Effect Of Clomiphene Citrate On Hematology And Serum Biochemistry Of Nigerian Indigenous Chicken

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Abstract: The effect of different doses of clomiphene citrate on haematology and serum biochemistry of Nigerian indigenous chicken were evaluated at the poultry Unit, Department of Animal Science and Fisheries, Abia State University, Umuahia, using 48 sexually matured (26 weeks old) local cocks, each group was divided into 4 treatment groups of 4 matured chickens, in a completely randomized design with 3 replications. Four levels of Clomiphene citrate treatments represented as T1 (0mg), T2 (10.00mg) T3 (20.00mg) and T4 (30.00mg) were administered to the birds. Haematology and Serum biochemical indices of the treated birds were determined at the end of the study. The result showed that there were significant differences (P<0.05) among the treatment groups in haematology and serum biochemical parameters except for the following parameters: hemoglobin red blood cell, mean corpuscular volume of the birds. It was concluded that the administration of Clomiphene citrate (Clomid®) led to changes in haematology and serum biochemistry in Nigerian indigenous chicken which showed that clomiphene citrate can be considered safe for chicken. 10mg and 20mg levels of clomiphene citrate can bring about improved hematological indices of Nigeria indigenous chicken

Keywords: Nigerian indigenous chicken, Cocks, Clomiphene citrate, Hematology, Serum biochemistry

1 INTRODUCTION

Indigenous chicken constitutes 80% of the 120 million poultry types raised in the rural areas in Nigeria [22]. They are known to possess qualities such as the ability to hatch on their own, brood and scavenge for major parts of their food and possess immunity from endemic diseases and are self-reliant and hardy birds with the capacity to withstand harsh weather conditions and adapt to adverse environments. Their products are preferred by the majority of Nigerians because of the pigmentation, taste, leanness and suitability for special distress [9]. Their outputs (egg and meat) are readily available for villagers and people in urban and semi urban areas and thus serve as a good source of protein in their diet and in the same vein, serve as a good source of income. Regrettably, reproduction efficiency among domesticated poultry is still very low in developing countries. These deficiencies have given rise to the development of the numerous techniques of reproduction such as artificial insemination and application of fertility drugs to enhance semen and egg production. One of such drugs is Clomiphene citrate (Clomid(R)).

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Clomiphene citrate is a non-steroidal anti estrogen, is a selective estrogen receptor modulator (SERM) of the tripheenylethylene group that has been the mostly prescribed drug for ovulation induction to reverse anovulation or oligoovulation (WHO, 2015), It also possesses weak estrogen properties. The degree of agonistic and antagonistic activity observed depends on the species, organ, tissues or cell type that is being examined and on the end point assay chosen. The effectiveness of Clomid® in super ovulation of WAD goats was demonstrated by Iheukwumere et al., [10]. The results of this study showed that Clomid® treatments were effective in making super ovulation in WAD goats in Nigeria but a higher dose of 0.40ml clomid® showed excellent result than the other dosage levels. Other studies [10],[7],[6],[19] [22],[11] have shown CC to be correlated with enhancement of embryo generation, induction of ovulation in cases of amenorrhea and oligomenorrhea in human and treatment of several clinical complications involving endometrial hyperplasia, and precocious puberty in the human female. However, the correlation of Clomiphene citrate with chicken haematology and serum biochemistry is scarce in literature. Blood, besides playing important role in the transportation of nutrients, metabolic wastes and gases around the body [23] also represents a means of assessing clinical and nutritional health status of animals [15]. It has been shown that data from blood profiles could be exploited in the improvement of chicken stocks (Ladikun et al., 2008). Studies have shown correlation of blood parameter (haemato-chemical parameter inclusive) with factors such as nutrition, gender and rearing temperature [3],[13] and other clinical status of animal. Moreover, the haemato-biochemical profiles are most commonly used in nutritional studies for chickens [1] and other birds [4]; [16],[17] but have been rarely applied in reproductive studies as it concerns use of clomiphene citrate in chickens. From the foregoing, how administration of clomiphene citrate to local chickens in Nigeria affects the hematology and serum biochemistry of the chickens needed to be determined. This study has therefore, evaluated some vital blood and serum biochemical parameters of local chicken administered with different levels of clomiphene citrate with a view to creating a baseline data on effect of

clomiphene citrate on the haematology and serum biochemistry of local chickens in Nigeria.

2. MATERIALS AND METHODS

2.1 Location of the Study

This experiment was conducted at the Poultry Unit of the Department of Animal Science and Fisheries of Abia State University, Umuahia Campus located within the South-Eastern Agro-ecological zone of Nigeria which lies between latitudes 50 and 60 N and longitude 60 and 70E at an altitude of 122m (400ft) above sea level [2].

2.2 Experimental animals

The experimental animals used were 48 matured local cocks, and 48 matured local hens purchased from an open market in Umuahia North Local Government of Abia State. The previous management background of the birds were not known, so the birds were housed in battery cage, quarantined, de-wormed and vaccinated to boost their immune system against any disease and then trained twice a day (morning and evening) for four weeks for semen collection by the massage technique as described by Hafez (1990). Grower mash diet (comprising Crude protein (15 %), Crude fibre (7% max), Calcium (1% min), Av. Phosphorus (0.45%), Lysine (7%), Methionine (0.35%min) and Energy Kcal/kg (2600 Kcal/kg) was fed to the birds ad-libitum for the duration of the experiment.

2.3 Experimental design and treatment application

The 48 cocks and the 48 Hens were randomly divided into 4 treatments each for the males and females. Each treatment group had 12 birds coded T1, T2, T3, T4each T1, T2, T3, and T4 for the males and females respectively in a completely randomized design. The twelve birds were further replicated three times with 4 birds per replicate. The birds received 4 levels of water administered Clomiphene citrate (0mg, 10.00mg, 20.00mg, and 30.00mg representing T1T2T3 and T4 respectively) as treatments with treatment one (T1), which contained no Clomid® serving as the control.The groups received the clomiphene citrate as follows:

Table 1 *Levels of Clomiphene citrate received by each treatment groups*

T_1	T_2	T_3	T_4
Day 1 – 0	10	20	30
Day 2 – 0	10	20	30
Day 3 – 0	10	20	30
Day 4 – 0	10	20	30
Day 5 – 0	10	20	30

Seven days after Clomiphene citrate administration, blood collection and hematological and serum biochemical evaluations were carried out. The model is represented as follows:

$Y_{ij} = \mu + G_1 + e_{ij}$

Where

Y_{ij} = single observation

G_1 = effect of the treatment (i= 1 2 3 & 4)

e_{ij} = random error

2.4Data Collection

Data were collected on two major parameters including: *Hematology* **(**PCV (%), hemoglobin (g/dl), Red Blood Cell ($x10^{12}$/L) and White Blood Cell differential count (/µl), and *Serum biochemistry*(alkali phosphate (iµ/l), alamine transminase(iµ/l), aspartate transaminase (iµ/l), creatinine (mmol/l), Urea (mmol/l), glucose (mmol/l), albumin (g/l), globulin(mmol/l) and serum protein(g/100ml),

2.5 Hematological Assay

2.5.1 Blood collection: Blood was aspirated from the cocks and hens following a wing vein puncture using a 2ml syringe. Blood was collected between 8am and 9am. 2mls of each blood sample were poured into a bijou bottle containing ethylene diamine tetra-acetic acid (EDTA) for hematological evaluation. The remaining samples were allowed to coagulate to produce sera for blood chemistry analysis.

2.5.2 Blood Analysis. The blood collected from the birds was analysed within 3 hours of collection for Packed Cell Volume (PCV) and hemoglobin (Hb). The hemoglobin and the PCV were determined according to method described by Jain (1986). Erythrocytes (RBC) and Leucocytes counts were done in a hemacytometer chamber under a light microscope with Henick's diluents to obtain a 1:200 dilution of blood. The number of leucocytes was thereafter estimated as total WBC = number of cells to total WBC x 2000. The various hematological indices like Mean Corpular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated from the result obtained from PVC Hb and RBC. The white blood cell indices were achieved using blood smear stained with Wright's dye and each type of cell (neutrophil, lymphocyte, eosinophil, monocyte and basophil) and counted with counter.

2.6 Blood Chemistry

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 mentioned earlier. Creatinine and urea concentration were determined following methods described by Baker and Silverton (1986), aspartate transaminase, alamine transaminase and alkaline phosphatase methods as described by Rej and Hoder [18]. The Standard flame Photometry using Gallenkamp analysis was used to determine serum sodium (Na^+), calcium(c^+) and potassium (K^+) ions, while bicarbonate and chloride ions were assayed according to the methods of Baker and Silverton [5]. Serum protein was determined by Goldbery refractometer method as described by Kohn and Allen [12]. Albumin and globulin were determined using bromocresol green (BCG) method as described by (Rodrguez 2006).

2.7 Data Analysis

Data collected on hematological and serum biochemical parameters of the local birds were subjected to analysis of

variance (ANOVA), using the technique of Steel and Torrie [21] and differences between paired means were tested using Duncan's New Multiple Range Test as Outlined by Obi [14].

3.0 RESULTS AND DISCUSSION

Effect of Clomiphene Citrate on Chicken Haematology

The result of the hematological parameters (Table 2) showed significant differences ($P < 0.05$) among the treatment groups in packed cell volume, hemoglobin, white blood cell, red blood cell, mean corpuscular volume, mean corpus hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values. Birds on 10mg clomiphene treatment showed highest value in PVC 30.24%, birds on 30mg clomiphene citrate treatment recorded the lowest 27.15% in PVC, birds on 30mg clomiphene citrate treatment recorded the highest value in hemoglobin (Hb) 15.85(g/dl). The highest value in Red blood cell was recorded by birds on 20mg clomiphene treatment; birds on 30mg clomiphene treatment recorded the highest value in Mean corpuscular volume 41.00.(fl) The highest value recorded in Mean corpuscular hemoglobin and Mean corpuscular hemoglobin concentration was in the bird on 30mg clomiphene treatment. While birds on control treatment recorded the highest value in lymphocytes 58%. The highest value was recorded in birds on T_4 (30mg) Clomiphene treatment in Neutrophils value 30%. Treatments T_1, T_2, T_3 and T_4 recorded the same value in eosinophils, (6%), basophils (1%) and monocytes (5%) respectively. The packed cell volume of local chicken treated with clomiphene citrate showed higher value at T_2 (10mg clomiphene), 30.24%. The values ranged from 27.15 – 30.24%. This range is comparable with the average value (28.00%) reported by Ameh (2004). However, it was lower than 35.54% reported by Iheukwumere*et al*., [10] in local chickens. The hemoglobin values in T_3 and T_4 were similar ($P > 0.05$). The values of haemoglobin T_1 (12.00g/dl) and T_2 (14.04g/dl) obtained in this study were not significantly different ($P < 0.05$) from each other. And these values were within the average of (9.37g/dl) reported by Islam *et al*., (2004) for local chicken reared in Sahel region of Africa and in Bangladesh and broiler chickens reported by Iheukwumere *et al*., (2002). These values were also within the average of 11.25mg/100ml reported by Iheukwumere and Herbert (2003) in broiler chickens. Hemoglobin concentration of blood has been associated with availability of nutrients in animal body (Esonu *et al*., 2001). The Red blood cells (RBC) value of the control T_1, T_3, and T_4 did not tally with the average value reported by Ikhimioya *et al*., (2000), Kundu *et al* (1993) and Arijeniwa *et al* (1999), but higher than values of 13.35% reported by Ameh (2004) and Iheukwumere *et al*., (2006) in local chicken. The differences observed in this study may be as a result of Clomiphene citrate administration, physiology and nutritional status of the birds. There were significant different ($P < 0.05$) among the treatment groups in mean corpuscular volume (MCV).Treatment four (T_4) which ranked highest in value (41.00fl) of MCV was significantly different ($P < 0.05$) from all other treatment groups.Treatment$_2$ (T_2) was not significantly different ($P > 0.05$) from T_3 but they differed significantly from T_1 Mean corpuscular volume of local chicken increased as the dosage of Clomid increased. Similar values of 41.00fl was recorded by Iheukwumere [10], but higher than 27.32fl reported by Ameh (2004) for local chicken administered with Clomid® Mean corpuscular volume (MCV) is an indicator of the average volume of Red blood cell, (Lazzaro, 2003). There were significant differences ($P < 0.05$) among the treatment groups in MCH. The values recorded for MCH followed the same trend with MCV. The highest value 33.60pg recorded in T_4, while the lowest value of 22.30pg was recorded in T_1. The value MCH in birds treated with clomiphene Citrate were comparable with 33.00pg reported by Iheukwumere *et al*., [10] in broiler chicken. The mean corpuscular hemoglobin concentration (MCHC) of birds in T_4 was not significantly different ($P > 0.05$) from T_3 (20mg) clomiphene treatment, though it(30mg treatment) had the highest value recorded, these values were lower than the average value of 41.02g/dl reported by Ikhimioya *et al*., (2000) and Kundu *et al*., (1993) and also not in line with the values(39.15g/dl) reported by Arijeniwe *et al*., (1999) but higher than values(18.0g/dl) reported by Ameh (2004) and Iheukwumere *et al*., (2006) in local cocks. The mean corpuscular hemoglobin concentrations (MCHC) were significantly different ($P < 0.05$) in all the treatment groups. Treatment 4(T_4) recorded the highest value of 35.0g/dl, followed by T_3, T_2 and T_1 respectively. The control was lower than the values reported by Islam *et al.,* (2004). This shows that the level of MCHC increase as the level of clomiphene citrate increases. The observed variations observed in MCV, MCH and MCHC values may not be unconnected to the differences in season, nutritional, and physiological status of the animals as suggested by Esonu *et al*., (2001). The values for white blood cell showed significant difference ($P < 0.05$) in the treatment groups, though they were not within the range reported by Iheukwumere *et al*., (2002) for chicken. For white blood cell differential counts (Lymphocytes, Neutrophils, Basophils, Monocytes and Eosinophils), higher value was observed for Lymphocytes in the control 58.05%. Basophils also recorded higher values within T_2 and T_4 whereas Neutrophils were highest in cocks on 20mg clomiphene treatment and 30mg clomiphene treatment (30.00). Eosinophils and Monocytes in all the groups did not differ significantly ($P > 0.05$). Neutrophils have phagocytic and bactericidal capabilities which mean that they play an important role in inflammatory condition. They are very important for defense whenever acute infection is present (Banerjee, 2007).

Effect of Clomiphene Citrate on Chicken Serum

Biochemistry

There were significant differences ($P < 0.05$) among the treatment groups in urea level in the serum biochemistry. Birds treated with 10mg clomid® recorded highest in the value for urea (32.62mmol/100ml) and this differed significantly ($P < 0.05$) from birds on control treatment, T_3 andT_4. The value for 20mg(T_3) clomiphene treated birds was not significantly different ($P > 0.05$) from the 30mg clomiphene treated birds(T_4) in urea value, but these two values T_3 and T_4 were significantly different from the value gotten from the control treatment. The lowest value for urea in serum biochemistry (29.35 mmol/100ml) was observed in birds on the control treatment. These values were lower than values recorded by Iheukwumere et al., (2006). Cortada et al., (2000) reported that sharp increase in serum urea level could result in gonadal degeneration and infertility, with reduced sperm production and loss of libido. There were significant differences ($P<0.05$) among the treatment groups in

creatinine content of serum. Birds onT1 were not significantly different (P > 0.05) from birds on T2, but T1 and T2 birds were significantly difference (P < 0.05) from T2 and T3 birds. 20mg clomiphene treated Chicken were not significantly different (P > 0.05) from T4. However, the lowest value (70.00mmol/L) for creatinine was observed on the T4 while the highest value (135.55mmol/L) was recorded in T1. A major source of creatinine in the blood is from the muscle when wasting occurs and creatinine phosphate catabolized, (Bale et al., 2000). There were significant differences (P < 0.05) among the treatment groups in glucose constituent of serum biochemistry. Birds in T1 recorded the highest value in serum glucose (160.55mmg/100ml) and this differed significantly (P< 0.05) from birds on T2, T3 and T4. Treatment2(T2 clomiphene citrate treated birds were also significantly different from 20mg clomiphene treated cocks, 20mg clomiphene treated birds were also significantly different (P < 0.05) from 30mg clomiphene treated birds their glucose serum level. Bird on T3 recorded the lowest value in serum glucose (75.75 g/100ml). There was significant difference (P < 0.05) between the control and the other three treatment groups in serum total protein. Cocks on 10mg clomiphene treatment, cocks on 20mg clomiphene treatment and birds on 30mg clomiphene treatment were not significantly different (P > 0.05) in serum total protein. Birds on 30mg clomiphene treatment recorded the highest value (6.61g/100ml) in total serum protein, while birds on control treatment recorded the lowest value (6.20g/100ml). Values recorded in this work were lower than the record (8.5g/100ml) reported by Iheukwumereet al., [10].) on serum total protein, it has also been reported that serum, urea, creatinine and total protein contents depend on the quality of protein supplied in the diet. Birds on T2 10mg clomiphene treatments recorded the highest value in Albumin level in the serum (3.80g/100ml) and this differed significantly (P < 0.05) from T3, and birds on T4. Treatment3 (T3) and T4 were significantly different from cocks in T1. The lowest level of Albumin level was recorded in T1, (2.29g/ml). The ranges of albumin level recorded in this experiment were in the same range reported by Iheukwumereet al., [10]. There were significant differences (P < 0.05) among the treatment groups in globulin constituent of serum biochemistry. Birds in T1 recorded the highest value (3.72g/ml) in globulin and this differed significantly (P < 0.05) from birds on T2, T3 and T4. Birds on T3 and T4 were similar. but differed significantly (P < 0.05) from T3 and T1. Birds on T2 recorded the lowest value (2.87g/ml) in globulin constituent of the serum. The recorded values were within the recorded values by Iheukwumere et al., [10].

Table 2.0 *Effect of Clomiphene Citrate on Hematology and Serum Biochemistry of Nigerian Indigenous Chicken*

Parameters	Treatment (clomiphene citrate)				
	T_1 0.00 mg	T_2 10mg	T_3 20mg	T_4 30 mg	SEM
Hematology					
PCV(%)	29.00^{b}	30.24^{a}	28.00^{c}	27.15^{d}	0.66
Hb (g/dl)	12.00^{c}	14.04^{b}	15.45^{a}	15.85^{a}	0.87
RBC (x10^{6}µ)	8.24^{b}	8.26^{b}	15.58^{a}	15.75^{a}	2.14
WBC (x10^{3}µ)	12.45^{a}	9.35^{b}	9.40^{b}	9.50^{b}	0.76
MCV(fl)	30.14^{c}	35.15^{b}	36.45^{b}	41.00^{a}	2.23
MCH(pg)	22.30^{b}	33.35^{a}	33.18^{a}	33.60^{a}	2.77
MCHC(g/dl)	27.00^{c}	33.00^{b}	34.15^{a}	35.00^{a}	1.81
Lymphocytes (%)	58.05^{a}	56.00^{b}	54.00^{c}	54.00^{c}	0.97
Neutrophils (%)	28.00^{c}	29.00^{b}	30.00^{a}	30.00^{a}	0.48
Eosinophils (%)	6.00^{a}	6.00^{a}	6.00^{a}	6.00^{a}	0.00
Basophils (%)	1.00^{b}	2.00^{a}	2.00^{a}	2.00^{a}	0.50
Monocytes (%)	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	0.63
Serum Biochemistry					
Urea (mmol/L)	29.35^{c}	32.62^{a}	30.65^{b}	30.55^{b}	0.44
Creatinine(mmol/)	85.55^{a}	84.50^{a}	70.20^{b}	70.00^{b}	2.65
Glucose(mmol/L)	160.55^{a}	98.40^{b}	75.75^{d}	76.50^{c}	13.00
Serum total protein (g/100ml)	6.20^{b}	6.55^{a}	6.60^{a}	6.61^{a}	0.07
Albumin (g/100ml)	2.29^{c}	3.80^{a}	3.30^{b}	3.40^{b}	0.29
Globulin (g/100ml)	3.72^{a}	2.87^{c}	3.35^{b}	3.37^{b}	0.00
Alkaline phosphate (µ/l)	73.50^{b}	75.65^{a}	43.50^{c}	44.50^{c}	5.79
	9.26^{b}	8.45^{c}	11.30^{a}	11.3^{a}	0.80
Alkaline transaminase (µ/l)	9.27^{b}	8.50^{c}	11.30^{a}	11.40^{a}	

^{Abcd} *means in the same row with different superscripts are significantly different (P < 0.05).*

There were significant differences (P < 0.05) among the treatment groups in alkaline phosphate content on the serum of birds Cocks on recorded the highest value (75.65 µ/l) which was significantly different (P< 0.05) from T1, T3 ,and T4. Treatment3 and T4 were similar (P > 0.05) to each group but difference significantly (P < 0.05) from T1. This report is in line with the report of alkaline phosphate recorded by Iheukwumereet al., [10] on Aspartase transaminase level in the serum biochemistry. There were significant differences (P < 0.05) among the treatment groups on alkaline transaminase. Cocks on T4 recorded the highest value (11.40µl). This is similar (P > 0.05) to cocks on T1, but differs significantly (P < 0.05) from cocks on T1 and cocks on T2 which are also significantly different (p<0.05) from each other. This record aligned with values recorded by Iheukwumereet al [10] on alkaline transaminase. Birds on T4 and T3 recorded highest values in aspartate transaminase 11.30 each and there was not significantly different (P > 0.05)

between them. These two treatments T3 and T4 were significantly different ($P < 0.05$) form T1 and T2 respectively. Birds treated with 10mg clomiphene citrate recorded the lowest value in aspartate transaminase. An increase in an alkaline phosphatase, alanine transaminase and aspartate transaminase values would signify necrosis or myocardial infection which is an indicator of drug toxicity or harmful chemical in the body (Nelson and Cox, 2005). Therefore, 10mg and 20mg level of Clomid can be considered safe for chicken since the level of alkaline phosphatase reduced with 10mg and 20mg clomiphene citrate inclusion.

CONCLUSION

The administration of Clomiphene citrate (Clomid®) led to changes in hematology and serum biochemistry in Nigerian indigenous chicken which showed that clomiphene citrate can be considered safe for chicken; and could be used to enhance hematology, serum biochemistry and semen production in Nigeria indigenous chicken. Therefore administration of Clomiphene Citrate at 10mg or 20mg level can bring about improved reproduction in Nigeria indigenous chicken without negative effects.

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