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Haematological and Serum Biochemical Parameters of Mature Harco Cocks Treated with Human Menopausal Gonadotrophin (Diclair®) For Spermatogenesis

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Abstract— *Twenty sexually matured (24 weeks old) healthy Harco cocks were used to determine the effect of Gonadotrophin (Diclair®) on haematology and serum biochemistry. The cocks were divided into 4 treatment groups of 5 cocks per group identified as T_1 (control) administered with 1ml physiological saline, T_2, administered with 6.75i.u Diclair® and T_4, administered with 20.25i.u Diclair®, with one cock per replicate in a completely Randomized Design (CRD). The injections were dividedinto three doses each and administered intramuscularly in the thigh for three consecutive days. One week after Diclair® treatments, five birds from each group were bled from the wing veins for haematology and serum biochemistry. Results of this study showed significant differences ($P<0.05$) among the treatment groups in all the haematological parameters except mean corpuscular haemoglobin concentration and monocytes which were similar ($P>0.05$) among the treatment groups. Basophils were not detected among the treatment groups. The results further showed significant differences ($P<0.05$) among the treatment groups in the serum biochemical parameters except total serum protein which was similar ($P>0.05$) among the treatment groups. However, the values were within the normal ranges, indicating that Diclair® had no deleterious effect on these parameters.*

Keywords— Harco cocks, haematology, serum biochemistry, Diclair®.

I. INTRODUCTION

For several decades, natural or synthetic hormones have been used to improve the productive and reproductive potentials of animals. In reproductive management of farm animals, human menopausal gonadotrophin is reputed to be effective in improving semen quality of local cocks (Abu *et al.,* 2006). Diclair® is a human menopausal gonadotrophin lyophilized in vials containing a mixture of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio of 1.1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH present in Diclair® play vital role in the initiation of spermatogenesis. The hormone preparation is cheap readily available and does not require cold chain storage (Iheukwumere, 2005).

Haematological and serum biochemical parameters provide valuable information on the health status of animals (Iheukwumere *et al.,* 2006) and also reflect an animal's responsiveness to its internal and external environment (Esonu *et al.,* 2001; Anyaehie and Madubuike, 2004). The effects of such steroid hormones as androgens and estrogens on haematological values are well documented (Iheukwumere *et al.,* 2004). Though studies have been conducted on the haematological parameters of Nigerian domestic chickens(Ikhimioya *et a.*,2000; Iheukwumere*et al.,* 2008), there is no information on the effect of human menopausal gonadotrophin (Diclair®) on such parameters in Harco cocks. Therefore, this study was carried out to evaluate the effect of Diclair® on haematological and serum biochemical parameters of Harco cocks as well as to contribute to knowledge on avian haematology.

II. MATERIALS AND METHODS

Experimental Birds and their Management:

Twenty clinically sound and sexually matured (24 weeks old) Harco cocks purchased from Elgibbor farms in Isuikwuato Local Government Area, Abia State Nigeria, were used for this study. The birds were dewormed and vaccinated soon after purchase. A two-week pre-experimental period was allowed to enable the animals acclimatize. The birds were housed and raised on a deep-litter system. They were fed commercial Grower mash containing 20% CP and 2000 Kcal ME/kg diet twice daily (in the morning and evening). Water was provided *ad libitum.*

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Experimental Design

The twenty sexually matured (2 weeks old) Harco cocks were divided into 4 treatment groups, identified as T_1, T_2, T_3, and T_4. Each treatment group consisted of 5 cocks with one cock per replicate in a Completely Randomized Design (CRD), with four levels of Diclair® as treatment. The levels of Diclair® were 0.00ml, 0.09ml, 0.18ml, and 0.27ml represented as T_1, T_2, T_3 and T_4 respectively. T_1 (Treatment 1) which contained no Diclair® served as the control. Diclair treatment was by intramuscular injection. The injection was administered as follows:

Diclair® was supplied in 2 vials each containing FSH 75i.u and LH 75i.u per ml.

Table.1: Doses of Diclair® Administered to Mature Harco Cocks.

Day	Treatment (Diclair® i.u)			
	T_1	T_2	T_3	T_4
1	0.00	0.03	0.06	0.09
2	0.00	0.03	0.06	0.09
3	0.00	0.03	0.06	0.09
Total	0.00	0.09	0.18	0.27

Table.2: Concentration of Diclair® Mature Harco Cocks.

Day	concentration of Diclair® (i.u)			
	T_1	T_2	T_3	T_4
1	0.00	4.50	9.00	13.50
2	0.00	4.50	9.00	13.50
3	0.00	4.50	9.00	13.50
Total	0.00	013.50	27.00	40.50

All treatments were administered intramuscularly on the thigh of each cock using a one ml syringe with 0.01ml graduation. Seven days after Diclair® injection, blood collection and haematological and serum biochemical evaluation were carried out.

Blood Collection and Haematological Analysis:

The cocks were bled one week after Diclair® injections between 9am and 10.30am from wing veins using needle and syringe and aspirated about 5ml of blood from each cock. Two millilitres of each blood sample were poured into Bijou bottles containing ethylene diamine tetra-acetic acid (EDTA) for haematological evaluation. The remaining 3ml of each blood sample were allowed to coagulate to produce sera for blood chemistry analysis.

Blood samples were analyzed within 2 hours of their collection for packed cell volume (PCV) and haemoglobin (Hb). Erythrocyte or red blood cells (RBC) and leucocyte counts were determined as described by Jain (1986). Erythrocyte count was dome in a haemocytometer chamber placed under a light microscope. Packed cell volume was determined by the micro haematocrit method (Jain, 1980) with 75x16mm capillary tubes filled with blood and centrifuged at 3000rpm for 5min. Haemoglobin concentration was also determined by the cyanmethemyoglobin method (Jain, 1986). Total leucocyte count was carried out using a neubaer haematocytometer placed under a light microscope under x 10 magnification, after using Natt and Henricks dilution to obtain a 1:200 blood dilution. Differential leucocyte count was achieved using blood smears stained with Wright's dye and each type of cell (neutrophil, lymphocyte, eosinophil, monocyte and basophil) were counted with a counter.

Blood Chemistry Analysis

The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for evaluation of serum biochemical parameters. Urea concentration was determined following method described by Baker and Silverton (1986). Aspertate transaminase, alanine transaminase and alkaline phosphatase activities were determined using spectrophotometric method as described by Rej and Hoder (1983). The standard flame photometry using Gallenkamp analysis was to determine serum calcium ion. Serum total protein was determined by Goldbery refractometer method as described by Kohn and Allen (1995). Albumin and globulin were determined using bromo cresol green (BCG) method as described by Randox (2006).

Data Analysis

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Data collected on haematological and serum biochemical parameters of mature Harco cocks were subjected to one - way analysis of Variance (ANOVA) using the technique of steel and Torrie (1980). Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi (1990).

III. RESULTS AND DISCUSSION

The results of Diclair® administration on haematology of mature Harco cocks are shown on Table 3.

There were significant differences (P<0.05) among the treatment groups in PCV, HB, RBC, WBC, MCV, MCH and MCHC values. Cocks on T_2 recorded the highest value of 38.40% in PCV and this differed significantly (P<0.05) from cocks on T_1 and T_4 which were similar (P>0.05) to each other in PCV values and also similar (P>0.05) to cocks on T_3. There was no significant difference (P>0.05) between cocks on T_2 and T_3 in PCV values. The PCV values obtained in T_1 (34.00%) and T_4 (32.40%) were within the range of 28 – 37% reported by Simara*ks et al.* (2004) and Iheukwume*e et al.* (2006). However, the PCV values obtained for the whole treatment groups were within the range of (25-45%) reported for birds by Banerjee (2005) and Islam *et al.* (2004).

Cocks on T_2 recorded the highest Hb value of 12.84(g/dl) and thus differed significantly (P<0.05) from cocks on T_1 and T_4 which were similar (P>0.05) to each other in Hb value. There was no significant difference (P>0.05) between cocks on T_2 and T_3 in Hb values. Similarly, there was no significant difference (P>0.05) between cocks on T_1 and T_3. The lowest value in Hb was observed in cocks on T_4 (10.80g/dl). The Hb values obtained in this study were within the normal range of 7.0 – 13.0g/dl reported for birds (Jain, 1993). However, the Hb values obtained in this study were higher than the range of 9.36 $\pm$ 0.01 – 9.39 $\pm$ 0.00 (g/dl) reported by Iheukwumere *et al*. (2006) for Nigerian indigenous chickens, but lower than the range of 11.00 $\pm$ 2.15 – 14.85 $\pm$ 1.42 (g/dl) reported by Iheukwumere *et al.* (2008) for Nigerian Local cocks. Haemaglobin concentration of blood has been associated with availability of nutrients to the animal body (Esonu *et al.,* 2001).

Table.1: Effect of Diclair on Haematology of Mature Harco Cocks

Parameters	Treatment (Diclair® i.u) T_1 0.00	T_2 6.75	T_3 13.50	T_4 20.25	SEM
PCV (%)	34.00^{b}	38.40^{a}	37.50^{ab}	32.40^{b}	1.28
Hb (g/dl)	11.30^{bc}	12.84^{a}	12.50^{ab}	10.80^{c}	0.41
RBC (x10^6/mm^3)	10.14^{b}	9.88^{b}	11.99^{a}	10.14^{b}	0.32
WBC (x10^3/mm^3)	6.86^{ab}	6.90^{ab}	7.00^{a}	6.50^{c}	0.05
MCV (fl)	33.40^{b}	39.90^{a}	35.20^{b}	32.00^{b}	1.35
MCH (pg)	11.10^{b}	13.30^{a}	11.80^{b}	10.70^{b}	0.39
MCHC (g/dl)	33.20	33.40	33.30	33.20	0.07

$^{abc:}$ Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Cocks on T_3 recorded the highest RBC value of 11.99 (x10^6/mm^3) and this differed significantly (P<0.05) from cocks on T_1 , T_2 and T_4 which which were similar (P>0.05) to each other in RBC values.The lowest value RBC was observed in cocks on T_1 and T_4 which had 10.14 x 10^6/mm^3 each. The RBC values obtained in this study were higher than the range of 2-4 (x10^6/mm^3) reported by Jain (1993) for birds but within the range of 8 – 11 x 10^{6}mm^3 reported by Simaraks *et al.* (2004). However, the RBC values obtained in this study were lower than the average 14.65 (x10^6/mm^3) reported by Kundu *et al.* (1993) and the highest values 13.35 x 10^6/mm^3 reported by Ameh (2004) and 14.85 $\pm$ 2.36 x 10^6 / µl) reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This disparity in the values of RBC may not be unconnected to the differences in breed and nutritional status of the birds (Esonu *et al.,* 2001)

Cocks on T_3 recorded the highest value of 7.00 x 10^3/mm^3 in WBC and this differed significantly (P<0.05) from cocks on T_4 (6.50x10^3/mm^3). There were no significant differences (P>0.05) among cocks on T_1, T_2 and T_3 in WBC values. Cocks on T_1 and T_2were significantly different (P<0,05) from those on T_4. The lowest value in WBC was observed in cocks on T_4 (6.50 x 10^3/mm^3). The WBC values obtained in this study were lower than the range of 9.30 $\pm$ 0.00 – 9.64$\pm$0.03 (x10^3/µl) reported by Iheukwumere *et al.* (2006) for Nigerian indigenous chickens.

Abnormal production of white blood cells in the blood of animals is usually associated with immune response by animals due to the presence of an antigen (foreign body) in

the body. Elevation of white blood cells suggests infection by microorganisms especially bacterial (Aka *et al.,* 2008; Sowande *et al.*, 2008).

Cocks on T_2 recorded the highest value in MCV 39.90 (fl) differed and this significantly (P<0.05) from cocks on the control treatment (T_1), cocks on T_3 and T_4 which were similar (P>0.05) to each other in MCV values. The lowest value in MCV was observed in cocks on T_4 (32.00fl). The MCV values obtained in this study were lower than the highest value 40.00 ± 7.8(fl) reported by Iheukwumere *et al.* (2008) in Nigerian local cocks and lower than the value 41.00 ± 6.5(fl) reported by Iheukwumere and Herbert (2002) in broiler chickens, but higher than the average 27.32 ± 1.58(fl) reported by Ameh (2004) in Nigerian local cocks. Mean corpuscular volume is an indication of the average volume of blood cells (Lazzaro, 2003).

Cocks on T_2 recorded the highest MCH value of 13.30(pg) and this differed significantly (P<0.05) from cocks on the control treatment (T_1), cocks on T_3 and T_4 which were similar (P>0.05) to each other in MCH values. The lowest value of 10. 70(pg) in MCH was observed in cocks on T_4. The MCH values obtained in this study were lower than the mean value 33.90(pg) reported by Iheukwunere *et al.* (2002) in broiler chickens, and lower than the range of 21.30 ± 2.52 – 33.50 ± 2.13(pg) reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This disparity in the values of MCV may be attributed to differences in breed, physiological and nutritional status of the birds.

Cocks on T_2 recorded the highest numerical value of 33.40(g/dl) in MCHC. The lowest value of 33.20(g/dl) in MCHC was observed in cocks on T_1 and T_4. The MCHC values obtained in this study were lower than the value 35. 70% reported by Iheukwumere *et al.* (2002) in broiler chickens, but higher than the value 30. 56% reported by Ameh (2004) in Nigerian local cocks. However, the MCHC values obtained in this study were within the normal range of 26.0 – 35.0(g/dl) reported by Banerjee (2005) for chickens and by Islam *et al.* (2004) for local chickens in Bangladesh.

The results of Diclair® administration on differential leucocyte count of mature Harco cocks are shown on Table 2.

There were significant differences (P<0.05) among the treatment groups in neutrophil and lymphocyte values. Cocks onT_2 recorded the highest value of 55.60% in neutrophil and this differed significantly (P<0.05) from cocks on T_1 and T_4 which were similar (P>0.05) to each other and also similar (P>0.05) to cocks on T_3 in neutrophil values. There was no significant difference (P>0.05) between cocks on T_2 and T_4 in neutrophil values. The lowest value of 53.40% was observed in cocks on T_1 and T_4. The neutrophil values obtained in this study were higher than the normal range of 25-30% reported by Banergee (2005) for chickens. Neutrophils have phagocytic and bactericidal capabilities which means that they play an important role in inflammatory condition. They are very important for defense whenever acute infection is present (Banerjee, 2005).

Table.2: Effect of Gonadotrophin (Diclair®) on Differential leucocyte count of Mature Harco Cocks.

	Treatment (Diclair® i.u)				
	T_1	T_2	T_3	T_4	
Parameters	0.00i.u	7.02i.u	14.01i.u	21.00i.u	SEM
Neutrophils (%)	53.40^{b}	55.60^{a}	54.40^{ab}	53.40^{b}	0.51
Lymphocytes (%)	42.60^{b}	41.00^{c}	42.00^{c}	43.00^{a}	0.41
Eosinophils (%)	2.50	2.50	2.50	2.50	0.00
Monocytes (%)	1.00	1.00	1.00	1.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00

abc: Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Cocks on T_4 recorded the highest value of 43.00% in lymphocyte and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar (P>0.05) to each other in lymphocyte value, but differed significantly (P<0.05) from cocks on the control treatment (T_1). The lowest value of 41.00% in lymphocyte was observe in cocks on T_2. The lymphocyte values obtained in this study were within the normal range of 35-60% reported by Banerjee (2005) for chickens, suggesting that these blood cells can still perform their phagocytic and immune functions.

There were no significant differences (P>0.05) among the treatment groups in Eosinophil and monocyte values. The values recorded for these parameters were 2.50% and 1.00% respectively across the treatment groups. Basophils were not detected among the treatment groups.

The results of Diclair® administration on serum biochemical parameters of mature Harco cocks are shown on Table 3.

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There were significant differences (P<0.05) among the treatment groups in urea, alkaline phosphatase (ALT), glucose, cholesterol, calcium, total serum protein, albumin and globulin values.

Cocks on T_4 recorded the highest value of 29.74(mg/dl) in serum urea and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3 which were similar (P>0.05) to each other in urea values. The lowest value of 10.20(mg/dl) in urea was observed on cocks on the control treatment (T_1). The urea values obtained in this study were lower than the range of 30.46 $\pm$ 2.51 – 54.08 $\pm$ 0.11 (mg/dl) reported by Iheukwumere *et al.* (2006) in Nigerian chickens. This disparity in urea values may be attributed to differences in bred and nutritional status of the birds. It has been observed that serum urea content depends on both the quantity and quality of protein supplied in the diet (Iheukwumere and Herbert, 2003).

Table.3: Effect of Gonadotrophin (Diclair®) on serum Biochemistry of Mature Harco Cocks.

Parameters	Treatment (Diclair® i.u) T_1 0.00	T_2 6.75	T_3 13.50	T_4 20.25	SEM
Urea (mg/dl)	10.20[b]	10.80[b]	11.44[b]	29.74[a]	0.95
Alkaline Phosphatase Aspartate (iu/L)	73.60[c]	80.00[b]	80.00[b]	81.00[a]	0.66
Transaminase (iu/L)	0.00	0.00	0.00	0.00	0.00
Alanine ttransaminase (iu/L)	0.00	0.00	0.00	0.00	0.00
Glucose (mg/dl)	151.40[b]	148.60[b]	176.40[a]	132.60[c]	3.17
Cholesterol (mg/dl)	109.60[d]	118.00[c]	120.00[b]	128.00[a]	0.66
Calcium (mg/dl)	8.06[b]	8.10[b]	8.70[a]	8.94[a]	0.18
Total serum protein (g/l)	6.66	6.24	6.30	6.70	0.25
Albumin (g/L)	3.40[b]	4.06[a]	4.16[a]	3.14[c]	0.06
Globulin (g/L)	3.26[b]	2.20[c]	2.14[c]	3.62[a]	0.09

[abc:] Means within row having different superscript are significantly (P<0.05) different.
SEM = Standard error of means.

Cocks on T_4 recorded the highest value of 81.00iu/L in Alkaline phosphatase and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar (P>0.05) to each other in Alkaline phosphatase value, but differed significantly (P<0.05) from cocks on T_1. The lowest value of 73.60iu/L in alkaline phosphatase was observed in cocks on T_1. The Alkaline phosphatase values obtained in this study were lower than the normal value 482.5u/L reported by Kaneko *et al.* (1997) for chicken. This disparity may not be unconnected to the differences in breed and physiological status of these birds. Alkaline phosphatase assay is useful in the diagnosis of obstructive liver disease (Murray *et al.,* 2003).

Aspartate transaminase and Alanine transaminase were not detected among the treatment groups. An increase in Alkaline phoshatase, Alanine transaminase and Aspartate transaminase values would signify necrosis or myocardial infarction which are all indicators of drug toxicity or harmful chemicals in the body (Nelson and Cox, 2005). In this regard Diclair® can be considered safe for the cocks as the values in the liver enzyme activity of the treatment groups were below the normal value 482.5u/L reported by Kaneko *et al.* (1997) for chicken.

Cocks on T_3 recorded the highest value of 176.40(mg/dl) in serum glucose and this differed significantly (P<0.05) from cocks on the control treatment, cocks on T_2 and T_4. Cocks on T_1 and T_2 were similar (P>0.05) to each other in glucose values, but they differed significantly (P<0.05) from cocks on T_4. The lowest value of 132.60mg/dl in serum glucose was observed in cocks on T_4. The glucose values obtained in this study were within the normal range of 125-200mg/dl reported by Banerjee (2005) for birds. Glucose is one of the metabolites measured as an indicator of the energy status of animals. Normal glucose levels in the cocks indicate adequate synthesis in the liver from propionate metabolism as the major glucose precursor (Sowande, *et al.,* 2008).

Cocks on T_4 recorded the highest value of 128.00mg/dl in serum cholesterol and this differed significantly ($P<0.05$) from cocks on the control treatment (T_1) cocks on T_2 and T_3 which were also significantly different ($P<0.05$) from each other in cholesterol values. The lowest value of 109.60mg/dl in cholesterol was observed in cocks on the control treatment.

The cholesterol values obtained in this study were within the normal range of 52-148mg/dl reported by Banerjee (2005) for birds. This implies that Diclair® injection was safe for the birds, so birds treated with Diclair® injection may not face the risk of myocardial infarction usually associated with high blood cholesterol content and emaciation due to low serum cholesterol (Frandson, 2002).

Cocks on T_4 recorded the highest value of 8.94(mg/dl) in serum calcium and this differed significantly ($P<0.05$) from cocks on T_1 and T_2 which were similar ($P>0.05$) to each other in calcium values. There was no significant difference ($P>0.05$) between cocks on T_4 and T_3 in calcium values. The lowest value of 8.06mg/dl in calcium was observed in cocks on the control treatment (T_1). The calcium values obtained in this study were lower than the mean value 28.4mg/dl reported by Kaneko *et al.* (1997) for chicken. The similarity observed in cocks on T_3 and T_4 indicates probable electrolyte balance in the cocks' body caused by gonadotrophin administration. This observation is in agreement with the report of Iheukwumere *et al.* (2004) in WAD goats.

Cocks on T_4 recorded the highest numerical value of 6.70g/dl serum total protein. The lowest value of 6.24g/dl in serum total protein was observed in cocks on T_2. The serum total protein values obtained in this study were lower than the range of 7.6 $\pm$ 0.27 – 8.2 $\pm$ 0.30 mg/dl reported by Iheukwumere *et al.* (2006) for Nigerian chickens. This variation in values of serum total protein may not be unconnected to the differences in breed and nutritional status of the birds (Esonu, *et al.,* 2001).

Cocks on T_3 recorded the highest value of 4.16(g/dl) in serum albumin and this differed significantly ($P<0.05$) from cocks on T_1 and T_4 which were also significantly different ($P<0.05$) from each other in albumin value.

There was no significant difference ($P>0.05$) between cocks on T_3 and T_2 in albumin values. The lowest value of 3.14g/dl in serum albumin was observed in cocks on T_4. The serum albumin values obtained in this study were higher than the range of 3.1 $\pm$ 0.27 – 3.5 $\pm$ 0.22mg/dl reported by Iheukwumere *et al.* (2006) for Nigerian chickens. Low albumin suggests poor clotting ability of blood and hence poor prevention of hemorrhage (Robert *et al.*, 2000).

Cocks on T_4 recorded the highest value of 3.62g/dl in serum globulin and this differed significantly ($P<0.05$) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar ($P>0.05$) to each other in serum globulin value, but differed significantly ($P<0.05$) from cocks on the control treatment (T_1). The lowest value of 2.14 g/dl in serum globulin was observed in cocks on T_3. The serum globulin values obtained in this study were within the range of 2.1 – 3.7g/dl reported for birds by Banerjee (2005). Babatunde and Oluyemi (2006) opined that the higher the value of globulin, the better the ability to fight against disease.This implies that cocks on T_4 which recorded the highest value of 3.62g/dl in globulin had the best ability to resist disease.

IV. CONCLUSION

From the results of this study, it can be concluded that human menopausal gonadotrophin (Diclair®) had no deleterious effect on haematological and serum biochemical parameters of Harco cocks. Though most of the values obtained fall within the normal ranges for chicken, the variations observed suggest the need to constantly monitor blood Profile of Harcococks under Diclair® treatment for spermatogenesis.

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