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 T₁ (control), administered with 1.00ml physiological saline, T₂, administered with 13.50i.u Diclair®, T₃, administered with 27.00 i.u Diclair® and T₄, 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 (Meleagris gallopavo) 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).

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 T1, T2, T3, and T4 respectively. The group which received 0.00i.u Diclair® (T1) 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 1ml syringe with 0.01ml graduation. The doses and concentration of Diclair® 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 subjected to coagulation. The bottles of coagulated blood were subjected to centrifugation at 1500 rpm for 10 minutes to obtain the plasma and the remaining 3ml of each blood sample were subjected to centrifugation at 1500 rpm for 10 minutes to obtain the plasma. The plasma and the harvested plasma were stored at -20°C. Hb and PCV (Lazzaro, 2003). Total leucocyte count was carried out using a Neubauer haematocytometer placed under a light microscope. Total leucocyte count was carried out using a Neubauer haematocytometer placed under a light microscope. 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 results of haematological parameters of mature male turkeys 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.

RESULTS AND DISCUSSION

The results of haematological parameters of mature 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.

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

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment Dosage (ml)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.03</td>
<td>0.06</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.03</td>
<td>0.06</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.00</td>
<td>0.03</td>
<td>0.06</td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>0.00</td>
<td>0.09</td>
<td>0.18</td>
<td></td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 2: Concentration of Diclair® on Mature Male Turkeys

<table>
<thead>
<tr>
<th>Day</th>
<th>Concentration of Diclair® (i.u)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>4.50</td>
<td>9.00</td>
<td></td>
<td>13.50</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>4.50</td>
<td>9.00</td>
<td></td>
<td>13.50</td>
</tr>
<tr>
<td>3</td>
<td>0.00</td>
<td>4.50</td>
<td>9.00</td>
<td></td>
<td>13.50</td>
</tr>
<tr>
<td>Total</td>
<td>0.00</td>
<td>013.50</td>
<td>27.00</td>
<td></td>
<td>40.50</td>
</tr>
</tbody>
</table>

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 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).

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 Neubauer 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) was determined with a counter.

Evaluation of blood chemistry

The results of haematological parameters of mature male turkeys 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.

RESULTS AND DISCUSSION

The results of haematological parameters of mature 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 T2 recorded the highest value of 40.33% in PCV and this differed significantly (P<0.05) from turkeys on T1 and T4 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment (Diclair®)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1:0.00</td>
<td>T1:13.50</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.92±</td>
<td>40.33±b</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>10.47±a</td>
<td>12.73±b</td>
</tr>
<tr>
<td>RBC (x10^6/mm³)</td>
<td>4.63±b</td>
<td>4.80±b</td>
</tr>
<tr>
<td>WBC (x10^9/mm³)</td>
<td>26.47±a</td>
<td>40.33±b</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>73.80±d</td>
<td>84.13±b</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>30.73±d</td>
<td>31.87±d</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment (Diclair®)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1:0.00</td>
<td>T1:13.50</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>48.50±b</td>
<td>29.23±c</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>51.50±b</td>
<td>50.37±b</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.00±b</td>
<td>1.07±b</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.00±b</td>
<td>1.33±a</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.00±b</td>
<td>0.00±b</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment (Diclair®)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1:0.00</td>
<td>T1:13.50</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>39.10±b</td>
<td>32.83±a</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>16.03±b</td>
<td>19.12±b</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>70.60±a</td>
<td>70.43±a</td>
</tr>
<tr>
<td>Alkaline phosphatase (iu/L)</td>
<td>38.07±d</td>
<td>41.07±b</td>
</tr>
<tr>
<td>Alanine transaminase (iu/L)</td>
<td>15.83±b</td>
<td>16.43±b</td>
</tr>
<tr>
<td>Aspartate transaminase (iu/L)</td>
<td>63.07±d</td>
<td>110.33±a</td>
</tr>
</tbody>
</table>

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

T2 and T4 in PCV values. The PCV values obtained in this study were within the range of 25.45% reported for birds (Banerjee, 2005; Islam et al., 2004).

Turkeys on T2 recorded the highest value of 12.73 (g/dl) in HB and this differed significantly (P<0.05) from turkeys on T1, T3 and T4 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 (Ihekwumere et al., 2006), but lower than the range of 11.00±2.15 – 14.85±1.42(g/dl) reported for Nigerian local cocks (Ihekwumere et al., 2008). Haemoglobin concentration of blood has been associated with availability of nutrients to the animal’s body (Esonuet al., 2001).

Turkeys on T3 recorded the highest value of 5.03 (x10^6/mm³) in RBC and this differed significantly (P<0.05) from turkeys on T1, T2 and T4. Turkeys on T2 and T1 were similar (P>0.05) to each other in RBC values, but differed significantly (P<0.05) from turkeys on T4. The RBC values obtained in this study were higher than the range of 2-3 (x10^6/mm³) reported for birds (Jain, 1993), but lower than the range of 8-11 (x10^6/mm³) reported in Thai indigenous chickens (Simaraksat et al., 2006) and lower than the highest values 13.35 x 10^6/mm³ and 14.85±2.36(x 10^6/mm³) reported by Ameh (2004) and Ihekwumere 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 (Esonuet al., 2001).

Turkeys on T3 had the highest value of 62.67(x 10^6/mm³) in WBC and this differed significantly (P<0.05) from turkeys on T1, T2 and T4 which 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 Ihekwumere 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; Sowandeet al., 2008).

Turkeys on T4 had the highest value of 87.43 (fl) in MCV and this differed significantly (P<0.05) from turkeys on T1, T2 and T3 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 (Ihekwumere et al., 2008) and higher than the value 41.00 ± 6.5 (fl) reported in broiler chickens (Ihekwumere 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 T4 had the highest value of 27.37(pg) in MCH and this differed significantly (P<0.05) from turkeys on T1, T2 and T3 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.
The result of serum biochemical parameters of male turkeys treated with Diclair<sup>®</sup> 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<sub>1</sub> recorded the highest neutrophil value of 48.50% and this differed significantly (P<0.05) from turkeys on T<sub>2</sub> and T<sub>3</sub> which were similar (P>0.05) to turkeys on T<sub>4</sub> in neutrophil values. There was no significant difference (P>0.05) between turkeys on T<sub>1</sub> and T<sub>4</sub> in neutrophil values. The neutrophil values obtained in T<sub>1</sub> and T<sub>4</sub> were higher than the normal range of 25 – 30% reported for chickens (Banerjee, 2005), whereas neutrophil values obtained in T<sub>2</sub> and T<sub>3</sub> 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 (Banerjee, 2005).

Turkeys on T<sub>3</sub> recorded the highest value of 70.00% in lymphocyte and this differed significantly (P<0.05) from turkeys on T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub> 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<sub>3</sub> 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<sub>4</sub> had the highest value of 4.04% in eosinophil and this differed significantly (P<0.05) from turkeys on T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> which were similar (P>0.05) to each other in eosinophil values. Turkeys on T<sub>1</sub> and T<sub>3</sub> recorded the lowest value in eosinophil (0.00%).

Turkeys on T<sub>2</sub> had the highest value of 1.33% in monocyte and this differed significantly (P<0.05) from turkeys on T<sub>1</sub> and T<sub>3</sub> which were similar (P>0.05) to each other in monocyte values, but differed significantly (P<0.05) from turkeys on T<sub>4</sub>. There was no significant difference (P>0.05) between turkeys on T<sub>2</sub> and T<sub>4</sub> in monocyte values. The lowest value in monocyte was observed in turkeys on T<sub>1</sub> and T<sub>3</sub> (0.00%). Basophils were not detected among the treatment groups.

The result of serum biochemical parameters of male turkeys treated with gonadotrophin (Diclair<sup>®</sup>) 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<sub>3</sub> recorded the highest value of 41.97 (mmol/L) in serum urea and this differed significantly (P<0.05) from turkeys on T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub> which were also significantly different (P<0.05) 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; Odugwu et al., 1999; Ahamefule et al., 2005).

Turkeys on T<sub>2</sub> recorded the highest value of 19.12 (mmol/L) in serum calcium and this differed significantly (P<0.05) from turkeys on T<sub>1</sub> and T<sub>3</sub> 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<sub>4</sub>. There was no significant difference (P>0.05) between turkeys on T<sub>1</sub>and T<sub>3</sub> 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<sub>2</sub> and T<sub>3</sub> indicates probable electrolyte 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<sub>1</sub> recorded the highest value of 70.60mg/dl in cholesterol and this differed significantly (P<0.05) from turkeys on T<sub>3</sub> which were similar (P>0.05) to turkeys on T<sub>4</sub> in cholesterol values. There were no significant differences (P>0.05) among turkeys on T<sub>1</sub>, T<sub>2</sub> and T<sub>4</sub> 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<sup>®</sup> injection was safe for the turkeys, so turkeys treated with Diclair<sup>®</sup> injection may not face the risk of myocardial infarction usually associated with high blood cholesterol content and emaciation due to low serum cholesterol (Frandsen, 2002).

Turkeys on T<sub>3</sub> recorded the highest value of 44.13iu/L in Alkaline phosphatase and this differed significantly (P<0.05) from turkeys on T<sub>1</sub> which were similar (P>0.05) to each other in Alkaline phosphatase values. The Alkaline phosphatase values obtained in this study were lower than the mean value 482.5(µ/L) reported for chickens (Kaneko et al., 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 Aspartate transaminase values would signify necrosis or myocardial infarction which are all indicators of drug toxicity or harmful chemical in the body (Nelson and Cox, 2005).

Turkeys on T<sub>3</sub> 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_3. 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 Aspartate 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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