P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

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

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Article History: Received: July 12, 2016 Revised: August 16, 2016 Accepted: September 05, 2016

ABSTRACT

Sixteen sexually matured (12 months old) healthy male turkeys were used to determine the effect of Gonadotrophin (Diclair®) on haematology and serum biochemistry. The turkeys were divided into 4 treatment groups of 4 turkeys per group, identified as T_1 (control), administered with 1.00ml physiological saline, T_2, administered with 13.50i.u Diclair®, T_3, administered with 27.00 i.uDiclair® and T_4, administered with 40.50i.u Diclair®, with one turkey per replicate in a Completely Randomized Design (CRD). The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. One week after Diclair® treatments, four turkeys from each group were bled from the wing veins for haematology and serum biochemistry. The results of the study showed significant differences ($P<0.05$) among the treatment groups in all the haematological parameters except eosinophils 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 all the serum biochemical parameters. However, the values were within the normal ranges indicating that Diclair® had no deleterious effects on these parameters.

Key words: Turkeys, Haematology, Serum Biochemistry Diclair®

INTRODUCTION

Turkeys (*Meleagrisgallopavo*) are birds that originated in North America, that were domesticated in Europe and are now an important source of food in many parts of the world (Brant, 1998). Turkey occupies an important position next to chicken, duck, guinea fowl and quail in contributing to the most evolving sector, which is playing a significant role in augmenting the economic and nutritional status of varied population (Katie and Frazer, 1988). All over the world turkeys are reared for their tasty and high quality meat (Probakaran, 2003). Hence they are kept because of the economic service they render (Okeudo, 2005) such as eggs, meat, feathers and sometimes pet.

In order to carry out any sustainable improvement in livestock, there should be methods of ensuring the repeatability and multiplication of desired traits in subsequent generations. To get the fullest benefits from the breeding turkeys therefore, a good knowledge of their sperm production is essential as well as their sperm output. In view of the increasing use of livestock for specialized production, there is need for more practical and better control methods of reproduction.

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).

Cite This Article as: Egu UN, 2016. Haematological and serum biochemical parameters of mature male turkeys treated with human menopausal gonadotrophin (Diclair®) for spermatogenesis. Inter J Vet Sci, 5(4): 274-279. www.ijvets.com

Though studies have been conducted on the haematological parameters of Nigerian domestic chickens (Ikhimioye *et al.*, 2000; Iheukwumere *et al*., 2008), there is no information on the effect of Human, menopausal gonadotrophin (Diclair®) on such parameters in male Turkeys. Therefore, this study was carried out to evaluate the effect of Diclair® on haematological and serum biochemical parameters of mature male turkeys.

MATERIALS AND METHODS

Experimental birds and their management

Sixteen healthy sexually matured male turkeys aged 12 months were used for this study. The turkeys were purchased from the local markets and housed in clean pens. Routine management practices were carried out which include deworming, daily observation of birds to identify sick ones, maintaining clean and dry litter and vaccination against diseases. The turkeys were fed Grower Mash. Feed and water were provided *ad libitum* throughout the 28 days duration of the experiment. They were weighed every week and their weights were recorded.

Experimental design and drug administration

Sixteen male turkeys were divided into 4 treatment groups consisting of 4 turkeys per group with one turkey per replicate in a Completely Randomized Design (CRD. These groups were assigned to 4 levels of Diclair® injection as treatments. The levels of Diclair® were 0.00i.u, 13.50i.u, 27.00i.u, and 40.50i.u Diclair® represented as T_1, T_2, T_3, and T_4 respectively. The group which received 0.00i.u Diclair® (T_1) served as the control.

Diclair® was supplied in 3 vials, each containing FSH 75i.u and LH 75i.u. The content of each vial was dissolved in 1ml of physiological saline solution immediately prior to use resulting in a solution of DFSH 75i.u plus DLH 75i.u per ml. All treatments were administered intramuscularly on the breast muscle of each turkey using a one ml syringe with 0.01ml graduation. The doses and concentration ofDiclar®administered are shown in Tables 1 and 2.

Blood collection and haematological analysis

The turkeys were bled one week after Diclair®injections between 9am and 10.30am from a punctured wing vein and aspirated about 5ml of blood from each turkey. 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 done in a haemocytometer chamber placed under a light microscope. Packed cell volume was determined by the microhaematocrit method (Jain, 1986) with 75 x 16mm capillary tubes filled with blood and centrifuged at 3000rpm for 5 minutes. Haemoglobin concentration was also determined by the cyanmethemyoglobin method (Jain, 1986). The various red cell indices like mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated from RBC, Hb and PCV (Lazzaro, 2003). Total leucocyte count was carried out using a Neubaerhaemocytometre 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) was determined with a counter.

Evaluation of blood chemistry

The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for biochemical evaluation: urea, calcium, cholesterol, aspartate transaminase, alanine transaminase and alkaline phosphatase concentrations were determined using the analytical kits of Randox Laboratories Limited Crumin. Co. Anthrax, UK at MOUAU Medical Laboratory Umuahia, Nigeria.

Table 1: Doses of Diclair® Administered to Mature Male Turkeys

Day	Treatment Dosage (ml)			
	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	0.06
Total	0.00	0.09	0.18	0.27

Table 2: Concentration of Diclar® on Mature Male Turkeys

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 breast muscle of each turkey using a 1ml syringe with 0.01ml graduation.

Statistical analysis

Data collected on haematological and serum biochemical parameters of the male turkeys were subjected to 0ne-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).

RESULTSAND DISCUSSION

The results of haematological parameters of male turkeys treated with gonadotrophin (Diclair®) are shown in Table 3. There were significant differences ($P<0.05$) among the treatment groups in packed cell volume (PCV), haemoglobin (HB), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values.

Turkeys on T_2 recorded the highest value of 40.33% in PCV and this differed significantly ($P<0.05$) from turkeys on T_1 and T_4 which were also significantly different ($P<0.05$) from each other in PCV values. There was no significant difference ($P>0.05$) between turkeys on

Table 3: Effect of Diclair® on Haematology of Mature Male Turkeys

Parameters	Treatment (Diclair®i.u)				
	$T_1$0.00	$T_2$13.50	$T_3$27.00	$T_4$40.50	SEM
PCV (%)	33.92[c]	40.33[a]	40.27[a]	35.07[b]	0.05
HB (g/dl)	10.47[d]	12.73[a]	12.23[b]	10.87[c]	0.04
RBC (x10^6/mm^3)	4.63[b]	4.80[b]	5.03[a]	4.17[c]	0.06
WBC (x10^3/mm^3)	26.47[d]	40.33[b]	62.67[a]	37.57[c]	0.03
MCV (fl)	73.80[d]	84.13[b]	80.37[c]	87.43[a]	0.09
MCH (pg)	22.50[d]	26.63[b]	24.43[c]	27.37[a]	0.06
MCHC(g/dl)	30.73[c]	31.87[a]	30.33[d]	31.13[b]	0.04

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

Table 4: Effect of Diclair® on Differential Leucocyte Count of Mature Male Turkeys

Parameters	Treatment (Diclair®i.u)				
	$T_1$0.00	$T_2$13.50	$T_3$27.00	$T_4$40.50	SEM
Neutrophils (%)	48.50[a]	29.23[b]	30.00[b]	39.10[ab]	4.52
Lymphocytes (%)	51.50[b]	50.37[b]	70.00[a]	55.83[b]	4.51
Eosinophils (%)	0.00[b]	1.07[b]	0.00[b]	4.04[a]	0.95
Monocytes (%)	0.00[b]	1.33[a]	0.00[b]	1.03[a]	0.35
Basophils (%)	0.00	0.00	0.00	0.00	0.00

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

Table 5: Effect of Diclair® on Serum Biochemical Parameters of Mature Male Turkeys

Parameters	Treatment (Diclair®i.u)				
	$T_1$0.00	$T_2$13.50	$T_3$27.00	$T_4$40.50	SEM
Urea (mmol/L)	39.10[b]	32.83[d]	41.97[a]	37.03[c]	0.16
Calcium (mmol/L)	16.03[b]	19.12[a]	18.53[a]	16.31[b]	0.02
Cholesterol (mg/dl)	70.60[a]	70.43[a]	51.77[b]	60.57[ab]	4.51
Alkaline phosphatase (iu/L)	38.07[c]	41.07[b]	44.13[a]	32.07[d]	0.07
Alanine transaminase (iu/L)	15.83[b]	16.43[b]	17.23[a]	11.83[c]	0.19
Aspertate transaminase (iu/L)	63.07[d]	110.33[a]	103.33[b]	95.33[c]	0.29

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

T_2 and T_3 in PCV values. The PCV values obtained in this study were within the range of 25-45% reported for birds (Banerjee, 2005; Isliam *et al.*, 2004).

Turkeys on T_2 recorded the highest value of 12.73 (g/dl) in HB and this differed significantly (P<0.05) from turkeys on T_1, T_3 and T_4 which were also significantly different (P<0.05) from each other in HB values. 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±0.01 – 9.39±0.00(g/dl) reported for Nigerian indigenous chickens (Iheukwumere*et al.*, 2006), but lower than the range of 11.00±2.15 – 14.85±1.42(g/dl) reported for Nigerian local cocks (Iheukwumere*et al*., 2008). Haemoglobin concentration of blood has been associated with availability of nutrients to the animal's body (Esonu*et al.*, 2001).

Turkeys on T_3 recorded the highest value of 5.03 (x10^6/mm^3) in RBC and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_4. Turkeys on T_2 and T_1 were similar (P > 0.05) to each other in RBC values, but differed significantly (P<0.05) from turkeys on T_4. The RBC values obtained in this study were higher than the range of 2-4 (x10^6/mm^3) reported for birds (Jain, 1993), but lower than the range of 8-11 (x10^6/mm^3) reported in Thai indigenous chickens (Simaraks*et al.*, 2006) and lower than the highest values 13.35 x 10^6/mm_3 and 14.85±2.36(x 10^6/mm^3) reported by Ameh (2004) and Iheukwumere*et al*. (2008) respectively 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).

Turkeys on T_3 had the highest value of 62.67(x 10^6/mm^3) in WBC and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_4which were also significantly different (P<0.05) from each other in WBC values. The WBC values obtained in this study were higher than the range of 9.30 ± 0.00 – 9.64 ± 0.03 (x 10^3/µl) reported by Iheukwumere*et al.* (2006) for Nigerian chickens. Abnormal production of white blood cell in the body 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 cell suggests infection by microorganisms especially bacteria (Aka *et al.*, 2008; Sowande*et al.*, 2008).

Turkeys on T_4 had the highest value of 87.43 (fl) in MCV and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_3 which were also significantly different (P<0.05) from each other in MCV values. The MCV values obtained in this study were higher than the highest value 40.00 ± 7.8 (fl) reported in Nigerian local cocks (Iheukwumere *et al.*, 2008) and higher than the value 41.00 ± 6.5 (fl) reported in broiler chickens (Iheukwumere and Herbert, 2003) and higher than the average value of 27.32 ± 1.58 (fl) reported in Nigerian local cocks (Ameh, 2004). Mean corpuscular volume is an indication of the average volume of blood cells (Lazzaro, 2003).

Turkeys on T_4 had the highest value of 27.37(pg) in MCH and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_3 which were also significantly different (P<0.05) from each other in MCH values. The MCH values obtained in this study were lower than the mean value 33.90(pg) reported in broiler chickens

(Iheukwumereb *et al.,* 2002), but within the range of 21.30±2.52 – 33.50±2.13 (pg) reported in Nigerian local cocks (Iheukwumere *et al.,* 2008). This disparity in the values of MCH may be attributed to differences in breed, physiological and nutritional status of the birds (Esonu *et al.*, 2001).

Turkeys on T_2 had the highest value of 31.87 (g/dl) in MCHC and this differed significantly (P<0.05) from turkeys on T_1, T_3 and T_4 which were also significantly different (P<0.05) from each other in MCHC values. The MCHC values obtained in this study were lower than the value 35.70% reported in broiler chickens (Iheukwumere *et al.,* 2002), but within the normal range of 26.0-35.0(g/dl) reported for chickens (Banerjee, 2005) and for local chickens in Bangladesh (Islam *et al.*, 2004). The results of differential leucocytes count of male turkeys treated with gonadotrophin (Diclair®) are shown in Table 4.

There were significant differences (P<0.05) among the treatment groups in neutrophil, lymphocyte, eosinophil and monocyte values.

Turkeys on T_1 recorded the highest neutrophil value of 48.50% and this differed significantly (P<0.05) from turkeys on T_2 and T_3 which were similar (P>0.05) to turkeys on T_4 in neutrophil values. There was no significant difference (P>0.05) between turkeys on T_1 and T_4 in neutrophil values. The neutrophil values obtained in T_1 and T_4 were higher than the normal range of 25 – 30% reported for chickens (Banerjee, 2005), whereas neutrophil values obtained in T_2 and T_3 were within the normal range. Neutrophils have phagocytic and bactericidal capabilities which means that they play an important role in inflammatory conditions. They are very important for defense whenever acute infection is present (Bnerjee, 2005).

Turkeys on T_3 recorded the highest value of 70.00% in lymphocyte and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_4 which were similar (P>0.05) to each other in lymphocyte values. The lymphocyte values obtained in this study were within the normal range of 35-60% reported for chickens (Banerjee, 2005) except turkeys on T_3 whose lymphocyte value (70.00%) was higher than the normal range. White blood cells and lymphocytes counts are known to increase during infection.

Turkeys on T_4 had the highest value of 4.04% in eosinophil and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_3 which were similar (P>0.05) to each other in eosinophil values. Turkeys on T_1 and T_3 recorded the lowest value in eosinophil (0.00%).

Turkeys on T_2 had the highest value of 1.33% in monocyte and this differed significantly (P<0.05) from turkeys on T_1 and T_3 which were similar (P>0.05) to each other in monocyte values, but differed significantly (P<0.05) from turkeys on T_4. There was no significant difference (P>0.05) between turkeys on T_2 and T_4 in monocyte values. The lowest value in monocyte was observed in turkeys on T_1 and T_3 (0.00%). Basophils were not detected among the treatment groups.

The result of serum biochemical parameters of male turkeys treated with gonadotrophin (Diclair®) are shown in Table 5. There were significant differences (P<0.05) among the treatment groups in urea, calcium, cholesterol, Alkaline phosphatase (ALP) Alanine transaminase (ALT) and Aspartate transaminase (AST) values.

Turkeys on T_3 recorded the highest value of 41.97 (mmol/L) in serum urea and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_4 which were also significantly different (P<0.050 from each other in serum urea. The serum urea values obtained in this study were within the range of 30.46 ± 2.51 – 54.08 ± 0.11 (mg/dl) reported in Nigerian chickens (Iheukwumere *et al.,* 2006). It has been observed that serum urea content depends on both the quantity and quality of protein supplied in the diet (Iheukwumere and Herbert, 2002). High levels of urea in the blood have been reported to indicate a lowered utilization of protein, poor protein quality or excess protein catabolism associated with protein deficiency (Oduye and Adadevoh, 1976; Oduguwa *et al.,* 1999; Ahamefule *et al.,* 2005).

Turkeys on T_2 recorded the highest value of 19.12 (mmol/L) in serum calcium and this differed significantly (P<0.05) from turkeys on T_1 and T_4 which were also significantly different (P<0.05) from each other in serum calcium values, but differed significantly (P<0.05) from turkeys on T_3. There was no significant difference (P>0.05) between turkeys on $T_2$and T_3 in calcium values. The serum calcium values obtained in this study were lower than the mean value 28.4mg/dl reported for chickens (Kaneko *et al.,*1997). The similarity observed in turkeys on T_2 and T_3 indicates probable electrocyte balance in the birds' body caused by gonadotrophin administration at those levels. This observation is in agreement with the report of Iheukwumere *et al.* (2004) in goats.

Turkeys on T_1 recorded the highest value of 70.60mg/dl in cholesterol and this differed significantly (P<0.05) from turkeys on T_3 which were similar (P>0.05) to turkeys on T_4 in cholesterol values. There were no significant differences (P>0.050 among turkeys on T_1, T_2 and T_4 in serum cholesterol values. The serum cholesterol values obtained in this study were within the normal range of 52-148mg/dl reported for birds (Banerjee, 2005). This implies that Diclair® injection was safe for the turkeys, so turkeys 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).

Turkeys on T_3 recorded the highest value of 44.13iu/L in Alkaline phosphatase and this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_4 which were also significantly different (P<0.05) from each other in Alkaline phosphatase values. The Alkaline phosphatase values obtained in this study were lower than the normal value 482.5(µ/L) reported for chickens (Kaneko *etal*., 1997). This disparity may not be unconnected to the differences inbreed and physiological status of these birds. Alkaline phosphatase assay is useful in the diagnosis of obstructive liver disease (Murray *et al.,* 2003). An increase in Alkaline phosphatase, Alanine transaminase and Aspertate transaminase values would signify necrosis or myocardial infarctsion which are all indicators of drug toxicity or harmful chemical in the body (Nelson and Cox, 2005).

Turkeys on T_3 recorded the highest value of 17.23iu/L in Alanine transaminase and this differed

significantly ($P<0.05$) from turkeys on T_1, $T_2$and T_4. The Alanine transaminase values obtained in this study were lower than the range of 22.10 – 22.20(µL) reported in broiler chickens (Iheukwumere and Herbert, 2003). This disparity in the value of ALT may be attributed to differences in breed, nutritional and physiological status of the birds.

Turkeys on T_2 recorded the highest value of 110.33iu/L in Aspertate transaminase and this differed significantly ($P<0.05$) from turkeys on T_1, T_3 and T_4 which were also significantly different ($P<0.05$) from each other in AST values. The AST values obtained in this study were higher than the mean value 31.00µL reported by Iheukwumere and Herbert (2003) in broiler chickens.

Conclusion

The results of this study showed that the haematological and serum biochemical parameters of male turkeys would be affected when 13.50i.u or more of Diclair® are used for induction of spermatogenesis. Though Diclair® had no deleterious effects on these parameters, the variations observed in the values suggests the need to constantly monitor blood profile of male turkeys under Diclair® treatment for spermatogenesis.

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