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Blood profile and Internal Organ Dynamics of Mature Nigerian Indigenous Cocks administered Exogenous Follicle Stimulating Hormone and Luteinizing Hormone (Menotropin)

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Abstract
This study was conducted to examine the effect of the intramuscular administration of Menotropin on the blood profile and internal organ dynamics of mature Nigerian Indigenous cocks during spermatogenic stimulation. 72 mature Nigerian indigenous cocks were randomly divided into 4 groups of 18 cocks in a completely randomized design (CRD) experiment. Varying doses of Menotropin at 0.0mL, 0.10mL, 0.20mL, and 0.30mL, representing T_1, T_2, T_3 and T_4, respectively were administered. At the start of the experiment, the red blood cell (RBC) ranged from 4.10–5.07x10^6mm^3, packed cell volume (PCV) ranged from 36.60 to 40.05%, the haemoglobin ranged from 11.30 to 12.20g/dl, White blood cell (WBC) 214.06-240.73x10^3mm^3, Platelet 128.00-157.00x10^9/l, Mean Corpuscular Volume (MCV) 128.56-158.56fl, while mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) ranges from 25.39-33.00pg and 31.00-32.75g/dl, respectively. At the end of the experiment, RBC, haemoglobin, platelet, and MCV showed significant improvement ($p < 0.05$) across the treatment groups; while the PCV, WBC, and MCHC significantly reduced ($p<0.05$) compared with the control group. At the start of the experiment, total protein ranges from 3.80 to 4.60g/dl, albumin 2.25 to 3.30g/dl, urea and cholesterol ranged from 9.65 to 12.30mg/dl and 121.50 to 138.00mg/dl, respectively. At the end of the trial, the total protein, albumen, and cholesterol values were significantly ($p < 0.05$) reduced, while the urea significantly ($p<0.05$) increased. The results indicated that apart from MCH, globulin, glucose, and creatinine, other haematological and serum biochemical parameters of mature Nigerian indigenous cocks may be affected by exogenous treatment with FSH and LH above 0.10ml. Increasing the Menotropin up to 0.20mL had deleterious consequences on the kidney and gallbladder. Although the values obtained fall within the normal range for chicken, the variations observed in the blood parameters suggest the need to constantly and closely monitor the blood profile of Nigerian indigenous cocks during treatment with exogenous FSH and LH to avoid deleterious consequences on the blood profile and internal organs.

Keywords: Menotropin, FSH, Luteinizing Hormone, Blood profile, Internal organs, Indigenous cocks.

Introduction

The poultry industry is one of the fastest-growing segments of animal agriculture in Nigeria. Rapid human population growth and low protein intake are some of the major problems facing developing countries. Poultry meat and eggs present one of the most affordable animal protein sources to mitigate the problems of protein malnutrition in Nigeria (Oguike, *et al.,* 2000; Ajayi, *et al*., 2014; Agida, *et al.*, 2020). The Nigerian indigenous chicken has many comparative advantages when compared to the exotic breeds (Nosike, *et al.,* 2017; Obike, *et al*., 2019). These indigenous breeds of cocks possessed desirable traits such as ease of adaptability to tropical and rural environments tolerant to heat stress, disease resistance, increased productive capacity, survival on little or no feed supplementation, and adjust favorably to fluctuation in feed availability (Oguike, *et al.,* 2000). These comparative advantages and many advantageous gene complexes could be harnessed in the development of meat and egg-type chicken suitable for use in the humid tropics (Ajayi, *et al*., 2011; Yasks, *et al.,* 2017). Among the major Nigerian indigenous cocks are the naked neck, frizzled, and normal feather chicken.

Blood profiles of animals provide valuable information on the behavior, overall physiology, and homeostatic mechanisms of an animal. (Amaduruonye, *et al*., 2021; Jiwuba, *et al*., 2022). Changes in blood profiles are routinely used to determine stress due to environmental, nutritional, and pathological factors. Blood profile and

the general physiology of farm animals have been reported to be largely affected by several factors such as nutrition, drugs, hormones, environment, and other physiological factors (Onunkwo, *et al*., 2019; Jiwuba, *et al*., 2021).

Menotropin, a gonadotrophin preparation is a mixture of pituitary gonadotropin, consisting of 75 International Unit (IU) follicles stimulating hormone (FSH) and 75 International Unit (IU) luteinizing hormone (LH) in a ratio of 1:1. In human, Menotropin is used in the treatment of infertility in both males and females. It is utilized in the treatment of hypogonadism, resulting in delayed puberty, low sperm count, and increases testosterone level in males. In females, Menotropin works by stimulating the ovary, follicular growth, and ovulation. The injection can be administered intramuscularly or subcutaneously (Alviggi, *et al.,* 2018). Gonadotrophins (Follicle Stimulating Hormone and Luteinizing Hormone) are gonadotrophic hormones produced by the anterior pituitary. Follicle-stimulating hormone (FSH) is a glycoprotein gonadotropin secreted by the anterior pituitary in response to gonadotropin-releasing hormone released by the hypothalamus. Luteinizing hormone (LH) stimulates the secretion of sex steroids from the gonads (Iheukwumere, *et al*., 2004; O'Shaughnessy, 2014). In male reproduction, LH stimulates testosterone production from the Leydig cells of the testes. FSH and LH bind to receptors in the testis and ovary and regulate gonadal function by promoting sex steroid production and gametogenesis (O'Shaughnessy, *et al.,* 2010a; Oduwole, *et al.,* 2021). FSH stimulates testicular growth and enhances the production of androgen-binding protein by the Sertoli cells, necessary for sustaining the maturation of sperm cells (O'Shaughnessy, *et al*., 2010b; Huhtaniemi, 2018). Hence, the initiation of spermatogenesis and maturation of spermatozoa requires FSH and LH. These hormones are chemical messengers produced by the endocrine gland, transported through the blood to the target organs and their receptors to affect a particular chemical or physiological process. Therefore, this study aimed to examine the combined effects of exogenous Gonadotrophins (FSH and LH) on the blood profile and internal organ dynamics of mature Nigerian indigenous cocks.

Materials and Methods

Experimental Location

This research was conducted at the Poultry Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The area is located in the South-Eastern part of Nigeria on latitude $5^0 27^1$ north, longitude 7^0 32' East, an altitude of 123m above sea level with an annual rainfall of 2177mm, temperature of 22^0C – 36^0C, and relative humidity of 50 – 90%. It is situated within the humid rainforest zone of West Africa, characterized by a long-duration of rainy season (March-October) and short period of dry season (November - February). Climatic data were collected from the Meteorological Center of the National Root Crop Research Institute, Umudike, Abia State (NRCRI, 2010).

Experimental Animals and Management

Ninety (90) matured indigenous cocks aged 7-8 months comprised of Frizzle feather, Naked Neck, and Normal feathered, purchased from the rural farmers in Ngwa, Ikwuano, and Umuahia, Abia State, Nigeria were used for this study. Two weeks of pre-experimental period were used to acclimatize the cocks to the experimental procedures. Thereafter, Seventy-two (72) indigenous cocks were selected and randomly assigned to 4 treatments replicated 3 times with 6 cocks per replicate in a completely randomized design (CRD) experiment that lasted for 2 months. They were managed in deep litter pens throughout the experimental period and concentrated diet with water-supplied *ad-libitum*. Routine management practices were carried out appropriately. The cocks in each treatment were fed the same experimental diet and administered different doses of FSH and LH injection intramuscularly on the thigh using a 1mL syringe with 0.1mL graduation at 2 weeks intervals. The blood samples were collected at the start and end of the experiment. The conduct of the experiment was in line with the ethical guidelines on the use of animals for experimentation provided by the Animal Welfare and Ethics Committee, Michael Okpara University of Agriculture, Umudike, Nigeria. The experimental diet for the cocks is shown in Table 1.

Experimental Design

The study was a Completely Randomized Design (CRD) with four treatments consisting of T_1, T_2, T_3 and T_4. T_1 administered no FSH and LH served as the control. Eighteen (18) matured cocks were randomly assigned to each treatment and replicated 3 times with 6 cocks per replicate. Menotropin, a gonadotropin preparation which is a mixture of pituitary gonadotropin, consisting of 75 IU follicles stimulating hormone and 75 IU luteinizing hormone in the ratio of 1:1 were sourced from a reputable pharmacy in Umuahia, Abia State. Thereafter, the FSH and LH were intramuscularly administered on the thigh of the cocks between 7.00 and 8.00 am local time after feeding on each day of administration at two weeks intervals for 8 weeks. Each cock on T_1 were intramuscularly administered Menotropin at 0.0mL, T_2 0.10mL Menotropin, T_3 0.20mL Menotropin, and T_4 0.30mL Menotropin respectively. The field work lasted for 12 weeks.

The experimental model is as follows:

$$Y_{ij} = U + T_i + e_{ij}$$

Where:

Y_{ij} = individual observation on the broiler characteristics.

μ = overall mean

T_i = treatment effect

e_{ij} = Experimental error assumed to be independently, identically and normally distributed with zero means and constant variances.

Data Collection

Haematology

Four (4) birds were randomly sampled from each replicate at the start and at the end of the experiment for blood collection and analysis. Four (4) blood samples of two milliliters each were collected from each replicate for hematological examination. A 5 ml syringe fitted with a sterile needle was used to collect 2 ml of blood from each cock and quickly transferred to ethylene diamine tetraacetic acid (EDTA) sample bottles. The EDTA sample bottles were shaken gently to prevent clotting. The following hematological indices were determined: hemoglobin, packed cell volume, red blood cell, and white blood cell, platelet, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Packed cell volume (PCV) was determined by the micro-hematocrit method as described by Kahn, *et al.* (2010). The platelets were determined according to the method as described by Bain *et al*. (2017). The Hemoglobin (Hb) concentration was determined using a spectrophotometer through the Cyanomethanoglobin method as described by Feldman *et al.,* (2000). Red blood cell (RBC) and white blood cell (WBC) counts were determined using the Neubauer-hemocytometer method as described by Bain *et al*. (2017). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean cor-puscular hemoglobin concentration (MCHC) were calculated as described by Douglas and Wardrop, (2010).

Serum Biochemistry

Four (4) birds were randomly sampled from each replicate at the start and at the end of the experiment for blood collection and analysis. Four (4) blood samples of two milliliters each were collected from the cocks in each replicate for serum biochemistry. The blood samples were immediately transferred into the sample bottles without anticoagulants. The blood samples were allowed to clot for 30 minutes, after which it was centrifuged at 3000 revolutions per minute for 10 min in order to separate the serum from the clot. After the centrifugation, the serum was carefully collected and transferred into a clean sample bottle, and the blood, and chemistry tests were performed thereafter. The following blood chemistry indices were collected, prepared, and analyzed colorimetrically, and spectrophoto-meterically according to standard clinical biochemistry procedure as described by Kahn, *et al.,* (2010): Total protein, Albumin, Globulin, Glucose, Blood Urea, Cholesterol, and Serum Creatinine. The blood samples for Hematology and Serum biochemistry were analyzed at the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike.

Internal Organ Dynamics

At the end of the experiment, four (4) cocks sampled from each replicate were weighed, slaughtered, and allowed to bleed. The internal organs; the Heart, Liver, Kidney, Gizzard, Spleen, Gallbladder, and pancreas were collected, weighed, and recorded. All weight measurements were done using a 5kg digital weighing scale (Camry EK 5055 Digital Scale) of 0.01 sensitivity. The absolute and relative organ weights were determined and recorded.

Statistical Analysis

Data collected on the different parameters were subjected to analysis of variance (ANOVA) in accordance with the methods of Steel and Torrie (1980). Significant means were separated according to Duncan's Multiple Range Test at a 5% level of significance (Duncan, 1955).

Results and Discussion

The effects of graded dosage of intramuscular administration of Menotropin on the Haematology of mature Nigerian indigenous cocks are presented in Table 2. At the start of the experiment, the red blood cell (RBC) ranged from 4.10–5.07x$10^6$$mm^3$, packed cell volume (PCV) ranged from 36.60 to 40.05%, the haemoglobin ranged from 11.30 to 12.20g/dl, White blood cell (WBC) 214.06-240.73x$10^3$$mm^3$, Platelet 128.00-157.00x10^9/l, Mean Corpuscular Volume (MCV) 128.56-158.56fl, while mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration ranges from 25.39-33.00pg and 31.00-32.75g/dl, respectively. At the end of the experiment, red blood cell, haemoglobin, platelet, and MCV significantly ($p<0.05$) increased; while the packed cell volume, white blood cell, and MCHC significantly reduced ($p<0.05$) following the intramuscular injection of FSH and LH on the cocks compared with the control group. The major function of red blood cells is the transport of hemoglobin, which in turn carries oxygen and carbon dioxide between the lungs, cells, tissues, and organs of the body. Platelets functioned in blood clotting, haemostasis, repair of the ruptured blood vessels, and in body defense mechanisms (Guyton and Hall, 2006; Sembulingam, and Sembulingam, 2012; Frandson, *et al*., 2018). The resultant increase in the red blood cell, haemoglobin, MCV, and platelet compared with the cocks in the control group inferred that the intramuscular administration of Menotropin at this dosage on the cocks impacted favorably on the oxygen-carrying capacity, the blood clotting efficiency, haemostasis and on the cock's body defense mechanisms. From these observations, it could also be seen that the intramuscular injection of Menotropin at 0.10ml, 0.20ml, and 0.30ml on the cocks reduced some haematological parameters; suggesting the need to constantly monitor these haematological parameters during the treatment with exogenous FSH and LH. Although these haematological parameters are within the normal range as observed by Banerjee, (1998a) and Ladokun, *et al*., (2008), the variations observed following the administration of Menotropin injection indicated that serious care should be taken when increasing the dosage of intramuscular administration of FSH and LH up to 0.30mL to stimulate spermatogenesis on mature Nigerian indigenous cocks to avoid deleterious consequences on some haematological parameters.

The effect of graded dosage of intramuscular injection of Menotropin on the serology of mature Nigerian indigenous cocks is presented in Table 3. At the start of the experiment, total protein ranges from 3.80 to 4.60g/dl, albumin 2.25 to 3.30g/dl, urea and cholesterol ranged from 9.65 to 12.30mg/dl and 121.50 to 138.00mg/dl, respectively. At the end of the experiment, significant differences ($p<0.05$) were observed on the total protein, albumin, urea, and cholesterol; while the other serological parameters were not significantly affected. The intramuscular injection of Menotropin at 0.10mL, 0.20mL, and 0.30mL significantly ($p<0.05$) reduced the serum total protein, albumen, cholesterol and significantly ($p<0.05$) increased the serum urea compared with the control group. This implied that the intramuscular administration of Menotropin at this dosage impacted negatively on these serum biochemical parameters. The serum parameters play key roles in the maintenance of the osmotic pressure, normal blood pressure, pH, and immune responses; which are very crucial for the survival of an animal. Although these serum biochemical parameters were within the normal range for healthy indigenous chicken, the variations observed herein indicated that the serum biochemical parameters should be closely monitored during the treatment with intramuscular exogenous FSH and LH above 0.10ml on mature Nigerian indigenous cocks to avoid deleterious consequences on the serum biochemical parameters.

The results of the graded dosage of intramuscular injection of Menotropin on the internal organ dynamics of mature Nigerian Indigenous cocks are presented in Table 4. The results of the relative organ weights of the cocks showed that only the kidney and gallbladder were statistically different ($P<0.05$) compared with the control group; indicating that the intramuscular injection of Menotropin at this experimental dose did not have deleterious effects on the weights of the heart, liver, lungs, gizzard, spleen, and pancreas of the cocks. The weights of the kidney administered 0.10mL, 0.20mL, and 0.30mL of Menotropin significantly reduced. The gallbladder of the cocks on T_2 and T_4 were statistically similar and significantly ($P<0.05$) higher compared to the gallbladder of cocks on T_1 and T_3. From these observations, it could be seen that the weights of the gallbladder increased as the dosage of Menotropin administration on the cocks increased from 0.10mL to 0.20mL and 0.30mL; while the weights of the kidney reduced. The reason for this is not clear. Therefore, care should be taken when increasing the doses of intramuscular injection of FSH and LH above 0.10mL on mature Nigerian indigenous cocks to avoid atrophic kidney and cholecystomegaly, respectively.

Conclusion

Based on the results and observations from this study, it is concluded that apart from MCH, globulin, glucose, and creatinine, other haematological and serum biochemical parameters of mature Nigerian indigenous cocks may be affected by the exogenous intramuscular injection of FSH and LH above 0.10mL when used to stimulate spermatogenesis. The exogenous intramuscular injection of FSH and LH up to 0.20mL on the mature Nigerian indigenous cocks reduced the weights of the kidney and increased the weight of the gallbladder. Increasing the exogenous administration of FSH and LH up to 0.20mL had deleterious consequences on the kidney, gallbladder, haematology and serum biochemistry of the cocks. From the results obtained, it is recommended that exogenous intramuscular injection of FSH and LH should not be used above 0.10mL to stimulate spermatogenesis on mature Nigerian indigenous cocks. The blood profile should be closely monitored during the treatment with exogenous FSH and LH. Although the values obtained fall within the normal ranges for chicken, the variations observed in the blood parameters suggest the need to constantly and closely monitor the blood profile of Nigerian indigenous cocks during intramuscular treatment with Menotropins for spermatogenesis at higher doses to avoid deleterious consequences on the blood profile and on the internal organs.

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Table 1: Composition of the Experimental diet

Ingredients	Percentage composition
Maize	60.00
Soya bean meal	18.00
Palm kernel cake	5.00
Fish meal	2.00
Blood meal	2.00
Wheat offal	8.00
Bone meal	4.00
Common salt	0.25
Lysine	0.25
Methionine	0.25
Vit/mineral Premix*	0.25
Total	100.00
Jatropha tanjorensis leaf meal	0%
Crude protein (%)	19.00
Energy (kcal/kg)	2920.00

Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200 mg.

Table 2: Haematology of mature Nigerian Indigenous cocks administered graded dosage of Menotropin

Parameter	Normal Range	T_1 (0.0mL)	T_2 (0.10mL)	T_3 (0.20mL)	T_4 (0.30mL)	SEM
At the start of the experiment						
Red blood cell ($x10^{12}$/l)	4.02-4.84	4.10	4.82	4.85	5.07	0.21
Packed cell volume (%)	33.5-41.00	38.33	38.40	36.60	40.05	0.63
Haemoglobin (g/dl)	4.02-13.68	11.30	11.68	12.20	11.60	0.18
White blood cell ($x10^9$/l)	200.00-400.00	228.10	214.06	240.73	221.33	3.03
Platelet ($x10^9$/l)	-	132.50	128.00	157.00	135.30	4.02
MCV (fl)	75.96-89.10	132.08	158.56	128.56	154.76	5.04
MCH (pg)	25.20-32.60	32.21	25.39	33.00	28.75	0.41
MCHC (g/dl)	32.19-33.37	32.75	32.25	31.42	31.00	0.25
At the end of the experiment						
Red blood cell ($x10^{12}$/l)	4.02-8.84	2.15^a	2.88^b	2.87^b	3.05^b	0.10
Packed cell volume (%)	33.5-41.00	40.87^c	38.80^{ab}	38.03^a	39.57^b	0.40
Haemoglobin (g/dl)	4.02-13.68	9.80^a	11.00^{ab}	11.97^c	11.40^b	0.18
White blood cell ($x10^9$/l)	200.00-400.00	307.80^c	282.50^a	287.33^a	293.40^b	2.30
Platelet ($x10^9$/l)	-	27.00^a	32.00^b	34.00^b	32.05^b	1.00
MCV (fl)	75.96-89.10	120.51^a	136.08^b	149.50^{bc}	152.88^c	9.03
MCH (pg)	25.20-32.60	38.10	37.53	39.80	38.60	0.38
MCHC (g/dl)	32.19-33.37	28.02^b	26.76^a	26.01^a	26.50^a	0.21

$^{abc:}$ Means with different superscripts along rows are significantly different ($p<0.05$). SEM= Standard error of means. MCV= mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration. Normal range as observed by Banerjee (1998a) and Ladokun et al. (2008)

Table 3: Serum biochemistry of mature Nigerian Indigenous cocks administered graded dosage of Menotropin

Parameter	Normal Range	T_1 (0.0mL)	T_2 (0.10mL)	T_3 (0.20mL)	T_4 (0.30mL)	SEM
At the start of the experiment						
Total protein (g/dl)	3.23-4.81	4.60	3.94	4.21	3.80	0.12
Albumin (g/dl)	1.38-3.48	3.30	2.74	3.14	2.25	0.07
Globulin (g/dl)	1.15-1.67	1.30	1.16	1.07	1.55	0.06
Glucose (mg/dl)	125.00-200.00	146.00	154.18	148.50	161.04	8.10
Urea (mg/dl)	0.50.-6.00	11.87	9.65	10.91	12.30	0.42
Cholesterol (mg/dl)	152.00-148.00	126.33	133.01	121.50	138.00	6.05
Creatinine (mg/dl)	-	0.96	0.78	0.64	0.57	0.03
At the end of the experiment						
Total protein (g/dl)	3.23-4.81	5.00^{b}	4.46^{a}	4.03^{a}	4.00^{a}	0.33
Albumin (g/dl)	1.38-3.48	3.10^{b}	2.30^{a}	2.05^{a}	2.43^{a}	0.18
Globulin (g/dl)	1.15-1.67	1.90	2.00	2.05	1.77	0.10
Glucose (mg/dl)	125.00-200.00	154.00	156.67	152.10	154.67	3.00
Urea (mg/dl)	0.50.-6.00	10.01^{a}	11.20^{b}	11.60^{b}	10.85^{ab}	0.29
Cholesterol (mg/dl)	152.00-148.00	148.53^{c}	127.57^{ab}	132.05^{b}	124.86^{a}	2.99
Creatinine (mg/dl)	-	0.68	0.67	0.70	0.72	0.04

$^{abc:}$ Means with different superscripts along rows are significantly different ($p<0.05$). SEM= Standard error of means. Normal range as observed by Banerjee (1998b) and Ladokun et al. (2008)

Table 4: Effects of graded dosage of Menotropin on the internal organ dynamics of mature Nigerian indigenous cocks

Parameter (%)	T_1 (0.0mL)	T_2 (0.10mL)	T_3 (0.20mL)	T_4 (0.30mL)	SEM
Live weight (g)	1866.67	1856.67	1700.00	1733.33	62.14
Heart	0.75	0.69	0.73	0.70	0.04
Liver	1.91	1.87	1.84	1.81	0.07
Lungs	0.82	0.78	0.81	0.86	0.05
Kidney	0.54^{b}	0.36^{a}	$.040^{a}$	0.43^{a}	0.02
Gizzard	3.45	3.32	3.94	3.68	0.20
Spleen	0.16	0.13	0.16	0.14	0.01
Gall bladder	0.06^{a}	0.09^{b}	0.08^{ab}	0.12^{b}	0.01
Pancreas	0.19	0.18	0.19	0.17	0.01

$^{abc:}$ Means with different superscripts along rows are significantly different ($p<0.05$). Internal organ dynamics are expressed as Relative organ weight. SEM= Standard error of means