215, 227, 195, 198, 228 and 192 respectively. These results may be explain the relationship between plasma glucose and production especially, egg production (Nady Esa, 1997).

2.4. Liver function enzymes (Transaminase activity)

Data of plasma glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) are presented in Table 2. The three breeds had near levels of GOT and GPT. Gharib (1998) reported very high levels of GOT and GPT in Fayoumi females during production period, the values for (F × F cross) ranged between 100.1 at 9 weeks and 134.4 I.U/L at 45 weeks of age. The Corresponding values in the present research ranged from 12.02 at 12 weeks of age to 12.04 at 23 weeks of age. This may be due to sexual hormones and productive status. Payne (1972) and Gildersleene *et al.*, (1982) demonstrated that steroid hormones affect liver transaminase activity, (Rosen *et al.*, 1958).

3. Graft versus host reaction splenomegaly or spleen index SI

Lymphocytes activity, expressed as Graft versus Host reaction (splenomegaly or spleen Index).

The averages of splenomegaly symptoms or spleen index (SI) are present in Table (3 and 4). The data show that grater splenomegaly was induced in Sena breed, followed by Fayoumi, while Dandarawi showed minor splenomegaly. These data suggest that Sena breed had more active lymphocyte cells. Johnson and Edgar (1986) studied splenomegaly in some genotypes and their combinations recombinants in Lighorn lines, they found that some genotypes show major splenomegaly (B^5B^5) when it is the donor of the cells" and other genotypes (B^2B^2) produce minor splenomegaly. This support the genetic control on the splenomegaly. We can suggest that the genetic differences between the local strains of chicks may cause this differences in splenomegaly (spleen index) among these strains. Also, the natural selection through the local environmental conditions may help in this concern. F region of MHC (Major histocompatability) controlled graft versus host, (Hala *et al.*, 1976).

Table 3. Relative weight of spleen in embryos received blood from Fayoumi, Dandarawi and Sena

Bird No.	Fayoumi	Dandarawi	Sena
1	0.586 *	0.529	0.507
	0.584	0.484	0.521
3	0.541	0.528	0.474
4	0.467	0.540	0.792
5	0.541	0.441	0.646
6	0.526	0.438	0.912
7	0.948	0.64	0.726
8	0.501	0.61	0.647
9	0.405	0.511	0.533
10	0.545	0.447	0.581
11		0.426	0.854
12			0.596
X	0.5644	0.5065	0.6491

* $\frac{\text{Spleen weight}}{\text{Embryo B. Wt.}}$ = (mg/g).

Table 2. Means ± S.E. of plasma total protein, Albumin, Glucose, Cholesterol contents, GOT and GPT activity level at different ages in Fayoumi, Dandarawi and Sena immature males (Mean ± SE)

Breeds	Age)	Total Protein (g/dl)	Albumin (g/dl)	Glucose (mg/100 ml)	Cholesterol (mg/dl)	GOT (U/ml)	GPT (U/ml)
		b	bc	a	a	bc	bc
Fayoumi	1 *	4.197 ±0.158	2.493 ±0.085	226.800 ±3.685	436.000 ±6.825	12.024 ±0.133	8.756 ±0.069
		b	Bc	ab	cd	a	d
	2	4.140 ±0.166	2.378 ±0.090	224.444 ±3.886	392.333 ±7.195	12.971 ±0.141	8.254 ±0.072
		a	a	c	d	bc	b
	3	5.128 ±0.176	3.569 ±0.095	193.250 ±4.122	389.375 ±7.631	12.044 ±0.149	8.953 ±0.077
		b	bc	b	bc	bc	bc
Dandarawi	1	4.111 ±0.166	2.308 ±0.090	215.444 ±3.886	408.667 ±7.195	12.034 ±0.141	8.798 ±0.072
		cd	bc	a	b	b	d
	2	3.333 ±0.176	2.434 ±0.95	227.625 ±4.122	415.125 ±7.632	12.253 ±0.149	8.246 ±0.077
		bc	bc	c	d	c	a
	3	4.073 ±0.158	2.404 ±0.085	195.300 ±3.686	380.800 ±6.825	11.796 ±0.133	9.313 ±0.069
		bc	c	c	bc	bc	b
Sena	1	3.951 ±0.176	2.190 ±0.095	198.500 ±4.122	407.625 ±7.631	11.854 ±0.149	8.926 ±0.077
		d	bc	a	d	a	d
	2	3.210 ±0.166	2.343 ±0.090	228.333 ±3.886	381.556 ±7.195	13.190 ±0.141	8.314 ±0.072
		cd	bc	c	e	bc	c
	3	3.636 ±0.166	2.382 ±0.090	192.778 ±3.886	340.667 ±7.195	12.179 ±0.141	8.600 ±0.072

* 1 = 12 weeks of age, 2 = 18 of age weeks and 3 = 23 weeks of age.

[a, b, c, d and e] Means in the same column with no common superscripts differ significantly ($P < 0.05$).

Table 4. Splenomegaly [Spleen Index (SI)[1]]for Fayoumi, Dandarawi and Sena

Bird No.	Fayoumi		Dandarawi		Sena	
	No.2	SI	No.2	SI	No.2	SI
1	7	0.973 [3]	8	1.021	6	0.979
2	10	1.127	5	1.934	3	1.006
3	6	1.044	7	1.021	6	0.915
4	12	0.902	8	1.042	4	1.529
5	13	1.044	8	0.851	5	1.247
6	9	1.015	6	0.846	3	1.761
7	8	1.830	8	1.185	8	1.402
8	5	0.967	8	1.185	8	1.249
9	4	0.781	9	0.986	7	1.029
10	8	1.052	5	0.863	6	1.122
11			6	0.822	6	1.649
12					6	1.151
X		1.0735		0.978		1.253

(1) Spleen Index (SI) = $\frac{\text{Cell-injected spleen weight / Cell-injected embryo weight}}{\text{Saline-injected spleen weight / saline-injected embryo weight}}$

(2) No. of embryo injected by blood from one male and control = 33 embryos injected by saline.

(3) The mean (average value) embryos injected of each bird divided by the control (embryos injected with saline).

4. Phytohemagglutinin (PHA) response "Wattle Index"

The wattle test is one of the very important methods available to determine cell-mediated immune response (Morita and Soekawa, 1972), but this test can only be used when chickens have wattles of sufficient size, this test is also, easier to inject and more convenient to measure (Timms *et al.*, 1987). The PHA and con-A belong to a class of mitogens that activate normal peripheral T-Lymphocytes, they have been used extensively in the study of immune response in normal and diseased animals.

This index was determined twice, at 12 and 20 weeks of age. The responses to PHA in Fayoumi, Dandarawi and Sena at 12 and 20 weeks age are presented in Table (5 a and b). The data show that PHA response at 12-week-old was lower than at 20 weeks. This is consistent with the concept that cellular immune competence, similar to the humoral immune competence, matures during development, (Bacon and Lee, 1981 and Schauenstein *et al.*, 1985).

Fayoumi and Dandarawi did not differ significantly from each other, although they generally responded much lower than Sena, but Dandarawi showed intermediate response between the two other breeds. The present values are in good agreement with that reported by Clare *et al.* (1985). But, that reported by Lamont and Smyth (1984) and Morita and Soekawa (1972), were much lower. Finally, we can suggest that Fayoumi breed has a hyperactivity in cell-mediated immunity (T-lympocytes) at 20 weeks of age, and Sena breed had a hyperactivity at early stage.

Table 5-a. PHA-response (Wattle Index) in local breeds of chickens at different ages, (A) At 12 weeks of age

Bird No.	Dandarawi		Fayoumi		Sena	
	Treated	Control	Treated	Control	Treated	Control
1	0.80 *	-	1.0	0.5	1.20	0.1
2	2.00	-	1.5	-	1.00	-
3	1.00	-	1.5	-	1.00	-
4	1.75	-	1.0	-	0.50	-
5	3.00	0.25	2.5	0.5	0.25	0.1
6	0.50	-	2.0	-	0.50	-
7	1.00	0.50	1.0	-	0.50	-
8	0.50	-	2.0	-	1.00	-
X	1.44	0.38	1.56	0.50	0.74	0.1
Wattle index	= 3.80		3.126		0.74	

* The values = [Post-injection wattle thickness (mm) – pre-injection wattle thickness (mm)].

Table 5-b. PHA response (wattle Index) in local breeds of chickens at different ages. (b) 20 weeks of age

Bird	Dandarawi		Fayoumi		Sena	
No.	Treated	Control	Treated	Control	Treated	Control
1	-	-	6.00	0.50	1.00	0.50
2	1.50 *	-	1.00	0.50	1.50	0.50
3	3.00	-	2.75	1.50	2.25	-
4	4.00	-	2.50	0.50	4.50	0.50
5	4.75	0.25	3.00	0.50	1.50	0.10
6	1.00	0.50	7.50	0.50	2.00	0.10
7	2.00	-	2.00	-	1.75	-
8	5.00	-	2.50	0.50	4.00	0.50
9	2.75	0.25	2.50	-	1.50	-
10	4.40	0.50	1.50	-	1.20	-
11	4.75	0.75	5.50	0.50	1.00	-
12	4.00	-				
X	3.09	0.45	3.063	0.62	2.018	0.3667
Wattle index	6.87		4.90		5.50	

* The values = (Post-injection wattle thickness – pre-injection wattle thickness).

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