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EFFECT OF BROILER BREEDERS AGEING ON HEMATOLOGICAL PARAMETERS, BIOCHEMICAL ANALYSES, AND MATERNAL IMMUNITY TRANSFER RATES

A.A. Abdelhamid; I. El-Wardany; F.B.A. Badri, and Ghada, G. Gad

Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

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SUMMARY

The present study was conducted to investigate the effect of flock age and sex on hematological and biochemical parameters as well as the transfer rate of maternal antibodies from female breeders to their one-day-old chicks. A total of 20 birds (10 males and 10 females) were used at 60, 62, and 64 weeks of age (WOA) in the study. The hematological parameters included hemoglobin concentration (Hb), red blood cells (RBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). While the biochemical parameters included plasma total protein (TP), albumin (A), globulin (G), A/G ratio, triglycerides (TG), total cholesterol (TC), uric acid (UA) and creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (GLU), calcium (Ca), and phosphorus (P). Results showed that, with the exception of RBCs, flock age had a significant effect ($P < 0.05$) on all hematological parameters and blood indices. Furthermore, sex had a significant effect on Hb, while it did not affect significantly ($P > 0.05$) the rest of the parameters and blood indices. Plasma TP, G, TG, UA, Ca, P, and GLU were significantly affected by flock age, while the rest of the biochemical parameters were not significantly affected by flock age. Moreover, sex had significant effects on A, A/G ratio, TG, TC, UA, Ca, GLU, and AST, whereas there was no significant effect of sex on the rest of the parameters. Furthermore, the results revealed that there were low levels of geometric mean titer (GMT) of Newcastle Disease Virus (NDV) and Avian Influenza Virus (AIV) in female breeders, accompanied by low levels of passive maternal antibodies (Mab) in their progeny. The previous results provided baseline data on hematological and biochemical parameters for Indian River broiler breeders. In conclusion, ageing had a detrimental effect on female breeders' immune systems, which could negatively affect their progeny at an early age.

Keywords: *Hematology, blood constituents, maternal immunity, age, sex, broiler breeders, one-day-old chicks, hemagglutination, antibodies.*

INTRODUCTION

The poultry production industry in Egypt is regarded as one of the most important contributors to the availability of animal protein (Afiffi *et al*., 2022). Hematological analyses are critical for providing crucial information and identifying the physiological and immunological state of an animal's body (Khan and Zafar, 2005). Moreover, age, sex, season, and nutrition have all been demonstrated to have an effect on hematological parameters in birds (Elagib and Ahmed, 2011).

The greatest protein fraction in healthy birds is represented by albumin. Elevated globulin fractions may contribute to an increase in total protein in acute or chronic inflammatory diseases. In such cases, albumin levels are frequently reduced. Glucose is a bird's most basic, most vital, and easily obtainable source of energy, and blood glucose levels are a sign of nutritional status (Dunbar *et al*. 2005). Most bird species have normal blood glucose levels ranging from 200–500 mg/dL (Harris, 1991).

Creatinine levels in birds are lower than those in mammals, and excessive creatinine levels are uncommon and indicative of serious renal impairment (Harr, 2002). Sex appears to be one of the key factors influencing the amount of plasma uric acid in birds (Sturkie, 1965). Aspartate aminotransferase is currently thought to be a very sensitive but nonspecific indication of hepatic disorders in other avian species. Birds' muscles and other tissues, as well as their hepatocyte cytosol, contain ALT, which has a

low specificity for liver damage (Harr, 2002). Many nutrients are required by embryos, but energy is of great importance to growth and development. During egg laying, estrogen enhances hepatic lipid synthesis, primarily triglycerides, to provide an energy reserve for the embryo (Walzem and Hamilton, 1999).

Maternal immunity is important in regulating commercial broiler flock vaccinations (live vaccines). For instance, maternal antibodies against Newcastle disease virus (NDV) reduce the intensity of live vaccines; nevertheless, they also reduce immunity following vaccination (Gharaibeh and Mahmoud, 2013). Suardana *et al*. (2022) reported that there is a necessity for an immediate vaccination for broiler chicks that lack maternal immunity because there will not be interference from maternal antibodies against vaccine antigens. Moreover, a good immune response will be acquired.

Therefore, the objectives of the present study were to establish a baseline for the hematological and biochemical profiles of the Indian River broiler breeder strain under closed-system housing, determine the transfer rate of maternal antibodies to progeny to aid in setting the appropriate vaccination programs, and determining whether or not the offspring needs an early or immediate vaccination.

MATERIALS AND METHODS

The current study was carried out on a private farm owned by the Al-Hurriya Company. Data were obtained from an Indian River broiler breeder's flock located at Khatatba, Monufia. The flock was 60 weeks of age at the beginning of the study, with an average body weight of 5289 and 4197 g for males and females, respectively. Hematological, biochemical, and immunological parameters were performed biweekly at 60, 62, and 64 WOA.

Blood collection:

Blood samples were being taken via wing vein puncture from randomly selected breeders (10 males and 10 females each time) from the two flocks using anticoagulants (heparin for biochemical analyses and EDTA for hematology and serology) using syringes with different volumes (5 and 3 ml), then gently drained out of the syringes into plastic tubes, then centrifuged at a speed of 3000 r.p.m. for 15 minutes, then plasma samples were decanted into a 2-ml ependorf and kept at -20^{o}C until performing the biochemical and immunological analyses. A 1-ml blood sample with EDTA as an anticoagulant was taken into a 2-ml ependorf in order to perform the hematological parameters right after blood collection.

Additionally, at hatching, 10 blood samples were collected from one-day-old chicks (as hatched) by heart puncture using 3 ml syringes (23 gauge), according to Rayan and Badri (2017), and EDTA as an anticoagulant. The blood samples were then gently drained out of the syringes into Ependorf, centrifuged at a speed of 3000 r.p.m. for 15 minutes, and then plasma samples were decanted into 2-ml Ependorf tubes and kept at -20^{o}C until performing the hemagglutination inhibition test to detect the maternal antibody titers against NDV and AIV.

Hematological parameters:

The number of red blood cells (RBCs) was counted manually using a hemocytometer slide, while Sahli's hemoglobinometer was used for the estimation of hemoglobin concentration (Hb). For packed cell volume (PCV %), microhaematocrit tubes were used, and it was calculated using this equation:

$$\text{Packed cell volume \%} = \frac{\text{length of the RBCs (cm)}}{\text{length of the RBCs,buffy coat and plasma (cm)}} \text{ x } 100$$

Blood indices:

In terms of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), these were also calculated using the following formulae, according to Muneer *et al*. (2021).

- MCV (fL) = $\frac{\text{PCV (\%)}}{\text{RBC counts}}$ x 10

- MCH (Pg) = $\frac{\text{Haemoglobin(g/dL)}}{\text{RBCs counts}}$ x 10

- MCHC (%) = $\boldsymbol{\frac{Haemoglobin(g/dL)}{PCV\ (\%)}}$ ***x 100***

Blood biochemical parameters:

All biochemical parameters were determined using Spectrum Diagnostic kits. Plasma total proteins were determined according to the method described by Gornall *et al*. (1949). Plasma albumin was determined using a colorimetric method described by Doumas *et al*. (1971), while globulin was calculated by subtraction of plasma albumin from plasma total protein, and then the A/G ratio was calculated. Plasma triglycerides were determined according to McGowan *et al*. (1983), and plasma total cholesterol was determined based on a method described by Roeschlau *et al*. (1974). The determination of plasma uric acid was done using the method described by Tiffany *et al*. (1972), and plasma creatinine was determined according to Bowers and Wong (1980). The activity of liver enzymes was measured according to Breuer (1996) and Zilva and Pannall (1979). Plasma calcium was determined according to Kessler and Wolfman (1964), while the method of Daly and Ertingshausen (1972) was used to determine plasma phosphorus. The method by which plasma glucose levels were determined was according to Weissman and Klein (1958).

Immunisation of broiler breeders and vaccine administration methods:

The flock was routinely vaccinated against the Avian Influenza virus (H9N2 strain), and the vaccine was given subcutaneously in the lower back part of the neck. Also, the flock was routinely vaccinated against the Newcastle Disease virus (Lasota strain), and the vaccine was administered via drinking water. Blood samples were collected via brachial vein at 60, 62, and 64 WOA in order to detect the total amount of antibodies in plasma against AIV and NDV. The immune responses were measured using a 96-well microplate (U-shape).

The hemagglutination test (HA) was carried out by using detection reagents for AIV and NDV viruses, which were adjusted to contain 4 Hemagglutinating units (HAU) just before use. Titration of viral HA units was done with serially 2-fold-diluted sterile phosphate-buffered saline (PBS) (pH 7.2) and 1% chicken RBCs on 96-well plates, and hemagglutination inhibition antibodies (HI) were determined in females' plasma and their matched day-old chicks' plasma, according to Alexander (1992). Antibody titers were calculated from the geometric mean according to Yeo *et al.* (2003).

Statistical analysis:

The obtained data on blood biochemical and hematological parameters were analyzed using a two-way ANOVA with interaction. The general linear model (GLM) procedure of IBM SPSS statistics 2010 (version 19.0) was used. While the obtained data on antibody titers were analyzed by one-way ANOVA, according to the following statistical models:

First statistical model:

$$Y_{ijk}= \mu + A_i + S_j + A_i*S_j + e_{ijk}$$

Where:
Y_{ijk} = an observation.
μ = Overall mean
A_i = Effect of flock age (i= 1, 2, 3)
Sj = Effect of sex (j=1, 2)
A_i*S_j = Interaction between flock age and sex
e_{ijk} = a random error assumed to be normally distributed

Second statistical model:

$$Y_{ik}= \mu + A_i + + e_{ik}$$

Where:
Y_{ik} = an observation.
μ = Overall mean
A_i = Effect of female breeder age (i= 1, 2, 3)
e_{ik} = a random error assumed to be normally distributed

When there were significant differences among means, they were separated using Tukey's multiple comparison test. Statistical significance was accepted at a probability level of 0.05.

RESULTS AND DISCUSSION

Hematological parameters:

Effect of flock age and sex on hematological parameters of Indian River broiler breeders:

Table (1) shows the mean ± standard error (SE) and overall means of Hb, RBCs, and PCV% at 60, 62, and 64 WOA for both males and females. It is apparent that flock age and sex had a significant effect on Hb concentration. The data showed a declining trend in Hb. Males had higher Hb values than females. It is noteworthy that flock age and sex had no effect on RBCs. While flock age had a significant effect on PCV%, there was no significant effect of sex on PCV%. For Hb, RBCs, and PCV%, statistically insignificant interactions were observed between flock age and sex.

Regarding the sex effect, the results disagree with Addass (2012), who reported that sex showed a significant effect on PCV and RBC values, with males having a higher value than females. While we are in agreement in terms of Hb concentrations that showed a significant effect of age. In addition, the mean value of Hb differs significantly by sex, with males having higher values than females.

The results are in disagreement with Orji *et al.* (1986) who reported that males had a significantly greater packed cell volume (39.6%) than females (34.6%), and males had a significantly higher mean RBCs count (2.58 x 10^6/mm^3) than females. However, the obtained results agree in terms of Hb concentrations that males showed a higher hemoglobin (Hb) concentration than females. The obtained results disagree with Chinenye *et al.* (2017) who observed that as the birds advanced in age, Hb and PCV levels elevated while RBCS levels declined. Also, the results disagree with those of Abdi-Hachesoo *et al.* (2011) who reported that PCV levels in cocks (48.80%) were significantly greater than in hens (32.60 %). Previous studies by Islam *et al.* (2004) and Tufan and Ramazan (2011) disagree with ours; both of them reported that PCV% and Hb elevate with age, while the results were in agreement with the latter, who observed that RBCs decreased with age.

Table (1): Effect of flock age and sex on hematological parameters of Indian River broiler breeders.

Traits	Sex	Flock age (weeks)			Overall mean	*P*-value		
		60	62	64		A	S	A*S
Hb	**F**	$10.20^{a} \pm 0.4$	$8.00^{b} \pm 0.4$	$7.66^{b} \pm 0.4$	$8.62^{B} \pm 0.2$	<0.01	<0.01	0.07
(g/dL)	**M**	$12.00^{a} \pm 0.4$	$9.78^{b} \pm 0.4$	$7.76^{c} \pm 0.4$	$9.85^{A} \pm 0.2$			
Overall mean		$11.10^{A} \pm 0.3$	$8.89^{B} \pm 0.3$	$7.71^{C} \pm 0.3$				
RBCs	**F**	2.91±0.2	3.24 ± 0.2	2.99 ± 0.2	3.05 ± 0.12	0.47	0.22	0.94
(X 10^6/mm^3)	**M**	3.18±0.2	3.37 ± 0.2	3.25 ± 0.2	3.27 ± 0.12			
Overall mean		3.05±0.2	3.30 ± 0.2	3.12 ± 0.2				
PCV	**F**	$41.20^{a} \pm 1.9$	$31.90^{b} \pm 1.9$	$38.20^{a} \pm 1.9$	37.10 ± 1.1	0.002	0.36	0.51
(%)	**M**	$40.90^{a} \pm 1.9$	$35.90^{b} \pm 1.9$	$38.90^{a} \pm 1.9$	38.57 ± 1.1			
Overall mean		$41.05^{A} \pm 1.4$	$33.90^{B} \pm 1.4$	$38.56^{A} \pm 1.4$				

*A = age; S = sex; A*S = interaction between age and sex; g/dL = grams per deciliter;* F= Females; M = Males.
$^{A-C}$ *Means as well as* $^{a-c}$ *Means within a row with different superscripts differ significantly (P ≤ 0.05).*
$^{A-B}$ *Means within a column with different superscripts differ significantly (P ≤ 0.05).*
Hb = Hemoglobin; RBCs= Red blood cells; PCV= Packed cell volume.

Effect of flock age and sex on blood indices of Indian River broiler breeders:

Table (2) shows the mean ± SE and overall means of MCV, MCH, and MCHC at 60, 62, and 64 WOA for both males and females. The data revealed that flock age had a significant effect on MCV, MCH, and MCHC, but there were no significant differences between males and females for MCV, MCH, and MCHC. MCV values fluctuated during the duration of the study, while both MCH and MCHC levels showed a declining tendency with age. Non-significant interactions were observed between flock age and sex for MCV, MCH, and MCHC.

The results are consistent with Afiffi *et al.* (2022) and Bora *et al.*, (2017), who found that there were no significant differences between males and females for MCH, MCV, and MCHC. Moreover, our results agree with those obtained by Rasheed (2017), who found that blood parameters such as Hb, MCH, and

MCHC values declined significantly with age. Furthermore, the findings are in agreement with Nyaulingon (2013), who observed that MCH and MCHC declined with chicken age. The obtained results disagree with those of Addass (2012), who found that MCHC was highly related to sex, with females having a greater value than males, and MCV was significantly affected by sex, with males achieving higher values than females. The findings disagree with those of Panigrahy *et al.* (2017) who found that MCV and MCH values were greater in female Vanaraja birds. The results contrast with those of Chinenye *et al.* (2017), who found that age had no effect on MCHC, while MCH results increased with the birds' age.

Table (2): Effect of flock age and sex on blood indices of Indian River broiler breeders.

Traits	Sex	Flock age (In weeks)			Overall mean	*P*-value		
		60	62	64		A	S	A*S
MCV	**F**	$147.60^{a} \pm 10.6$	$102.70^{b} \pm 10.6$	$130.30^{a} \pm 10.6$	126.86 ± 6.11	0.01	0.78	0.50
(fL)	**M**	133.00 ± 10.6	113.20 ± 10.6	127.30 ± 10.6	124.50 ± 6.11			
Overall mean		$140.30^{A} \pm 7.5$	$107.95^{B} \pm 7.5$	$128.80^{AB} \pm 7.5$				
MCH	**F**	$36.50^{a} \pm 2.2$	$26.20^{b} \pm 2.2$	$26.50^{b} \pm 2.2$	29.73 ± 1.3	<0.01	0.44	0.42
(Pg)	**M**	$38.50^{a} \pm 2.2$	$30.30^{b} \pm 2.2$	$24.70^{b} \pm 2.2$	31.17 ± 1.3			
Overall mean		$37.50^{A} \pm 1.6$	$28.25^{B} \pm 1.6$	$25.60^{B} \pm 1.6$				
MCHC	**F**	$25.20^{ab} \pm 1.5$	$25.90^{a} \pm 1.5$	$20.90^{b} \pm 1.5$	24.00 ± 0.9	<0.01	0.17	0.30
(%)	**M**	$29.40^{a} \pm 1.5$	$27.50^{a} \pm 1.5$	$20.30^{b} \pm 1.5$	25.73 ± 0.9			
Overall mean		$27.30^{A} \pm 1.1$	$26.70^{A} \pm 1.1$	$20.60^{B} \pm 1.1$				

*A = age; S = sex; A*S = interaction between age and sex;* F= Females; M = Males.
$^{A-B}$ Means as well as $^{a-b}$ Means within a row with different superscripts differ significantly ($P \leq 0.05$).
MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; fL = Femtoliter; Pg = Picogram.

Effect of flock age and sex on blood biochemical constitutes of Indian River broiler breeders.

Total protein, Albumin, Globulin (g/dL), and A/G ratio:

The mean ± SE and overall means of the plasma concentrations of total protein, albumin, and globulin (g/dL) and A/G ratio at 60, 62, and 64 WOA for both males and females are presented in Table (3). It is clearly observed that the total protein and globulin were significantly affected by the experimental ages, whereas there was no significant effect of flock age on albumin or the A/G ratio.

The highest concentration of total protein was recorded at 64 WOA (5.65 g/dL), whereas the lowest was 4.64 g/dL at 60 WOA. In the case of globulin, the lowest concentration was 3.04 g/dL at 60 WOA, and the highest was 3.88 g/dL at 64 WOA.

The findings show an upward trend in total protein and globulin concentrations with ageing, while a downward trend was found in the A/G ratio. The highest ratio accounted for 0.55 at 60 WOA, and the lowest reached 0.48 at 64 WOA. The current results show that sex had no effect on total protein and globulin. On the contrary, the results display a significant effect of gender on albumin concentration and A/G ratio: females had a higher concentration (1.92 g/dL), whereas males had 1.47 g/dL; moreover, females had a higher increase in A/G ratio over males (0.60, 0.46, receptively). A statistically significant interaction was observed between age and gender for total protein, albumin, and globulin (P = 0.001, 0.008, and 0.015, respectively). While the interaction was not significant for the A/G ratio.

Griffin *et al.* 1984; Lumeij, (2008) reported that proteins are the precursors to the oocyte's yolk, such as vitellogenin and lipoproteins, which are generated in the liver and transported to the ovary. Schmidt *et al.* (2008) observed that, compared to adult males and young birds, mature females' plasma total protein values were higher, contrasting with our results. These current results are in agreement with Rezende *et al.* (2021) who found that serum albumin values obtained by females were higher than those obtained by males and reported that the high levels of protein required by females for egg development and the lower proportions of protein and amino acids in male diets also contributed to the lower values discovered for male serum albumin. The present findings are also in agreement with the results of Dunbar *et al.* (2005), who reported that total protein concentrations may be reduced as a result of lower albumin or globulin levels, which in turn may affect the amount of proteins deposited per egg. The results also closely match those obtained by Rezende *et al.* (2021), who observed a trend of higher globulin values with ageing (up to 52 weeks of age).

Table (3): Effect of flock age and sex of Indian River broiler breeder on plasma total protein (TP), albumin (A), globulin (G) concentrations and A/G ratio.

Traits	Sex	Flock age (In weeks)			Overall mean	*P*-value		
		60	62	64		A	S	A*S
TP	**F**	$5.1^{b} \pm 0.30$	$4.49^{b} \pm 0.29$	$6.24^{a} \pm 0.29$	5.27 ± 0.17			
(g/dL)	**M**	$4.19^{b} \pm 0.29$	$5.41^{a} \pm 0.29$	$5.07^{a} \pm 0.29$	4.89 ± 0.16	0.004	0.114	0.001
Overall mean		$4.64^{B} \pm 0.21$	$4.95^{B} \pm 0.20$	$5.65^{A} \pm 0.20$				
A	**F**	$1.97^{ab} \pm 0.07$	$1.75^{b} \pm 0.06$	$2.05^{a} \pm 0.06$	$1.92^{A} \pm 0.04$	0.262	<0.01	0.008
(g/dL)	**M**	1.39 ± 0.07	1.56 ± 0.06	1.48 ± 0.06	$1.47^{B} \pm 0.04$			
Overall mean		1.68 ± 0.05	1.65 ± 0.04	1.76 ± 0.04				
G	**F**	$3.12^{b} \pm 0.29$	$2.78^{b} \pm 0.29$	$4.2^{a} \pm 0.28$	3.40 ± 0.16	0.015	0.715	
(g/dL)	**M**	2.96 ± 0.28	3.84 ± 0.28	3.57 ± 0.28	3.45 ± 0.16			
Overall mean		$3.04^{B} \pm 0.21$	$3.31^{AB} \pm 0.21$	$3.88^{A} \pm 0.20$				
A/G	**F**	0.62 ± 0.05	0.66 ± 0.04	0.51 ± 0.04	$0.60^{A} \pm 0.02$	0.264	0.001	
ratio	**M**	0.48 ± 0.04	0.43 ± 0.04	0.46 ± 0.04	$0.46^{B} \pm 0.02$			
Overall mean		0.55 ± 0.03	0.54 ± 0.03	0.48 ± 0.03				

*A = age; S = sex; A*S = interaction between age and sex; A/G = Albumin to Globulin ratio; g/dl = grams per deciliter*; F= Females; M = Males.
$^{A-B}$ *Means as well as* $^{a-b}$ *Means within a row with different superscripts differ significantly (P ≤ 0.05).*
$^{A-B}$ *Means within a column with different superscripts differ significantly (P ≤ 0.05).*

Triglycerides and total cholesterol (mg/dL):

The means ± SE and overall means ± SE of the plasma concentrations of triglycerides and cholesterol at 60, 62, and 64 WOA for both males and females are presented in Table (4). The results demonstrate a significant effect (P = 0.028) on triglycerides between flock ages (60, 62, and 64), with the middle age (62 WOA) achieving the highest levels of triglyceride concentrations for both males and females (312.14 and 51.80 mg/dL, respectively). Female triglyceride levels indicate a significant increase, suggesting a wide variance between males and females. The results clearly reveal that flock age had no significant effect on cholesterol levels, but there was a significant increase in male cholesterol levels, indicating a significant effect of sex on cholesterol levels. Males had a significantly higher increase in cholesterol levels than females (129.50 and 82.55 mg/dL, respectively). A statistically non-significant interaction was observed between age and sex for triglycerides and cholesterol.

Table (4): Effect of flock age and sex of Indian River broiler breeder on plasma triglycerides (TG) and total cholesterol (TC) concentrations.

Traits	Sex	Flock age (In weeks)			Overall mean	*P*-value		
		60	62	64		A	S	A*S
TG	**F**	247.42 ± 11.13	312.14 ± 20.8	239.61 ± 9.31	$266.40^{A} \pm 8.46$	0.02	<0.01	0.07
(mg/dL)	**M**	47.04 ± 11.13	51.80 ± 13.17	45.27 ± 10.41	$48.04^{B} \pm 6.71$			
Overall mean		$147.23^{AB} \pm 7.87$	$181.99^{A} \pm 12.32$	$142.44^{B} \pm 6.98$				
TC	**F**	155.47 ± 12.48	164.73 ± 16.11	164.86 ± 17.64	$161.64^{A} \pm 8.98$	0.98	0.02	0.90
(mg/dL)	**M**	135.20 ± 12.48	132.73 ± 13.95	136.27 ± 13.15	$134.73^{B} \pm 7.63$			
Overall mean		145.35 ± 8.82	148.73 ± 10.66	150.56 ± 11.00				

*A = age; S = sex; A*S = interaction between age and sex; mg/dL = milligrams per deciliter*; F= Females; M = Males.
$^{A-B}$ *Means within a row or a column with different superscripts differ significantly (P ≤ 0.05).*

Similar results were obtained by Chapman *et al.* (1977), who found that the range of serum triglycerides (240–672 mg/dL) was much larger than the range of cholesterol (58–125 mg/dL) in laying Rhode Island Red chickens (8–11 months old). Additionally, Rezende *et al.* (2021) reported that Cobb broiler breeder females had considerably greater serum triglyceride and cholesterol levels than males at various ages (28, 36, 44, 52, and 60). One interpretation of this would be the dramatically increased oestrogen secretion at the onset of egg production in laying hens, which increases serum levels of triglycerides and phospholipids (Chaikoff *et al.*, 1956; Dashiti *et al.*, 1983). The results agree with the findings of Afiffi *et al.* (2022), who observed that the triglyceride levels in the females are significantly greater than those in the males. However, they contrast in terms of cholesterol levels. We are also in

disagreement with Saeki (1971), who noted that males had plasma cholesterol levels that were much lower than those of laying chickens.

Uric acid and creatinine (mg/dL):

The means ± SE and overall means of the plasma concentrations of uric acid and creatinine at 60, 62, and 64 for both males and females are presented in Table (5). It is conspicuous that flock age and sex had a significant effect on uric acid levels, whereas they had no significant effect on creatinine levels (P = 0.564 and P = 0.140, respectively). It could be seen that uric acid concentrations for both sexes revealed considerable fluctuations from week 60 to week 64.

Moreover, from week 60 to week 62, there was a dramatic decline in uric acid concentrations from 5.86 to 5.07 mg/dL, followed by a sharp increase (7.31 mg/dL). Furthermore, males had the highest levels of uric acid, which were approximately 7.02 mg/dL compared to females' (5.14 mg/dL). Additionally, it is apparent that males and females roughly had the same levels of creatinine, which were 0.50 and 0.40 mg/dL, respectively. Moving to the interaction effect, the current results showed a statistically non-significant interaction between age and gender for uric acid and creatinine (P = 0.09) and (P = 0.166), respectively.

The results coincide with those of Rezende *et al.* (2021), who conducted a study on Cobb broiler breeders and revealed that at 60 weeks, males had higher uric acid levels than females. Furthermore, the uric acid levels in these birds increased with age, and the values seen in birds that were 52 and 60 weeks old were greater than those seen in birds that were 28 and 36 weeks old. The results disagree with those obtained by Afiffi *et al.* (2022), who found that plasma uric acid levels in females of the Silver Sabhia strain were significantly higher than in males. On the other hand, the results agree on plasma creatinine levels, which did not show any significant differences with regard to sex. Moreover, the findings are consistent with those of Albokhadaim *et al.* (2012), who observed that sex had no effect on creatinine levels of local chicken in Saudi Arabia.

Table (5): Effect of flock age and sex of Indian River broiler breeder on plasma uric (UA) acid and creatinine (Cr) concentrations.

Traits	Sex	Flock age (In weeks)			Overall mean	*P*-value		
		60	62	64		A	S	A*S
UA	F	$5.44^{a} \pm 0.39$	$4.11^{b} \pm 0.37$	$5.88^{a} \pm 0.37$	$5.14^{B} \pm 0.21$	<0.01	<0.01	0.090
(mg/dL)	M	$6.28^{b} \pm 0.48$	$6.04^{b} \pm 0.44$	$8.74^{a} \pm 0.52$	$7.02^{A} \pm 0.28$			
Overall mean		$5.86^{B} \pm 0.31$	$5.07^{C} \pm 0.29$	$7.31^{A} \pm 0.32$				
Cr	F	0.37 ± 0.07	0.50 ± 0.07	0.34 ± 0.08	0.40 ± 0.04	0.564	0.140	0.166
(mg/dL)	M	0.43 ± 0.09	0.47 ± 0.08	0.61 ± 0.07	0.50 ± 0.04			
Overall mean		0.40 ± 0.06	0.48 ± 0.06	0.47 ± 0.05				

*A = age; S = sex; A*S = interaction between age and sex; mg/dL = milligrams per deciliter*; F= Females; M = Males.

$^{A-C}$ Means as well as $^{a-b}$ Means within a row with different superscripts differ significantly (P ≤ 0.05).

$^{A-B}$ Means within a column with different superscripts differ significantly (P ≤ 0.05).

Aspartate aminotransferase and alanine aminotransferase:

The means ± SE and overall means of the AST and ALT at 60, 62, and 64 for both sexes are presented in Table (6). It could be noticed that there was a non-significant effect of flock age on AST and ALT activity; moreover, there was a highly significant effect (P = 0.001) of sex on AST activity, but it had no significant effect on ALT (P = 0.726). It is apparent that there was an upward trend in AST activity with ageing, and in contrast with that, a downward trend was found in ALT. From Table (6), it can be observed that males had the highest AST values compared to females, which had the lowest values (324.17 and 199.43 IU/L, respectively). While the values of alanine aminotransferase activity between males and females were roughly the same at 56.13 and 58.9 IU/L, respectively. Regarding the interaction, the obtained results revealed that there was no significant interaction between age and sex for ALT and AST (P = 0.540 and P = 0.234, respectively).

Aspartate aminotransferase activity is currently thought to be a very sensitive but nonspecific indication of hepatic illness in other avian species (Harr, 2002). The results closely match those obtained by Rezende *et al.* (2021), who stated that AST activity levels in birds from a 60-week-old flock were significantly greater, and there was also an upward trend consistent with our results. One interpretation of

this would be that hepatic and muscular damage increase with ageing, elevating AST values. The results are not consistent with those of Afiffi *et al.* (2022) and Shanmathy *et al.* (2020), who found no significant effect of sex on ALT activity. Regarding their AST results, they observed no significant effect of sex on AST activity, contrary to ours.

Table (6): Effect of flock age and sex of Indian River broiler breeder on plasma Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity.

Traits	Sex	Flock age (In weeks)			Overall	*P*-value		
		60	62	64	mean	A	S	A*S
AST	F	205.38 ± 30	209.52 ± 33	183.39 ± 31	$199.43^{B\pm}$ 18	0.417	0.001	0.234
(IU/L)	M	261.31^{b} ± 31	326.79^{ab} ± 46	384.41^{a} ± 58	324.17^{A} ± 29			
Overall mean		233.35 ± 22	268.15 ± 29	283.90 ± 36				
ALT	F	75.46 ± 12	47.87 ± 7.0	53.42 ± 10	58.92 ± 6.0	0.152	0.726	0.540
(IU/L)	M	63.43 ± 8.0	56.64 ± 9.0	48.30 ± 8.0	56.13 ± 5.0			
Overall mean		69.45 ± 7.7	52.25 ± 6.1	50.86 ± 6.6				

*A = age; S = sex; A*S = interaction between age and sex; IU/L = International units per liter*; F= Females; M = Males.

[a-b] Means within a row with different superscripts differ significantly (P ≤ 0.05).

[A-B] Means within a column with different superscripts differ significantly (P ≤ 0.05)

Calcium and phosphorus (mg/dL):

The means ± SE and overall means of the plasma concentrations of calcium and phosphorus at 60, 62, and 64 WOA for both sexes are presented in Table (7). It is clearly noted that flock age had a highly significant effect on plasma concentrations of phosphorus (P = 0.002), while it had no effect on plasma concentrations of calcium. Also, a highly significant effect of flock sex was observed for plasma levels of calcium, while it had no effect on plasma concentrations of phosphorus. Females had higher concentrations of calcium than males, at 24.23 mg/dL, compared to 17.41 mg/dL for males.

Table (7): Effect of flock age and sex of Indian River broiler breeder on plasma concentrations of calcium and phosphorus.

Traits	Sex	Flock age (In weeks)			Overall	*P*-value		
		60	62	64	mean	A	S	A*S
Ca	F	22.80 ± 5.14	26.55 ± 12.02	23.32 ± 6.61	24.23^{A} ± 1.67	0.304	0.002	0.680
(mg/dL)	M	17.82 ± 5.46	19.74 ± 3.42	14.68 ± 3.77	17.41^{B} ± 1.12			
Overall mean		20.31± 1.47	23.14 ± 2.19	19.00 ± 1.45				
P	F	11.29 ± 1.40	15.92 ± 1.23	12.73 ± 1.17	13.31 ± 0.73	0.002	0.118	0.253
(mg/dL)	M	7.29^{b} ± 1.85	13.91^{a} ± 1.31	13.35^{a} ± 1.17	11.52 ± 0.85			
Overall mean		9.29^{B} ± 1.16	14.91^{A} ± 0.90	13.04^{A} ± 0.82				

*A = age; S = sex; A*S = interaction between age and sex; mg/dL = milligrams per deciliter;* F= Females; M = Males.

[A-B] Means as well as [a-b] Means within a row with different superscripts differ significantly (P ≤ 0.05).

[A-B] Means within a column with different superscripts differ significantly (P ≤ 0.05).

Furthermore, it is apparent that plasma concentrations of phosphorus at weeks 62 and 64 roughly had the same levels and accounted for 14.91 and 13.04 mg/dL, respectively. While week 60 had the lowest levels (9.29 mg/dL) compared to the remaining two weeks. It is conspicuous that the current results reveal a statistically non-significant interaction between age and sex for calcium and phosphorus (P = 0.680 and P = 0.253, respectively). The results are in agreement with those obtained by Rezende *et al.* (2021), who observed that serum calcium concentrations found in females were significantly higher than those in males. One way of interpreting this is that females in the reproductive period exhibit marked increases in plasma total calcium levels as a result of oestrogen-stimulated synthesis of calcium-binding proteins such as vitellogenin and albumin (Harr, 2002).

The findings are in harmony with those of Afiffi *et al.* (2022), who observed that females in the laying phase (33WOA) had plasma calcium values significantly greater than males. Moreover, the present findings agree in terms of plasma phosphorus levels. The results agree with Bora *et al.* (2017) who stated that Kadaknath birds' males and females did not differ significantly in their plasma levels of phosphorus.

Glucose (mg/dL):

The means ± SE and overall means ± SE of the plasma concentrations of glucose 60, 62, and 64 WOA for both sexes are presented in Table (8).

The obtained results reveal a significant effect of flock age and sex ($P < 0.01$) on glucose levels. A significant interaction was observed between age and sex for glucose ($P < 0.01$). Glucose is a bird's most basic, most vital, and easily obtainable source of energy, and blood glucose levels are a sign of nutritional status (Dunbar *et al.* 2005). The results are mostly consistent for both males and females with Harris (1991), who reported that most bird species have normal blood glucose levels ranging from 200–500 mg/dL.

Table (8). Effect of flock age and sex of Indian River broiler breeder on plasma glucose concentrations.

Traits	Sex	Flock age (In weeks)			Overall	*P*-value		
		60	62	64	mean	A	S	A*S
GLU	F	$281.25^{a \pm}$ 12	$170.44^{b \pm}$ 12	206.56^{b} ± 12	219.41^{B} ± 7.0	<0.01	<0.01	<0.01
(mg/dL)	M	255.19^{b} ± 12	218.06^{b} ± 12	384.27^{a} ± 13	285.84^{A} ± 8.0			
Overall mean		268.22^{B} ± 9 .0	194.25^{B} ± 9.0	295.41^{A} ± 9.0				

*A = age; S = sex; A*S = interaction between age and sex; mg/dL = milligrams per deciliter;*
F= Females; M = Males
$^{A-B}$ Means as well as $^{a-b}$ Means within a row with different superscripts differ significantly ($P \leq 0.05$).
$^{A-B}$ Means within a column with different superscripts differ significantly ($P \leq 0.05$).

Effect of Indian River broiler breeder females' age on immune response and maternal antibodies transfer rate

Data on the geometric mean titer (GMT) of Newcastle disease virus (NDV) and Avian Influenza (AIV) in Indian River broiler breeder females and their progeny are presented in Table (9), and the results of maternal antibody transfer rates are presented in Table (10). The CV% of GMT in female titers reached 29.46% for NDV, whereas the CV of GMT in chicks was 14.16%. Additionally, the CV% values of females and chicks GMT for AIV were 5.31 and 11.45%, respectively. The transfer rates of maternal antibodies against NDV were 14.13, 11.36, and 19.35% at weeks 60, 62, and 64, respectively. While maternal antibody transfer rates of AIV accounted for 48.44, 61.10, and 66.67 at 60, 62, and 64 weeks of age, respectively. In addition, the CV% of transfer rates for both NDV and AIV was 27.16 and 15.90%, respectively. Also, the findings were consistent with those of Wyeth and Cullen (1978), who indicated the possibility of decreasing antibody transfer with age (the mean transfer rate of maternal antibodies against NDV was recorded at just 14.95% with a CV% value of 27.16%). The present study revealed that there were low levels of GMT in female breeders accompanied by low passive maternal antibodies in their progeny, and this agrees with Suardana *et al.* (2022) who recently added that maternal immunity in chickens will be greatly impacted by the females' titer and age. As a result of reduced maternal immunity, chicks from older breeders must be vaccinated at an early age.

Table (9): Effect of flock age on Indian River broiler breeder females' and their progeny's Geometric mean titer (GMT) of Newcastle disease virus (NDV) and Avian Influenza (AIV)

	Flock age (In Weeks)							
	60		62		64		CV%	
Virus	Females	Chicks	Females	Chicks	Females	Chicks	Females	Chicks
NDV	210.29	29.71	211.20	24.00	118.85	23.00	29.46	14.16
AIV	128.00	62.00	123.43	75.42	115.20	76.80	5.31	11.45

Coefficient of variation (CV %) = (Standard deviation/sample average × 100)

Table (10): Transfer percentages of maternal antibodies against NDV and AIV from Indian River broiler breeder females and their progeny.

Virus	Flock Age (In weeks)			Mean transfer (%)	CV%
	60	62	64		
NDV	14.13	11.36	19.35	14.95	27.16
AIV	48.44	61.10	66.67	58.74	15.90

Coefficient of variation (CV %) = (Standard deviation/sample average × 100)

CONCLUSION

According to the obtained results, it could be concluded that ageing had a detrimental effect on female breeders' immune systems and maternal antibody transfer rate, which could negatively affect their progeny at an early age. Therefore, chicks from older breeders must be vaccinated at an early age. In addition, both flock age and sex had a significant influence on some hematological and biochemical analyses in birds.

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تأثير تقدم عمر أمهات اللحم علي قياسات الهيماتولوجي ، المؤشرات البيوكيميائية للدم و معدلات انتقال المناعة الأمية

عبد الحميد أحمد عبد الحميد، إبراهيم الورداني السيد، فيصل بيومي عبد السلام وغادة جودة راشد

قسم إنتاج الدواجن – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة – مصر.

أجريت هذه الدراسة خلال الفترة من فبراير إلى أبريل 2023 بهدف دراسة تأثير تقدم العمر في أمهات اللحم على صفات الهيماتولوجي، المؤشرات البيوكيميائية للدم ومعدلات انتقال المناعة الأمية. تم اختيار إجمالي 20 طائر (10 ذكور و10 إناث) عشوائيًا عند ثلاثة أعمار مختلفة: وهي 60 و62 و64 أسبوعًا من العمر لسلالة (Indian River) وتم جمع عينات الدم لإجراء تحليلات الهيماتولوجي ومكونات بلازما الدم وتقدير الأجسام المضادة عند هذه الأعمار. وقد أظهرت نتائج التجربة أنه كان هناك تأثير معنوي لعمر القطيع علي الهيموجلوبين وحجم المكونات الخلوية ودلائل الدم، بينما لم يكن هناك أي تأثير لعمر القطيع علي عدد كرات الدم. تأثر تركيز هيموجلوبين الدم معنويا بالجنس، بينما لم تتأثر عدد كرات الدم، حجم المكونات الخلوية ودلائل الدم بالجنس. أثر عمر القطيع بشكل معنوي على تركيز البروتين الكلي في البلازما، الجلوبيولين، الدهون الثلاثية، حمض اليوريك، الكالسيوم، الفوسفور والجلوكوز، بينما لم تتأثر تركيزات باقي مكونات بلازما الدم بعمر القطيع. أظهرت النتائج أن الجنس كان له تأثيرات معنوية على مستويات الألبيومين، ونسبة A/G، والدهون الثلاثية، والكوليسترول الكلي، وحمض اليوريك، والكالسيوم، والجلوكوز، وإنزيم AST، وكان لدى الإناث اختلافاً معنوياً في حين لم يكن هناك تأثير معنوي للجنس على بقية المكونات. كانت هناك مستويات منخفضة في الأجسام المناعية لفيروس النيوكاسل (NDV) وفيروس أنفلونزا الطيور (AIV) في الإناث مصحوباً بمستويات منخفضة من المناعة الأمية (Mab) المنقولة في الكتاكيت عمر يوم.

الكلمات المفتاحية: *أمهات اللحم – العمر- الجنس - كتاكيت عمر يوم - المناعة الأمية - مكونات الدم - أجسام مضادة.*