**Egyptian Poultry Science Journal** 

http://www.epsj.journals.ekb.eg/



## ISSN: 1110-5623 (Print) – 2090-0570 (Online)

## EFFECTS OF DIETARY PREDNISOLONE SUPPLEMENTATION ON EGG PRODUCTION, COMPLETE BLOOD COUNT, AND HUMORAL IMMUNITY IN BROILER BREEDERS

## M.A. M. Sayed, R.M. Abu-Elmagd and M. El-Sagheer M.

Dep. of Poult.Prod., Fac. of Agric., Assiut Uni., Assiut 71526, Egypt. **Corresponding Author:** M.A. M. Saved Email: mohamed.saved4@agr.au.edu.eg

	mani monanioaisa you i e agriaaioaalog
Received: 06/07/2024	Accepted: 11 /08 /2024

**Abstract:** This study investigated the effects of dietary prednisolone supplementation on various parameters in Arbor Acres Plus broiler breeders. One hundred and twenty birds were randomly assigned to four treatment groups, each with three replicates of 10 birds. Birds in 4 groups received a basal diet supplemented with 0, 5, 10, or 20 mg prednisolone/kg feed for a period of 45 days. Egg production metrics, hematological indices, and antibody titers against Infectious bronchitis virus (IBV) and Newcastle disease virus (NDV) were estimated.

Results indicated no significant differences in egg number and hen day production across groups (P > 0.05). However, average egg weight decreased significantly with increasing prednisolone levels compared to the control (P < 0.05). Egg mass did not differ significantly among groups (P > 0.05).

Chickens on the basal diet (control) tended to have lower red blood cell counts (P = 0.064) and higher mean corpuscular hemoglobin levels (P = 0.083) compared to those supplemented with prednisolone. Furthermore, broiler breeders in control group exhibited higher mean corpuscular volume values than the normal range, which were normalized by dietary prednisolone supplementation. Additionally, prednisolone supplementation increased mean corpuscular hemoglobin concentration compared to the control group.

No significant differences were observed in white blood cell count and percentages of various cell types (P > 0.05). However, birds supplemented with 10 and 20 mg prednisolone/kg tended to have a higher H/L ratio (P = 0.09) and percentage of monocytes (P = 0.08) compared to controls.

Antibody titers against IBV and NDV did not differ significantly between prednisolone-supplemented and control groups (P > 0.05).

In conclusion, dietary supplementation with prednisolone for 45 days had minimal impact on egg production parameters. Hematological parameters indicated potential benefits in erythrocyte indices in case of existing anemia without adverse effects on the immune response.

Keywords: synthetic glucocorticoids, broiler breeder chickens, macrocytic anemia, CBC, humoral immunity

## INTRODUCTION

In poultry industry, the ability to control offspring sex ratios could eliminate the need for euthanizing surplus animals of unfavorable sex, particularly in egg-producing poultry breeds (Kaleta and Redman, 2008). Additionally, given the growth and feed efficiency advantages of male broiler chickens, biasing sex ratios towards males in broiler breeder populations becomes increasingly significant.

Various studies, including those by Clout et al. (2002) and Whittingham et al. (2005), have linked the maternal condition to the primary offspring sex ratio, suggesting a potential mediation by steroid hormones, as proposed by Pike and Petrie (2005). Multiple studies have indicated a correlation between maternal health and the sex ratio of offspring. Healthy mothers in optimal conditions tend to produce more male offspring, whereas those in poorer conditions tend to have more female offspring (Cameron and Linklater, 2002). Additionally, Petrie et al. (2001) proposed that maternal steroids might play a role in influencing sex chromosome segregation. Correa et al., (2005) found that injecting laying hens with 2 mg corticosterone 5 h prior to ovulation reduced the number of produced males by 38% compared to the control group. Injecting laying with corticosterone shortly before hens ovulation alters gene expression related to meiotic processes, indicating a potential influence on offspring sex ratios in birds (Wrobel et al., 2020).

Administering hormones via injection requires precise timing before ovulation, which is difficult to manage in large flocks where individual hen ovulation times are hard to track. This limitation prevents the method from being feasible on a commercial scale. Alternatively, delivering steroids through feed allows for a elevation more consistent of hormone concentration in the bloodstream. Nevertheless, maintaining a chronic elevation of hormones through feed may pose risks to egg production, immunity, and overall well-being. Exogenous corticosterone is commonly used to induce stress responses in chickens to study its effects. For instance, in laying hens, corticosterone

administration results in increased feed intake, decreased weight gain, elevated corticosterone levels, altered heterophil/lymphocyte ratios, increased feather pecking behavior, prolonged tonic immobility, and diminished immune functions (El-lethey et al., 2001; Shini et al., Additionally, corticosterone 2009). administration delays the onset of egg laying and shortens the peak production period, thereby reducing overall hen day egg production (Shini et al., 2009).

Prednisolone is a synthetic corticosteroid medication commonly used to treat a variety of inflammatory conditions, autoimmune disorders, allergic reactions, and certain types of cancers. It works by reducing inflammation and suppressing the immune system's response. Prednisolone is similar to cortisol, a hormone naturally produced by the adrenal glands, but it has more potent anti-inflammatory effects Center for Biotechnology (National Information, 2024).

Although, it remains uncertain whether this sustained elevation would effectively bias the offspring sex ratio, increasing corticosterone levels using silastic implants caused homing pigeons to generate more female progeny (Goerlich, 2009). Silastic implants can provide a steady release of hormones that can help maintain stable levels in the body over a prolonged period.

This preliminary study aims to assess the impact of administering prednisolone through feed on multiple facets of broiler breeders' egg production, humoral immunity, and complete blood count (CBC). This initial investigation precedes further exploration into the potential use of this method for altering the sex ratio of offspring.

## MATERIALS AND METHODS

This study was conducted at a farm belonging to the Dakahlia Poultry Company in Gharbiya Governorate, Egypt. The study aimed to incorporating evaluate the effects of Prednisolone into the feed of Arbor-Acres plus broiler breeders on various parameters including performance, humoral immunity, and whole blood composition. Chickens were subjected to a 15-day treatment regimen with Prednisolone prior to the initiation of egg collection. This treatment regimen was maintained for an additional 30 days during the egg collection phase.

#### Animals, diets and experimental design

One hundred and twenty healthy, vaccinated, Arbor Acres Plus broiler breeder chickens were randomly selected. Each bird was individually weighed, leg banded for identification, and then evenly allocated across four treatment groups, with each group comprising three replicates of 10 birds each. Throughout the experimental phase, spanning from 53 to 59 weeks of age, the birds were provided with a commercial diet formulated to fulfill all their nutritional requirements as per the guidelines outlined by the NRC (1994). Feeding was restricted to a recommended daily amount of 163g per bird. Reported in Table 1 the composition of the commercial diet, while Table 2 presents the proximate chemical analysis of the diet.

Birds in groups 1 to 4 were provided with the basal diet supplemented with 0, 5, 10, or 20 mg prednisolone per kilogram of diet, respectively.

## **Birds' management**

Birds were housed in deep litter floor pens each with dimensions of 100 cm  $\times$  200 cm  $\times$  1500 cm (L  $\times$  W  $\times$  H) in a closed house under strict hygienic and optimal environmental conditions. Birds were subjected to a constant lighting program comprising 15 h light and 9 h dark cycles.

## **Traits under study**

#### **Egg production**

#### **1- Hen-day production (HDP %)**

The laid eggs were collected and recorded daily. Hen day production was calculated according to the following equation;

HDP % = Total number of daily eggs laid X 100/ Total number of daily survived hens

#### 2- Average egg weight (g)

Eggs were individually weighed daily for each replicate and the average was calculated.

#### 3- Egg mass

Egg mass (g/hen/day) was calculated as according to the following equation;

Egg mass (g/hen/day) = Average egg weight (g)  $\times$  number of eggs throughout 30 days/ number of hens

#### **Blood parameters**

At the end of the experiment, blood samples were collected from the brachial vein of ten randomly selected birds per treatment. These samples were promptly placed on ice in EDTA containing tubes. Subsequently, we assessed various hematological parameters including hematocrit, hemoglobin levels, red blood cell count, differential leukocyte count, and thrombocyte count.

## Total white blood cells (WBC) count

The staining solution used for birds, Natt-Herrick solution, was prepared following the methods by Natt and Herrick (1952). After filtration, it was stored at room temperature, shielded from light. For staining, EDTA blood samples were mixed with Natt-Herrick solution at a 1:100 ratio. This involved pipetting 990  $\mu$ L of staining solution and 10  $\mu$ L of blood into a sample vial using a micropipette, followed by thorough mixing. The mixture was then incubated and agitated for 5–10 minutes on an automated mixer at room temperature.

The mixture was loaded into a Neubauer hemocytometer. Placed in a petri dish with damp filter paper, the hemocytometer was allowed to incubate for 2 minutes to facilitate cell sedimentation. Granulocytes were counted using a light microscope (Labomed, CXL, USA) at 400x magnification, tallying cells in the four large outer squares containing sixteen small squares. The average count from both chamber grids was calculated and multiplied by 250 to determine the non-corrected white blood cell count (NCLC), specifically accounting for eosinophils, basophils, and heterophils. To include lymphocytes and monocytes in the total white blood cell count (TLC), the NCLC was multiplied by 100 and divided by the sum of the percentages of heterophils, eosinophils, and basophils as determined through microscopic differentiation.

## **Differential leukocyte count**

To determine the differential leukocyte count, blood was smeared onto a glass slide using the cover glass technique (Campbell, 1988). The blood films were fixed in methanol for 5 minutes and allowed to air dry before staining with Giemsa stain (GESCA Co., Shanghai, China). One hundred white blood cells per slide were counted and classified based on their morphological and staining characteristics. The differential white blood cell count is reported as a percentage of each cell type. Leukocytes were counted using a light microscope (oil immersion lens) at 1000x magnification, following Wintrobe's method (1967).

#### Red blood cell (RBC) count

Using an automatic dispenser, 4 mL of Natt and Herrick's solution was transferred into a 5 mL tube. The working solution was allowed to reach room temperature. Using a micropipette, 20  $\mu$ L of whole blood was dispensed onto the side of the working solution tube to achieve a dilution of 1:200. The sample tube was placed on a roller mixer for 3 minutes.

A small aliquot of the diluted sample was loaded into one chamber of the Neubauer hemocytometer using a micropipette. The hemocytometer was then placed in a petri dish with damp filter paper. The dish was covered, and the hemocytometer was allowed to incubate for 5 minutes to allow the cells to settle.

The red blood cell count was calculated using the formula:  $N/100 = RBC \ge 10^{12}/L$ , where N equals the number of cells counted in 160 small squares.

#### Hematocrit (Packed cell volume; PCV)

To determine Hematocrit or packed cell volume (PCV), the micro-hematocrit method was employed. Two heparinized capillary tubes were filled with blood, and their ends were sealed before centrifuging in a microcapillary centrifuge at 1200 rpm for ten minutes. PCV measurements were determined using a circular reader as described by Daice and Lewis, (1991).

## Hemoglobin

Hemoglobin was determined using a hematology analyzer (Mindray device, model Bc-3200; Altra Lab, Zakaziq, Sharqia governorate).

## Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV) is the expression of the average volume of individual erythrocytes calculated with the following formula:  $MCV = (PCV \times 10)/RBC = MCV$  femto liters (fl)

#### Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin (MCH) is calculated using the following formula: MCH =(Hb x 10)/RBC = MCH picogram (pg)

# Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) is calculated using the following formula:

 $MCHC = (Hb \times 100)/PCV = MCHC (g/L)$ 

## Antibody titers against Newcastle disease (ND) and Infectious bronchitis (IB) viruses

After 45 days of steroid supplementation, blood samples were collected to assess antibody titers against Newcastle disease (ND) and Infectious bronchitis (IB) viruses. Antibody titers were determined using the hemagglutination inhibition (HI) test, following the method described by Villegas (1998). The HI titer was reported as the log<sub>2</sub> value of the highest reciprocal dilution.

## **Statistical analysis**

The data underwent statistical analysis using the least squares analysis of variance with the GLM procedure in SAS software (SAS Institute, 1999). Differences among treatment means were assessed using Duncan's new multiple-range test (Duncan, 1955) at the 5% level of significance. The statistical model used was: Yijk=  $\mu + T_i + E_{ij}$ 

Where:  $Y_{ijk}$  represents the individual observation,  $\mu$  is the overall mean,  $T_i$  is the effect of treatment i, (where, i = 1, 2, 3 and 4), and  $E_{ij}$  denotes the experimental error.

## **RESULTS & DISCUSSION**

The effects of prednisolone supplementation on egg number, hen day egg production (HDP %), egg weight (EW), and egg mass (EM) are summarized in Table 3.

No significant differences in egg number and HDP% were observed among the groups receiving prednisolone supplementation in their feed and the control group (P > 0.05). However,

#### synthetic glucocorticoids, broiler breeder chickens, macrocytic anemia, CBC, humoral immunity

adding prednisolone to diets at levels of 5, 10, and 20 mg/kg significantly (P < 0.05) decreased average egg weight by 2.59%, 2.82%, and 2.73%, respectively, compared to the control group. Nonetheless, no significant differences (P > 0.05) in egg mass were found among all groups.

Corticosterone has been shown to decrease egg production by affecting energy metabolism through reduced feed and calorie efficiencies (Lin et al., 2006). Additionally, it reduces the weight of ovaries and oviducts in laying hens (Shini et al., 2009). Elevated corticosterone levels in the blood are linked to increased expenditure, proteolysis, energy and gluconeogenesis (Lin et al., 2004), leading to reductions in body weight (Shini et al., 2009) and subsequently decreasing the egg weight (Wang et al., 2013). Chickens exposed to corticosterone during the rearing phase experienced significant reproductive challenges, including an approximate 8-day delay in the onset of first egg laying and reduced egg production throughout the entire laying period (18 to 35 weeks of age; Shini et al., 2009). Other researchers reported rapid and dramatic declines in egg production due to corticosterone administration which ended up with a complete halt of laying. Kim et al. (2015) reported that egg production in laying hens treated with corticosterone (30 mg/kg feed) began to decline sharply, dropping by approximately 30% by day 5 and reaching nearly 0% by day 11, whereas, the control group maintained a steady rate of around 90%.

On the other hand, other studies reported no significant changes in laying rate due to glucocorticoid supplementation. Our findings align with those of Aslam et al. (2014), who reported that corticosterone administered at 20 mg/kg diet had no significant effect (P > 0.05) on the laying rate of Isa Brown hens between 40 and 42 weeks of age. Similarly, our results are consistent with Hanafy and Hassan (2021), who found no significant differences (P > 0.05) in laying rate and egg number between birds receiving 0.25 mg dexamethasone/kg diet and the control group in Japanese quails during the period 6-9 weeks of age.

Regarding the effect of steroids on egg weight, our results disagree with those reported by Kim et al. (2015), who found that supplementing laying hens' diets with 30 mg corticosterone/kg did not affect egg weight compared to the control group. Similarly, Hanafy and Hassan (2021) demonstrated that dietary administration of dexamethasone at 0.25 mg/kg from 6 to 9 weeks of age had no significant effect (P > 0.05) on egg weight in Japanese quails.

The effects of prednisolone supplementation on hematological parameters are shown in Table 4. Chickens fed the basal diet (control) tended to have lower RBC counts (P = 0.064) and higher MCH levels (P = 0.083) compared to those supplemented with prednisolone. Dietary prednisolone administration did not affect PCV or hemoglobin concentration (P > 0.05). However, broiler breeders in the control group had higher MCV values than the normal range. Dietary prednisolone administration normalized MCV and increased MCHC compared to the control group.

The avian CBC is a most important component of a diagnostic panel and the best indicator of a bird's general health. The results of the CBC are indicative of the activity of the immune system, as it examines and evaluates the red and white blood cells that make up the cellular component of blood.

Mean corpuscular volume (MCV) measures the average size of red blood cells and is an important indicator of their function. Changes in MCV can affect oxygen distribution in the body and may signal blood disorders such as macrocytic anemia. This type of anemia involves the production of unusually large red blood cells in the bone marrow, often due to deficiencies in vitamin B12 and B9 (folate), which are essential for red blood cell formation (Aslinia et al., 2006). In deficiencies of these vitamins, the bone marrow produces large, immature cells called megaloblasts that do not divide normally. As a result, fewer mature red blood cells are produced, and those that are often do not survive as long in the bloodstream. This leads to a decrease in red blood cell count and causes anemia (Aslinia et al., 2006).

In domestic fowl (Gallus domesticus), normal ranges for hematological parameters include RBCs: 2.5-3.9 x 10^6/µL, MCV: 104-135 fL, MCH: 32-43.9 pg, MCHC: 30.2-36.2 g/dL, PCV: 30-49%, Hb: 10.2-15.1 g/dL, and WBCs: 1.9-9.5 x 10<sup>3</sup>/µL (Samour, 2006). Comparing these ranges with our findings, the higher MCV observed in the control birds and the tendency for decreased RBC counts in both the control and P5 (5 mg prednisolone supplementation / kg diet) groups, falling below the normal range, suggests that birds in the control group may have experienced mild megaloblastic anemia, prednisolone supplementation which This analysis highlights how attenuated. prednisolone might impact hematological parameters in the context of potential anemic conditions. Chickens in the control group had higher MCH and lower MCHC than those prednisolone. supplemented with The combination of low RBC count, low MCHC, higher MCV, and higher MCH seen in the control birds suggests macrocytic anemia. This type of anemia can be caused by deficiencies in vitamin B12 or folate, which are essential for red blood cell production and maturation. In these deficiencies, the bone marrow produces larger, immature red blood cells (megaloblasts) that do not function effectively in oxygen transport (Hariz and Bhattacharya, 2023).

According to the CBC values obtained in the current study, it can be assumed that prednisolone administration in feed for a period of 45 days had no detrimental effect on the general health of the birds but it helped them to recover from anemia. Voorhees et al. (2013) sustained elevations reported that in glucocorticoid levels may enhance erythroid progenitor proliferation and positively influence erythropoiesis.

No significant differences in PCV were observed among the different groups. Additionally, although hemoglobin concentrations in chickens supplemented with 10 and 20 mg of prednisolone were 5.98% and 10.3% higher, respectively than those in control birds, these differences did not reach statistical significance. Despite the lack of statistical differences, hemoglobin concentrations in the

control and 5 mg prednisolone groups were lower than the normal range, suggesting anemia. These findings align with Aengwanich's study, where (2007)no significant changes in hemoglobin concentrations were reported in broiler chickens fed diets supplemented with dexamethasone at levels of 4 and 6 mg/kg (P >0.05) compared to the control group. Similarly, Adam et al. (2019) found no significant effect (P > 0.05) on hemoglobin levels in rabbits oral following daily administration of dexamethasone at 1.8 mg/kg body weight for 45 days.

Table 5 depicts the effects of prednisolone in diets on WBC and differential leukocyte counts. Across treatment groups, no significant differences were observed in WBC count, absolute and percentage of heterophils (bands segmented cells), lymphocytes, and eosinophils, basophils, and monocytes. There was а tendency for broiler breeders with supplemented 10 and 20 mg prednisolone/kg to exhibit a higher H/L ratio (P = 0.09) and a higher percentage of monocytes (P = 0.08) compared to control birds and those supplemented with 5 mg prednisolone/kg.

Our findings align with Aengwanich (2007), who also noted no significant differences in WBC counts with dexamethasone treatment in broilers. However, our results contrast with other studies reporting increased heterophil numbers with glucocorticoid supplementation in chickens (Gross et al., 1980), House Finches (*Carpodacus mexicanus*; Davis, 2005), and broilers (Puvadolpirod and Thaxton, 2000).

A strong relationship between increased plasma corticosterone concentrations and the H/L ratio exists (Puvadolpirod and Thaxton, 2000; Mumma et al., 2006; Shini et al., 2008). Glucocorticoids, such as prednisolone, can increase the heterophil to lymphocyte (H/L) ratio primarily due to their immunosuppressive effects and stress response modulation (Cain and Cidlowski, 2017: Dhabhar, 2014). Glucocorticoids suppress the immune system by inhibiting various immune responses, particularly those involving lymphocytes. This suppression leads to a relative increase in the synthetic glucocorticoids, broiler breeder chickens, macrocytic anemia, CBC, humoral immunity

heterophils proportion of compared to lymphocytes in the blood (Cain and Cidlowski, 2017; Dhabhar, 2014). This explains why chickens receiving 10 and 20 mg of prednisolone/kg tend to show an increase in the H/L ratio in their blood. Daily intake of 10 and 20 mg prednisolone/kg tended to increase the percentage of monocytes. Chronic stress has been reported to increase monocyte levels in humans (Heidt et al., 2014) and in mice (Zheng et al., 2016).

Our study found that dietary supplementation with prednisolone had no significant effects (P>0.05) on antibody titers against Infectious bronchitis virus (IB) and Newcastle disease virus (Table 6). Corticosteroids are known to primarily suppress cell-mediated immunity, with a lesser impact on humoral immunity. Although transient lymphocytopenia occurs shortly after corticosteroid administration due to lymphocyte redistribution (Fauci et al., 1976), B lymphocytes generally maintain their circulation levels despite exposure. However, prolonged corticosteroid use can lead to significant lymphopenia, resulting in a immunodeficiency combined that affects humoral immunity, predominantly impairing the body's ability to mount effective immune responses mediated by antibodies (Fedor and Rubinstein, 2006).

Additionally, environmental stressors affect chickens' susceptibility to viral and bacterial infections differently. Socially stressed chickens showed increased vulnerability to viral infections but enhanced resistance to bacterial infections (Gross and Colmano, 1969). Studies on chickens treated with corticosteroneblocking chemicals revealed altered infection susceptibilities, suggesting complex interactions between corticosterone levels and immune responses (Gross and Colmano, 1971). Overall, while corticosterone levels correlated significantly with lymphocyte counts and antibody titers (Gross et al., 1980), the effectiveness of circulating antibodies may remain unaffected by corticosteroid exposure, demonstrated in experiments involving as Newcastle disease virus challenge (Gross et al., 1980). The present results indicate that prednisolone administration via feed for 45 days did not impair humoral immune response against Newcastle and Infectious bronchitis viruses in broiler breeders.

Table (1): The composition of the basal diet.

Ingredient	%
yellow corn	62.77
Wheat bran	12.11
Soybean meal 47%	14.31
Sunflower meal	0
Dicalcium Phosphate	1.01
Limestone	8.1
potassium chloride	0.1
Sodium chloride	0.2
Sodium bicarbonate	0.2
L-Methionine	0.15
L-Threonine	0.07
L-tryptophan	0.015
L-lysine	0
Choline chloride 60%	0.10
Breeders Premix <sup>1</sup>	0.168
Hi Phoss <sup>2</sup>	0.006
Antioxidant	0.015
Biotronic Top 3 <sup>3</sup>	0.3
manganese sulfate	0.026
Mycofix Plus <sup>4</sup>	0.3
Poultry Star MI <sup>5</sup>	0.05
Total	100

<sup>1</sup>Each kg of vitamin- mineral premix with Vit. A 12000 IU; Vit. D3 2000 IU; Vit. E. 100mg; Vit. k3 2mg; Vit.B1 100mg; Vit. B2 40mg; Vit. B6 15 mg; Pantothenic acid 100mg; Vit.B12 0.01mg; Folic acid 10mg; Niacin 20mg; Biotin 0.05mg; Choline chloride (50% choline) 500 mg; Zn 55mg; Fe 30mg; I 1mg; Se 0.1mg; Mn 55mg; Ethoxyqain 3000 mg.<sup>2</sup>

<sup>2</sup> Hi-Phoss is a liquid formulation containing magnesium, potassium and phosphorus in carefully balanced proportions.

<sup>3</sup> Biotronic Top3 is a compounds working Amnionium fornate Formic acid Acetic acid: Propionic acid Flavouring cormpounds

<sup>4</sup> Mycofix<sup>®</sup> Plus 5.Z with ZENzyme<sup>®</sup> is an innovative, all-in-one feed additive providing next-generation mycotoxin risk management for breeding animals and their offspring.

<sup>5</sup> PoultryStar® is a well-defined, poultry-specific, multi-species synbiotic product that promotes a beneficial gut microbiota through the combined action of carefully selected probiotic microorganisms and prebiotic fructooligosaccharides.

Ta	ble	(2):	The	proximate	chemical	analysis	of the	basal	diet*.
----	-----	------	-----	-----------	----------	----------	--------	-------	--------

Nutrients	g/kg
Crude Protein	132.96
ME Poultry, MJ/kg	11.69
Lysine	6.18
Methionine	3.68
Calcium	35.11

\*The proximal analysis was calculated according to A. O. A. C. (1998).

**Table (3):** Effect of prednisolone supplementation on egg number, Hen-Day Production (HDP%), egg weight, and egg mass of broiler breeders.

Treatment	*Eggs number	HDP (%)	Egg weight (g)	Egg Mass
P0 (C)	18.83±0.46	62.77±1.52	66.64a±0.25	41.82±1.04
P5	19.31±0.47	64.36±1.57	64.91b±0.27	41.78±0.99
P10	18.93±0.46	$62.82 \pm 1.50$	64.76b±0.24	$40.86 \pm 0.98$
P20	17.93±0.67	59.77±2.23	64.82b±0.28	38.74±1.38
P-value	0.2920	0.3026	< 0.0001	0.1594

<sup>a-b</sup> Means in the same column with different superscripts are significantly different ( $P \le 0.05$ ) P0= Control (without prednisolone supplementations)

P5= 5 mg prednisolone/ kg diet

P10= 10 mg prednisolone/ kg diet

P20= 20 mg prednisolone/ kg diet

HDP%= Hen Day Production %

\*Eggs number per treatment group per day

**Table (4):** Effects of prednisolone supplementation on red blood cell count  $(10^{6}/\mu L)$ , mean corpuscular volume (fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/dL), hemoglobin (g/dL), and packed cell volume (%) of broiler breeders

Treatment	RBCs	MCV	МСНС	МСН	Hemoglobin	PCV
Control P0	2.17±0.03	142.57 <sup>a</sup> ±1.07	31.37 <sup>b</sup> ±0.03	44.70±0.30	9.70±0.10	30.93±0.27
P5	2.29±0.09	$124.32^{b}\pm 2.09$	34.24 <sup>a</sup> ±0.26	42.58±0.64	9.72±0.28	28.38±0.74
P10	$2.44 \pm 0.08$	123.60 <sup>b</sup> ±1.66	34.14 <sup>a</sup> ±0.37	42.14±0.22	10.28±0.38	30.12±1.03
P20	2.46±0.08	$125.84^{b} \pm 1.57$	$33.54^{a}\pm0.60$	42.84±0.80	10.70±0.51	30.80±1.35
P-value	0.0645	< 0.0001	0.0029	0.0838	0.2595	0.3191

<sup>a-b</sup> Means in the same column with different superscripts are significantly different (P $\leq$ 0.05)

P0= Control (without supplementations)

P5= 5 mg prednisolone/ kg diet

P10= 10 mg prednisolone/ kg diet

P20= 20 mg prednisolone/ kg diet

PCV%= Packed Cell Volume

MCV= Mean red blood cell Volume

MCH= Mean cell Hemoglobin

MCHC= Mean cell hemoglobin Concentration

**Table (5):** Effects of prednisolone supplementation on white blood cell count, differential leukocyte count, and H/L ratio of broiler breeders.

Parameter	C (P0)	P5	P10	P20	P-value
WBCs, $x10^3/mm^3$	5.37±1.23	4.44±0.13	4.87±0.18	4.85±0.20	0.56
Heterophils%	46.67±1.76	$43.40 \pm 5.50$	51.60±1.99	$55.20{\pm}6.38$	0.34
Heterophils Absolute	$2.47 \pm 0.48$	$1.91 \pm 0.20$	$5.52 \pm 0.17$	$2.95 \pm 0.54$	0.26
Lymphocyte%	$38.00 \pm 6.81$	$48.60 \pm 6.09$	$38.20{\pm}1.98$	39.20±1.16	0.34
Lymphocyte Absolute	$1.90 \pm 0.32$	$2.17 \pm 0.34$	$1.78 \pm 0.08$	$2.05 \pm 0.33$	0.83
H/L ratio	$1.23 \pm 0.26$	$0.89 \pm 0.18$	$1.35 \pm 0.30$	$1.40 \pm 0.29$	0.09
Monocyte%	$6.33 \pm 1.76$	$5.60 \pm 0.24$	$6.40 \pm 0.60$	$8.60 \pm 0.81$	0.08
Monocyte Absolute	$0.32 \pm 0.09$	$0.24{\pm}0.01$	$0.30 \pm 0.03$	$0.44 \pm 0.07$	0.22
Eosinophil %	$2.67 \pm 0.88$	$2.40{\pm}0.51$	$3.40 \pm 0.93$	$2.60 \pm 0.24$	0.82
Eosinophil Absolute	$0.13 \pm 0.04$	$0.10 \pm 0.02$	$0.16 \pm 0.05$	$0.14{\pm}0.03$	0.83
Basophils%	$0.67 \pm 0.33$	$0.40 \pm 0.24$	$0.80 \pm 0.37$	$0.80 \pm 0.20$	0.48
Basophils Absolute	$0.03 \pm 0.02$	$0.02 \pm 0.01$	$0.04 \pm 0.02$	$0.04 \pm 0.01$	0.32

<sup>a, b and c</sup> Means in the same row with different superscripts are significantly different ( $P \le 0.05$ )

C (P0) = Control (without supplementations)

P5 = 5 mg prednisolone/ kg diet

P10 = 10 mg prednisolone/ kg diet

P20 = 20 mg prednisolone/ kg diet

WBCs= Total Leucocytes Count

**Table (6):** Effect of Prednisolone Supplementation on Antibody Titers Against Infectious Bronchitis Virus (IBV) and Newcastle Disease Virus (NDV)

Treatment	IBV	NDV
Control P0	12100.00±173.21	10.37±0.13
Р5	12240.00±72.11	10.33±0.15
P10	12133.33±88.19	10.33±0.20
P20	12366.67±88.19	10.20±0.35
P-value	0.3896	0.9534

Means in the same column with different superscripts are significantly different (P $\leq$ 0.05)

P0 = Control (without prednisolone supplementations)

P5 = 5 mg prednisolone/kg diet

P10 = 10 mg prednisolone/ kg diet

P20 = 20 mg prednisolone/ kg diet

IBV = Infectious bronchitis virus

NDV = Newcastle disease virus

synthetic glucocorticoids, broiler breeder chickens, macrocytic anemia, CBC, humoral immunity

#### REFERENCES

- **A.O. A. C. 1998.** Official Method of Analysis. 15th Edition, Association of Official Analytical Chemists, Washington DC.
- Adam Ismail, A., Saba Siddig, A., Awad Mohammed, B. 2019. The impacts of medications misused for body weight gain on complete blood count (CBC) concentration of female rabbits. Am J Biomed Sci & Res. 1(6): 242-249.
- Aengwanich, W. 2007. Effects of dexamethasone on physiological changes and productive performance in broilers. Asian J Anim Vet Adv. 2:157-161.
- Aslam, M. A., Groothuis, T. G., Smits, M. A., Woelders, H. 2014. Effect of corticosterone and hen body mass on primary sex ratio in laying hen (Gallus gallus), using unincubated eggs. Biol Reprod. 90(4):76-1.
- Aslinia, F., Mazza, J. J., Yale, S. H. 2006. Megaloblastic anemia and other causes of macrocytosis. Clin Med Res. 4(3):236-241. doi: 10.3121/cmr.4.3.236.
- Cameron, E. Z., Linklater, W. L. 2002. Sex bias in studies of sex bias: the value of daughters to mothers in poor condition. Anim Behav. 63(2):F5-F8.
- Cain, D., Cidlowski, J. 2017. Immune regulation by glucocorticoids. Nat Rev Immunol. 17:233-247. https://doi.org/10.1038/nri.2017.1.
- **Campbell, T. W. 1988.** Avian Hematology and Cytology. Iowa: Iowa State University Press.
- Clout, M. N., Elliott, G. P., Robertson, B. C. 2002. Effects of supplementary feeding on the offspring sex ratio of kakapo: a dilemma for the conservation of a polygynous parrot. Biol Conserv. 107(1):13-18.
- Correa, S. M., Adkins-Regan, E., Johnson, P. A. 2005. High progesterone during avian meiosis biases sex ratios toward females. Biol Lett. 1:215-218.
- Daice, S. J., Lewis, S. M. 1991. Practical Hematology. (7th ed.). Churchill Livingstone.
- **Davis, A. K. 2005.** Effect of handling time and repeated sampling on avian white blood cell

counts. J Field Ornithol. 76(4):334-338. http://www.jstor.org/stable/4151321.

- Dhabhar, F. S. 2014. Effects of stress on immune function: the good, the bad, and the beautiful. Immunol Res. 58(2-3):193-210. doi: 10.1007/s12026-014-8517-0.
- **Duncan, D. B. 1955.** Multiple range and multiple F tests. Biometrics. 11(1):1-42.
- El-lethey, H., Jungi, T. W., Huber-Eicher, B. 2001. Effects of feeding corticosterone and housing conditions on feather pecking in laying hens (Gallus gallus domesticus) Physiol Behav. 73:243–251.
- Fauci, A. S., Dale, D. C., Balow, J. E. 1976. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. Ann Intern Med. 84:304-315.
- Fedor, M. E., Rubinstein, A. 2006. Effects of long-term low-dose corticosteroid therapy on humoral immunity. Ann Allergy Asthma Immunol. 97(1):113-116. doi: 10.1016/S1081-1206(10)61380-4.
- Gross, W. B., Colmano, G. 1967. Further studies on the effects of social stress on the resistance to infection with Escherichia coli. Poultry Sci. 46:41-46.
- Gross, W. B., Colmano, G. 1971. Effect of infectious agents on chickens selected for plasma corticosterone response to social stress. Poultry Sci. 50(4):1213-1217. https://doi.org/10.3382/ps.0501213.
- Gross, W. B., Siegel, P. B., DuBose, R. T. 1980. Some effects of feeding corticosterone to chickens. Poultry Science, 59(3), 516-522.
- Goerlich, V. C. 2009. Manipulative mothers: maternal steroid hormones and avian offspring sex ratio. PhD thesis, University of Groningen, The Netherlands.
- Hanafy, A. M., Hassan, S. A. 2021. Effects of dietary dexamethasone on productive and reproductive performance of premature Japanese quail (Coturnix coturnix japonica). Egyptian Poultry Science Journal. 41(1):129-145.
- Hariz, A., Bhattacharya P. T. 2023. Megaloblastic Anemia. [Updated 2023 Apr 3]. In: StatPearls [Internet]. Treasure Island

(FL): StatPearls publishing Available from: https://www.ncbi.nlm.nih.gov/books/NBK53 7254/

- Heidt T, Sager HB, Courties G, Dutta P, Iwamoto Y, Zaltsman A, von Zur Muhlen C, Bode C, Fricchione GL, Denninger J, Lin CP, Vinegoni C, Libby P, Swirski FK, Weissleder R, Nahrendorf M. 2014. Chronic variable stress activates hematopoietic stem cells. Nat Med. 20(7):754-758. doi: 10.1038/nm.3589.
- Kaleta, E. F., Redmann, T. 2008. Approaches to determine the sex prior to and after incubation of chicken eggs and of day-old chicks. Worlds Poultry Sci J. 64(3):391-399.
- Kim, Y. H., Kim, J., Yoon, H. S., Choi, Y. H. 2015. Effects of dietary corticosterone on yolk colors and eggshell quality in laying hens. Asian-Australas J Anim Sci. 28(6):840-846. doi: 10.5713/ajas.14.0849.
- Lin, H. S., Sui, J., Jiao, H. C., Buyse, J., Decuypere, E. 2006. Impaired development of broiler chickens by stress mimicked by corticosterone exposure. Comp Biochem Physiol A Mol Integr Physiol. 143(3):400-405.
- Lin, H., Decuypere, E., Buyse, J. 2004. Oxidative stress induced by corticosterone administration in broiler chickens (Gallus gallus domesticus): 2. Short-term effect. Biochem Physiol B Biochem Mol Biol. 139(4):745-751.
- Mumma, J. O., Thaxton, J. P., Vizzier-Thaxton, Y., Dodson, W. L. 2006. Physiological stress in laying hens. Poultry Sci. 85:761-769.
- Natt, M. P., Herrick, C. A. 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Sci. 31:735-739.
- National Center for Biotechnology Information.PubChemCompound Summary for CID 5755, Prednisolone. Available from: http://pubchem.ncbi.nlm.nih.gov/compound/ Prednisolone. Accessed July 4, 2024.
- Nutrient Requirements of Poultry. 1994. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Petrie, M., Schwabl, H., Brande-Lavridsen, N., Burke, T. 2001. Maternal investment:

sex differences in avian yolk hormone levels. Nature. 412(6846):498-499.

- **Pike, T. W., Petrie, M. 2005.** Maternal body condition and plasma hormones affect offspring sex ratio in peafowl. Anim Behav. 70(4):745-751.
- **Puvadolpirod, S., Thaxton, J. P. 2000.** Model of physiological stress in chickens 2. Dosimetry of adrenocorticotropin. Poultry Sci. 79:370-376.
- Samour, J. 2006. Diagnostic value of hematology. In: Olsen, G. (Ed.). Clinical Avian Medicine. pp. 587-610. Spix Publishing.
- SAS Institute. 1999. SAS user's guide: version 6.12. SAS Institute Inc., Cary, NC.
- Shini, S., Kaiser, P., Shini, A., Bryden, W. L. 2008. Biological response of chickens (Gallus gallus domesticus) induced by corticosterone and a bacterial endotoxin. Comp Biochem Physiol B Biochem Mol Biol. 149(2):324-333.
- Shini, S., Shini, A., Huff, G. R. 2009. Effects of chronic and repeated corticosterone administration in rearing chickens on physiology, the onset of lay and egg production of hens. Physiol Behav. 98(1-2):73-77.
- Villegas, P. 1998. Titration of biological suspensions. In: Swayne, D. E., Glisson, J. R., Jackwood, M. W., Pearson, J. E., Reed, W. M. (Eds.). A laboratory manual for the isolation and identification of avian pathogens (4th ed.). The American Association of Avian Pathologists, Kennett Square, PA. pp. 248-253.
- Voorhees, J. L., Powell, N. D., Moldovan, L., Mo, X., Eubank, T. D., Marsh, C. B. 2013. Chronic restraint stress upregulates erythropoiesis through glucocorticoid stimulation. PLoS One. 8(10):e77935. doi: 10.1371/journal.pone.0077935.
- Wang, X. J., Li, Y., Song, Q. Q., Guo, Y. Y., Jiao, H. C., Song, Z. G., & Lin, H. 2013. Corticosterone regulation of ovarian follicular development is dependent on the energy status of laying hens. Journal of lipid research, 54(7), 1860-1876.

**K. J. 2020.** Corticosterone and testosterone treatment influence expression of gene pathways linked to meiotic segregation in preovulatory follicles of the domestic hen. PLoS One. 15(5):e0232120. doi: 10.1371/journal.pone.0232120.

- Zheng, X., Ma, S., Kang, A., Wu, M., Wang, L., Wang, Q., Wang, G., Hao, H. 2016. Chemical dampening of Ly6C(hi) monocytes in the periphery produces.
- Whittingham, L. A., Dunn, P. O., Nooker, J. K. 2005. Maternal influences on brood sex ratios: an experimental study in tree swallows. Proc Biol Sci 272(1574):1775-1780.
- Wintrobe, M. M. 1967. Clinical Hematology (6th ed.). Lea and Febiger, Philadelphia, Kimpton, London.
- Wrobel, E. R., Bentz, A. B., Lorenz, W. W., Gardner, S. T., Mendonca, M. T., Navara,

الملخص العربي تأثير اضافة البريدنيزولون في العلف على إنتاج البيض، وصورة الدم الكاملة، والمناعة الخلطية في أمهات دجاج التسمين (أربر ايكرز بلس)

محمد عبد الحميد محمد سيد و رمضان محمود أبو المجد ومحمد الصغير محمد حسن

قسم انتاج الدواجن – كلية الزراعة جامعة أسيوط

تناولت هذه الدراسة تأثير إضافة البريدنيزولون الى العليقة على بعض المقاييس في أمهات دجاج التسمين Arbor Acres Plus. تم توزيع مائة وعشرين طائراً عشوائياً على أربع مجموعات، كل مجموعة بها ثلاث مكررات تحتوي كل منها على ١٠ طيور. تلقت الطيور علفاً أساسيًا مضافًا إليه ٥، ٥، ١٠، أو ٢٠ ملجم بريدنيزولون / كجم علف لمدة ٤٥ يومًا. شملت المقاييس الرئيسية التي تم تقييمها مقاييس إنتاج البيض، ومؤشرات الدم، ومعايرة الأجسام المضادة ضد فيروس التهاب الشعب الهوائية المعدي (IBV) وفيروس مرض النيوكاسل (NDV).

أشارت النتائج إلى عدم وجود فروق ذات دلالة إحصائية في عدد البيض والإنتاج اليومي للدجاجة بين المجموعات (P > 0.05). ومع ذلك، انخفض متوسط وزن البيضة معنويا مع زيادة مستويات البريدنيزولون مقارنة بالتحكم (P <0.05). ولم تختلف كتلة البيض بشكل كبير بين المجموعات (P > 0.05).

وجد أن عدد خلايا الدم الحمراء تميّل الى الانخفاض (P = 0.064) و متوسط مستويات الهيموجلوبين الكريوي (P = 0.083) تميل الى الارتفاع في الدجاج المغذى على النظام الغذائي الأساسي (التحكم) مقارنة بتلك المغذاة على علف مضاف اليه بريدنيز ولون. علاوة على ذلك، كانت لأمهات دجاج التسمين في المجموعة الضابطة قيم لمتوسط حجم كريات الدم الحمراء أعلى من المعدل الطبيعي، والتي تم رجوعها الى المعدل الطبيعي عن طريق اضافة البريدنيز ولون في العلف. بالإضافة إلى ذلك، أن المعدل الطبيعي عن طريق المحموعة الضابطة قيم لمتوسط حجم كريات الدم الحمراء أعلى من المعدل المعدل ألمي المعدل المعدل المعدل المعدل المعدل الطبيعي، والتي تم رجوعها الى المعدل الطبيعي عن طريق اضافة البريدنيز ولون في العلف. بالإضافة إلى ذلك، أدت المعدل الطبيعي عن طريق اضافة البريدنيز ولون ألمي العلف.

لم يلاحظ أي فروق ذات دلالة إحصائية في عدد خلايا الدم البيضاء والنسب المئوية لأنواع الخلايا المختلفة (P > 0.05). ومع ذلك، فإن الطيور المغذاة على علف مضاف اليه ١٠ و ٢٠ ملغ بريدنيزولون / كغ كانت تميل لاظهار نسبة H / L (0.09 = 9) ونسبة وحيدات أعلى (P = 0.08) من مثيلاتها في مجموعة التحكم.

لم تختلف معايرة الأجسام المضادة ضد IBV و NDV بشكل كبير بين المجموعات المغذاة على علف مضاف اليه البريدنيزولون والمجموعة الضابطة (P > 0.05).

في الختّام، كان لاضافة البريدنيز ولون لمدة ٤٥ يوما في العلف تأثير ضئيل على معابير إنتاج البيض أشارت مقابيس الدم إلى فوائد محتملة لاضافة البريدنيز ولون في تحسين مؤشرات كريات الدم الحمراء في حالة فقر الدم دون آثار ضارة على الاستجابة المناعية.