

## Evaluation of reproductive hormones and sperm characteristics of five strains of male broiler chicken raised in the derived savanna zone of Nigeria

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### Abstract

One of the challenges of broiler production in Nigeria is lack of technical information on improving broiler breeding. Reproductive profiles of some of the available strains could bridge the knowledge gap. Thus, evaluating the reproductive profiles of the few available breeder strains in our locality is essential. This study evaluated strain effects on the reproductive hormones and sperm variables of breeder broiler chicken in the derived savanna of Nigeria. A total of one hundred and twenty five (125) day old male broiler chick strains comprising of Abor Acre (T1), Marshall (T2), Cobb (T3), Ross (T4) and Anak (T5) of 25 birds per strain were the experimental birds. Each strain was further divided into 5 replicates of 5 birds per replicates in a Completely Randomized Design. The duration was twenty-eight (28) weeks. Reproductive hormones (Follicle stimulating hormone-FSH, Testosterone and Luteinizing hormone-LH) and sperm parameters (sperm count, motile sperm, non-motile sperm, normal sperm, abnormal sperm, live sperm and dead sperm) were evaluated. Significant ( $p < 0.05$ ) differences were observed in all the hormonal variables assayed. FSH, testosterone and LH were significantly ( $p < 0.05$ ) higher for T5 (Anak strain) than the rest of the treatments and the least mean concentration for LH, FSH and Testosterone was consistently observed in T3. Furthermore, the strain does not have a significant ( $p > 0.05$ ) effect on any of the sperm variables. However, the mean value for sperm count ranged from  $44.68 \times 10^6$  to  $82.45 \times 10^6$  with T5 having the highest mean value while T3 was the least. Similar trend was observed in percentage motile sperm, percentage normal sperm and percentage live sperm. The study confirms existence of strain differences in terms of FSH, LH and Testosterone concentration in broiler raised in the derived savanna zone of Nigeria. Moreover, the five considered strains in this study have similar sperm quality. It is therefore recommended that Anak can be used effectively in breeding schemes aimed at improving Nigeria broiler production.

**Keywords:** Broilers, reproduction, sperm, hormones, breeders, poultry

## Évaluation des hormones de reproduction et des caractéristiques du sperme de cinq souches de poulets à griller mâles élevés dans la zone de savane dérivée du Nigeria



### Résumé

L'un des défis de la production de poulets de chair au Nigeria est le manque d'informations techniques sur l'amélioration de l'élevage de poulets de chair. Les profils de reproduction de certaines des souches disponibles pourraient combler le manque de connaissances. Ainsi, l'évaluation des profils de reproduction des quelques souches de reproducteurs disponibles dans notre localité est essentielle. Cette étude a évalué les effets de la souche sur les hormones de reproduction et les variables du sperme de poulets à griller reproducteurs dans la savane dérivée du Nigeria. Un total de cent vingt-cinq (125) souches de poussins de chair

## *Evaluation of reproductive hormones and sperm characteristics of five strains of male broiler chicken*

mâles âgés de 125 jours comprenant Abor Acre (T1), Marshall (T2), Cobb (T3), Ross (T4) et Anak (T5) de 25 oiseaux par souche ont été les oiseaux expérimentaux. Chaque souche a ensuite été divisée en 5 répétitions de 5 oiseaux par répétition dans une conception entièrement randomisée. La durée était de vingt-huit (28) semaines. Les hormones de reproduction (hormone folliculo-stimulante-HFS, testostérone et hormone lutéinisante-HL) et les paramètres du sperme (nombre de spermatozoïdes, spermatozoïdes mobiles, spermatozoïdes non mobiles, spermatozoïdes normaux, spermatozoïdes anormaux, spermatozoïdes vivants et spermatozoïdes morts) ont été évalués. Des différences significatives ( $p < 0,05$ ) ont été observées dans toutes les variables hormonales analysées. La HFS, la testostérone et la HL étaient significativement ( $p < 0,05$ ) plus élevées pour la T5 (souche Anak) que pour le reste des traitements et la concentration moyenne la plus faible pour la HL, la HFS et la testostérone a été systématiquement observée dans la T3. De plus, la souche n'a d'effet significatif ( $p > 0,05$ ) sur aucune des variables spermatiques. Cependant, la valeur moyenne du nombre de spermatozoïdes variait de  $44,68 \times 10^6$  à  $82,45 \times 10^6$ , T5 ayant la valeur moyenne la plus élevée tandis que T3 était la plus faible. Une tendance similaire a été observée pour le pourcentage de spermatozoïdes mobiles, le pourcentage de spermatozoïdes normaux et le pourcentage de spermatozoïdes vivants. L'étude a confirmé l'existence de différences de souches en termes de concentration de HFS, de LH et de testostérone chez les poulets de chair élevés dans la zone de savane dérivée du Nigeria. De plus, les cinq souches considérées dans cette étude ont une qualité de sperme similaire. Il est donc recommandé qu'Anak puisse être utilisé efficacement dans les programmes de sélection visant à améliorer la production de poulets de chair au Nigeria.

**Mots-clés:** Poulets de chair, reproduction, sperme, hormones, reproducteurs, volaille

### **Introduction**

Poultry plays a significant role in human economy through food provision while also creating wealth through job creation for our teeming population (Alders *et al.*, 2019). In order to reduce malnutrition and most importantly protein deficiency in Nigeria, poultry production is considered as one of the most viable and popular options (Ezeh *et al.*, 2012). A broiler chicken (*Gallus gallus domesticus*) is known to be of fast-growing genotype (Riber *et al.*, 2018) and nowadays, broiler chicken can attain slaughter weight with decreasing age of maturity (Sleman *et al.*, 2015). Wilson (2005) and Zuidhof *et al.* (2014) reported that the ability of broiler chicken to grow faster has been as result of improved breeding, nutrition and good husbandry practices. The common broiler strains in the Nigeria market include Marshall, Abor Acre Plus, Cobb, Ross, Hubbard, Anak, Cockerel, Noiler/Kroiler (Amao *et al.*,

2015; Olawumi and Fagbuaro, 2011).

The endocrine system plays an imperative role in the management of animal reproductive cycle which includes semen production for male (Belanga *et al.*, 2019). Semen evaluation by macroscopic and microscopic methods is the tool to evaluate male fertility in broilers (Peter *et al.*, 2004). The microscopic traits like sperm motility, abnormal sperms and percent dead sperms are used in semen evaluation (Moce and Graham, 2008). The semen characteristics of avian species are different to a greater degree from that of mammals owing to the diverse physiology and anatomy like the intra-abdominal placement of testes and absence of accessory sex glands (Murugesan, 2013). Despite the numerous advantages of broiler chicken, the challenges cannot be overemphasized. One of such challenges is the lack of technical information and know-how on improving broiler breeding (Ajala *et al.*, 2007).

Adequate knowledge about the reproductive profile of some of the available strains may bridge that knowledge gap. Hence, it is imperative to evaluate the strain effects on the reproductive hormones and sperm variables of breeder broiler chicken in the derived savanna of Nigeria.

## **Materials and methods**

### ***Experimental site***

The experiment was carried out at the Poultry Production and Research unit, Teaching and Research Farm of the Ladoko Akintola University of Technology, Ogbomoso. Ogbomoso is situated in a derived savannah zone, southwest of Nigeria and lies on latitude 8° 8' 31.7940" N and longitude 4° 14' 42.6696" E. The altitude is between 300 m and 600 m above the sea level while the mean temperature and annual rainfalls are 27 °C and 1247 mm respectively (Google earth map, 2021)

### ***Source of experimental birds***

Five strains of male breeder broiler chicken namely Abor Acre, Anark, Cobb, Marshall and Ross were purchased from Obasanjo Farms in Abeokuta, Ogun State, Nigeria.

### ***Experimental birds and management***

A total of 125 day old male broiler chick strains comprising of Aboracre, Marshall, Cobb, Ross and Anark strains (25 each) were used to compare sperm characteristics and reproductive hormone concentration in the derived savanna zone of Nigeria. Each strain was identified by wing tag, randomized and allotted to pens in a brooder house. The brooder house floor was covered using wood shavings which was kept dry throughout the experimental period by replacing the litter regularly. The birds were brooded with the aid of kerosene stoves and charcoal as heat source and reared on deep litter from day-old to 8 weeks of age. All the chicks were fed *ad libitum* with a broiler starter feed containing 23.75% Crude Protein (CP) and 3,038.64 MEKcal/kg up to 4 weeks of age. Thereafter

the birds were given broiler finisher diet containing 19.95% CP and 3,102.00 MEKcal/kg for the remaining part of the experiment in accordance with NRC (1994) nutrient standard for broiler birds. Fresh, cool drinking water was also given *ad libitum*. Vaccination and other routine medication were carried out as at and when due.

### ***Experimental design***

The experiment was conducted in a Completely Randomized Design (CRD). Each strain serves as a treatment. Strains were replicated five times with five birds representing a replicate/pen. These strains are: Abor Acre (T1), Marshall (T2), Cobb (T3), Ross (T4), and Anark (T5)

### ***Semen evaluation***

Prior to actual semen collection, the broiler were primed twice weekly from 20 weeks of age, using the abdominal massage techniques stipulated by Hafez (1987). Spermogram of the five strains was done at the end of the experiment (week 28) and 15 ejaculates were evaluated per treatment i.e three (3) replicates per treatment. To achieve this, individual ejaculates were collected into sterile labeled calibrated collection vial of 2-mL capacity. Sperm count was done by counting any sperm head that fell within the sub-divided smaller squares at the four edges and center of the haemocytometer and recording the average. Semen motility was done using a light microscope under ×400 magnification on the basis of the proportion of sperm showing progressive forward movement (Ax *et al.*, 2000). The eosin-negros in differential staining techniques described by Burke (1984) was used to estimate the percentage of live or dead sperms and sperm morphology. Sperm morphology was categorized as either normal form or abnormal form depending on the characteristics of the head, mid-piece and tail region, and also on stage of maturity of the germ cells.

### **Hormonal assays**

On the 28<sup>th</sup> week, blood sample was collected from the wing vein of thirty (15) broilers per strain/treatment and the following reproductive hormones were analyzed; Follicle stimulating hormone, Testosterone and Luteinizing hormone. Blood was collected into plastic tubes that did not contain anticoagulant. They were centrifuged at 1000 rpm for 10 minutes to obtain the plasma. Glass beads were introduced into the plasma to coagulate the fibrinogen to give the serum used for hormonal assay.

FSH was determined by the method described in Accu-Bind ELISA (Microwells) FSH test system. The microplates were well formatted then 0.05 ml sample was pipetted into the assigned well. 0.1 ml of FSH-enzyme reagent solution was added to all the wells and the microplate was swirled gently for 30 seconds to enhance proper mixing. It was then incubated for 60 minutes at room temperature before the content of the microplate was decanted. 0.35ml of washing buffer was added and 0.1 ml of working substrate solution to all the wells. It was then incubated at room temperature for 15minutes, 0.05 ml of stop solution was added to each well and mixed gently for 20 minutes. The absorbance was then read at 450 nm. The concentration of FSH in the sample was then ascertained from dose-response curve. Testosterone and luteinizing hormones were assayed using the same procedure with testosterone luteinizing enzyme reagent solution respectively.

### **Data analysis**

Data obtained were subjected to one-way analysis of variance (ANOVA) using SAS (2003). Separation of means was accomplished by Duncan's multiple range test of the same statistical package.

### **Results and discussion**

#### ***Reproductive hormone concentration of broiler chicken strains raised in the derived savanna zone of Nigeria***

Table 1 shows the result of the strain effects on the reproductive hormone concentration. Significant ( $p < 0.05$ ) differences were observed in all the hormonal variables assayed. Follicle stimulating hormone (FSH), testosterone and Luteinizing hormone (LH) were significantly ( $p < 0.05$ ) higher for T5 (Anark strain) than the rest of the treatments i.e other strains (Abor acre, Marshall, Cobb and Ross). However, the least mean concentration for LH, FSH and Testosterone were consistently observed in T3. Luteinizing hormone (LH) is secreted by the anterior pituitary and plays a role in reproductive functions such as synthesis of testosterone in male animals (Belanger *et al.*, 2019). LH along with FSH are both stored in the anterior segment of the pituitary gland and their secretion is stimulated by gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus into the pituitary via the hypophyseal portal system (Martini *et al.*, 2012). The high levels of FSH, LH and testosterone observed in T5 confirmed existence of strain differences and this corroborates the finding of Ajayi and Ejiofor (2009). Hence, it can be said that T5 has superior genetic make-up than the rest of the strains. Furthermore, high concentration of these hormones is an indication of an efficient hypothalamic-pituitary-gonadal hormone axis (Bergada *et al.*, 1999). This further suggests a better pubertal development that might thus influence testes and genital growth, as testosterone may be directly or indirectly involved in the production/development and proliferation of Leydig and Sertoli cells (Main *et al.*, 2000) which in turns enhances sperm production.

Rose *et al.*, (2000) reported that FSH, in

combination with testosterone (which is under the control of LH), is required for the initiation and maintenance of qualitatively and quantitatively normal spermatogenesis hence the significant elevation observed in these variables for T5 implies a better reproductive performance of Anark strain.

***Sperm characteristics of broiler chicken strains raised in the derived savanna zone of Nigeria***

The strains effect of broiler chicken on the sperm characteristics was shown in Table 2. Strain does not have significant ( $p>0.05$ ) effect on all the sperm parameters (sperm count, motile sperm, non-motile sperm, normal sperm, abnormal sperm, live sperm and dead sperm). However, the mean value for sperm count ranged from  $44.68 \times 10^6$  to  $82.45 \times 10^6$  with T5 having the highest mean value while T3 was the least. Similar trend was observed in percentage motile sperm, percentage normal sperm and percentage live sperm. The present investigation agreed with Siudzinska and Lukaszewicz (2008) who confirmed that breed had no significant effect on semen characteristics. It however disagree with the findings of Peters *et al.*, 2008 who postulated that the semen quality in chickens was found to be varied among different genotypes like breed, strain and line. Sperm quality is extremely important in poultry breeding because it is mostly used in estimating the fertilizing ability of the sperm cells (Lukaszewicz, 1988). Baskt and Brillard (1994) reported that only sperms with normal morphology can ascend through the vagina of the hen to the sperm storage tubules. Adeoye *et al.* (2017) further stated

that abnormal spermatozoa are found in normal semen analysis but it becomes challenging when there is high proportion of these sperms in the sample, as they possess defects which could affect their ability to fertilize an egg or result in poor hatchability if the egg is fertilized. The findings of this study on sperm morphology attests to Ameen *et al.* (2014) who reported non-significant variation in the sperm morphology in different breeds/strains of chicken. The study however disagrees with Tabatabaei *et al.* (2010) who observed an increase in rate of sperm morphological defect in Iranian indigenous broiler breeder chickens. Tanemura *et al.* (1993) attributed such defects to impaired spermatids and spermatocytes resulting in interrupted spermatogenesis. The total motile sperm suggests the total number of sperms assumed to be capable of progressing from the site where semen is deposited in the course of insemination to the sperm storage tubules in the hen. It is a good indicator of a sire's reproductive potential and is often used in predicting fertility (Donoghue *et al.*, 1998). Decreased sperm motility has been associated with abnormal spermatogenesis and epididymal sperm maturation problems (Rengaraj *et al.*, 2015). Sperm motility of the five strains studied was within the reference range of 60-80% reported for chicken by Getachew (2016). The non-significant variation observed in sperm motility in the present study disputes the report of Molekwa and Umesiobi (2009) who reported strain variations in sperm motility of South African indigenous chicken breeds.

**Table 1: Strain effect on the reproductive hormone levels of male broiler chickens raised in derived savanna zone of Nigeria**

Reproductive Hormones (ng/mL)	T1 (Abor acre)	T2 (Marshall)	T3 (Cobb)	T4 (Ross)	T5 (Anak)	±SEM
LH	1.48 <sup>b</sup>	1.55 <sup>b</sup>	1.38 <sup>b</sup>	1.45 <sup>b</sup>	2.00 <sup>a</sup>	0.65
FSH	2.08 <sup>b</sup>	2.07 <sup>b</sup>	2.07 <sup>b</sup>	2.08 <sup>b</sup>	2.13 <sup>a</sup>	0.01
Testosterone	0.27 <sup>b</sup>	0.27 <sup>b</sup>	0.26 <sup>b</sup>	0.29 <sup>b</sup>	0.40 <sup>a</sup>	0.12

ab: Means on same row with different superscripts differ significantly ( $P<0.05$ )

SEM: Standard Error of Mean

**Table 2: Strain effect on the sperm characteristics levels of male broiler chickens raised in derived savanna zone of Nigeria.**

Sperm Characteristics	T1 (Abor acre)	T2 (Marshall)	T3 (Cobb)	T4 (Ross)	T5 (Anak)	±SEM
Sperm Count ( $\times 10^6$ )	74.60	76.35	44.68	77.55	82.45	2.51
Motile sperm (%)	82.51	82.37	81.57	82.60	83.78	1.11
Non-Motile sperm (%)	17.50	17.63	18.43	17.42	16.22	1.11
Normal Sperm (%)	87.17	87.21	85.99	86.15	87.88	0.35
Abnormal sperm (%)	12.83	12.80	14.01	13.85	12.12	0.35
Live Sperm (%)	84.01	84.80	83.40	85.70	86.16	0.68
Dead Sperm (%)	15.98	15.20	16.60	14.31	13.84	0.68

### Conclusion

The study showed that strains used in this study have a significant effect on reproductive hormone concentration of male breederbroiler chicken raised in the derived savanna zone of Nigeria. Furthermore, the five considered strains in this study had similar sperm quality. It is therefore inferred that Anak can be used effectively in breeding programs aimed at improving Nigeria broiler production.

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