

BLOOD CONSTITUENTS AND IMMUNITY RESPONSE IN MUSCOVEY AND SUDANI DUCKS AT DIFFERENT AGES.

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ABSTRACT

A number of 300-day-old ducklings was used to compare blood constituents and immunity response between Muscovy and Sudani breeds under the Egyptian environmental conditions. Results indicated no significant breed differences in concentrations of total protein, albumin, globulin, total lipids, cholesterol, calcium, inorganic phosphorus, T3 and E2 and hematocrit value. Concentrations of total lipids and T3 decreased in both breeds with advance of age. Activity of GOT and GPT and count of RBC's counts and hemoglobin concentration were significantly higher in Muscovy than Sudani breed. While proportion of heterophils and H/L ratio were significantly higher in Muscovy than Sudani breed, count of WBC's and proportion of lymphocytes showed an opposite trend. The primary and secondary responses against SRBC's were significantly higher in Sudani (93.49 and 274.73) than in Muscovy ducks (64.21 and 236.46), respectively. The current study may indicate higher immunity responsiveness for Sudani compared with Muscovy ducks.

INTRODUCTION

Blood protein fractions were known to be influenced by genetic and non-genetic factors (Agag, 1983). Ress and Nordskog (1981) found that the breed effects on globulin concentration may be the cause of marked variation in total serum protein fractions between chicken strains. Radwan *et al.*, (1989) noted significant breed differences in serum calcium content. Sturkie (1986) suggested that there is a positive relation between the RBC's number and hemoglobin concentration in blood. He revealed that the corpuscular volume (Ht) is influenced by age and other factors, which affect cell number.

Zuprizal and Geraert (1993) reported that the effect of breed on immune response may reflect genetic differences between strains. El-Badry (2004) observed marked differences in counts of white blood cells, lymphocytes, heterophils and H/L ratio between Domyati and Muscovy ducks.

Aim of this study is to compare some blood constituents and immunity response between Muscovy (foreign) and Sudani (local) duck breeds at different ages under the Egyptian environmental conditions. Concentration of some blood metabolites, hormones, enzymes activity and hematological traits were determined. Lymphoid organs, WBC's count, primary and secondary response against Sheep red blood cells (SRBC's) were recorded as an 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 Muscovy and Sudani breeds were used to compare some blood constituents and immune 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 to 16 weeks of age and layer ration (15.67% crude protein and 2746.00 ME Kcal/Kg ration) from 16-38 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 week of age and to natural light, thereafter.

To determine blood constituents, 12 blood samples/breed (2 males and 2 females/rep.) were collected in to heparinized tubes at 12, 26 and 38 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. Concentration of Triiodothyronine (T3) and estradiol hormones were determined using RIA technique.

In fresh blood samples collected at 12 weeks of age the total count of red (RBC's) and white blood cells (WBC's) were determined according to Winteroba (1967). Also, hemoglobin concentration (Pilaski, 1972), hematocrit value (Hunsaker, 1969), and count of lymphocytes and heterophils (Haddad and Mashaly, 1990) were performed.

At 16 weeks for 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 lymphoid organs, 12 ducklings from each breed (2 males and 2 females/rep.) were taken randomly for slaughtering either at 12 or at 38 weeks of age and eviscerated. Spleen, thymus and pancreas were separated and weighed. Relative weights to live body weight were calculated.

Statistical analysis:

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

Results in Table (1) showed no significant differences in concentration of total protein, albumin, globulin and A/G ratio between Muscovy and Sudani breeds at all ages. However it can be observed that the total protein, albumin (except Muscovy at 26 weeks) and globulin (except Sudani at 38 weeks) concentration increased with advancement of age. In contrast to the present results, Agag (1983) noted that the protein fraction was 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.

The present concentration of total protein, albumin, globulin and A/G ratio at 12 weeks of age in Muscovy ducks are nearly similar to those reported by El-Badry (2004), being 5.88, 3.97, 1.91 g/dl and 1.48, respectively. Generally wide variation was found in concentration of total protein, albumin and globulin, being 6.42, 3.54 and 2.88 g/dl (Yassein, 1995) and 5.81, 3.9 and 1.91, respectively, in Muscovy ducks (El-Badry, 1998).

Total lipids and Cholesterol:

Also, no significant differences were observed in concentration of total lipids and cholesterol in serum (Table 1). In general, concentration of total lipids decreased in both breeds with advance of age, while cholesterol concentration showed pronounced increase. Desoke (1986) observed that concentration of total lipids were 450.6, 348.3 to 348.3 mg/100ml at 7, 9 and 11 weeks of age, respectively. El-Bogdady *et al.*, (1987) showed slight increase in cholesterol concentration from 1.9, 2.0 to 2.2 g/dl with advance of age from 23, 32 to 38 weeks of age, respectively.

The obtained concentration of total lipids in Muscovy ducks at 12 weeks of age, is nearly similar to that reported by El-Kaiaty *et al.*, (2004) in Muscovy ducks at 11 weeks of age. However, higher concentration of total lipids was recorded by Nematallah *et al.*, (2003). In our study, serum cholesterol concentration (123.06 mg/dl) in matured Sudani ducks is lower than 171.52 mg/100ml as reported by Salem *et al.*, (1983). Serum cholesterol concentration in Muscovy ducks at 12 weeks of age (Table 1) is different than: 86.0 mg/dl (Samy *et al.*, 2001) and 132.0 mg/dl (El-Ghamry, 2004) at 10 weeks of age and 98.89 mg/dl (Nematallah *et al.*, 2003), 129.61 mg/dl (El-Kaiaty *et al.*, 2004) and 120.0 mg/dl (El-Ghamry *et al.*, 2004) at 11 weeks of age. The differences in biochemical concentration in blood plasma

in this study and those previously reported on ducks may be attributed to the method of analysis, period of sample storage and storage temperature as well as the individual errors.

Calcium and phosphorus:

Results revealed no significant differences in calcium and inorganic phosphorus concentration at all ages studied (Table 1). It was observed that concentration of Ca and P in both breeds increased with advance of age. Radwan *et al.*, (1989) found significant breed differences in serum calcium content. This may be attributed to the increase in estrogen secretion during those periods which increase calcium release in blood stream.

In the present study, calcium and phosphorus levels ranged from 9.98 to 13.74 and from 4.39 to 7.75 mg/100 ml, respectively. These are within a range between 9.2 and 13.0 mg/100 ml for calcium and between 4.2 and 6.10 mg/100 ml for inorganic phosphorus in ducklings as reported by Lin and Shen (1979). However, El- Tehiti (2001) obtained 10.12 and 4.70 mg/100 ml for calcium and phosphorus, respectively, in Domyati ducks at 12 weeks of age.

Table (1): Concentration of some biochemicals and minerals In blood plasma of Muscovy (MU) and Sudani (SU) ducks at different ages (Means ± SE).

Item	Age/weeks					
	12		26		38	
	(MU)	(SU)	(MU)	(SU)	(MU)	(SU)
Total Protein (g/dl)	5.97 ±0.47	5.58 ±0.47	6.99 ±1.14	8.17 ±1.14	9.23 ±0.52	8.84 ±0.52
Albumin (g/dl)	3.98 ±0.34	3.66 ±0.34	3.61 ±0.59	3.99 ±0.59	5.23 ±0.36	4.90 ±0.36
Globulin (g/dl)	1.98 ±0.16	1.92 ±0.16	3.38 ±0.62	4.18 ±0.62	4.01 ±0.20	3.95 ±0.20
A\G ratio	2.02 ±0.14	1.98 ±0.14	1.06 ±0.10	1.09 ±0.10	1.29 ±0.06	1.25 ±0.06
Total Lipids (g/dl)	1.30 ±0.17	1.27 ±0.17	0.93 ±0.11	1.09 ±0.11	1.08 ±0.17	1.05 ±0.17
Cholesterol (mg/dl)	104.34 ±8.57	104.29 ±8.57	124.53 ±9.10	133.02 ±9.10	120.11 ±8.57	123.06 ±8.57
Calcium (mg/dl)	10.89 ±1.08	9.98 ±1.08	12.11 ±1.02	11.46 ±1.01	13.74 ±1.11	12.83 ±1.11
Phosphors (mg/dl)	4.39 ±0.26	4.17 ±0.26	6.47 ±0.34	5.58 ±0.34	7.75 ±0.37	5.91 ±0.37

All breed differences are not significant (P<0.05).

Calcium and phosphorus levels at 12 weeks of age in Muscovy ducks were 10.89 and 4.39 mg/dl, respectively (Table 1). These values are

higher than 16.2 and 6.3 mg/100 ml (Abdel-Samee, 1995), and 16.2 and 6.28 mg/dl (Samy *et al.*, 2001) in Muscovy ducks at 10 weeks of age for Ca and P, respectively. Serum calcium level in Muscovy was 12.51 mg/dl (Nematallah *et al.*, 2003) and 11.37 mg/100ml (El-Kaiaty *et al.*, 2004) at 11 weeks of age.

Enzyme activity:

Results in Table (2) declared significant breed differences in GOT and GPT activity at all ages. Both GOT and GPT activity were higher in Muscovy than Sudani ducks. There was inconsistent trend in GOT and GPT activity with age progress in each breed. The highest activities appeared at 26 weeks of age in Muscovy, and at 12 weeks of age in Sudani ducks. Geraert *et al.*, (1996) noted that activity of both enzymes was depended upon pathological conditions, in case of the abnormal values.

Activity of GOT and GPT in Muscovy ducks were 99.5 and 31.67 IU/L at 12 weeks of age (Table 2). Higher activity of both enzymes (117.5 and 37.97 IU/l respectively) was reported by El-Badry (2004) for the same breed and age. At 38 weeks of age, activity of GOT and GPT in Muscovy ducks was 71.25 and 27.67 IU/l, respectively (Table 2). Fattouh (2001) indicated lower activities, being 26.0 and 10.0 IU/l, respectively in Muscovy ducks at 44 weeks of age.

Table (2): Activity of transaminases (GOT and GPT) and hormones concentration in plasma of Muscovy (MU) and Sudani (SU) ducks at different ages.

Item	Age/weeks								
	12			26			38		
	(MU)	(SU)	Sign.	(MU)	(SU)	Sign.	(MU)	(SU)	Sign.
GOT (IU/l)	99.5	76.67	**	103.5	41.17	**	71.25	61.17	**
	±1.67	±1.67		±1.68	±1.86		±1.87	±1.87	
GPT (IU/l)	31.67	26.75	*	33.67	26.25	**	27.67	19.25	**
	±1.36	±1.36		±1.37	±1.37		±1.37	±1.37	
T3 ng/ml	83.08	60.67	NS	77.44	74.98	NS	71.80	69.30	NS
	±7.52	±7.52		±3.33	±3.33		±6.30	±6.30	
Estrogen (E2) ng/ml	22.62	22.93	NS	28.94	26.26	NS	29.07	26.90	NS
	±2.64	±2.64		±4.84	±4.84		±5.93	±5.93	

NS: Non significant * Significant at P<0.05; ** Significant at P<0.01;

c. Hormonal assay:

Results indicated insignificant differences in concentration of T3 and E2 between Muscovy and Sudani ducks (Table 2), but concentration of both hormones tended to be higher in Muscovy than Sudani ones. Concentration of T3 decreased in both breeds with advance of age, while E2 showed the opposite trend.

Plasma T3 concentration in Muscovy ducks was 83.08 ng/ml (Table 2), being lower than 95.08 ng/ml (Nagwa *et al.*, 2004), and higher than 70.40 ng/ml (El-Badry, 2004) and 69.95 ng/ml (El-Badry 2004) in Muscovy ducks at 12 weeks of age. Darras *et al.*, (1995) found that at a relatively low

metabolic demand, the peripheral conversion of T4 to T3 (most active from thyroid hormone) is impaired.

Hematological traits:

There were significant breed differences in hemoglobin concentration and RBC's count, but hematocrit value did not differ significantly between Muscovy and Sudani breeds. Concentration of hemoglobin and RBC's count were significantly ($P<0.05$) higher in Muscovy than Sudani ducks. However, hematocrit value tended to be slightly higher in Muscovy than Sudani ducks (Table 3). In Muscovy ducks at 12 weeks of age, hemoglobin and RBC's values are nearly in agreement with 18.10 g/dl, $2.94 \times 10^6/\text{mm}^3$ (El-Badry 2004) and hematocrit value was lower than 35.32% (Nagwa et al., 2004). Vo et al (1978) and Sturkie (1986) suggested that there is a positive relation between the RBC's number and hemoglobin concentration in blood, which was indicated in this study.

Table (3): Hematological traits in blood of Muscovy and Sudani ducks at 12 weeks of age.

ITEM	Muscovey	Sudani	Significance
RBC.s $\times 10^6(\text{mm}^3)$	2.90 ± 0.02	2.36 ± 0.02	**
Hemoglobin g/100ml	17.9 ± 0.03	9.62 ± 0.03	**
Hematocrit %	32.74 ± 0.97	30.60 ± 0.97	NS

NS: Not significant

** Significant at ($P \leq 0.01$).

Immune Response:

Lymphoid organs:

Results in Table (4) declared significant breed differences in all absolute (except spleen at 38 weeks) and relative (except thymus at 12 weeks) weights of lymphoid organs at both 12 and 38 weeks of age. The absolute weight of spleen was higher in Sudani than Muscovy, being significant ($P<0.05$) at 12 weeks and insignificant at 38 weeks of age. However, the absolute weight of spleen and pancreas was significant ($P<0.01$) higher in Muscovy than Sudani at 12 and 38 weeks of age. In general, all weights of organs studied relative to live body were significantly higher in Sudani than Muscovy ducks (Table 4). The relative weight of spleen was 0.10/% in Muscovy ducklings at 12 weeks of age, being higher than 0.08% at 11 weeks of age that reported by El-Ghamry et al., (2004).

Data in Table (4) showed significant sex differences in all absolute and relative (except spleen at 38 weeks) weight at both 12 and 38 weeks in the two breeds between males and females. While males increased at both ages and breeds in all absolute weights, females increased in all relative weights. It may be due to the lighter weights of females.

In this study, the absolute weight of spleen, thymus and pancreas in Muscovy ducks at 12 weeks of age were 3.43, 17.61 and 7.12g in males

Table (4): Absolute and relative weight of lymphoid organs in Muscovy (MU) and Sudani (SU) ducks at different ages.

Item	(MU)			(SU)			(MU)	(SU)	Sign.
	Male	Female	Sign.	Male	Female	Sign.	Av.	Av.	Breed
At 12 weeks of age									
Absolute weight (g)									
Spleen (g)	3.43 ±0.17	2.77 ±0.17	*	4.15 ±0.17	2.88 ±0.17	*	3.10	3.51	*
Thymus (g)	17.61 ±0.60	13.43 ±0.60	**	13.08 ±0.60	10.25 ±0.60	*	15.52	11.66	**
Pancreas (g)	7.12 ±0.32	5.84 ±0.32	**	6.54 ±0.32	4.70 ±0.32	*	6.48	5.62	*
Relative weight (%)									
Spleen %	0.092 ±0.005	0.109 ±0.005	**	0.152 ±0.005	0.166 ±0.005	**	0.100	0.159	**
Thymus %	0.470 ±0.017	0.530 ±0.017	*	0.480 ±0.017	0.590 ±0.023	*	0.500	0.535	NS
Pancreas %	0.190 ±0.011	0.230 ±0.011	**	0.240 ±0.011	0.270 ±0.011	*	0.210	0.255	**
At 38 weeks of age									
Absolute weights (gm)									
Spleen (g)	4.29 ±0.19	3.32 ±0.19	*	5.14 ±0.19	3.13 ±0.19	**	3.80	4.13	NS
Thymus (g)	20.61 ±0.59	15.31 ±0.59	**	15.65 ±0.59	12.17 ±0.59	*	17.96	13.91	**
Pancreas (g)	9.66 ±0.37	7.49 ±0.37	*	8.76 ±0.37	6.10 ±0.37	**	8.58	7.43	**
Relative weights (%)									
Spleen %	0.098 ±0.005	0.115 ±0.005	*	0.158 ±0.005	0.155 ±0.005	NS	0.106	0.157	**
Thymus %	0.471 ±0.017	0.531 ±0.017	*	0.48 ±0.017	0.602 ±0.017	**	0.500	0.542	*
Pancreas %	0.220 ±0.011	0.260 ±0.011	**	0.270 ±0.011	0.302 ±0.011	*	0.240	0.286	**

NS: Non significant

* Significant at (p≤ 0.05)

** Significant at (p≤ 0.01)

and 2.77, 13.43 and 5.84g in females. These values at 11 weeks of age were reported as 3.33, 10.83 and 5.58g in males and as 2.45, 5.72 and 4.42g in females by El-Kaiaty *et al.*, (2004). In this study, the relative weight of Muscovy spleen, thymus and pancreas at 12 weeks of age were 0.092, 0.470 and 0.190% in males and 0.109, 0.530 and 0.230% in females, respectively. El-Kaiaty *et al.*, (2004) reported these values at 11 weeks of age as 0.075, 0.243 and 0.12% in males and 0.102, 0.238 and 0.19% in females, respectively.

Count of WBC's counts, lymphocytes %, heterophils % and H/L ratio:

Table (5) show significant breed differences in frequency distribution of lymphocytes (L), and heterophils (H), and H/L ratio, but count of WBC's was not affected significantly by breed. Distribution of lymphocytes was lower and of heterophils was higher in Muscovy than Sudani ducks. With the respect to the effect of breed on immune response Zuprizal and Geraert (1993) found that breeds may reflect genetic differences between strains. El-Badry (2004) reported that count of WBC's, proportion of lymphocytes and heterophils and H/L ratio significantly increased in Domyati compared with in Muscovy ducks. This may indicate that the Domyati strain had higher immunity responsiveness compared with Muscovy ducks.

Table (5): Traits of immune response in Muscovy and Sudani ducks at 12 weeks of age.

Item	Muscovey	Sudani	Significance
WBC,s ($\times 10^3$)	26.22 ± 1.03	29.11 ± 1.03	NS
Lymphocyte (%)	59.25 ± 1.04	62.55 ± 1.04	*
Heterophils (%)	27.55 ± 0.47	26.03 ± 0.47	*
H/L ratio	0.47 ± 0.007	0.41 ± 0.007	**

NS: Not significant *Significant at ($P \leq 0.05$). **Significant at ($P \leq 0.01$).

The present values of WBC's, lymphocytes, heterophils and H/L ratio in Muscovy ducks at 12 weeks of age are in agreement with the corresponding values ($25.90 \times 10^3/\text{mm}^3$, 59.38%, 28.73% and 0.48, respectively) as reported by El-Badry (2004). Nagwa et al., (2004) reported 61.22%, 28.26% and 0.45 as values of lymphocytes, heterophils and H/L ratio in Muscovy ducks at 12 weeks of age, respectively.

Danial and Terr (1991) reported that the changes in lymphocytes and heterophils percentages are attributed to the redistribution of cells out of the circulation and into secondary lymphoid organs. Trout et al., (1988) indicated that on early step in the initiation of humoral immunity there is an increase in serum hormones (especially corticosterone); this shows that physiological status may play an important role in immune response.

Sheep Red Blood Cells (SRBC's):

Data in table (6) cleared significant ($P \leq 0.01$) breed differences in both primary and secondary responses. The primary (at 16 weeks of age) and secondary responses (at 20 weeks of age) against Sheep Red Blood Cells (SRBC's) were higher in Sudani (93.49 and 274.73, respectively) than in Muscovy ducks (64.21 and 236.46, respectively). This may indicate higher immunity responsiveness in Sudani than Muscovy ducks. Antibody production against SRBC's for Muscovy ducks was reported to be 63.97 (Nematallah et al., 2003) and 63.973 (El-Kaiaty et al., 2004) at 11 weeks of age.

Table (6): Antibody production against SRBC's in Muscovy and Sudani ducks.

Immune response	Age (week)	Muscovey	Sudani	Significance
Primary response	16	64.12 ±1.01	93.49 ±1.01	**
Secondary response	20	236.46 ±1.01	274.73 ±1.01	**

** Significant at (P≤ 0.01).

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مكونات الدم والاستجابة المناعية في البط المسكوفي والسوداني على أعمار مختلفة

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أستخدم في هذه التجربة ٣٠٠ بطة عمر يوم من كل من سللتي البط المسكوفي كسلالة أجنبية و السوداني كسلالة محلية لمقارنة مكونات الدم والاستجابة المناعية تحت الظروف المحلية. أظهرت النتائج أنه لا توجد اختلافات معنوية بين النوعين في تركيز البروتين الكلي والالبومين والجلوبيولين والدهون الكلية والكوليسترول والكالسيوم والفوسفور وهرموني E_2 , T_3 وقيمة النييماتوكريت، انخفض تركيز الدهون الكلية وهرمون T_3 بتقدم العمر بينما ارتفع تركيز جميع الصفات الأخرى مع التقدم في العمر، كما ارتفع نشاط كل من GOT و GPT وعدد كرات الدم الحمراء و تركيز الهيموجلوبين معنويا في المسكوفي عن السوداني. كما لوحظ زيادة معنوية في النسبة المئوية لكريات الدم البيضاء (heterophils) ونسبة هذه الخلايا إلى الخلايا اللمفاوية في المسكوفي، بينما زاد عدد كرات الدم البيضاء و الخلايا اللمفاوية في السوداني، لوحظ ارتفاع كلا من الاستجابة المناعية الأولية (سند ١٦ أسبوع) والاستجابة المناعية الثانوية (عند ٢٠ أسبوع) ضد كريات الدم الحمراء للأغنام في البط السوداني (٩٣،٤٩ و ٢٧٤،٧٣) عن مثيله في البط المسكوفي (٦٤،٢١ و ٢٣٦،٤٦) على التوالي.