

RELATIONSHIP BETWEEN BLOOD CONSTITUENTS AND IMMUNITY IN KHAKI-CAMPBELL AND DOMYATI DUCKS.

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ABSTRACT

A number of 300 day-old ducklings from each Khaki-Campbell and Domyati breeds was used to compare both blood constituents and immunity response under the Egyptian environmental conditions. In general, concentrations of total lipids, cholesterol, calcium, hemoglobin and hematocrit value were significantly higher in Khaki-Campbell than Domyati ducks. However, concentrations of total protein, albumin, globulin, inorganic phosphorus and activity of GOT and GPT significantly increased in Domyati as compared to Khaki-Campbell ducks. Relative weights of pancreas, thymus and spleen and absolute weight of thymus and spleen were higher in Domyati than Khaki-Campbell ducks. Count of WBC's and distribution of lymphocytes significantly increased in Domyati ducks, while heterophils distribution and H/L ratio increased in Khaki-Campbell ducks. Both primary and secondary responses against SRBC's were significantly higher in Domyati ducks (92.2 and 272.6) than Khaki-Campbell ones (66.1 and 241.5), respectively.

INTRODUCTION

Negative relationship between growth rate and level of total protein in serum of chicken was observed by Kolataj (1964). El-Sayiad *et al.*, (1988) noted a significant positive increase of serum protein in Domyati with the advance of age. Ress and Nordskog (1981) suggested that the breed effects on globulin concentration may be the cause of significantly variation in total serum protein fraction between the strains. Comparing between two breeds, El-Badry (2004) determined total protein, albumin, globulin and A/G ratio in both Domyati and Muscovy at 12 weeks of age. The values were 5.44, 3.11, 2.33 g/dl and 1.43 in Domyati and 5.91, 3.53, 2.37 and 1.61 in Muscovy, respectively. Nagwa *et al.*, (2004) obtained levels of 28.13, 18.59 IU/L and 85.74 ng/ml in Domyati and 93.44, 29.62 IU/L and 95.08 ng/ml in Muscovy for GOT, GPT and T3 at 12 weeks of age, respectively.

Inooka *et al.*, (1994) reported an attribution between genetic regulation and antibody production which involved in general antibody production and resistance, namely common genetic control system, exists among mechanisms of disease resistance and antibody production. El-Badry (2004) obtained $29.02 \times 10^3/\text{mm}^3$, 62.47%, 28.06% and 0.45% in Domyati ducks and $25.9 \times 10^3/\text{mm}^3$, 59.38%, 28.74 and 0.48% in Muscovy for WBC's, lymphocyte%, heterophil% and H/L ratio at 12 weeks of age, respectively. The antibody production against SRBC's for Muscovy was reported as 63.97 (Nematallah *et al.*, 2003) and 63.973 (El-kaiaty *et al.*, 2004) at 11 weeks of age.

The aim of this study is to evaluate differences in both blood constituents and immunity response between Khaki-Campbell ducks (foreign) and Domyati ones (local) under the Egyptian environmental conditions. Some

blood metabolites, enzymes, hormones and hematological traits were determined. Lymphoid organs, WBC's count, primary and secondary response against SRBC's were recorded to evaluate the immunity response in both breeds.

MATERIALS AND METHODS

The experimental work was carried out at El-Serw Research Station for Waterfowls, Animal Production Research Institute, Ministry of Agriculture, during the season 2004/2005. A number of 300 day-old ducklings from each of Khaki-Campbell and Domyati breeds were used to compare some blood constituents and immunity response under Egyptian environmental conditions. Day-old ducklings were banded, weighed and divided randomly to 3 similar replicate. Each replicate (100 ducklings) was housed in well-ventilated brooding pens. Birds were fed starter ration (19.20% crude protein and 2868.00 ME Kcal/kg ration) from one-day up to 6 weeks of age, grower ration (15.20% crude protein and 2690.00 ME Kcal/kg ration) from 6-16 weeks of age and layer ration (15.67% crude protein and 2746.00 ME Kcal/kg ration) from 16-36 weeks of age. Water and feed were offered *ad-libitum*. Ration was slightly moistured by fresh water to minimize feed losses. Birds were exposed to continuous light during the first weeks of age and to natural light, thereafter. To determine blood constituents, 12 blood samples/breed (2 males and 2 females/replicate) were collected into heparinized tubes at 12, 24, 36 weeks of age. Samples were centrifuged at 3000 rpm for 15 minutes to separate blood plasma, which was kept at -15°C until the chemical analysis. Concentration of total protein, albumin, total lipids and cholesterol, calcium and inorganic phosphorus and activity of GOT and GPT were determined using spectrophotometer. Concentrations of Triiodothyronine (T3) and estradiol hormones were determined using RIA technique.

Fresh blood samples collected at 12 weeks of age to determine the total count of red (RBC's) and white blood cells (WBC's) were determined according to Wintrobe (1967). Also, hemoglobin concentration (Pilaski, 1972), hematocrit value (Hunsaker, 1969), count of lymphocytes and heterophils (Haddad and Mashaly, 1990) were performed. At 16 weeks of age for primary immune response (PR) and at 20 weeks of age for secondary immune response (SR) against Sheep red blood cells, (SRBC's), 12 birds of each breed were injected intravenously with one ml from 7% suspension of SRBC's. Seven days later, blood samples were collected, clotted and centrifuged to obtain the sera which were frozen until the measurements of the humeral response according to Van der zijpp *et al.*, (1983) and Bachman and Mashaly (1986). To determine weights of lymphoid organs, 12 ducklings from each breed (2 males and 2 females/replicate) were taken randomly for slaughtering either at 12 or at 36 weeks of age and eviscerated. Spleen, thymus and pancreas were separated and weighted. Relative weights to the live body weight were calculated.

Data were analyzed by using Mixed Model least squares and Maximum likelihood Mean weighted program of Harvey (1990) with following model:

$$Y_{io} = \mu + B_i + e_{io}$$

Where:-

Y_{io} = Y_{io} observation;

μ = Overall mean;

B_i = fixed effect due to i^{th} breed ($i = 1 \& 2$)

e_{io} = residual random effect.

RESULTS AND DISCUSSION

Blood constituents:

Total protein, albumin, globulin and A/G ratio:

Results in Table (1) showed significant differences in all traits (except A/G ratio) between Khaki-Campbell and Domyati breeds at all ages. Agag (1983) noted that the protein fraction was known to be influenced by genetic and non-genetic factors. Ress and Nordskog (1981) reported that the breed effects on globulin concentration may be the cause of significantly variation in total serum protein fraction between the strains. Zimmerman (1976) found that the decrease in A/G ratio may be due to a decrease in production of albumin by the liver reflecting malhepatic function. In both breeds, it can be observed that all traits increased with the advancement of age. This trend agrees that reported by El-Sayiad *et al.*, (1988) who noted significant and positive increase of serum protein in Domyati with the advance of age.

The present concentration of plasma protein for Khaki-Campbell was 4.51 g/dl at 12 weeks of age is nearly similar to 4.1 g/100 ml protein in male Khaki-Campbell at 8 weeks of age by Attia (2003). Concentration of plasma protein, albumin, globulin and A/G ratio in Domyati ducklings at 12 weeks of age agreed with El-Badry (2004), being 5.44, 3.11, 2.33 g/dl and 1.43, respectively. While, the corresponding values were 5.56, 3.69, 1.87 mg/100 ml, respectively, in Domyati at 12 weeks of age (El-Tehiti, 2001).

Total lipids and cholesterol:

Results in Table (1) declared significant increase in concentration of serum total lipids and cholesterol at all ages in Khaki-Campbell than Domyati ducks. Total lipids concentration in Khaki-Campbell ducks increased from 0.66, 0.72 to 0.74 g/dl with advance of age from 12, 24 to 36 weeks of age, respectively. The corresponding values in Domyati ducks were 0.33, 0.39 to 0.44 g/dl. In agreement with the results of Abd El-Moty (1992), total lipids and cholesterol concentrations increased in Pekin drakes with advance of age. In contrast, cholesterol concentration was 152 mg/100 ml at 22 weeks of age (Ghonim 1998) and 154 g/dl at 33 weeks of age (Awad 2004). In Domyati ducklings plasma cholesterol concentration increased from 157 to 165 mg/dl at 24 and 36 weeks of age (Table 1).

Calcium and inorganic phosphorus:

There were significant breed differences in concentration of calcium and inorganic phosphorus (Table 1). Plasma calcium concentration increased at all ages in Khaki-Campbell as compared to Domyati, while inorganic phosphorus showed an opposite trend. Concentration calcium and inorganic phosphorus increased in both breeds with advance of age. Radwan *et al.*, (1989) found a significant breed difference in serum calcium content. Plasma calcium concentration increased also with the advance of age. This was attributed to the increase in estrogen secretion during those periods which increase calcium release in blood stream as a result of medullary bone formation.

In this study, calcium and inorganic phosphorus concentration ranged from 10.35 to 39.3 mg/dl and from 4.17 to 7.30 mg/dl, respectively. Abd El-Ghary (2002) found that serum calcium and inorganic phosphorus concentrations in Khaki-Campbell ducks were 13.8 and 4.23 mg/dl at 22 weeks of age, increased to be 39.0 and 7.78 mg/dl at the peak of production, respectively. The corresponding values for Domyati ducks were 11.5 and 6.52 mg/dl at 22 weeks of age and 39.6 and 8.31 mg/dl at the peak of production. El-Tehiti (2001) reported 10.12 and 4.70 mg/100 ml for concentration of calcium and inorganic phosphorus in Domyati at 12 weeks of age, while Awad (2004) reported these values as 10.63 and 5.06 mg/dl at 33 weeks of age, respectively.

Table (1): Average concentration of some biochemical in plasma of Khaki-Campbell (KC) and Domyati (DO) ducks at different ages (Means \pm SE).

Item	Age (weeks)								
	12			24			36		
	(KC)	(DO)	Sign.	(KC)	(DO)	Sign.	(KC)	(DO)	Sign.
Total protein (g/dl)	4.51	5.59		4.68	5.77		4.78	5.91	
	± 0.01	± 0.01	**	± 0.08	± 0.08	**	± 0.01	± 0.01	**
Albumin (g/dl)	2.61	3.27		2.72	3.40		2.81	3.51	
	± 0.08	± 0.08	**	± 0.08	± 0.08	**	± 0.09	± 0.09	**
Globulin (g/dl)	1.90	2.32		1.96	2.37		1.97	2.41	
	± 0.04	± 0.04	NS	± 0.05	± 0.05	NS	± 0.05	± 0.05	NS
A/G ratio	1.38	1.42		1.39	1.44		1.43	1.46	
	± 0.04	± 0.04	**	± 0.04	± 0.04	**	± 0.05	± 0.05	**
Total lipids (g/dl)	0.66	0.33		0.72	0.39		0.74	0.44	
	± 0.01	± 0.01	.	± 0.01	± 0.01	.	± 0.01	± 0.01	.
Cholesterol (mg/dl)	178.65	146.50		186.43	156.95		193.61	165.41	
	± 8.60	± 8.60	**	± 8.64	± 8.64	**	± 8.57	± 8.57	**
Calcium (mg/dl)	11.07	10.35		14.12	11.93		39.32	35.79	
	± 0.17	± 0.17	**	± 0.19	± 0.19	**	± 0.21	± 0.21	**
Inorganic Phosphorus (mg/dl)	4.17	4.74		4.41	6.78		6.61	7.30	
	± 0.12	± 0.12		± 0.13	± 0.13		± 0.14	± 0.14	

* = significant at (P \leq 0.05).

NS = Not significant.

** = significant at (P \leq 0.01).

Enzymes activity and hormones:

GOT and GPT:

Results in Table (2) revealed significant breed differences in activity of GOT at 12 weeks of age, and in GPT actives at 12 and 24 weeks of age, but not at 36 weeks of age. Activity of both enzymes increased with advance of age. The highest activities were observed at 36 weeks of age in both breeds. Both GOT and GPT activities were higher in Domyati than Khaki-Campbell ones. Geraert *et al.*, (1996) reported that both enzymes releases depended upon pathological conditions. El-Tehiti (2001) obtained 118.17 GOT and 38.40 U/L in Domyati at 12 weeks of age, while Awad (2004) reported 48.0 and 32.30 U/L in Domyati at 33 weeks of age, respectively. These results indicated that all enzyme activities at all ages are within normal values of ducklings.

Triiodothyronine (T3) and estradiol (E2):

There were no significant differences in T3 and E2 at all ages between Khaki-Campbell and Domyati breeds (Table 2). Both hormones concentration were slightly higher in Domyati than Khaki-Campbell ducks. Concentration of T3 decreased in both breeds with advance of age, while E2, increased with advance of age. It is well known that thyroid activity is important in controlling both metabolic rate and lipogenesis. Colin *et al.*, (1986) found strong relationship between thyroid hormones and the secretion of growth hormones in birds. Also, Scheele *et al.*, (1992) reported a positive effect of T3 on lipogenesis. Plasma T3 concentration in Domyati observed in this study at 12 weeks of age was nearly similar to 69.41 ng/ml (El-Badry 2004) and 85.74 ng/ml (Nagwa *et al.*, 2004). Darras *et al.*, (1995) found that during the relatively low metabolic demand, the peripheral conversion of T3; that is most active from thyroid hormone; is impaired.

Table (2): Activity of transaminase (GOT and GPT) and concentration of T3 ad estradiol in plasma of Khaki-Campbell (KC) and Domyati (DO) at different ages (Means ± SE).

Item	Age (weeks)								
	12			24			36		
	(KC)	(DO)	Sign.	(KC)	(DO)	Sign.	(KC)	(DO)	Sign.
GOT (U/L)	29.25	39.67		38.75	46.83	NS	47.42	51.50	NS
	±2.80	±2.80		±3.30	±3.30		±3.35	±3.35	
GPT (U/L)	18.75	27.92	**	23.41	32.17	**	30.58	36.67	NS
	±2.01	±2.01		±2.11	±2.11		±2.43	±2.43	
T3 (ng/ml)	67.08	67.44	NS	57.21	57.92	NS	48.74	49.62	NS
	±0.58	±0.58		±0.65	±0.65		±0.88	±0.88	
Estradiol (ng/ml)	22.06	24.98	NS	37.75	40.14	NS	34.93	38.29	NS
	±1.16	±1.16		±4.39	±4.39		±4.32	±4.32	

* = significant at (P≤0.05).

NS = Not significant.

** = significant at (P≤0.01).

Hematological traits:

Data in (Table 3) revealed that count of RBC's, concentration of hemoglobin and hematocrit values were higher in Khaki-Campbell and Domyati breeds, but The differences were significant only for hemoglobin concentration and hematocrit value.

The present concentration of RBC's count, hemoglobin and hematocrit value in Khaki-Campbell and Domyati ducks at 12 weeks of age are compared with $1.78 \times 10^6/\text{mm}^3$, 17.6 mg/100 ml and 37.0% in Khaki-Campbell ducks at 9 weeks of age (Abdalla *et al.*, 2004). While, it was $2.34 \times 10^6/\text{mm}^3$, 9.16 mg/100 ml and 30.41% in Domyati ducks at 12 of age (El-Badry 2004), respectively. Sturkie (1986) reported that the corpuscular volume (hematocrit) was affected by age.

Table (3): Hematological traits in Khaki-Campbell (KC) and Domyati (DO) ducks at 12 Weeks of age (Means \pm SE).

Item	(KC)	(DO)	Sign.
RBC,s ($\times 10^6/\text{mm}^3$)	2.48 ± 0.05	2.36 ± 0.05	ns
Hemoglobin (mg/100 ml)	17.35 ± 0.34	9.73 ± 0.34	**
Hematocrit%	36.43 ± 0.47	32.60 ± 0.47	**

NS= Not significant

** = significant at ($p \leq 0.01$).

Immune Response:

Lymphoid organs:

Results in (Table 4) declared that the absolute weights of thymus gland increased in Domyati as compared to Khaki-Campbell, insignificantly at 12 weeks and significantly ($p \leq 0.05$) at 36 weeks of age. Absolute weights of spleen did not differ significantly at both ages. Meanwhile, absolute weight of pancreas significantly ($p \leq 0.05$) increased in Khaki-Campbell compared with Domyati at both ages. All relative weights to the body weights were higher at both ages in Domyati than Khaki-Campbell. Such trends may indicate that Domyati ducks had more immunity response than Khaki-Campbell ones. The relative weights of thymus, spleen and pancreas in Khaki-Campbell at 12 weeks of age were comparable with 0.00449, 0.04709 and 0.52% at 9 weeks of age, respectively as reported by Abdalla *et al.*, (2004). Also, absolute and relative weights of spleen in Domyati ducks at 12 weeks of age (Table 4) compared with 0.84 g and 0.05% (El-Tehiti, 2001), respectively.

Data in Table (4) showed significant sex differences in all relative and absolute weights at 36 weeks, but not at 12 weeks of age (except absolute weights of pancreas). At 36 weeks of age, males had higher absolute and relative weights for all traits than females, which may indicate that males had more immunity response at 36 weeks of age than females. At 12 weeks of age, males had also higher pancreas weight than females, i.e. had more immunity.

Relative weight of pancreas in male Khaki-Campbell ducks was 0.42% at 12 weeks of age compared with 0.36% at 8 weeks of age reported by Attia (2003).

Table (4): Absolute and relative weights of lymphoid organs in Khaki-Campbell (KC) and Domyati (DO) ducks at different ages (Means ± SE).

Item	(KC)			(DO)			(KC)	(DO)	Sign.
	Male	Female	Sign.	Male	Female	Sign.	Av.	Av.	
At 12 weeks of age									
Absolute weight (g)									
Thymus (g)	0.06 ±0.01	0.04 ±0.01	NS	0.09 ±0.01	0.07 ±0.01	NS	0.05	0.08	NS
Spleen (g)	1.07 ±0.19	0.81 ±0.19	NS	1.27 ±0.19	0.81 ±0.19	NS	0.94	1.04	NS
Pancreas (g)	8.98 ±0.26	8.19 ±0.26	**	8.11 ±0.26	6.91 ±0.26	**	8.58	7.51	**
Relative weight to live body weight (%)									
Thymus %	0.0030 ±0.0008	0.0020 ±0.0008	NS	0.0050 ±0.0008	0.0040 ±0.0008	NS	0.0025	0.0045	*
Spleen %	0.05 ±0.01	0.04 ±0.01	NS	0.07 ±0.01	0.05 ±0.01	NS	0.05	0.06	NS
Pancreas %	0.42 ±0.01	0.41 ±0.01	NS	0.45 ±0.01	0.43 ±0.01	NS	0.42	0.44	*
At 36 weeks of age									
Absolute weights (g)									
Thymus (g)	0.10 ±0.01	0.06 ±0.01	**	0.14 ±0.01	0.09 ±0.01	**	0.08	0.11	*
Spleen (g)	1.69 ±0.16	1.11 ±0.16	**	1.49 ±0.16	1.00 ±0.16	**	1.40	1.25	NS
Pancreas (g)	10.42 ±0.17	9.31 ±0.17	**	8.69 ±0.17	7.33 ±0.17	**	9.86	8.01	**
Relative weights to live body weight (%)									
Thymus %	0.0038 ±0.0006	0.0027 ±0.0006	*	0.0073 ±0.0006	0.0051 ±0.0006	*	0.0032	0.0063	**
Spleen %	0.07 ±0.008	0.05 ±0.008	*	0.08 ±0.008	0.06 ±0.008	*	0.06	0.07	NS
Pancreas %	0.44 ±0.007	0.42 ±0.007	**	0.47 ±0.007	0.44 ±0.007	**	0.43	0.46	**

* = significant at (P≤0.05). ** = Not significant. * = significant at (P≤0.01).

White blood cells count and its frequency distribution:

It was observed from Table (5) that there were significant breed differences in all studied traits. Count of WBC's and lymphocyte distribution increased in Domyati ducks, while distribution of heterophils and H/L ratio increased in Khaki-Campbell ducks. This may reflect that higher immunity in Domyati more than Khaki-Campbell. Zuprizal and Geraert (1993) reported the effect of breed on immunity response, which may reflects genetic differences between strains. El-Badry (2004) reported that WBC's count, distribution of lymphocytes and heterophils as well as H/L ratio significantly

increased in Domyati than in Muscovy ducks. This may indicate that the Domyati strain had higher immunity responsiveness than Muscovy ducks. Abdalla *et al.*, (2004) noted a higher value of WBC's $54.88 \times 10^3/\text{mm}^3$ in Khaki-Campbell at 9 weeks of age which was more than 2-fold of value obtained in our study ($26.61 \times 10^3/\text{mm}^3$) at 12 weeks of age. This may suggest increasing the immunity response in ducks by age progress.

In this study, count of WBC's , distribution of lymphocytes and heterophils as well as H/L ratio in Domyati ducks at 12 weeks of age are nearly similar to $29.02 \times 10^3/\text{mm}^3$, 62.47 and 28.06% and 0.45 as reported by El-Badry 2004. However, Nagwa *et al.*, (2004) reported 64.42, 27.69% and 0.43 for lymphocyte, heterophil distibution and H/L ratio in Domyati at 12 weeks of age, respectively. Danial and Terr (1991) reported that the change in lymphocytes and heterophil distribution was attributed to the redistribution of cells out of the circulation and to secondary lymphoid organ wightes. Trout *et al.*, (1988) indicated that on early step in the initiation of humeral immunity (Ab's production) appear to be increased as a result of serum hormones release (especially, corticosterone). This shows that physiological status may play an important role in immune response.

Table (5): Immune response in Khaki-Campbell (KC) and Domyati (DO) ducks at 12 weeks of age (Means \pm SE).

Item	(KC)	(DO)	Sigr.
WBC,s ($\times 10^3/\text{mm}^3$)	26.61 ± 0.44	29.49 ± 0.44	**
Lymphocyte%	58.32 ± 0.84	62.87 ± 0.84	**
Heterophils%	28.87 ± 0.37	26.69 ± 0.37	**
H/L%	0.50 ± 0.01	0.43 ± 0.01	**

** = significant at ($p \leq 0.01$).

Antibody against sheep red blood cells (SRBC's):

Data in Table (6) cleared significant breed differences in both primary (at 16 weeks) and secondary responses (at 20 weeks) against SRBC's. Both traits were significantly higher in Domyati (92.15 and 272.61) than Khaki-Campbell (66.13 and 241.52), respectively. This may indicate that Domyati ducks had higher immunity responsiveness than Khaki-Campbell ducks. The antibody production against SRBC's for Muscovy ducks was reported as 63.97 (Nematallah *et al.*, 2003) and 63.97 (El-kaiaty *et al.*, 2004) at 11 weeks of age.

Table (6): Primary and Secondary responses of antibody production against SRBC's in Khaki-Campbell (KC) and Domyati (DO) ducks at 12 weeks of age(Means \pm SE).

Item	(KC)	(DO)	Sign.
Primary at 16 wks	66.13 ± 0.67	92.15 ± 0.67	..
Secondary at 20 wks	241.52 ± 0.67	272.61 ± 0.67	..

** = significant at ($p \leq 0.01$).

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العلاقة بين مكونات الدم والمناعة في بط الكامبل والدمياطي

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استخدمت في هذه الدراسة ٣٠٠ بطة عمر يوم من كل من سلتي الكاكي الكامبل (كسلالة أجنبية) والدمياطي (كسلالة محلية) لتقييم العلاقة بين مكونات الدم والصفات المناعية تحت الظروف المصرية. وتميز دم البط الكاكي الكامبل بارتفاع معنوي في تركيز الدهون الكلية والكلوسترول والكالسيوم والهيموجلوبين وقيم الهيماتوكريت، بينما تميز دم البط الدمياطي بارتفاع معنوي في تركيز البروتين الكلي والاليومين والجلوبيولين والفسفور ونشاط انزيمات GOT و GPT. لوحظ زيادة في الوزن المطلق للغدة الثيموسية والطحال والوزن النسبي لكل من الغدة الثيموسية والطحال والبنكرياس في البط الدمياطي عن الكاكي الكامبل، أيضا ارتفع عدد كرات الدم البيضاء والخلايا اليمفاوية معنويا في البط الدمياطي، بينما يلاحظ ارتفاع heterophils ونسبة H/L في البط الكاكي الكامبل، كما لوحظ ارتفاع معنوي لكل من الاستجابة المناعية الأولية والثانوية ضد دم الأغنام SRBC's في البطة الدمياطي (٩٢،٢، ٢٧٢،٦) عن البط الكاكي الكامبل (٢٤١،٥، ٦٦،١) على التوالي.