

**PHYSIOLOGICAL AND REPRODUCTIVE RESPONSES OF YORUBA ECOTYPE
CHICKENS TO DIETARY AND HORMONAL MANIPULATIONS**

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DEDICATION

This thesis is dedicated to the Almighty God, the creator of heaven and earth, for His mercy and grace that sustained me through the research work, and to the loving memory of my late father, Pa Akanbi Amos Remilekun who left a lasting legacy of hardwork and dedication to children training and education.

And My Wife, Dr. (Mrs) Akanbi Ibukun Mary and children, Glory Deborah, Goodness Abraham and Godwin Israel Akanbi, you are great.

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To all who have contributed in one way or the other to the success of this work, but whose name was not been mentioned, I thank you and God bless you all. I am Grateful to you all.

Akanbi A.S.

DECLARATION

I declare that the work described in this thesis is original and has not been previously submitted to any University or similar Institutions for the award of any degree or certificate.

Name of candidate: Akanbi, Ademola Samuel.

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CERTIFICATION

This is to certify that this research work was carried out by AKANBI, ADEMOLA SAMUEL of the Department of animal production, University of Ilorin and the report of the research work has been read and approved for the award of Doctor of Philosophy (Ph.D.) in Animal Production, having met the requirement of the Department of Animal Production, Faculty of Agriculture and the Post Graduate School, University of Ilorin, Ilorin, Nigeria.

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ABSTRACT

Male chicks are less desirable outputs of hatchery production from layer lineage. There is flat information on the manipulation of the physiology of chicken to achieve reasonable profit in Yoruba ecotype chicken (YEC). Achievement of profitable sex ratio is important for planning and decision making. The objectives of this study were to determine the effects of: (i) artificial insemination on fertility, hatchability and morphometric characteristics in YEC; (ii) dietary palm kernel oil (PKO); and levels of estradiol and testosterone administration (i.m) on serum biochemistry, haematology, hormonal status, reproductive responses, egg characteristics and offspring sex ratio of YEC; and (iii) *in-ovo* estradiol and testosterone administration on sex ratio of YEC chicks.

Four experiments were conducted. Experiment 1 investigated the effects of number of days post artificial insemination on fertility and hatchability in a completely randomized design; alternative methods on the percentile accuracy of sexing; sex on weekly body weights and morphometric measurements in YEC using a t-test. Experiment 2 examined the effects of dietary PKO (0, 1, 2, 3 and 4 percent) inclusion on serum biochemistry, haematology, hormonal status, reproductive responses and offspring sex ratio of YEC in a 2x5 factorial design. Experiment 3 evaluated the effects of estradiol and testosterone administration (i.m) on serum biochemistry, haematology, hormonal status, reproductive responses, egg characteristics and offspring sex ratio of YEC in a 2x2x5 factorial design. Experiment 4 investigated the *in-ovo* estradiol and testosterone administration (0, 1, 2, and 4 IU) on hatchability and offspring sex ratio of YEC in a 2x4 factorial design. Data obtained were subjected to analysis of variance. Means were separated by the new Duncan's multiple range test at $p < 0.05$.

The findings of this study were that:

- i. fertility was stable from day 1 to 12 except for a transient increase on day 6 and decrease from day 13 onwards;
- ii. male chicks had longer ($p < 0.05$) keel, shank and thigh length than female chicks at seventh and eighth weeks;
- iii. dietary PKO; and levels of estradiol and testosterone administration (i.m) had no effect on serum biochemistry, haematology, hormone status and sex ratio in YEC;
- iv. estradiol administration (i.m) resulted in lower feed per dozen egg ($p < 0.05$) than testosterone administration;
- v. dietary 4% PKO decreased ($p < 0.05$) feed intake, weight gain, feed per dozen egg, fertility and egg hatchability of YEC; and
- vi. *in-ovo* estradiol administration resulted in higher ($p < 0.05$) hatchability and percentage female chicks than testosterone administration.

The study concluded that fertile eggs can be collected within day 1 and 12 after artificial insemination in YEC. Male chicks can be separated from female YEC from 7 weeks and above using body weight and morphometric traits. *In-ovo* estradiol administration increased hatchability and the percentage of female chicks hatched. *In-ovo* estradiol administration of up to 2 IU is therefore recommended for increased female chicks production in YEC.

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CHAPTER ONE

INTRODUCTION

Yoruba ecotype chicken is one of the two main types of indigenous chicken that is reared among the Yoruba tribe in the Western part of Nigeria (Atteh, 1990; Ige and Salako, 2014). They have outstanding advantage in being hardy, diseases resistant, good forage ability and survival on low nutritious food (Ajayi, 2010). However, they have problems of sexing at early age (day old) and their offspring sex ratio is at variance to Fisher's postulation of equality of sex because they are noted for low percentage of male to female in the flock (Yakubu *et al.*, 2014).

In the exotic breed, sexing of chicks are usually carried out either at embryonic stage (Ricks *et al.*, 2003) to separate male from female through detection of the W-specific chromosome by PCR mechanism (Wang *et al.*, 2008; Chang *et al.*, 2008), the application of Embrex's automated gender sorting device that sort out male from female embryonic egg by identification of estrogenic content of the allantois' fluid (Tran *et al.*, 2010), or at day old using plumage colour (Coyne *et al.*, 2007), feather sexing (Kalina *et al.*, 2012) and Japanese vent sexing (Tran *et al.*, 2010) to ensure that farmers obtained the desired sex of exotic chicks for production.

People living in rural areas are the major producer of indigenous chicken in Nigeria which constitutes 80% of poultry production. In the Nigerian indigenous chicken production, none of these chick sexing methods have been adopted by farmers and as such male and female chickens are kept together till maturity. This practise gives room for indiscriminate mating of the flock that makes selection process more difficult. Phenotypically, at maturity, the males are bigger, taller and heavier (Sola-ojo *et al.*, 2008) than their female counterpart and as such

command more market price (Shafey *et al* 2013). Also, meats of indigenous chicken are tough, pigmented and have desirable flavour that make the chicken acceptable for special dishes (Ajayi, 2010). All these attributes make the male (cocks) more preferable for meat purpose than the female (hen) of Yoruba ecotype chicken. Despite this preference for male, the population ratio of female (hen) is greater than male (Yakubu *et al.*, 2014) which negate the Fisher's proposition of equal male and female offspring number (Radford and Blakey, 2000). Therefore the separation of male chicks from female chicks will enhance consumer preferences during the production of indigenous chickens because consumer preference is vital in decision making for profitable income generation by producers (Yakubu *et al.*, 2012). The two ecotypes indigenous chicken mostly reared in Kwara state Nigeria are the Fulani and the Yoruba ecotype chickens. The Fulani ecotypes are found in the Kraals that are Fulani domicile, while the Yoruba ecotype chickens are reared by the poultry farmers living in both rural (farmsteads and villages) and urban (towns, cities and metropolises) areas of Kwara state (Sola-Ojo *et al*, 2013a).

People living in rural areas are the major producers of indigenous chicken which constitutes 80% of poultry production in Nigeria (Ajayi, 2010). The sex determining mechanisms in birds and mammals appear to be different. In the avian species, unlike the case in mammals, female birds are the heterogametic sex (Lin *et al.*, 1995; Smith and Sinclair, 2004; Osinowo, 2006) that carry ZW chromosome while the male birds are the homogametic sex that carry ZZ chromosome. In mammals, the presence of the Y chromosome provides the primary genetic switch towards the formation of the testis which leads to development along the male sex. This gene for sex determination (SRY) is located within the sex determination region of the Y chromosome that is present on all mammalian Y chromosome (Sinclair *et al*, 1990; Johnson, 1991; Osinowo, 2006; Ogbuewu, *et al.*, 2010) but there is no (SRY) gene in avian

spices. This implies that the Mendelian postulation of chromosomes segregation would absolutely be controlled by female (hen) (Thomas and Marion, 2013) in avian species.

Different hypotheses had been formulated concerning sex manipulation in avian species. Thomas and Marion (2013) suggested that the follicles that fundamentally lead to production of male and female offspring develop at different time. Another suggestion was based on the environmental conditions that can affect male and female offspring sex ratio in birds which include parental condition (Nager *et al.*, 2000) and hormone circulation during the time of sex determination in laying hen (Pike and Petric, 2003). The sudden increase in glucocorticoids hormone of laying hen during egg production had been reported to bias offspring sex toward female (Bonier *et al.*, 2007) by causing male mortality in the embryonic stage (Love and Williams, 2008). Also, maternal diet and photoperiod were reported to influence offspring sex ratio in avian and reptiles (Rutstein *et al.*, 2004; Sturkie, 2000).

There has been increasing attention on the hormonal manipulation of sex ratio (Sarah *et al.*, 2011) which was attributed to the yolks hormonal content of avian eggs. This was likened to maternal hormone production that reflects the hormonal status of the birds in egg production period (Petric *et al.*, 2001). Among the different hormone, testosterone has been reported to adjust avian offspring sex towards male (Petric *et al.*, 2001). The findings of Muller *et al.*, (2002) posited that eggs that develop to male contained more testosterone than those that produced female, which had more oestrogen. Exposure of the developing embryos inside the egg to changes in estradiol concentration can cause genetic males sex reversal (transgender) to phenotypic females at the gonad, gonaducts, anatomy and conformation of birds (Thomas and Marion, 2013).

The basis of steroid hormone formation in the body is cholesterol which is lipid. Lipid metabolism is very crucial in cell differentiation (Zuo *et al.*, 2015). In chicken, addition of

fats and oils in the diet of laying hen produce a special scent in the yolk that developing embryos normally smell and utilise through chemosensory actions of the surrounding (Aigueperse *et al.*, 2013). Among other oils, Palm kernel oil find its usefulness in containing saturated fatty acids of about 80% and unsaturated fatty acid of 20% (Mukherjee and Mitra 2009) which is the basis for steroid hormones via cholesterol formation. Linoleic acid is among the two basic necessary fatty acids needed by the chicken for proper physiological functioning of the body (Senkoylu *et al.*, 2004).

Although, experimental demonstrations have shown that avian species in the wild bias their offspring sex through sex hormones (Muller *et al.*, 2002) and dietary oil inclusion (Rutstein *et al.*, 2004), the manipulation of the physiological state and sex ratio of Nigerian indigenous chickens still remain unclear. Sex means different things in different contexts; sex in this study refers to male and female phenotype (phenotypic sex). This study therefore intended to examine how Yoruba ecotype chicken can be manipulated for improved reproduction and offspring sex ratio through the use of palm kernel oil in the diet and hormone approaches.

OBJECTIVES

The aim was to investigate the effects of dietary palm kernel oil (PKO) inclusion and hormonal injection on reproductive responses and offspring sex ratio of Yoruba ecotype chicken.

SPECIFIC OBJECTIVES

1. To investigate the effects of artificial insemination on fertility, hatchability and chicks sexing methods in Yoruba ecotype chicken.

2. To examined the effects of dietary palm kernel oil on serum biochemistry, haematology, hormone, reproductive response, egg characteristics and offspring sex ratio of the Yoruba ecotype chicken.
3. To investigate the effects of oestrogen and testosterone on serum biochemistry, haematology, hormone, reproductive response, egg characteristics and offspring sex ratio of the Yoruba ecotype chicken.
4. To evaluate the effects of *in-ovo* hormonal administration on offspring sex ratio of Yoruba ecotype chicken

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INDIGENOUS CHICKEN PRODUCTION

The stocks of indigenous chicken widely contribute to the animal protein consumption of the under developed and developing nations of the world. Indigenous chicken are characterized by geographical location where they are found or by tribe keeping them in Nigeria (Oluyemi, 1979, Sola-Ojo *et al*, 2013a). Local chickens are varied in shape, size, colour and feathering and within geographical locations namely: the Nsukka, Owerri and Agwu strains (Nwosu *et al*, 1985), Ibadan strain (Hill and Mobede, 1961) and the Ife strain (Akinokun and Dettmers, 1979) etc.

Indigenous chicken have numerous uses and advantages and each ecotype originated as the product of genetic deviation, mutation, change, adaptation and evolution with diverse selection stresses imposed through weather condition, available nutrition, endemic diseases, and selection criteria enforced by man (Ige and Salako, 2014). Sola-Ojo *et al* (2013a) categorized indigenous chicken majorly domicile in Kwara state of Nigeria as Fulani and Yoruba strain (ecotype) chicken. The former is found in the Kraal where Fulani people domicile and the later (Yoruba ecotype) is reared in the yards of farmers living in the rural and urban areas of the state.

Indigenous chicken production plays a crucial role in providing the ever increasing human population of the developing countries with required good protein quality and also serves as sources of income for the people at the grass root (kingori *et al.*, 2010). High demands are placed on poultry products especially chickens because they are essentially the widely reared species of in the universe (Yakubu *et al.*, 2012). Indigenous chicken are reared in the rural areas where they serve as source of income (Moreki *et al.*, 2010) for the rural dwellers. Indigenous chicken is about 70% of the total chicken production in Africa (Kingori *et al.*,

2010) and 80% poultry production in Nigeria (Ajayi 2010). Eggs and meat of indigenous chicken serves as important animal protein source in many household in Nigeria. Revenue generation from poultry products increases the economic diversity of livestock farmers in the country. It also creates employment opportunities for rural dwellers.

Indigenous chicken as well as their products found relevant traditional importance and uses in tradiomedicine as they are use in appeasing “gods” and “goddess” (Moreki *et al.*, 2010). Chickens have advantage of quick returns on investment and easy to manage (Kingori, 2004). The start-up capital and maintenance cost of indigenous chicken is relatively low.

The productivity of indigenous chicken is very low as a result of poor feed conversion efficiency, system of rearing and genetic make-up (Yakubu *et al.*, 2014). They are generally characterised as been hardy, having good adaptive nature to the rural areas, surviving on little inputs, possessing good foraging ability and adjust to irregular feed and water availability (Ajayi 2010). Nigerian indigenous chickens are mostly abandoned to source for feed for themselves around the houses, backyards and nearby bushes and fields.

2.2 TYPES OF NIGERIAN INDIGENOUS CHICKENS

Nigerians indigenous chickens have been characterised based on plumage colour (black, white, mottled), feather appearance (normal feather or frizzle feather) and body structure (naked neck and dwarf type) (Ajayi 2010). Among all these classification, the normal feathered bird account for about 91.8% while frizzle feathered account for about 5.2% and naked neck has 3.0% (Ajayi and Agaviezor, 2009). Classification on body size and weight according to Momoh *et al.*, (2008) revealed that Nigerian indigenous chicken can broadly be categorized into two, namely: heavy strains (Fulani ecotype) which are found in guinea savannah, mountainous regions, sahel savannah and all cattle kraals in Nigeria. Their body weight ranges between 0.9Kg to 2.5Kg at table size. The second type is the light strain which is found in the rainforest and derived savannah of Nigeria. They have body weight of about

0.68-1.5kg (Momoh *et al.*, 2008). The light strains found in Kwara State, Nigeria are the Yoruba ecotype chickens (Sola-Ojo *et al.*, 2013a).

2.3 GROWTH PERFORMANCE OF NIGERIAN INDIGENOUS CHICKEN

Nigerian indigenous chicken grows slowly and has small body weight (Olawunmi *et al.*, 2008). Usually, the Fulani strains of chicken are heavier and bigger in body size and weight than the Yoruba strains (Sola-Ojo, 2013). The cockerels (males) of Yoruba ecotype chicken are also bigger in size than hens (Sola-Ojo, 2008, Ajayi and Agavierzor, 2009). Indigenous chicken lack gene for better feed conversion (Gunn, *et al.*, 2016) and this could be the major factor responsible for their slow growth rate. Matured indigenous chickens are smaller in size and weight when compared with the exotic breeds. Notwithstanding indigenous chickens are prominent among the basic local treasures and wealth of the poor rural dwellers living in Africa (Momoh *et al.*, 2008). Over 90% of households in villages and towns and cities of Nigeria raise indigenous chicken in small units of 20-30 birds per flock (Adebambo, 2005). All families living in the rural areas of kwara State, Nigeria will normally rear some local chickens to occasionally provide egg and meat for the family (Ayorinde, 1990).

Yoruba ecotype chickens are among the important sources of quality protein producing eggs and meat for the rural people. They are poor in both growth performance and egg production (Olawunmi *et al.*, 2008). This low productivity may result from the exposure to extreme climatic conditions, low nutrient intake and total reliance on scavenging feed resources, which can be responsible for their seasonal egg production and broody characteristic in hen (Ndegwa *et al* 1998). Similarly, Musharaf *et al* (1990) reported that low productivity in the indigenous chickens are evident from poor growth performance, poor weights at maturity, high mortality and low egg production. Gueye (2000) and Sola-Ojo *et al* (2013a) however posited that regardless of the poor productivity in local chickens, they play crucial function in

alleviating poverty among the local poultry farmers. The age in days of indigenous chicken at table size (maturity) ranges from 133days to 169 days when raised extensively (Gunn, 2008).

2.4 CONSUMERS PREFERENCE FOR INDIGENOUS CHICKEN

Consumers' preference for indigenous chicken for meat is higher for males than females because of the bigger size of the males (Sola-Ojo, 2008). The meat is characterised by good leanness, flavour and organoleptic attributes (Kingori *et al.*, 2010).

The morphometrical characteristics are relatively larger in male chicken than the female. The average comb length of 6.36cm and height of 4.88cm was reported for cockrels as against comb length of 3.64 cm and height of 1.63 cm reported for hens (Kingori *et al.*, 2010). The cockerels' body weight during maturity is reported to be 2.1kg as against 1.2kg reported for hens (Kingori *et al.*, 2007).

Majority of Nigerian indigenous chickens are kept for meat purpose as over 50% of the poultry farmers leave the eggs for hatching rather than eating the eggs directly (Kingori *et al.*, 2010). Most of the birds are sold at 5-6 months (Upton, 2000) with average live weight of 1.3-1.8kg and dressed weight of 72% (Yakubu *et al.*, 2014).

2.5 EGG PRODUCTION OF NIGERIAN INDIGENOUS CHICKEN

The average egg production of Nigerian indigenous chicken is 40eggs to 100 eggs annually (Yakubu *et al.*, 2014). Hens laid their eggs in clutches (3 to 4) in a year and 1 clutch consists of 12eggs to 20 eggs (Oluwande *et al.*, 2009). The average weight of the egg is 25-49g. This low egg production (40-100 eggs per year) is generally caused by poor feeding and management. Under intensive management and improved feeding, egg production can improve to 150 eggs per year (Kingori, 2009).

The decrease in egg production of Nigerian indigenous chicken can be attributed to broodiness. This is natural instincts that allows the hen sit on the egg and hatch it to chicks. In

order to achieve improvement in egg production of indigenous chickens, broodiness must be eradicated (Kingori *et al.*, 2009). This can be actualized by keeping the affected hen inside a cage that is over one metre height from the ground; adequate feeding and supply of water *ad libitum*. Broodiness is suppressed in cage system of rearing. The egg weight of a heavy strain chicken was reported to be heavier than the light strain chicken (Fayeye *et al.*, 2005), however, the light strain lays more egg than the heavy strain.

2.6 EGG FORMATION

The continuous ovulation has contributed immensely to increase egg production by enabling commercial layers to produce 300 eggs in a year and above 500 eggs within their life cycle (Kasper, 2016). Inside the ovary of a day old female chicks, over 12,000 oocytes are resident and not more than 250-500 oocytes will grow and become fully developed ovulatory that form matured follicles for egg formation (Nys and Guyot, 2011).

The initiation of sexual maturity gives rise to the growth of follicles having various sizes. This is formed by collection of lipids coming together that resulted into hierarchical order where only one pre-ovulatory follicle developed daily. The hens that are sexually mature have various primordial ovarian follicles at the ovary cortex with the size of about 1 mm (Flock and Havenstein, 2015). The initial ovarian follicles accumulation develops to a limited number of primary oocytes that undergoes further developmental process to become pre-hierarchical oocytes among which only one oocyte is chosen daily to attain peak matured size (Johnson and Woods, 2007). The regulation mechanism is not fully elusive as there is assumption that the follicle stimulating hormone (FSH) receptor that is produced from the pituitary gland is prevented by inhibitory action (Johnson and Woods, 2007). The prevention of the inhibitory mechanism in each oocyte results in production of follicle stimulating hormone receptor and follicle stimulating hormone synthesis of sex hormone and systems in favour of ovarian follicular cells survival. This action of FSH mediation subsequently

diminishes thereby giving chance for luteinizing hormone (LH) secretion to further control ovarian follicular development and maturation of yolk (Johnson, 2015). The primordial oocytes are initially transported to the cortex and afterward joined together with the blood vessels, veins and nerve through follicular cavity (Johnson and Woods, 2007). The development of primordial oocytes to the primary oocytes through the pre-hierarchical oocytes is closely related to the development of whitish yolk containing high quality lipoproteins (Mann and Mann, 2008). The oocytes are mostly 6mm to 8 mm in diameter and can be identified easily at the ovary duct. The identification of such oocyte from the pre mature ovarian follicles will take approximately 4 to 6 days and is accompanied with heavy yolk weight gain of about 2g daily (Johnson, 2015). Yolk formation process is completed within 24 hours before to ovulation in birds (Johnson, 2015). The yolks normally consist of 36% fat, 17% protein and water. The fat component of the yolk consist of triacylglycerols (65-70%), phospholipids (25-31%) and cholesterol (4-5%)(Mann and Mann, 2008). The protein fractions consist of 14% alpha livetin, 41% beta livetin and 45% gamma livetin (Jonchere, 2010). Vitamins, minerals and carbohydrates are not up to 1% of the egg yolk (Mann and Mann, 2011). The characteristic yellow yolk colour during egg formation resulted from xanthophyll (Nys and Guyot, 2011). The yolk composition such as yolk fats and yolk proteins are produced from the liver by the action of oestrogen and testosterone mediation (Mann and Mann, 2008). The fat composition of the blood is transported in the form of very low density lipoprotein (Kaspers, 2016).

The egg formation process is an hierarchical order that takes approximately 27 hours from ovulation to oviposition (Johnson *et al.*, 2015). The reproductive tract receives the matured ovum that is a mere yolk with germ disc, and other egg compositions (egg shell, protective membranes and other nutrients) are added as the egg yolk moves from one point to the other along the tract (Hartle *et al.*, 2014).

2.7 MAJOR PROCESSES IN EGG FORMATION

Kaspers, 2016 described the processes of egg formation as given below:

Ovulation is the process whereby a matured ovum is released from the ovary. The released egg is received by the oviduct and enters the infundibulum. Ovulation normally takes place within half an hour after each successive egg is laid.

Fertilization is the fusion of male with female to produce a zygote and it normally takes place at the infundibulum of birds, which has potentials in storing sperm cells and retaining fertility up to fourteen days after mating or insemination (Froman *et al.*, 2011).

Egg formation takes place as the egg moves from one place to the other in the oviduct and is encompassed by all layers consisted in the egg of chicken.

Oviposition refers to the process of egg laid in birds. The contraction of the uterus usually results in pressure build up that pushes out the egg from the cloaca (vent). Egg is mostly laid through the broader end.

2.8 TIMING IN EGG FORMATION PROCESS

The mean time spent by egg inside the oviduct during egg formation process is summarized below:

Organs for egg formation	Time of egg formation
At the infundibulum	one hour
At the magnum	Two to three hours
At the isthmus	One hour
At the uterus	Twenty to twenty six hours
At the vagina	within few minutes

Source: Kasper, 2016

2.9 SPERM STORAGE TUBULE

Birds have Uterovaginal Junction (UVJ) that performs significant role as Storage Tubules for Sperm cells (SSTs). The ejaculated sperm cells from cock after natural mating or artificial insemination ascend through the vaginal and enter the SSTs (Bakst, 2011, Froman *et al.*, 2011). The storage time of sperm in the SSTs defer from one species of animal to the other (Christensen and Bagley, 1989). Chickens have ability to retain semen with potent sperm motility for three weeks, while turkeys have ability to retain semen for ten weeks in the tubules that store sperm cells (Brillard 1993). Most variation that occurred in their semen storage periods may be associated to the varying number of sperm storage tubules located on Uterovaginal Junction. The report of Bakst *et al.*, (2010) showed that turkeys have about twenty to thirty thousand SSTs whereas chickens have only five to thirteen thousand SSTs. Although, Brillard *et al* (1998) suggested that the identifiable quantity of sperm storage tubules on the UVJ may be directly correlated to percentage egg fertility, but different strains of broiler with the same number of SSTs exhibited different levels of egg fertility. Fertility is the most critical consideration in poultry production.

2.10 FERTILITY AND HATCHABILITY OF EGGS

Fertility is the percentage of fertile egg set per the total egg set inside the incubator while hatchability is defines as the percentage hatched egg per total fertile egg (Adedeji, 2016). In poultry production and breeding, increase percentage egg fertility and hatchability as well as low embryonic mortality are keen considerations in increasing production and maximizing profit (Çağlayan *et al.*, 2010). Fertility and hatchability of egg can be affected by the weight of egg (Abiola *et al.*, 2008), storage period (Sari *et al.*, 2010) and feed composition of hen diet (Praes *et al.*, 2014). The dietary composition of feed was reported to have direct influence on egg production (Perez-Bonilla *et al.*, 2012) and the weight of egg in birds (Shim *et al.*, 2013). Fertility and hatchability of Nigerian indigenous chicken are similar to edd

fertility and percentage hatchability of all local chickens of the world kept under traditional system of rearing (Ajayi, 2010). Fertility of 76% and hatchability of 48% are reported by Adedeji *et al* (2016) in Fulani strains of chicken and these values were within the normal recommended range of 82.0 - 93.% and 52.0 - 87.0% documented for Bangladesh local chicken (Islam and Nishibori, 2009).The normal feathered birds of Nigerian indigenous chicken were reported to have about 45% hatchability (Ajayi *et al.*, 2008).

2.11 FACTORS AFFECTING EGG FERTILITY AND HATCHABILITY IN NIGERIAN INDIGENOUS CHICKEN.

The percentages fertile eggs and hatchable eggs are the essential consideration in the evaluation of reproductive performance of breeders stock (Kingori, 2011) that is affected by genetic components and environment. The estimates of heritability for percentage fertile and hatchable eggs in birds are within 0.06-0.13 revealing that other factors (aside genetic) have higher effects on these traits (Haghighi *et al.*, 2016).

Factors such as storage periods, age of hen, storage condition, mating ratio and system, temperature and relative humidity of the incubator and egg turning angle and frequency (Kingori, 2011) have been reported to influence hatchability of egg.

The diet of breeders' birds should be of good quality and quantity to prevent excessive weight gain that can cause poor quality semen and ovulation (Brillard, 2007). The estimated dietary protein recommendation of 14-18% for exotic chickens has been proved to exert no significant influence on fertility and hatchability of egg (Kingori, 2011). The report on indigenous chicken from Kenya showed that dietary crude protein requirement of 12% showed no significant effect on percentage hatched eggs (Kingori *et al.*, 2010). Among all the nutritional factors, Linoleic acid was reported to affect embryonic mortality as well as hatchability (Kingori, 2011). Ayidin and Cook (2004) reported that embryonic mortality in quail depends on conjugated linoleic acid (CLA) dosage and duration of feeding.

Embryonic mortality occurs as a result of modification in the profile of fatty acid of the egg yolk. Javanka *et al* (2010) on the other hand documented improved egg fertility and hatchability in breeder's egg fed brewery by-products. Brewery by-products contain 4-5% linoleic acid. Supplementation of organic selenium in layers diet improved hatchability and fertility of eggs (Hanafy *et al.*, 2009). Similar increased fertility and hatchability of egg was reported by Osman *et al* (2010) who fed laying hen with 200mg of selenium.

2.12 SEX MANIPULATION IN AVIAN SPECIES

The skew in offspring sex ratio of avian species are significantly influence by diet and hormones of birds (Gam *et al.*, 2017 and Okekpe, 2009). The sex allocation theories stated that the changes from 50:50 expected sex ratio of the offspring will be affected by the surrounding environment during reproduction. Hens will adjust the sex of offspring in favour of environmental condition in order to optimize success in reproduction and survival of offspring (Wild and West, 2007). Birds adjust sex ratios by different resources allocation post-natally through offspring protection and behavioural characteristics (Okekpe, 2009, Gam *et al.*, 2017). Birds are capable of negating the costly losses relating to sex specific postnatal mortality by primary adjustment of sex ratio before fertilization (Pike and Petric, 2003).

Hens can adjust offspring sex ratio before ovulation owing to their heterogametic sex allocation of W or Z chromosome that determines offspring sexes. The offspring sex of avian species is fixed during first meiotic cell division in which haploid sex chromosome is allocated to the ovum and the second chromosome to the polar surface of the body which take place within 2-4hrs before ovulation in chicken (Aslam *et al.*, 2014).

Circulating hormone are the major concerns of environmental and social condition which act as signal mediators in physiological conditions that control sex ratio manipulation of birds (Pike and Petrie, 2006 and Bonier *et al.*, 2007). The common mediating hormones that are implicated in offspring sex determination in birds are testosterone (Goerlich, *et al.*, 2009),

Oestrogen (Bonier *et al.*, 2007), Progesterone (Correa *et al.*, 2005) and Corticosterone hormone (Pike and Petrie, 2006).

Sex determination theory in most vertebrate animals derives benefit from current advancement in the physiological studies and mechanisms of sex variation caused by factors such as maternal stress, glucose, estradiol and testosterone hormone (Helle, *et al.*, 2012). In the monotocous and polytocous animals, the effects of high concentration in maternal testosterone hormone usually occur during embryo development or fertilization process and were observed to skew offspring sex of animal towards males (Grant and Chamley, 2010 and Grant *et al.*, 2011). Maternal glucocorticoids and estradiol were also reported to bias the sex of offspring through sex-skewing embryo mortality in mammals (Navara, 2010). Similarly in the ground squirrels (*Uroditellus richardsonii*), matured females with high concentration of glucocorticoids hormone skew their offspring litter size toward male during gestation period (Ryan *et al.*, 2012). Also, high concentration of glucose in the mother was reported to adjust sex ratio of offspring in favour of males (Dunn *et al.*, 2010). Previous study by Cameron *et al* (2008) suggested that sex-skewed embryonic mortality is a mechanism responsible for male-bias offspring.

Steroid hormones such as testosterone and glucocorticoids in the vertebrate animals are directly associated with post-delivery growth, reproduction and survival in animal which may be prominent in one sex over the other (Warner *et al.*, 2009 and Monclús and Blumstein, 2012). Testosterone hormone is implicated in the development of muscle and growth of skeletal structure in males (Cox *et al.*, 2009).

These hormones are implicated and found in the ovulatory site and peaked daily at the meiotic cell segregation (Rutkowska *et al.*, 2011). These hormones have potentials to manipulate sex ratio at follicular levels and are such stimulate specific bias in primary

offspring sex. Experiment has shown that female off springs are produced when plasma levels of maternal corticosterone are higher and experimentally elevated (Bonier *et al.*, 2007). The administration of progesterone at the time of meiotic segregation induced a sex ratio towards females (Correa *et al.*, 2005).

2.13 LIPID METABOLISM IN LAYING HEN.

The main metabolism pathway where lipid metabolism and synthesis occur in chicken is liver (Wang *et al.*, 2014). The synthesis of about 70% *in vivo* fatty acids with its degradation and transportation occurred in the liver (Li *et al.*, 2015). During egg formation in laying hen, the synthesized hydrophobic lipids like triacylglycerol, free fatty acids and cholesterol are assembled to form precursors of egg-yolk like vitellogenin substances and very low density lipoprotein (Li *et al.*, 2015). These particles are discharged into the circulatory system from where they are transported into the developing embryo to supply their nutrient requirement for growth and development (Nikolay *et al.*, 2013). This important physiological process was investigated and generally believed that synthesis and transportation of lipids are mainly controlled by oestrogen in the liver of laying birds (Schneide, 2009). Oestrogen regulates gene transcription through oestrogen receptors consisting of Alpha Oestrogen receptor and Beta Oestrogen (Schneide 2009). The roles of the genes that are closely related to hepatic lipid metabolism are insubstantially different in poultry when compared with mammals (Li *et al.*, 2014). In mammals, it was reported that the microsomal triglyceride transfer protein aids lipoproteins assemblage to produce low density lipoproteins (Hussain *et al.*, 2012 and Soh *et al.*, 2013) whereas the production of very low density lipoprotein (VLDL) in birds are tightly controlled by estrogen (Trapnell *et al.*, 2013). Ivessa *et al* (2013) affirmed that the microsomal triglyceride transfer proteins regulation inside the liver was not necessary in the increment of VLDL assemblage during the laying cycle in birds. The endoplasmic reticulum of the hepatic cells performs the function of VLDL assemblage (Li *et al.*, 2015) while the

microsomal triglyceride transfer proteins perform the function of lipids transportation in chicken (Rava and Hussain, 2007)

2.14 FATTY ACID

Fats and Oils are grouped as saturated, monosaturated and polysaturated according to the fatty acids that are dominant (Ratnayake and Galli, 2009). Fatty acids from the plant are basically unsaturated at the two positions of the glyceride structure (Lee and Wang, 2008).

Palm oil as well as cocoa butter can be seen as highly structured possessing two positional fatty acids mainly unsaturated at the 1,3-fatty acids mainly saturated (Haug *et al.*, 2008).

Long chain fatty acids are poorly soluble and form insoluble calcium and magnesium salts. These acids require greater concentrations of bile than the unsaturated fatty acids for absorption. The saturated fatty acids like palmitic and stearic acids do not elevate blood cholesterol in people with cholesterol level within normal ranges (Mukharjee and mita 2009). It structure the synthesis of protective HDL cholesterol and removal of harmful low density lipoprotein(LDL)cholesterol.

Lipolytic hydrolysis of palm oil glycerides containing predominantly oleic acid at the 2 position and palmitic and stearic acids at the 1 and 3 positions which aids ready absorption of the 2-monoacylglycerols, while the saturated fatty acids remain poorly absorbed (Bruce and Cora, 2004). Dietary PKO generally decrease total blood cholesterol by reducing low density lipoproteins (LDL) and triglycerides while increasing high density lipoproteins in the balanced diet (Food and Nutrition Board, 2002).

Docosaheanoic acids (DHA, 22:6 n-3) are fundamental in the efficient functioning of cells, tissues, organs and systems of the body . DHA is also crucial in early development, especially

for eye and brain development (Koppenol *et al.* 2014). Eicosapentanoic acids (EPA 20:5 n-3) are precursors of anti-inflammatory eicosanoids (Ajuyah *et al.* 2003).

2.15 LIPIDS METABOLISM AND EGG YOLK

Hens have ability to deposit dietary lipids into the yolk and modify the fatty acids (FA) component of the egg (Kappenal *et al.*, 2014). The composition of the egg will determine the success of the embryo development (Sural, *et al.*, 2016).

Linoleic acid has been reported to stimulate the synthesis of lipoproteins that engulf the developing yolk and increase the weight of egg (Koppenol *et al.*, 2014). The intake of polyunsaturated fatty acids (PUFA) increases the stimulation of low density lipoproteins VLDL which are precursors for egg yolk lipids. Successful embryonic development depends on the composition of the egg which is largely influenced by maternal nutrition.

2.16 LIPIDS HORMONES AND ENDOCRINE SYSTEMS

The endocrine controls ductless gland that secretes hormones in the body. Hormones are carried by the bloodstream to different cells of the body. The ability to sustain homeostasis and irritability is carried out by hormones in the body. Hormones are needed for growth, constant temperature maintenance, offspring production and other basic life essentials. The endocrine secret two types of hormones namely steroidal (lipid contained) and the none steroidal (protein contained) hormones. Hormones are the chemical substances that are produced in the cell and influence specific changes in the cellular functioning of other cells. In birds, the steroids hormone from the yolk affects developing offspring (Elf and Fivizzani, 2002). Increasing level of estradiol (E2) transport from maternal circulation system to the yolk was reported to permanently alter sex differentiation of the offspring (Adkins-Reagan *et al* 1995). Sex differentiation of yolk hormone levels at early stages of development of the pea fowl has been reported by Petric *et al* (2001) to affect chromosomal segregation which

determines the sex of the hatchling. Some specific hormones like androstenedione, dihydrotestosterone, estradiol and testosterone has been shown to influence gene expression and sex differentiation (Elf and Fivizzani, 2002). Among other hormones, corticosterone exogenous administration prior to the onset of sex chromosome segregation adjusts sex ratio skew towards males by 28% (Pike and Petrie, 2014).

2.17 LIPIDS, CHOLESTEROL AND STEROIDAL HORMONE ACTION

Steroidal hormones are obtained from cholesterol (Miller and Auchus, 2011). Cholesterol is produced from acetates (approximately 90%) or from diet (approximately 10%). Cholesterols are basically synthesized and stored in the liver, and are transferred to other cells in form of high density lipoproteins (HDL) or low density lipoproteins (LDL) (Miller and Bose, 2011). In animals, the production and the assimilation of cholesterol into the specified cells are regulated strictly by the liver. Cholesterol is a hydrophobic molecule that is insoluble in water. The larger portion of cholesterol produced function with cell membranes in the body and it performs an essential role in maintaining membrane fluidity (Mesmin and Maxfield 2009). During transportation of high density lipoproteins, low density lipoproteins and their storage in intracellular lipid droplets, the three position hydroxyl is altered to enlarge the hydrophobicity site of molecule by fatty acids ester formation.

Miller and Auchus (2011) reported that steroidal hormones which influence reproduction, sexual growth and development in avian species are categorized into three hormones: progestins, androgens, and estrogens. The progestin is known as progesterone, that has 21 carbon chains (pregnane), 3 keto Δ^4 chain steroids like cortisol and aldosterone. Progesterone usually acts as a precursor to other steroid hormone because of slight adjustment from the main steroidal template (Miller and Auchus, 2011).

The steroid hormone gain access into the cell possibly through inactive diffusion, and attaches itself to the receptor. The joining together of hormone stimulates a hormone dependent activation of the receptor (change in conformation, and probably phosphorylation). The excited receptor forms reaction with DNA and nuclear proteins causing a change in the transcription of mRNA (Rone *et al.*, 2012). The mRNA is transformed into different proteins that may directly change or return to the nucleus to cause transcription of other genes. These changes are responsible for the biological influences related to steroidal hormone.

2.18 CIRCULATING HORMONES AND SEX BIAS MECHANISM

Hormones in the actively reproducing female animals within the period of sex differentiation influence the region of sex determination region (Pike and Petric 2006). The amounts of hormones circulation in avian species undergo frequent changes which are indication of both seasonal alteration and environmental variation response of birds. Observation shows that female zebra finches (*Taeniopygia guttata*) based secondary sex ratio through injections of 17 β -estradiol (Williams 1999). The female spotless starling (*Sturnus unicolor*) implanted or injected with testosterone gave birth to more males than females (Veiga *et al.*, 2004). The suggestion of Pike and Petrie (2005) stated that the proportion of male progeny reproduced by female peafowl (*Pavo cristatus*) was directly related to the amount of maternal testosterone levels and inversely related to corticosterone hormone.

There are relationship between plasma hormones and egg-yolk hormones of hen reflecting that elevated androgen led to reproduction higher number of male progeny while high 17 β -oestradiol may end in reproduction of many females (Pilz *et al.*, 2005).

CHAPTER THREE

3.0 PRELIMINARY EXPERIMENT

3.1 Effects of days post artificial insemination on fertile egg collection period, hatchability and chicks sexing methods in Yoruba ecotype chicken

Yoruba ecotype chickens are mainly kept by the rural people living in the tropical and sub-tropical areas of the world. This chicken is one of the two main types of indigenous chicken that is reared among the Yoruba tribe in Kwara state (Ige and Salako, 2014). They are prominent indigenous chicken that are hardy and easily adjust to different diets. They are believed to constitute 80% of the poultry numbers kept in Africa (Ajayi, 2010). In the rural areas of Nigeria, indigenous chickens constitute about 80% of 120 million poultry production. This is so because of intrinsic beneficial ability of the birds to withstand harsh weather, laying and hatching chicks on their own, scavenging for food and the observed innate immunity against endemic diseases (Ajayi, 2010).

The male and female of Yoruba ecotype chickens are usually kept together which gives room for indiscriminate mating. Keeping the male and female together have significant effect on the systematic differences in the growth performance and aggressiveness in feeding behaviour (Sola-Ojo *et al.*, 2008), marketing age, management and nutrition (Alasahan and Akpınar, 2014) between both sexes.

Chicks sexing in the commercial hatcheries are carried out on day old chicks using skilled animal breeder with the aim of separating the faster growth male line from female of lower performance (Shafey *et al.*, 2013). This can be achieved through various methods which this study would like to exploit three. The first is vent sexing (Tran *et al.*, 2010). This is based on the observable difference in the rudimentary genitalia organ of male termed ‘bumps’ (Cerit and Avanus, 2007). The method was first practiced in 1925 by the Japanese and has now been found to be the most reliable method of sexing day old chicks with an accuracy of 95%

(Cerit and Avanus, 2007). Although, Alasahan and Akpinar (2014) reported that the method has its difficulty in being dependent on trained expert sexer for this accuracy (95%) to be achieved.

The second method is feather sexing which is based on observable differences in the feather length characteristic at hatching (Kalina *et al.*, 2012). This requires specific crosses between rapid feathering male and slow feathering female to produce slow feathering male chicks and rapid feathering female chicks at hatching.

Apart from these two methods of chick sexing, sexually dimorphicized trait can be employed over a particular period to separate male from female chicks. This is based on variation in sizes between male and female animals kept together which may be in term of expression of some alleles which are male-limited in such a way that they conditioned to physiological or hormonal differences in growth pattern of male and female birds (Coyne *et al.*, 2007). On the other hand, both sexes could express the sexual dimorphic trait initially but later disappeared in female (Coyne *et al.*, 2007). This is why in most indigenous chicken, the male and female are mostly similar in physical appearance at their early stage of development. However, at some particular growth stage, male and female growths are governed by hormonal and physiological control (Alexander, 2002) and this result in phenotypic and genetic variation between the two sexes.

Reports on sexual dimorphism revealed that female quails are heavier than male quails at six weeks and above (Ojo *et al.*, 2014). The report of Sola-Ojo *et al* (2008) affirmed that the male of Fulani ecotype chicken can be separated from their female at eight weeks of rearing and the male is heavier than female at all ages.

There is paucity of information on sexing Yoruba ecotype chicken because bulks of birds that make up indigenous chickens have unrelated genetic population and diversity. Thus, male

and female chickens are reared together in every household backyard farm in Nigeria and can only be separated at maturity through prominent comb and wattle of the male.

This pilot study investigated the possibility of separating Nigerian' indigenous chicken (Yoruba ecotype) at day old and the common sexual dimorphic practice among local farmers to determine the best time male chicks can be separated from the females. Also, it is not uncommon among local farmers to rear both male and female together, the practice that gives room for uncontrolled mating among the flock. This practice makes it factually impossible to know how long Yoruba ecotype chicken can retain fertility after mating or artificial insemination.

3.2 Objectives

1. To investigate the periods of fertile eggs collection after artificial insemination
2. To examine various methods of separating the male from female of Yoruba ecotype chicken before sexual maturity.

3.3 Materials and methods

This experiment was carried out at Blessed Samaka farm along Asa-Dam road and Animal Production Laboratory, Faculty of Agriculture, University of Ilorin, Ilorin, Kwara State.

A total of seventy five (75) Yoruba Ecotype Chicken (YEC) (fifteen (15) males and sixty (60) females) of thirty six (36) weeks of age were used for this study. Commercial diet of 17.00 percent crude protein and 2700Kcal/kg metabolizable energy were fed to the birds throughout the experimental periods. The female chickens were randomly allotted to twenty units cages such that one unit (1m x1m) consisted of three birds while the males were allotted to five units cages (3 birds to a unit). The twenty cage units for female were divided into four divisions (replicates) such that each division consisted of five units. Fresh semen was collected from male through massage method. The semen were pooled and immediately withdrawn using 1ml syringe which was inserted into the left opening (cervices) of the female reproductive tract. Two (2) drops (0.2ml) of the semen were released into the cervices of laying hens in each insemination. The insemination was done consecutively for three days after which egg collection started on the fourth day and lasted until the twenty fifth day. Twenty (20) eggs (1 egg/ unit) laid were collected, labelled and weighed using electronic weighing scale daily for seven days and incubated on the seventh day. This was repeated weekly for three weeks (making a total of 420 eggs set in 21 days). Egg fertility test was carried out on the fifth day of incubation using candling machine.

The percentage fertility was determined as

$$\% \text{ fertility} = \frac{\text{Number of fertile egg} \times 100}{\text{Number of egg set}}$$

The percentage hatchability was determined as

$$\% \text{ Hatchability} = \frac{\text{Number of chicks hatched}}{\text{Number of egg set}} \times 100 \text{ (Adeyina et al., 2014)}$$

At the end of the incubation period, a total of 130 day old chicks were tagged immediately after hatching and their sex was determined through vent sexing at day old (Cerit and Avanus, 2007). This involves observing chick genital organ for the presence of ‘bumps’ (males) or absence of ‘bump’ (females). Feather sexing was carried out to observe the chicks wing for the height of covert and primary wing feather. Subsequently, weight of the chicks were determined weekly and body linear measurement were recorded weekly using the methods described by Sola-Ojo *et al* (2013) for 8 weeks to determine the differences in sexual dimorphism of linear body measurements.

The weight of the body (BW) was determined in gram using electronic sensitive scale and the linear measurement of body parts were determined using tape rule and vernier calliper. Body length (BL) was determined as the distance between the nasal opening to the top of pygostyle; Body girth (BG) was determined by winding a tape rule around the region of the breast; shank length (SL) was measured as the distance from the foot pad to the hock joint; thigh length (TL) was measured from the tip of the tarsus to the ball joint; Keel length (KL) was measured from the cranial to the caudal terminals of the keel bone; wing length (WL) was measure by stretching the wing and the measurement taken from humerous-coracoids junctions to the tip of the digit while the drumstick length (DL) was measured from the tip of the hock joint to the ball joint of femur.

The accuracy of vent sexing at day 3 and sexual dimorphism using body weight and linear measurements from 1-8 weeks were tested by comparing the data collected from day old with the morphometric characters at 18 weeks of age when the birds were clearly distinct in their sexes.

3.4 Statistical analysis

All data obtained from the periods of fertile eggs collection post artificial insemination (AI) were subjected to Analysis of Variance (ANOVA) procedure suitable for a Completely Randomize Design model (SAS, 1999). Significantly different means at $p < 0.05$ were separated using Duncan Multiple Range Test (Duncan 1955); Data on vent sexing of chicks were subjected to descriptive statistics (percentile) while data on weekly body weight and morphometric measurements were subjected to Student *t*-test.

3.5 Results

The effects of post artificial insemination time on fertile egg collection in Yoruba ecotype chickens are shown in Table 3.1. The percentage egg fertility was significantly ($p<0.05$) influenced by post artificial insemination collection time. This egg fertility result can be divided into three phases: not steady (days 1 to 6), steady (days 7 to 12), and decline (13 to 21). In the non-steady phase, the percentage fertility showed significant ($p<0.05$) increase (11.8%) from day 5 to day 6 (74.59^{abc} - 84.59^a) followed by a decrease of 10.34% on the 7th day (84.59^a - 75.84^{abc}).

In the steady phase, the fertility of egg was constant from day 7 to 12 (75.84^{abc} - 77.50^{abc}). While at the decline phase, there was 35.5% decrease in percentage fertility from days 12 to 13 (77.50^{abc} – 50.00^{cdef}). Egg fertility also decreased ($P<0.05$) by 34.6% from days 15 to 16 (42.05^{ef} – 27.50^{fg}) and 71.9% decrease from days 18 to 19 (26.68^{fg} – 7.50^{gh}). Finally, the egg fertility decreased by 100% from days 20 to 21 (7.50^{gh} – 0.00^h). The highest percentage fertility (84.59^a) was recorded at the 6th day post artificial insemination while the least fertility was on day 20.

The effects of post artificial insemination collection time on hatchability was not consistent from days 1 to 12, however there was significant decrease ($p<0.05$) from days 13 to 21.

Table 3.1: Effects of post artificial insemination collection time on egg fertility and hatchability of Yoruba ecotype chicken.

Days of egg collection	Fertility (%)	Hatchability (%)
1	70.83 ^b	65.00 ^d
2	75.84 ^b	70.00 ^c
3	70.83 ^b	70.00 ^c
4	80.84 ^{ab}	75.00 ^b
5	74.59 ^b	70.00 ^c
6	84.59 ^a	80.00 ^a
7	75.84 ^b	75.00 ^b
8	73.34 ^b	70.00 ^c
9	70.01 ^b	70.00 ^c
10	70.00 ^b	65.00 ^d
11	72.50 ^b	65.00 ^d
12	77.50 ^b	70.00 ^c
13	50.00 ^c	40.00 ^e
14	44.42 ^c	30.00 ^f
15	42.05 ^c	25.00 ^g
16	27.50 ^d	20.00 ^h
17	26.25 ^d	10.00 ⁱ
18	26.68 ^d	5.00 ⁱ
19	7.50 ^e	0.00 ^k
20	7.50 ^e	0.00 ^k
21	0.00	0.00 ^k
SEM	8.52	4.93

Means with different superscript on the same column are significantly different (P<0.05) SEM=standard error of means.

Table 3.2 showed the result of sex prediction using vent sexing method, feather sexing and sexual dimorphism over 8 weeks. The percentage of Vent sexing method was higher (73.58% males and 75.08% females) than feather sexing method (62.26% males and 64.20% females).

The percentage accuracy for dimorphic indices was 95.00 for male and 92.31% for female chicks at 8 weeks.

Table 3.2: Accuracy of sexing Yoruba ecotype chicken using vent sexing, feather sexing and sexual dimorphism.

Methods of sex prediction	Percentage accuracy at 18 weeks old	
	Male (%)	Females (%)
Vent sexing	73.56	75.08
Feather sexing	62.26	64.20
Dimorphic indices at 8 weeks	95.00	92.31
Actual sex at week 18	100.00	100.00

The results of sexual dimorphism in Yoruba ecotype chickens are presented in Table 3.3. Generally, male chicks had significantly higher ($p < 0.05$) values for body length, body girth and wing length than female chicks from 1 to 8 weeks of age. The result of other body linear measurements such as keel length and shank length showed that males were significantly ($p < 0.05$) longer, bigger and taller than the females at weeks five to eight. The thigh length of male was longer ($p > 0.05$) than the female at six to eight weeks while drumstick of male was longer than the female at week eight.

There was no significant ($p > 0.05$) difference in body weight between males and female chicks from week 1 to 6, however significant differences ($p < 0.05$) existed between male and female chicks in body weight at week 7 and 8.

Table 3.3: Mean Weekly Body Weight and Morphometric Measurements in Yoruba Ecotype

Chicken

Age(Wks)	Sex	BW(g)	BL(cm)	BG(cm)	WL(cm)	DL(cm)	KL(cm)	TL(cm)	SL(cm)
1	M	53.20 ±13.82	13.77 ±1.55 ^a	8.43 ±1.21 ^a	5.52 ±0.81 ^a	2.84 ±0.43	3.34 ±0.72	3.76 ±0.39	2.50 ±0.45
	F	48.82 ±10.40	13.36 ±1.59 ^b	8.24 ±0.93 ^b	5.51 ±0.78 ^b	2.84 ±0.46	3.41 ±0.74	3.68 ±0.42	2.42 ±0.47
2	M	87.61 ±23.97	16.59 ±1.77 ^a	9.96 ±1.07 ^a	7.72 ±0.96 ^a	3.45 ±0.09	4.80 ±0.75	4.59 ±0.57	3.16 ±0.67
	F	81.02 ±23.15	16.34 ±1.87 ^b	9.69 ±0.96 ^b	7.51 ±0.85 ^b	3.33 ±0.08	4.54 ±0.75	4.46 ±0.62	2.95 ±0.59
3	M	142.57 ±30.87	19.62 ±2.00 ^a	12.43 ±1.17	9.65 ±0.76 ^a	4.00 ±0.56	5.73 ±0.73	5.54 ±0.60	3.65 ±0.68
	F	124.05 ±28.74	18.87 ±1.98 ^b	11.94 ±1.34	9.28 ±0.85 ^b	3.95 ±0.63	5.39 ±0.67	5.28 ±0.62	3.49 ±0.65
4	M	198.66 ±44.15	22.51 ±1.74 ^a	15.15 ±1.11 ^a	11.44 0.85	4.45 ±0.37	6.61 ±0.76	6.28 ±0.59	4.06 ±0.30
	F	164.25 ±37.38	21.33 ±1.75 ^b	13.90 ±1.57 ^b	10.69 ±1.04	4.24 ±0.38	6.10 ±0.70	5.82 ±0.67	3.80 ±0.39
5	M	221.05 ±48.10	24.10 ±1.98 ^a	14.98 ±1.03 ^a	12.51 ±0.93 ^a	4.89 ±0.40	7.10 ±0.89 ^a	6.71 ±0.59	4.32 ±0.28 ^a
	F	185.64 ±40.90	22.71 ±1.99 ^b	13.95 ±1.09 ^b	11.64 ±1.06 ^b	4.58 ±0.44	6.47 ±0.84 ^b	6.34 ±0.64	3.99 ±0.29 ^b
6	M	300.20 ±65.77	26.25 ±2.14 ^a	16.52 ±1.09 ^a	13.83 ±1.28 ^a	5.43 ±0.50	7.79 ±0.86 ^a	7.35 ±0.63 ^a	4.90 ±0.45 ^a
	F	241.75 ±52.25	24.66 ±2.14 ^b	15.08 ±1.22 ^b	12.70 ±1.31 ^b	5.04 ±0.55	7.02 ±0.77 ^b	6.82 ±0.73 ^b	4.50 ±0.44 ^b
7	M	358.64 ±68.46 ^a	28.28 ±2.31 ^a	17.57 ±1.25 ^a	14.76 ±1.21 ^a	5.87 ±0.45	8.30 ±0.85 ^a	7.90 ±0.60 ^a	5.41 ±0.45 ^a
	F	283.21 ±61.38 ^b	26.09 ±2.33 ^b	16.16 ±1.28 ^b	13.61 ±1.16 ^b	5.41 ±0.52	7.44 ±0.79 ^b	7.30 ±0.69 ^b	4.90 ±0.49 ^b
8	M	404.00 ±76.01 ^a	30.51 ±2.02	18.52 ±1.25 ^a	15.95 ±1.27 ^a	6.34 ±0.52 ^a	8.86 ±0.74 ^a	8.40 ±0.62 ^a	5.84 ±0.50 ^a
	F	325.82 ±71.31 ^b	28.10 ±2.54	17.04 ±1.34 ^b	14.62 ±1.34 ^b	5.82 ±0.53 ^b	8.04 ±0.77 ^b	7.76 ±0.75 ^b	5.22 ±0.49 ^b

Means with different superscript on the same column between M and F were significantly different ($P < 0.05$). wks, M, F, BW, BL, BG, WL,

DL, KL, TL and SL represent weeks, male, female, body weight, body length, body gait, wing length, drumstick length, keel length, thigh length and shank length respectively.

3.6 Discussion

The observed percentage of egg fertility from days 1 to 20 post artificial insemination strongly suggests that Yoruba ecotype chicken could store sperm and retain fertility for three weeks. The Nigerian indigenous chicken may have sperm storage tubules (SST) that could store sperm for 20 days. This report concurs with the finding of Brillard and Motter, (1992) who posited that laying hen can retain viable sperm cells in their sperm storage tubules for about 6 to 45 days depending on their types. Chicken has been reported to have tubules that could store sperm for three weeks while the turkey could store sperm for 10 weeks (Bakst *et al.*, 2010). Adeyina *et al* (2015) observed that Japanese quail has the capacity to store sperm cells in female reproductive tract for about 10 days. The variation between the current findings and that of Adeyina *et al* (2015) may be related to the differences that exist between types and varieties of birds as Yoruba ecotype chickens are bigger than Japanese quail.

The highest percentage fertility (on day 6) obtained in this study suggests that the spermatozoa are best capacitated at this period. This corroborates the report of Suarez (2008) that sperm undergoes a biochemical transition process called capacitation that allowed motility hyperactivation of sperm in the SSTs to fertilize oocyte in mammals.

It has been reported that reduction in fertility of birds is connected with reduction in the capacity to retain sperm in the SST and a decrease in volume of sperm transported into the fertilization site (Santos *et al.*, 2013). The observed reduction in egg fertility from days 12 (77.50%) to 20 (7.50%) may be due to decrease in volume and metabolism rate of sperm transported into the fertilization site over some periods of time.

It can therefore be concluded that Yoruba ecotype chicken has the capacity to store sperm cells in female reproductive tract for up to 20 days but fertile eggs are better collected between day 1 and day 12 post artificial insemination to obtain 70% or more in egg fertility.

Female chicken need not be daily mated or inseminated and the male can be separated until they are needed to be introduced for production of fertile eggs.

The method of sex prediction through Japanese vent sexing reflected greater potential tools for separating males from females local chickens than feather sexing method. Separating heterogeneous population of local chicken into male and female with 73.58% accuracy on day 3 post hatching of egg may solve the problem of inequality in growth between male and female chicken and aggressiveness towards feeding that make female inadequately fed (Sola-Ojo *et al.*, 2013).

The lower percentage (73.58%) of prediction through Japanese vent sexing method when compared with the recommended 95% (Alaşahan and Akpınar, 2014) accuracy may be related to close association of observed organ of male and female at this stage of development.

The equality in body weight of chicks from weeks 1 to 6 may suggest reason while Yoruba ecotype chickens are very difficult to separate in their early ages using body weight alone. However, from week seven and above, the males could be separated from the females since at this stage, the males have pronounced combs and higher body weight than their female counterpart. This observation is consistence with the findings of Sola-Ojo *et al* (2008) who observed that morphologically males appear bigger and taller than the females and their comb are well pronounced at 8 weeks of age.

The lower body weight recorded in this experiment when compared with the body weight reported by Sola-Ojo *et al* (2008) may be ascribed to differences in the strains of birds used as Fulani ecotype chicken are characterized by uniform, heavy and faster growth rate than other Nigerian indigenous chickens (Fayeye and Adesiyan, 2008).

It can therefore be concluded that vent sexing method seems to be 73.58% accurate. Male chicks could be separated from female chicks at week 7 using body weight.

CHAPTER FOUR

4.0 EXPERIMENT 1

4.1 Hormonal, Egg characteristics and Reproductive responses of Yoruba ecotype chicken to dietary inclusion of palm kernel oil

4.2 Introduction

The Trivers-Willard postulation predicates laying hens in good dietary state should skew their offspring sex ratio in favour of male chicks while those in poor dietary state should adjust the sex of their chicks in favour of females (Trivers and Willard, 1973, Cameron and Linklater, 2002). In this postulation, the good and poor dietary conditions are referred to as parental condition. Therefore, factors such as parental condition (Nager *et al.*, 2000), dietary composition (Bradbury and Blakey, 1998), feeding regime (Kilner, 1998) and incubation temperature (Ferguson, 1994a, b) have been hypothesised to affect the offspring sex produced. These aforementioned factors were suggested to act by stimulating an endogenous change in the laying hen which may be responsible for the release of a particular hormone such as testosterone, oestrogen and other sex hormone. Similarly, fat and oil inclusion in the diet of layers was reported to increase egg weight, production and hatching efficiency in breeding stocks (Sosin-Bzducha and Krawczyk, 2012) while Thomas and Marion (2013) reported that low-fat and oil diet affected the litter size of mice with a significant sex ratio distortion (~1:3, females : male) relative to controls where the offspring sex ratio was 1:1.

Palm kernel oil (PKO) has been reported to have high saturated fat approximately 80% (Chow and Ching, 2012). The most abundant fatty acids in Palm kernel oil is lauric acid (Hudson and Knight, 2012) and it is about 40%. Lauric acid in PKO has the potentials of raising the blood cholesterol levels both as low density lipoproteins and high density lipoproteins (Rakel, 2012). The template for sex (steroids) hormone formation in the body is

cholesterol and this is formed from lipids that are derivatives of fat and oil. Zuo *et al* (2015) reported that fat and oil (lipids) metabolism is crucial factor in cell differentiation. PKO also contains linoleic acid which is one of the two fundamental fatty acids needed by chicken for proper physiological functioning of the body (Senkoylu *et al.*, 2004). Fats and oils inclusion in the diet of laying hen was reported to produce a special scent in the yolk that developing embryos normally smell and utilise through chemosensory actions of the surrounding (Aigueperse *et al.*, 2013). Palm kernel oil is very rich in vitamins K (Chad, 2014) which functions in bone health and blood coagulation.

The objective of this study was to assess the effects of graded levels of palm kernel oil (PKO) inclusion in the diet on the serum biochemistry, haematology, hormone, reproductive response and offspring sex ratio of Yoruba ecotype chicken.

4.3 Materials and Methods

4.3.1 Palm kernel oil

Forty litres of palm kernel oil (PKO) was obtained from lucky palm kernel oil processing industry at airport area of Ilorin, Kwara state, Nigeria.

4.3.2 Determination of fatty acid composition of palm kernel oil

A triplicate sample containing 100mg PKO each was taken to the central research laboratory and chemical engineering laboratory of University of Ilorin, Ilorin for de-esterification process and Gas Chromatography Mass Spectrometry (GC-MS) analysis respectively (Cristina *et al.*, 2012). Briefly, 100mL of PKO was treated with 5ml methanol.BF₃ solution and refluxed for 2 minutes on a water bath, and then 5ml hexane was added; after another one minute of reflux, the solution was treated with 15mL saturated Sodium Chloride (NaCl) solution under vigorous stirring. The fatty acid methyl esters (FAMES) extracted were used for Gas Chromatography Mass Spectrometry (GC-MS) analysis to determine the fatty acids composition of PKO (Liguori *et al.*, 2015). The results are presented in Table 6.

4.3.3 Experimental design

One hundred and seventy five (100 females and 75 males), twenty weeks old Yoruba ecotype chicken raised in experiment 1 were used for this experiment. One hundred (100) females were assigned to five (5) dietary treatments of five (5) replicates and four (4) birds per replicate while the males were allotted to five (5) treatment groups of five (5) replicates and three (3) birds per replicate in a Randomized Complete Block Design (RCBD). The five dietary treatments A, B, C, D and E consisted of palm kernel oil at 0, 1, 2, 3 and 4%, respectively to the diet as presented in Table 5. All the birds were fed with control diet for the

first two weeks to allow them accustom to the environment before changing to the experimental diets.

Each bird was offered 120g of the feed per day and water was supplied *ad-libitum*. The experiment lasted eleven (11) weeks.

Palm kernel oil (1%, 2%, 3% and 4%) was added daily to already compounded feed in order to avoid rancidity. 120g of feed was offered per bird per day and the left over weighed the following morning. All animals were weighed at the commencement of the experiment before allocating them to the treatments and subsequent weights were obtained on weekly basis to determine growth performance of birds

Table 4.1: Feed composition of the experimental diets

Ingredients	0%	1%	2%	3%	4%
Palm kernel oil	-	1.00	2.00	3.00	4.00
Soya bean meal	16.50	16.00	15.50	15.00	14.50
Maize	53.50	49.00	45.00	41.00	37.00
Wheat offal	15.00	19.00	22.50	26.00	29.50
Groundnut cake	4.50	4.50	4.50	4.50	4.50
Oyster shell	4.30	4.30	4.30	4.30	4.30
Bone meal	5.00	5.00	5.00	5.00	5.00
Methionine	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
C.P (%):	16.86	16.89	16.86	16.85	16.83
M.E (Kcal/Kg):	2681.99	2677.16	2680.15	2683.14	2686.13
E.E (%):	3.51	4.44	5.38	6.33	7.27
C.F (%):	3.97	4.27	4.36	4.53	4.71

4.3.3 Hen day production

Records of daily egg production on replicate basis were taken for eleven weeks. Hen-day at a given period was estimated as the percentage of the ratio of number of egg laid to the number of hens.

$$\text{Hen Day Egg Production (\%)} = \frac{\text{number of egg laid in a day}}{\text{number of hen alive}} \times 100$$

While the record of mortality (10) was used to calculate hen-house production (HHP): This is the ratio of eggs laid in a day divided by the number of birds originally housed multiplied by 100

$$\text{Hen house production (\%)} = \frac{\text{number of egg laid in a day}}{\text{number of birds originally housed}} \times 100$$

4.3.4 Feed efficiency per dozen eggs

This takes into consideration the feed intake and egg production. It is the ratio between the feed consumed and the number of eggs produced.

$$\text{FCR (Feed per dozen eggs)} = \frac{\text{Kg of feed consumed} \times 12}{\text{Total eggs produced}}$$

4.3.5 Quality assessment of settable eggs

Twenty (20) eggs (four (4) from each treatment) were collected weekly for seven weeks of the experiment, external and internal qualities of eggs were evaluated.

4.3.6a External qualities of egg

The parameters assessed include: egg weight, egg length, egg breadth and egg-shape index.

4.3.6ai Egg weight

It was measured with the aid of electric sensitive scale.

4.3.6aⁱⁱⁱ Egg shape index

This was evaluated as the ratio of the egg breadth to the egg length. The egg breadth was measured as the distance between two ends of the egg at the widest cross sectional region while the length was measured as the distance between the broad and narrows ends of the egg using Vernier callipers.

4.3.6aⁱⁱⁱ Shell thickness

The thickness of the individual dry shells was measured to the nearest 0.01mm using micrometer screw gauge.

4.3.6a^{iv} Shell weight

Egg shells were air-dried and the weight measured with the aid of an electric sensitive scale.

4.3.6b Internal qualities of egg

All the eggs sampled for external egg quality assessment were used for the assessment of albumen height, albumen weight, yolk weight, shell weight, shell thickness and Haugh unit).

4.3.6bⁱ Albumen height:

This was measured using the p6085 spherometer (tripod micrometer) with 0.01mm accuracy in a flat dish.

4.3.6bⁱⁱ Percentage albumen weights:

This was determined using the difference between the weight and the sum of weight of yolk and dry egg shell as a percentage of the whole egg.

4.3.6bⁱⁱⁱ Yolk weight:

This was measured with the aid of mettle top-loading weighing balance.

4.3.6biv Yolk colour:

It was determined for individual yolk by comparing the yolk with the colour chips of a Hoffman-Laroche (R) Yolk colour fan. The yolk colour fan has different scores for different colour ranging from pale yellow (score 1) to deep orange (score 15)

4.3.6bv Haugh unit (HU):

The HU was calculated using the values obtained for egg weight and albumen height as expressed by Card *et al* (1979) in the formula shown below

$$HU = 100 \log (H + 7.5 - 1.7W^{0.37})$$

Where H= Albumen height in mm

W= Egg weight in gram

4.3.6 Fertility, hatchability and sex ratio

At the ninth week of feeding trials, semen was collected from male in each treatment and used to inseminate the female within the same treatment daily for three consecutive days after which fertile egg collection started on the fourth day and lasted for twelve days. A total of 600 fertile eggs (120 eggs from each treatment) were collected and set two times within 12 days inside the incubator. Fertility test was carried out at day 5 and 18 of incubation to determine fertile eggs and live germ, respectively. The number of hatched eggs and unhatched eggs were also recorded. Percentage male and female chicks were determined at week eight after hatching.

4.3.7 Semen collection and analysis

Semen was collected after feeding Palm Kernel Oil for eleven weeks from twenty five males (1 male in each replicate) through massaging of the dorso-lateral lumbosacral region of the

cockerels to cause erection of the male copulatory organ (papilla) and subsequent ejaculation (Peter *et al.*, 2008). The semen was subjected to physical evaluations to determine semen colour and volume. Sperm motility was determined by placing a drop of the semen on a clean glass slide under the cover slip and viewed on a binocular microscope. Sperm motility was determined by counting both motile and immotile spermatozoa per unit area and sperm concentration was determined using the haemocytometer (Patrick *et al.*, 2009).

4.3.8 Hormonal, serum biochemical and haematological indices of birds

At the end of the eleventh week of feeding PKO, blood samples were collected from the wing web of experimental birds (1 bird in each replicate) into bottles containing EDTA (Ethylene Diamine Tetra acetic) which acts as an anticoagulant. The uncoagulated blood samples were analyzed for haematological parameters such as Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Platelets Pack Cell Volume (PCV) and others. All haematological analysis were carried out using HA 6000 machine (autohaematology analyzer) at the Faculty of Veterinary Medicine, University of Ilorin. While Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) values were calculated using the formula described by Schalm *et al* (1975).

Another blood was collected into labeled sterile sample bottles without anticoagulant and used to determine the serum biochemical contents such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total protein, Albumin, Total cholesterol and its' components (Triglycerides, High Density Lipoprotein and Low Density Lipoprotein). These serum biochemical parameters were analyzed using the procedure of monobind microplate immune enzymetric assay method using semi-auto

chemical analyzer (Rayto RT-1904C). The blood was analyzed for the concentration of reproductive hormones (luteinizing hormone, Follicle Stimulating Hormone, testosterone, oestrogen and progesterone) following the instructions of monobind microplate immunoassay (IEMA/ELISA) method using microplate reader (Rayto RT-2100C) as described by Mitruka and Rawnsley (1977) and Jain (1986).

4.4 Statistical analysis

The data obtained on serum biochemistry, haematology, hormonal indices, egg qualities and performance characteristics were subjected to analysis of variance (ANOVA) as appropriate for factorial design using SAS (1999), while the sex limiting parameters such as semen quality, fertility, hatchability and sex ratio were subjected to completely randomized design of the same package.

4.5 Results

The composition of fatty acid in the palm kernel oil is shown in Table 4.1. Lauric acid was presented at the highest percentage (36.53%), followed by Palmitic acid (18.59%). Myristic acid had 18.18%, Oleic acid had (18.09%), stearic acid had 5.33% and linoleic acid had 3.28%.

Table 4.1: Fatty acids composition of palm kernel oil

Fatty acids	Composition (%)
Lauric acid	36.53
Myristic acid	18.18
Palmitic acid	18.59
Stearic acid	5.33
Oleic acid	18.09
Linoleic acid	3.28

The performance characteristics of male Yoruba Ecotype Chicken (YEC) fed diet supplemented with graded levels of palm kernel oil are shown in Table 4.2. The feed intake was significantly ($p<0.05$) influenced by levels of Palm Kernel Oil (PKO) inclusion in the diet. The feed intake increased with inclusion levels of PKO such that Cocks on 3% (65.56^a) and 4% (65.56^a) consumed higher quantity of feed than those on 1% (55.40^c) and 0% (55.57^c) respectively. The final weight of male were not significantly ($p>0.05$) affected. The weight gain of cocks on 0% to 3% PKO inclusion was not significantly ($p>0.05$) different from one another but decreased ($p<0.05$) at 4% PKO inclusion. The feed to gain ratio was significantly ($p<0.05$) higher at 4% PKO (41.23^a) than other levels of inclusion (22.50^b - 25.50^b)

Table 4.2: Performance characteristic of male Yoruba ecotype chicken fed diet supplemented with graded levels of palm kernel oil

Trt/parameters	0%PKO	1% PKO	2% PKO	3% PKO	4% PKO	SEM
Initial weight(g)	1436.80	1437.00	1436.80	1436.80	1437.00	2.95
Final weight(g)	1627.10	1613.90	1621.00	1635.00	1559.90	20.29
Total weight gain (g)	190.30 ^a	176.90 ^{ab}	184.20 ^a	198.20 ^a	122.90 ^c	20.57
Weight gain(g/bird/day)	2.47 ^a	2.30 ^{ab}	2.39 ^a	2.57 ^a	1.59 ^c	0.28
Feed intake(g/b/d)	55.57 ^c	55.40 ^c	60.90 ^b	65.56 ^a	65.56 ^a	0.98
Feed:Gain	22.50 ^b	24.09 ^b	25.48 ^b	25.50 ^b	41.23 ^a	3.68

a,b,c: means with different superscript on the same column are significantly different (P<0.05). Trt=treatment,

PKO= Palm Kernel Oil, SEM=standard error of means

The performance characteristics of female YEC fed diet supplemented with graded levels of PKO (Table 4.3) showed that feed intake, feed per dozen egg and egg weight were significantly ($p<0.05$) affected by the levels of PKO inclusion in the diet. The feed intake of hens on 0-1% and 3% PKO inclusion were the same and significantly ($p<0.05$) different from those on 2% and 4% PKO. The efficiency of feed conversion (feed per dozen egg) for hens on 0-3% PKO inclusion were the same ($p>0.05$) and significantly ($p<0.05$) different from those on 4% PKO. Egg weight increased ($p<0.05$) with increase in inclusion levels of PKO.

Table 4.3: Performance characteristic of female Yoruba ecotype chicken fed diet supplemented with graded levels of palm kernel oil

Trt/parameters	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO	SEM
Initial weight (g)	1049.20	1048.20	1049.20	1049.00	1048.00	7.45
Final weight (g)	1164.00 ^a	1103.7 ^d	1122.9 ^c	1091.8 ^e	1148.2 ^b	12.33
Feed intake (g/b/d)	78.22 ^a	78.90 ^a	74.35 ^b	78.09 ^a	68.37 ^c	1.11
Feed/dozen egg(Kg)	2.13 ^b	1.97 ^b	2.14 ^b	2.16 ^b	2.93 ^a	0.56
Egg weight(g)	34.58 ^d	35.85 ^b	35.30 ^c	36.47 ^a	36.30 ^{ab}	0.17

a,b,..e: means with different superscript on the same column are significantly different ($P<0.05$). Trt=treatment, SEM=standard error of means

The effects of PKO on weekly egg laying performance of YEC (Table 4.4) showed that egg weight increased ($p<0.05$) from week 1 (30.30g) to week 11 (35.21g) of laying. Similarly, percentage hen day production increased from week 1 (19.14%) to week 4 (54.14%) and decreased from week 6 (47.99%) to week 9 (37.38%). The hen house followed similar trend as hen day. Egg weight showed significant ($p<0.05$) increase with increase in inclusion level of PKO. Hen day and hen house reflected significant ($p<0.05$) increase from control diet to 1% PKO inclusion which was followed by decrease ($p<0.05$) in both hen day and hen house production from 2% PKO inclusion and above.

Table 4.4: Weekly laying performance of Yoruba ecotype chicken fed diet supplemented with graded levels of palm kernel oil

Week	Egg Weight (g)	Hen day (%)	Hen house (%)
1	30.300 ^f	19.140 ^f	19.140 ^d
2	31.574 ^e	27.880 ^e	27.880 ^c
3	32.574 ^d	45.288 ^b	45.288 ^b
4	33.294 ^c	54.140 ^a	54.140 ^a
5	33.326 ^c	50.140 ^a	53.220 ^a
6	34.024 ^b	47.996 ^b	51.380 ^a
7	34.700 ^b	39.140 ^d	42.688 ^b
8	35.896 ^a	44.432 ^b	48.716 ^{ab}
9	35.896 ^a	37.380 ^d	41.464 ^b
10	35.092 ^a	38.424 ^d	44.660 ^b
11	35.212 ^a	42.428 ^c	48.716 ^{ab}
SEM	0.377	3.024	3.215
Treatment			
0% PKO	33.241 ^c	43.731 ^{ab}	48.575 ^{ab}
1% PKO	33.915 ^{abc}	49.184 ^a	51.838 ^a
2% PKO	33.390 ^{bc}	40.458 ^b	43.033 ^{bc}
3% PKO	34.042 ^{ab}	31.305 ^c	33.067 ^d
4% PKO	34.170 ^a	38.225 ^b	41.040 ^c
SEM	0.254	2.039	2.168
Week*Trt	S	S	S

a,b,...f: means with different superscript on the same column are significantly different (p<0.05).
Trt=treatment, SEM=standard error of means and S=significant

Table 4.5: Interaction of dietary palm kernel oil and weeks on egg weight of Yoruba ecotype chicken

Week/Trt	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	30.54 ^d	29.52 ^d	30.40 ^d	30.56 ^c	30.48 ^d
2	31.64 ^{cd}	31.62 ^{cd}	31.78 ^{cd}	31.76 ^{bc}	31.07 ^{de}
3	31.92 ^{bcd}	32.72 ^{bc}	33.08 ^{bc}	33.26 ^{abc}	31.96 ^{cde}
4	31.55 ^{cd}	34.32 ^{ab}	33.88 ^{ab}	33.56 ^{abc}	33.16 ^{bcd}
5	32.38 ^{abcd}	34.16 ^{ab}	33.32 ^{bc}	33.42 ^{abc}	33.34 ^{bc}
6	33.32 ^{abcd}	34.50 ^{ab}	33.16 ^{bc}	34.48 ^{ab}	34.66 ^{ab}
7	33.52 ^{abcd}	35.06 ^{ab}	33.58 ^{bc}	34.86 ^a	36.48 ^a
8	34.72 ^{abc}	35.26 ^{ab}	34.32 ^{ab}	35.72 ^a	36.04 ^a
9	35.10 ^{ab}	35.40 ^{ab}	33.64 ^{bc}	35.44 ^a	36.80 ^a
10	35.58 ^a	35.02 ^{ab}	34.56 ^{ab}	35.00 ^a	35.30 ^{ab}
11	35.38 ^a	35.48 ^a	35.64 ^a	36.40 ^a	36.58 ^a
SEM	0.37	0.33	0.25	0.35	0.35

a,b,...e: means with different superscript on the same column are significantly different ($p < 0.05$).
Trt=treatment, SEM=standard error of means, egg weight was measured in grams.

Table 4.6: Interaction of dietary palm kernel oil and weeks on Hen day of Yoruba ecotype chicken

Week/Trt	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	21.000 ^a	21.000 ^b	17.900 ^c	17.900 ^c	17.900 ^c
2	28.600 ^{ab}	28.600 ^{cd}	27.880 ^{bc}	27.880 ^{bc}	26.440 ^{bc}
3	42.140 ^{ab}	55.000 ^{ab}	46.420 ^{ab}	37.000 ^{ab}	45.000 ^{ab}
4	53.580 ^a	61.420 ^a	60.720 ^a	47.840 ^a	47.140 ^{ab}
5	52.140 ^{ab}	66.440 ^a	39.300 ^{abc}	47.840 ^a	44.980 ^{ab}
6	49.280 ^a	65.000 ^a	48.580 ^{ab}	30.000 ^{bc}	47.120 ^{ab}
7	46.440 ^{ab}	49.280 ^{ab}	35.720 ^{abc}	22.860 ^{bc}	41.400 ^{ab}
8	49.980 ^a	48.580 ^{ab}	41.420 ^{abc}	30.740 ^{bc}	51.440 ^a
9	45.000 ^{ab}	42.840 ^{bc}	37.860 ^{abc}	29.280 ^{bc}	31.920 ^{abc}
10	46.440 ^{ab}	48.580 ^{ab}	43.520 ^{abc}	23.580 ^{bc}	30.000 ^{bc}
11	46.440 ^{ab}	54.280 ^{ab}	45.720 ^{ab}	28.560 ^{bc}	37.140 ^{abc}
SEM	2.529	2.403	2.715	1.772	2.202

a,b,c: means with different superscript on the same column are significantly different (p<0.05). Trt=treatment, SEM=standard error of means

Table 4.7: Interaction of dietary palm kernel oil and weeks on Hen house of Yoruba ecotype chicken

Week/Trt	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	21.000 ^b	21.000 ^c	17.900 ^c	17.900 ^c	17.900 ^c
2	28.600 ^{ab}	28.600 ^c	27.880 ^{bc}	27.880 ^{bc}	26.440 ^{bc}
3	42.140 ^{ab}	55.000 ^{ab}	46.420 ^{ab}	37.880 ^{ab}	45.000 ^{ab}
4	53.580 ^a	61.420 ^{ab}	60.720 ^a	47.840 ^a	47.140 ^{ab}
5	59.220 ^a	70.960 ^a	43.100 ^{abc}	47.840 ^a	44.980 ^{ab}
6	55.000 ^a	68.820 ^a	53.800 ^{ab}	32.160 ^{abc}	47.120 ^{ab}
7	54.520 ^a	49.280 ^{ab}	40.480 ^{abc}	26.100 ^{bc}	43.060 ^{ab}
8	56.900 ^a	53.820 ^{ab}	46.420 ^{ab}	33.820 ^{abc}	52.620 ^a
9	51.440 ^a	47.120 ^{ab}	41.640 ^{abc}	33.300 ^{abc}	33.820 ^{abc}
10	52.620 ^a	54.280 ^{ab}	45.940 ^{ab}	27.360 ^{bc}	43.100 ^{ab}
11	59.300 ^a	59.920 ^{ab}	49.060 ^{ab}	31.660 ^{abc}	50.260 ^a
SEM	3.010	2.493	2.724	1.862	2.287

a,b,c: means with different superscript on the same column are significantly different (P<0.05). Trt=treatment, SEM=standard error of means

The external egg quality traits of YEC fed diet supplemented with PKO are shown in Table 4.8. Egg weight, egg length and egg width of hens on PKO supplementation were significantly ($p<0.05$) higher than those on control diet. Egg shape (0.72 - 0.74) was not significantly ($p>0.05$) influenced with the inclusion of PKO. Shell thickness, shell weight and shell ratio were significantly ($p<0.05$) influenced with no specific trend. All the external egg qualities like egg weight, egg length, egg width, shell thickness, shell weight and shell ratio increased ($p<0.05$) with weeks of egg collection.

Table 4.8: Effects of dietary palm kernel oil (PKO) on external egg qualities of Yoruba ecotype chicken

Parameter/ treatment	Egg wt(g)	Egg length (cm)	Egg width (cm)	Egg shape	Shell thickness (mm)	Shell weight(g)	Shell ratio
0% PKO	35.63 ^c	4.49 ^c	3.30 ^b	0.74	0.58 ^{ab}	4.33 ^b	0.12 ^{ab}
1% PKO	37.73 ^{ab}	4.60 ^{ab}	3.38 ^a	0.74	0.71 ^a	4.50 ^{ab}	0.12 ^{bc}
2% PKO	36.98 ^b	4.56 ^{bc}	3.37 ^a	0.74	0.51 ^b	4.73 ^a	0.13 ^a
3% PKO	37.88 ^{ab}	4.65 ^{ab}	3.37 ^a	0.73	0.52 ^b	4.35 ^b	0.11 ^c
4% PKO	38.65 ^a	4.67 ^a	3.41 ^a	0.73	0.51 ^b	4.70 ^a	0.12 ^{ab}
SEM	0.45	0.03	0.02	0.01	0.05	0.10	0.2x10 ⁻²
Week							
1	33.15 ^e	4.40 ^e	3.22 ^f	0.73	0.35 ^c	3.75 ^e	0.11 ^{cd}
2	34.50 ^{de}	4.45 ^{de}	3.28 ^{ef}	0.74	0.38 ^c	4.15 ^{bcd}	0.12 ^{bcd}
3	35.95 ^{cd}	4.51 ^{cde}	3.35 ^{cde}	0.74	0.64 ^{ab}	4.10 ^{cd}	0.12 ^{cd}
4	34.55 ^{dc}	4.45 ^{dc}	3.28 ^{ef}	0.74	0.44 ^{bc}	3.80 ^e	0.11 ^d
5	36.45 ^c	4.58 ^{cd}	3.33 ^{dc}	0.73	0.57 ^{abc}	4.40 ^{bc}	0.12 ^{bc}
6	38.35 ^b	4.59 ^c	3.41 ^{bc}	0.75	0.77 ^a	4.55 ^b	0.12 ^{bcd}
7	36.60 ^{bc}	4.56 ^{cd}	3.36 ^{cd}	0.74	0.44	4.25 ^{bc}	0.12 ^{cd}
8	40.50 ^a	4.73 ^b	3.48 ^{ab}	0.73	0.66 ^{ab}	5.20 ^a	0.13 ^{ab}
9	42.30 ^a	4.87 ^a	3.50 ^a	0.72	0.66 ^{ab}	5.40 ^a	0.13 ^{ab}
10	41.35 ^a	4.84 ^{ab}	3.47 ^{ab}	0.72	0.79 ^a	5.60 ^a	0.14 ^a
SEM	0.63	0.04	0.24	0.01	0.07	0.14	0.3x10 ⁻²
Treatment	S	NS	NS	NS	S	NS	NS
*week							

a,b,...e: means with different superscript on the same column are significantly different (p<0.05).

SEM=standard error of means. NS=not significant and S=significant

Table 4.9: Interaction of dietary palm kernel oil and weeks on egg weight (g) of Yoruba ecotype chicken

Treatments/ weeks	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	31.75 ^d	34.25 ^c	31.00 ^d	35.75 ^b	33.00 ^d
2	32.35 ^d	34.25 ^c	35.00 ^{cd}	35.25 ^b	35.75 ^{cd}
3	33.50 ^{cd}	39.75 ^b	34.50 ^{cd}	36.75 ^b	35.25 ^{cd}
4	32.00 ^d	34.50 ^c	34.50 ^{cd}	35.00 ^b	36.75 ^{cd}
5	35.75 ^{bcd}	37.25 ^{bc}	36.00 ^{cd}	37.00 ^b	36.25 ^{cd}
6	36.00 ^{bcd}	37.75 ^{bc}	37.50 ^{bc}	39.75 ^{ab}	40.75 ^{ab}
7	35.50 ^{cd}	38.25 ^b	34.50 ^{cd}	35.50 ^b	39.25 ^{bc}
8	40.50 ^{ab}	42.25 ^a	41.25 ^{ab}	37.50 ^b	41.00 ^{ab}
9	37.25 ^{abc}	42.00 ^a	44.00 ^a	43.75 ^a	44.50 ^a
10	41.75 ^a	37.00 ^{bc}	41.50 ^{ab}	42.50 ^a	44.00 ^a
SEM	0.66	0.03	0.75	0.63	0.69

a,b,...d: means with different superscript on the same column are significantly different ($p < 0.05$).

SEM=standard error of means, Egg weight was measured in grams.

Table 4.10: Interaction of dietary palm kernel oil and weeks on yolk width (cm) of Yoruba ecotype chicken

Treatments/ weeks	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	3.28 ^c	3.36 ^b	3.29 ^d	3.41 ^{cde}	3.25 ^e
2	3.25 ^c	3.43 ^b	3.41 ^{bcd}	3.22 ^e	3.31 ^{de}
3	3.44 ^{abc}	3.52 ^b	3.50 ^{abc}	3.42 ^{cde}	3.45 ^{cde}
4	3.25 ^c	3.39 ^b	3.32 ^{cd}	3.37 ^{de}	3.34 ^{de}
5	3.52 ^{ab}	3.50 ^b	3.49 ^{abcd}	3.57 ^{bc}	3.59 ^{bc}
6	3.45 ^{abc}	3.46 ^b	3.81 ^{2a}	3.63 ^{ab}	3.69 ^{abc}
7	3.31 ^{bc}	3.34 ^b	3.24 ^d	3.36 ^{de}	3.55 ^{cd}
8	3.57 ^a	3.78 ^a	3.72 ^{ab}	3.54 ^{bc}	3.92 ^a
9	3.47 ^{abc}	3.55 ^b	3.67 ^{abc}	3.82 ^a	3.92 ^a
10	3.45 ^{abc}	3.42 ^b	3.69 ^{abc}	3.76 ^{ab}	3.85 ^{ab}
SEM	0.03	0.03	0.04	0.04	0.05

a,b,...e: means with different superscript on the same column are significantly different ($p < 0.05$).

SEM=standard error of means

The effects of dietary PKO supplementation on internal egg qualities of YEC (Table 4.11) showed that albumen height, yolk weight, yolk width and albumen weight were significantly affected such that hens on dietary PKO had higher (0.05) qualities than hens on control diet. Yolk ratio was similar across diet with no significant ($p>0.05$) different in values on PKO inclusion basis. However, yolk ratio increased ($p<0.05$) with weeks of egg collection.

Table 4.11: Effects of dietary palm kernel oil (PKO) on internal egg qualities of Yoruba ecotype chicken

Param eter/tre atment	Yolk height (mm)	Albume n height (mm)	Yolk width (cm)	Yolk weight (g)	Albume n wt(g)	Yolk colour	Albume n ratio	Yolk ratio	Yolk index	Haugh unit
0%PKO	13.24 ^b	2.59 ^b	3.40 ^c	11.03 ^b	19.05 ^c	2.90 ^c	0.54 ^{ab}	0.31	0.76 ^b	55.64
1%PKO	13.36 ^{ab}	2.73 ^{ab}	3.47 ^{ab}	11.85 ^a	20.13 ^b	6.05 ^a	0.53 ^b	0.31	0.79 ^{ab}	55.46
2%PKO	13.79 ^a	3.01 ^{ab}	3.51 ^{ab}	11.80 ^a	20.08 ^b	4.70 ^b	0.54 ^{ab}	0.32	0.86 ^{ab}	59.44
3%PKO	13.14 ^b	3.22 ^a	3.51 ^{ab}	11.90 ^a	21.05 ^{ab}	6.33 ^a	0.56 ^a	0.31	0.92 ^a	60.43
4%PKO	13.53 ^{ab}	3.16 ^a	3.59 ^a	12.23 ^a	21.50 ^a	2.30 ^d	0.56 ^a	0.32	0.88 ^{ab}	59.69
SEM	0.15	0.17	0.03	0.17	0.33	0.17	0.01	4x10 ⁻²	0.01	1.76
Week										
1	12.68 ^b	3.30 ^{ab}	3.32 ^d	9.80 ^e	18.65 ^f	2.30 ^d	0.56 ^a	0.30 ^b	0.99 ^a	64.95 ^a
2	13.29 ^{ab}	2.53 ^b	3.32 ^d	10.70 ^d	19.05 ^{ef}	4.00 ^d	0.55 ^{ab}	0.31 ^b	0.76 ^b	55.75 ^{cd}
3	13.72 ^a	2.83 ^{ab}	3.47 ^c	10.95 ^{cd}	20.25 ^{cde}	3.35 ^{bc}	0.56 ^a	0.30 ^b	0.81 ^{ab}	57.13 ^{abcd}
4	13.44 ^a	2.90 ^{ab}	3.33 ^d	10.45 ^{de}	19.45 ^{dcf}	5.15 ^a	0.56 ^a	0.30 ^b	0.88 ^{ab}	59.96 ^{abc}
5	13.49 ^a	2.99 ^{ab}	3.53 ^{bc}	11.15 ^{cd}	20.00 ^{cdef}	5.40 ^a	0.55 ^{ab}	0.31 ^b	0.85 ^{ab}	59.96 ^{abc}
6	13.55 ^a	2.89 ^{ab}	3.61 ^{ab}	11.50 ^c	21.00 ^{bc}	5.55 ^a	0.55 ^{ab}	0.30 ^b	0.79 ^{ab}	56.77
7	13.53 ^a	3.38 ^a	3.36 ^d	11.05 ^{cd}	19.50 ^{dcf}	2.70 ^{cd}	0.53 ^{bc}	0.30 ^b	1.00 ^a	64.32 ^{ab}
8	13.14 ^a	3.15 ^{ab}	3.70 ^a	13.45 ^b	22.85 ^a	5.15 ^a	0.56 ^a	0.33 ^a	0.84 ^{ab}	58.11 ^{abcd}
9	13.81 ^a	2.81 ^{ab}	3.69 ^a	14.30 ^a	22.05 ^{ab}	5.40 ^a	0.52 ^{cd}	0.34 ^a	0.76 ^b	53.28 ^{cd}
10	13.23 ^{ab}	2.64 ^{ab}	3.63 ^{ab}	14.25 ^a	20.80 ^{bcd}	5.55 ^a	0.50 ^d	0.35 ^a	0.73 ^b	51.03 ^d
SEM	0.21	0.24	0.04	0.24	0.47	0.24	0.01	0.01	0.07	2.47
TRT*	NS	NS	S	NS	NS	S	NS	NS	NS	NS
week										

a,b,...e: means with different superscript on the same column are significantly different (p<0.05).

SEM=standard error of means, NS=not significant and S=significant

Table 4.12: Interaction of dietary palm kernel oil and weeks on shell thickness (mm) of Yoruba ecotype chicken

Treatments/ weeks	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	0.28 ^e	0.47	0.31 ^c	0.35 ^b	0.32 ^d
2	0.35 ^{de}	0.55	0.31 ^c	0.36 ^b	0.32 ^d
3	0.43 ^{de}	1.49	0.39 ^{bc}	0.44 ^b	0.47 ^{cd}
4	0.45 ^{cde}	0.35	0.47 ^{bc}	0.39 ^b	0.53 ^{bcd}
5	0.53 ^{bcd}	0.59	0.59 ^b	0.50 ^b	0.63 ^{abc}
6	1.25 ^a	1.14	0.48 ^{bc}	0.50 ^b	0.49 ^{cd}
7	0.49 ^{bcd}	0.41	0.46 ^{bc}	0.41 ^b	0.40 ^d
8	0.70 ^{bc}	0.68	0.53 ^{bc}	0.68 ^a	0.72 ^{ab}
9	0.60 ^{bcd}	0.76	0.61 ^b	0.83 ^a	0.51 ^{cd}
10	0.75 ^b	0.70	0.96 ^a	0.78 ^a	0.75 ^a
SEM	0.05	0.11	0.03	0.03	0.03

a,b,...e: means with different superscript on the same column are significantly different ($p < 0.05$).
SEM=standard error of means

Table 4.13: Interaction of dietary palm kernel oil and weeks on yolk colour of Yoruba ecotype chicken.

Treatments/ weeks	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO
1	1.00 ^d	2.00 ^d	1.50 ^d	3.00 ^c	4.00 ^b
2	2.00 ^c	3.75 ^c	4.25 ^{bc}	5.00 ^{bc}	5.00 ^a
3	2.00 ^c	4.00 ^c	3.25 ^{cd}	3.75 ^c	3.75 ^b
4	3.25 ^b	7.25 ^b	6.00 ^{ab}	8.00 ^a	1.25 ^d
5	2.00 ^c	7.75 ^b	7.00 ^a	9.00 ^a	1.25 ^d
6	4.50 ^a	9.25 ^a	5.50 ^{abc}	7.25 ^a	1.25 ^d
7	4.50 ^a	2.25 ^d	1.00 ^d	3.00 ^c	2.75 ^c
8	3.25 ^b	7.25 ^b	6.00 ^{ab}	8.00 ^a	1.25 ^d
9	2.00 ^c	7.75 ^b	7.00 ^a	9.00 ^a	1.25 ^d
10	4.50 ^a	9.25 ^a	5.50 ^{abc}	7.25 ^a	1.25 ^d
SEM	0.21	0.43	0.39	0.42	0.23

a,b,c,d: means with different superscript on the same column are significantly different ($p < 0.05$). SEM=standard error of means

The semen quality parameters of YEC were not significantly ($p>0.05$) affected by PKO inclusion (Table 4.14). Sperm motility ranges between 85.25-88.75%.

Table 4.14: Effects of dietary palm kernel oil on semen quality in Yoruba ecotype chicken

Treatment/ parameters	0%PKO	1% PKO	2% PKO	3% PKO	4% PKO	SEM
Semen volume (ml)	0.28	0.24	0.23	0.17	0.28	0.02
Total sperm count x10 ⁶ /ml	2836.00	2878.25	2865.50	2895.50	2885.50	228.94
Sperm motility (%)	87.75	88.75	88.00	86.75	85.25	2.29
Dead sperm (%)	3.75	1.50	1.75	2.25	2.25	1.58
Sluggish (%)	8.25	9.50	9.75	9.25	10.25	1.33
Abnormal (%)	0.25	0.25	0.50	1.75	2.25	0.33

The effects of palm kernel oil inclusion on egg fertility, hatchability and sex ratio of YEC (Table 4.15) showed that fertility and hatchability of egg were not influenced except for 4% PKO inclusion where significant decrease ($p < 0.05$) was observed in both egg fertility and hatchability. The percentage of male offspring increased with increasing PKO inclusion, while inverse relationship was observed in the female offspring.

Table 4.15: Effects of dietary Palm kernel oil on fertility, hatchability and sex ratio of Yoruba ecotype chicken

Treatment/ parameters	0% PKO	1% PKO	2% PKO	3% PKO	4% PKO	SEM
Fertility (%)	76.81 ^a	72.62 ^a	70.37 ^a	67.80 ^a	42.50 ^b	7.22
Hatchability (%)	36.03 ^a	44.24 ^a	32.50 ^a	36.08 ^a	14.31 ^b	5.39
Male (%)	33.33 ^c	36.93 ^c	42.93 ^b	46.67 ^b	66.67 ^a	6.19
Female (%)	66.67 ^a	63.07 ^a	57.07 ^b	53.33 ^b	33.33 ^c	6.19

a,b,c: means with different superscript on the same row are significantly different ($P<0.05$), SEM=standard error of means.

Similarly, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Protein, Albumin, Total Cholesterol, Triglyceride, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) were not significantly affected by dietary PKO (Table 4.16).

Table 4.16: Effects of dietary Palm Kernel Oil on serum biochemical indices of Yoruba ecotype chicken

	AST	ALT	ALP	T.Pro.	ALB	T.Chol.	Trig.	HDL	LDL
	(Iu/L)	(Iu/L)	(Iu/L)	(g/L)	g/L	mmol/L	mmol/L	mmol/L	mmol/L
0%PKO	18.00	12.83	22.50	48.67	34.83	4.44	0.63	0.78	3.03
1%PKO	16.50	12.83	22.50	49.00	35.33	4.51	0.72	0.77	3.02
2%PKO	17.33	13.17	24.83	48.67	34.50	4.46	0.68	0.73	3.05
3%PKO	17.33	12.33	25.00	48.67	35.50	4.43	0.68	0.65	3.10
4%PKO	17.00	13.83	24.33	49.00	35.00	4.38	0.63	0.73	3.02
SEM	0.89	0.72	1.12	0.84	0.81	0.08	0.05	0.07	0.04
Sex									
M	17.33	13.00	24.33	48.53	35.27	4.56	0.67	0.81	3.08
F	17.13	13.00	24.33	49.07	34.60	4.34	0.67	0.66	3.01
SEM	0.56	0.45	0.71	0.53	0.51	0.05	0.03	0.04	0.03
Trt*sex	NS	NS	NS	NS	NS	NS	NS	NS	NS

AST=Aspartate Transaminase, ALT= Alanine Transaminase, ALP= Alkaline Phosphatase,T.Pro=Total Protein, ALB=Albumin, T.Chol=Total Cholesterol, Trig=Triglyceride, HDL=High Density Lipoprotein and LDL=Low Density Lipoprotein, M= Male, F=female, SEM=standard error of means.

The sex hormones (Follicle Stimulating Hormone, Luteinizing Hormone, Progesterone, Testosterone and oestrogen) were not affected by dietary PKO (Table 4.17).

Table 4.17: Effects of dietary Palm Kernel Oil on hormonal indices of Yoruba ecotype chicken

	FSH	LH	Progesterone	Testosterone	Oestrogen
	(mIU/ml)	(mIU/ml)	(ng/ml)	(ng/ml)	(ng/ml)
0% PKO	6.02	5.00	5.95	0.57	56.67
1% PKO	6.32	4.97	5.85	0.57	59.50
2% PKO	6.18	5.18	5.73	0.60	61.00
3% PKO	5.78	5.37	5.57	0.60	60.33
4% PKO	6.32	5.40	5.85	0.57	58.83
SEM	0.32	0.15	0.18	0.05	2.17
Sex					
M	6.18	5.25	5.69	0.59	60.87
F	6.07	5.11	5.89	0.58	57.67
SEM	0.20	0.09	0.11	0.03	1.37
Trt*Sex	NS	NS	NS	NS	NS

FSH=Follicle Stimulating Hormone, LH= Luteinizing Hormone, M= Male, F=female, SEM=standard error of means.

The haematological parameters (Table 4.18) showed that variation in White Blood Cell (WBC), heterophil, monocyte, eosinophil, Red Blood Cell (RBC), Haemoglobin (Hb), Pack Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and basophil were not ($p>0.05$) affected by PKO inclusion. There were significant differences ($P<0.05$) in the lymphocyte and platelet of Yoruba ecotype chicken fed PKO. The highest lymphocyte was at 2% while the lowest was at 3% dietary inclusion of PKO. The platelet had the highest value at 3% PKO inclusion and the least at 2% dietary PKO. Both 2% and 3% PKO inclusion had the same values as control diet.

Table 4.18: Effects of dietary Palm Kernel Oil on haematological indices of Yoruba ecotype chicken

Parameters/ Treatment	WBC	Hete	Lymph	Mono	Eosin	RBC	Hb	PCV	MCV	MCH	MCHC	Plt
0%	14.63	45.83	51.67 ^{ab}	2.17	0.50	2.86	13.65	34.67	83.50	26.50	30.13	317.17 ^a
1% PKO	13.61	49.00	49.33 ^{ab}	1.33	0.33	2.38	12.15	27.83	81.67	26.18	31.55	225.83 ^{ab}
2% PKO	12.91	45.67	52.67 ^a	1.33	0.33	2.77	13.57	31.50	86.33	27.90	32.12	165.00 ^b
3% PKO	17.05	51.33	45.67 ^b	1.83	0.50	2.99	11.43	26.33	89.17	27.27	30.42	315.50 ^a
4% PKO	14.03	50.67	47.00 ^{ab}	1.33	1.00	2.79	14.23	33.00	89.83	30.13	33.55	296.83 ^a
SEM	1.24	1.99	1.93	0.42	0.27	0.38	0.91	3.51	3.25	1.35	1.21	35.48
Sex												
M	12.50	48.87	49.27	1.33	0.27	2.76	13.85	34.73	92.40	28.63	30.17	253.07
F	16.39	48.13	49.27	1.87	0.81	2.36	12.18	26.60	79.80	26.50	32.93	275.07
SEM	0.78	1.26	1.22	0.27	1.70	0.24	0.58	2.22	2.06	0.85	0.77	22.43
Trt*Sex	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means with different superscript on the same column are significantly different ($P < 0.05$). Where WBC=White blood cell ($\times 10^6/\text{mm}^3$), Hete= Heterophil (%), Lymph=Lymphocyte (%), Mono=Monocyte (%), Eosin=Eosinophil (%), RBC=Red blood cell ($\times 10^6/\text{mm}^3$), Hb= Haemoglobin (g/mm³), PCV= Pack cell volume (%), MCV= Mean corpuscular volume (fL/mm³), MCH= Mean corpuscular haemoglobin concentration (pg/mm³), Plt= Platelet ($\times 10^9/\text{mm}^3$), M= Male, F=female, SEM=standard error of means.

4.6 Discussion

Palm kernel oil (PKO) was high in saturated fatty acids which consist of lauric acid (36.53%), palmitic acid (18.59%), myristic acid (18.18%) and stearic acid 5.33%. The total percentage of saturated fatty acids in palm kernel oil was 78.63% while the unsaturated fatty acid (e.g Oleic and linoleic acid) was 21.27%. Similarly, Mukherjee and Mitra (2009) reported that palm kernel oil contains saturated fatty acids of about 80% and unsaturated fatty acid of 20%. Lauric acid has 12 carbon chain and was implicated in raising low density lipoproteins (LDL) cholesterol level (Rakel, 2012). Stearic acid on the other hand has no effect on cholesterol level (Chow, 1992). Linoleic acid is one of the two fundamental fatty acids needed by the animals for proper physiological functioning of the body. It is also an unsaturated omega-6-fatty acid, which is directly absorbed from the blood in the liver and promptly provide adequate lipid source for immediate delivery inside the egg yolk (Senkoylu *et al.*, 2004). In this study, PKO had 3.28% linoleic acid. The high saturated PKO fatty acids obtained confirms the report of Evangelista and Cermak (2007) that PKO contain 75-80% saturated fatty acids.

Palm kernel oil was readily accepted and consumed by both cocks and hens, and did not retard growth in Yoruba ecotype chicken up to 4% inclusion levels. The increased feed intake of male YEC with increasing inclusion of PKO could be due to increase in palatability and texture of the diet as well as reduction in feed dustiness (Parsons *et al.*, 1993). Dietary fat and oil has been reported to increase palatability of the diet, decrease dustiness, improve texture of feed, improve nutrient utilization and feed intake (Parsons *et al.*, 1993, Harms *et al.*, 2000 and Leeson *et al.*, 2001). This report is in tandem with Afolabi *et al* (2012) who observed increase in the average daily feed intake with increasing inclusion levels of Palm Kernel Cake with added palm oil in Nigerian local hens. Also, the weight gain of cocks on PKO dietary

treatment was not adversely affected as the levels of PKO inclusion up to 3% were comparable with control diet.

The feed conversion ratio (feed per dozen egg) of hens on 0-3% were lower, better and more efficient than those on 4% PKO. The reason for the poor feed conversion ratio of the hens on 4% PKO could be as a result of higher final weight which could suggest that hens on this inclusion (4% PKO) probably used the diet to gain more weight rather than laying more eggs. This findings follow similar trend with the observation of Afolabi *et al* (2012) who asserted lower, better and more favourable feed conversion ratio at 20 to 40% inclusion of Palm Kernel Cake with 3% addition of Palm oil than 50% Palm Kernel Cake (with 4% palm oil).

The increase in egg weight as a result of increasing inclusion level of PKO could be caused by higher intake of linoleic acid in the diet. This finding concurs with the observation of Keshavarz and Nakajima (1995) that dietary fat intake increase egg weight and other egg component in laying bird. Whitehead *et al* (1993) observed that dietary oil and fat inclusion in laying birds regulate plasma oestrogen and increase egg weight in commercial layers.

Basically, male chickens are expected to be heavier than their female counterparts (Ajayi and Agaviezor, 2009) since they convert their food directly to flesh while the females convert their feed to eggs. This assertion was true for male and female of YEC. Female consumed more feed than their male counterpart. This finding is in line with the report of Khobondo *et al* (2015) who observed that layers tend to eat more than the cockerels.

The similar in weight gained in cocks fed diet 0% to 3% PKO inclusion level suggested that male YEC could perform best at inclusion of PKO up to 3% and any further addition might result in reduced weight gained. Fats and oils are high in energy which their inclusion are expected to increase weight gain by increasing adipose tissues and abdominal fats (Bohnsack

et al., 2002), PKO inclusion did not increase weight gain of cocks better than control in this study.

Conventionally, smallest eggs (peewee) are usually collected at earlier ages of lay in bird and the weight of eggs should improve with laying age (Christians, 2002; Aslan and Yavuz, 2010). The results of this study followed a similar trend. However, the fact that the egg weights of 1-4% PKO inclusion were heavier than the weight of eggs from control diet suggest that the weekly increase in eggs weight for over 11 weeks were directly related to PKO inclusion since assimilation of dietary lipids into the yolk of egg increase the egg weight (Senkoylu, *et al.*, 2004). The increased egg weight pattern is in harmony with the observation Bhonzack *et al* (2002) whose findings revealed that corn oil increase egg weight in laying birds. The authors also revealed that fat inclusion in poultry diet increased egg weight as the levels increased from 1% to 4%. The egg weights recorded in this experiment are within the recommended range of 31.78-37.32g reported by Adebambo (2005) for white and light ecotype Nigerian indigenous chicken reared inside cages.

Hen day and hen house followed normal egg production pattern. The increase in percentage hen day production obtained concur with Afolabi *et al* (2012) who observed increased trend in hen day in Nigerian indigenous laying hens fed varied dietary palm kernel cake levels with addition of palm oil.

The observed increase in external egg characteristics with inclusion of PKO suggests hatchlings advantage of settable egg since egg characteristics such as egg weight, egg size and shape has been reported to influence egg water loss, gas exchange and egg shell porosity of incubated eggs (Schmid *et al.*, 2009). The external qualities of egg perform vital function in the growth and development of embryo and hatching success. Narushin and Romanov (2002) reported the weight of egg, thickness of egg shell, shape index of egg and porosity of

egg as the most important egg parameters in embryonic development and successful hatching. Although, egg internal and external characteristic like weight of the egg, egg length and albumen height increased with age of birds (Ulmer-Franco *et al.*, 2010), the increase in both egg external and internal characteristic in this experiment were synergetic effects of both age and PKO inclusion as birds on control diet had the lowest values in most of the parameters measured.

Similarly, the increase in egg weight with increasing PKO inclusion is suggestive of beneficial advantages of PKO in the production of high quality day old chicks which is of importance to the farmer and commercial hatchery as egg weight affects the weight of chicks which is a predictor of post hatch performance and market body weight of chicks (Sklan *et al.*, 2003). Egg weight may also affect the selling price of chicks as heavier weight commands better market price than the smaller chicks (Ulmer-Franco *et al.*, 2010).

The egg yolk and albumen parameters increased with increased PKO suggesting reasons for increased egg weight. The yellow colour of egg yolk is caused by the amount of fat-soluble xanthophyll pigmentation present in the diet (Aziz and Mustafa, 2010). Yolk and albumen ratio can be linked with the quality of chicks as 90% of energy required by the developing embryos would normally be supplied by the yolk lipids (Cherian, 2015). The albumen weight (19.05 – 21.50g) obtained in this study was within the range of 17.2 – 23.17g reported by Oluyemi *et al* (1982), Olori and Sonaiya (1992) Udeh and Omoje (2005) and Afolabi *et al* (2012). The weights of albumen and yolk obtained in this study suggest that albumen weight was heavier than yolk weight, a relationship that is true for most egg. This finding is in tandem with the assertion of Rose (1997) who observed that eggs with higher weight have relatively less yolk and more albumen than smaller eggs. Shell quality in term of shell thickness and pore number has been shown to cause greater percentage of moisture losses from eggs during incubation (Peebles *et al.*, 2001) and reduced hatchability (Narushin and

Romana, 2002). The shell thickness recorded in this study (0.35-0.79mm) was better (higher) than 0.32-0.34mm reported by Afolabi *et al.* (2012) who fed varying dietary levels of palm kernel cake with palm oil to Nigerian local hens.

The reduction in egg fertility and hatchability at 4% PKO inclusion suggests that PKO may exhibit adverse effect on egg fertility and hatchability. This may be due to high composition (78.63%) of saturated fatty acid contents which have been shown to influence embryogenesis and hatchability of egg (Peebles, 2000). This was also reported by Brake (1990) who found a significantly lower egg fertility and hatchability when 5% poultry fat was fed to hens. On the other hand, Pirsaraei *et al* (2011) reported no adverse effect on broiler breeders' egg fertility and hatchability when dietary tallow was fed up to 1.5%. The non-significant effect recorded by this author may be due to the lower level of tallow inclusion in the diet. Thus, the current results suggest that PKO inclusion should not exceed 3% for acceptable fertility and hatchability.

The observed higher male chicks upon PKO dietary supplementation may imply that farmers targeting increase number of male in the flock may include PKO in the diet of laying birds. This finding is consistent with the finding of Thomas and Marion (2013) who observed that fat supplementation affected litter size with a significant sex ratio distortion (1:3, females: Male) relative to control where the offspring sex ratio was 1:1.

Considering only male factor in fertility, it has been reported that potent sperm count might result in higher fertility (Oyeyemi *et al.*, 2008). The sperm motility (85.25-88.75%) of cockerels was enhanced at all levels of PKO inclusion. These values of sperm motility were higher than 71.25-74.75% motility reported by Adeyina *et al* (2016). This observation suggests that the reduced fertility and hatchability recorded could not be attributed to low sperm count and sperm motility but rather intrinsic female effect through dietary manipulation. The high

fertility in this study is consistent with the findings of Pirsaraei *et al* (2011) who reported that fatty acid enhances spermatozoa potency when graded levels of dietary tallow was fed to broiler breeder.

According to Ahamefule *et al.* (2008), liver and kidney metabolites are used as evidence of toxicity in animal. The non-significant differences in liver enzymes (AST, ALT and ALP), blood proteins and blood lipids of both male and female YEC showed that PKO inclusion did not have deleterious effect on liver function as well as cholesterol level of YEC. Cholesterols are mainly produced in the liver from the diet (~10%) or from acetate (~90%) (Miller and Auchus, 2011) and transported to the cell in the form of high density lipoprotein (HDL) and low density lipoprotein (LDL) (Rone *et al.*, 2012). The pattern of blood total cholesterol, HDL and LDL observed suggest that dietary PKO may not increase cholesterol formation in the liver despite the high composition of lauric acid. This finding negates the report of Rakel (2012) that Lauric component of fatty acid raised LDL cholesterol. Steroid hormones are produced from cholesterol (Venugopal *et al.*, 2016) but the observed hormones in this study revealed that dietary PKO did not affect steroid hormone production in NIC. The reason for this observed trend may be attributed to the non-significant effect of PKO on cholesterol in NIC. Therefore, the increase in the percentage of male chicks with increasing level of PKO in this study could not be related to increase in any particular hormone as observed by Petric *et al.* (2001) that increase testosterone resulted in increasing male offspring.

Haematological indices are crucial investigation of numerous metabolic and pathological abnormalities picturing the health status of chickens (Afolabi *et al.*, 2012). The values obtained from haematological examination often serves as investigatory measures of stress from environment, nutrition and disease infection in the body of an animal. Variations in the PCV, Hb and RBC of NIC revealed that inclusion of PKO was not detrimental to the health of birds as the values obtained were within the normal recommended range of $27.38 \pm 0.46\%$ -

34.60±0.64% PCV reported by Ikhimioya *et al* (2000) for Nigeria local chicken. The level of haemoglobin was consistent with Mitruka and Rawnsley (1977) who reported 11.60g/dl-13.68g/dl for haemoglobin of birds. The red blood cell count obtained in this study was within the range of 2.39-3.2x10⁶/mm³ RBC reported by Ikhimioya *et al.* (2000) and Pampori and Igbal (2007) for local chicken.

The WBC plays paramount role in disease resistance through phagocytosis process in generating antibodies against foreign bodies. The WBC obtained in this study (12.91-16.39x10⁶mm³) were within the normal range of 6.95-18.65x10⁶mm³ reported by Ikhimioya *et al.* (2000) for Nigerian local chicken.

Palm Kernel has been reported to have 30-35% β-mannan (Chemgen, 2002) producing manose in the gizzard that increases immunity of birds (Sundu *et al.*, 2006). PKO could be exhibiting similar mechanism of immunity booster since most of the anti-nutrients are concentrated in the oil. The immunomodulatory effect of PKO may be revealed by the response of lymphocytes, heterophils, eosinophils and monocytes, which are common differentials of WBC (Bounous *et al.*, 2000). The lymphocyte values (47.00- 52.67%) obtained in this study were within the normal range of 47.00- 85.0% reported by Mitruka and Rawnsley (1977) for healthy chicken. The lymphocytes are reactive cells, which are implicated in the production of antibodies (Afolabi *et al.*, 2012), so the status of antibodies and health of the birds remain unaffected with PKO inclusion.

In conclusion, the inclusion of PKO up to 4% in the diet of YEC revealed no detrimental effects on the haematological parameters of the experimental birds. The best level of inclusion was from 0-3% PKO for better performace, egg fertility and hatchability. The inclusion of PKO increased external and internal egg characteristics on weekly basis and did

not have adverse effect on sperm quality characteristics. PKO inclusion up to 4% increased percentage male chicks and reduced percentage female chicks of NIC.

CHAPTER FIVE

5.0 EXPERIMENT 2

5.1 Effects of hormone (testosterone and estradiol) injection on reproductive responses and offspring sex ratio of Yoruba ecotype chicken

5.2 INTRODUCTION

Testosterone is the chief male sex hormone that promotes the development and maintenance of characteristics associated with manhood, virility, libido, muscular mass, excitement and aggressiveness in animal (Kiefer, 2008). Testosterone hormone increases leptin in animal which is an appetite increasing hormone (Zou *et al.*, 2007).

Hormone (estradiol and testosterone) injection could activate folliculogenesis and ovulation (Mohammadi and Ansari-Parsaraie, 2016) in birds. Estradiol and progesterone injections also exert regulatory effects on follicular development and have positive association with egg production (Mohammadi and Ansari-Parsaraie, 2016).

Egg yolk of birds contain hormones of maternal origin that reflect the hormonal state of laying hen during egg production (Petrie *et al.*, 2001) and exposure of embryos in the egg to pharmacological changes in oestradiol concentration was reported to result in sex reversal of genetic males into phenotypic females (Elbrecht and smith, 1992). The embryo that developed into female chick was reported to contain more oestrogen than those that developed into males (Muller *et al.*, 2002).

Increase in maternal testosterone has been reported to be associated with male biased sex ratio in the breeding females (Rutkowska and Cichon, 2006; Pike and Petrie, 2005). Testosterone is associated with maleness and responsible for secondary sexual characteristic in male animal. In female, testosterone is present in little amount and researchers have

reported higher concentration of testosterone in mother to increase male offspring (Shargal *et al.*, 2008; Grant, 2011).

The objective of this study was to investigate the effects of dosage levels of estradiol and testosterone injection on serum biochemistry, haematology, hormone, reproductive response and offspring sex ratio of Yoruba ecotype chicken.

5.3 Materials and Methods

5.3.1 Estradiol and Testosterone hormones

Estradiol and Testosterone propionate injectable were purchased from Aromokeye Veterinary Shop, opposite 'A' Division, Ilorin, Kwara state, Nigeria.

5.3.2 Experimental animals and design

Ninety (90) laying hens and ninety (90) cocks of Yoruba ecotype chickens were used for this study. The male and female birds were thirty-six weeks old. The hens were randomly allotted to six (6) treatment groups of five (5) replicates and three (3) birds per replicate while the male birds were allotted to six (6) treatment groups of five (5) replicates and three (3) birds per replicate in a factorial (2x2x3) design. The three treatment groups consisted of 0IU, 2IU and 4IU levels of estradiol and testosterone propionate independently administered (intramuscularly) once in 21 days following manufacturer's prescription. The factors consisted of 2 sex (male and female), 2 hormone types (testosterone and estradiol injection) and 3 levels of hormone administration.

The birds were offered Commercial diet of 17.00% crude protein and 2700Kcal/kg energy at 120g/bird/day. Water was supplied *ad-libitum* throughout the experimental periods of eight (8) weeks.

5.3.3 Performance parameters

120g of feed was offered per bird per day and the left over weighed in the following morning. All animals were weighed at the commencement of the experiment before allocating them to the treatments and subsequent weight were obtained on weekly basis to determine the body weight changes in birds

5.3.4 Hen day production

Records of daily egg production on replicate basis were taken and hen-day at a given period was calculated as the percentage of the ratio of number of egg laid to the number of hen.

$$\text{Hen Day Egg Production (\%)} = \frac{\text{number of egg laid in a day}}{\text{number of hen alive}} \times 100$$

Hen-house production (HHP): this is the ratio of eggs laid in a day divided by the number of birds originally housed multiplied by 100

$$\text{Hen house production (\%)} = \frac{\text{number of egg laid in a day}}{\text{number of birds originally housed}} \times 100$$

5.3.5 Feed efficiency per dozen eggs

This takes into consideration the feed intake and egg production. It is the ratio between the feed consumed and the number of eggs produced.

$$\text{FCR (Feed per dozen eggs)} = \frac{\text{Kg of feed consumed} \times 12}{\text{Total eggs produced}}$$

5.3.6 Quality assessment of settable eggs

A total of fifteen (15) eggs (five (5) from each treatment) were collected weekly for seven weeks of the experiment, external and internal qualities of eggs were evaluated.

5.3.6a External qualities of egg:

The parameters assessed included: egg weight, egg length, egg breadth and egg-shape index.

5.3.6ai Egg weight:

It was measured with the aid of electric sensitive scale.

5.3.6aii Egg shape index:

This was evaluated as the ratio of the egg breadth to the egg length. The egg breadth was measured as the distance between two ends of the egg at the widest cross sectional region while the length was measured as the distance between the broad and narrow ends of the egg using Vernier callipers.

5.3.6aiii Shell thickness:

The thickness of the individual dry shells was measured to the nearest 0.01mm using micrometer screw gauge.

5.3.6aiv Shell weight:

Egg shells were air-dried and the weight measured with the aid of an electric sensitive scale.

5.3.6b Internal qualities of egg

All the eggs sampled for external egg quality assessment were used for the assessment of albumen height, albumen weight, yolk weight, shell weight, shell thickness and Haugh unit).

5.3.6bi Albumen height:

This was measured using the p6085 spherometer (tripod micrometer) with 0.01mm accuracy in a flat dish.

5.3.6bii Percentage albumen weights:

This was determined using the difference between the weight and the sum of weight of yolk and dry egg shell as a percentage of the whole egg.

5.3.6biii Yolk weight:

This was measured with the aid of mettler top-loading weighing balance.

5.3.6biv Yolk colour:

It was determined for individual yolk by comparing the yolk with the colour chips of a Hoffman-Laroche (R) Yolk colour fan. The yolk colour fan has different scores for different colour ranging from pale yellow (score 1) to deep orange (score 15)

5.3.6bv Haugh unit (HU):

The HU was calculated using the values obtained for egg weight and albumen height as expressed by Card *et al.*, (1979) in the formula shown below

$$HU = 100 \log (H + 7.5 - 1.7W^{0.37})$$

Where H= Albumen height in mm

W= Egg weight in gram

5.4 Semen collection and analysis

Semen was collected three days after hormonal injection at the sixth week from twenty five males (1 male in each replicate) through massaging of the dorso-lateral lumbosacral region of the cockerels to cause erection of the male copulatory organ (papilla) and subsequent ejaculation (Peter *et al.*, 2008). The semen was subjected to physical evaluations to determine semen colour and volume. Sperm motility was determined by placing a drop of the semen on a clean glass slide under the cover slip and viewed on a binocular microscope. Sperm motility was determined by counting both motile and immotile spermatozoa per unit area and sperm concentration was determined using the haemocytometer (Patrick *et al.*, 2009).

5.5 Fertility, hatchability and sex ratio

At the sixth week of feeding trials, semen were collected from males in each treatment and used to inseminate the female of the same treatment once in a week for two consecutive weeks. The semen were withdrawn from collecting funnel using 1ml syringe which was inserted into the left opening (cervices) of the female reproductive tract. Two (2) drops (0.2ml) of the semen were released into the cervices of laying hens in each insemination. A total of 600 fertile eggs (120 eggs from each treatment) were collected and set two times within 12 days inside the incubator. Fertility test was carried out at days 5 and 18 of incubation to determine fertile eggs and live germ, respectively. The number of hatched eggs and un-hatched eggs were also recorded. Percentage male and female chicks were determined at weeks eight after hatching.

5.6 Hormonal, serum biochemical and haematological indices of birds

At the end of the eight week of this study, blood samples were collected from the wing web of experimental birds (1 bird in each replicate) into bottles containing EDTA (Ethylene Diamine Tetra acetic) which acts as an anticoagulant. The uncoagulated blood samples were analyzed for haematological parameters such as Red Blood Cell Count (RBC), White Blood Cell Count (WBC) , Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Platelets Pack Cell Volume (PCV) and others. All haematological analysis were carried out using HA 6000 machine (autohaematology analyzer) at the Faculty of Veterinary Medicine, University of Ilorin.

Another blood was collected into labeled sterile sample bottles without anticoagulant and used to determine the serum biochemical contents such as Aspartate Transaminase (AST), Alkaline Phosphate (ALP), Total protein, Albumen, Total cholesterol and its'

components (Triglycerides, High Density Lipoprotein and Low Density Lipoprotein). These serum biochemical parameters were analyzed using the procedure of monobind microplate immune enzymetric assay method using semi-auto chemical analyzer (Rayto RT-1904C). The blood was analyzed for the concentration of reproductive hormones (luteinizing hormone, Follicle Stimulating Hormone, testosterone, oestrogen and progesterone) following the instructions of monobind microplate immunoenzymometric assay (IEMA/ELISA) method using microplate reader (Rayto RT-2100C) as described by Mitruka and Rawnsley (1977) and Jain (1986).

5.7 Statistical analysis

All data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) procedure following a factorial (2x2x3) model (SAS 1999) and the level of interaction were determined using the same procedure while, sex limited data were subjected to factorial (2x3) analysis of the same model.

$$Y_{ijk} = \mu + S_i + H_j + L_k + (SH)_{ij} + (SL)_{ik} + (HL)_{jk} + (SHL)_{ijk} + e_{ijk}$$

Y_{ijk} = The parameters of interest

μ = Over all mean for parameters of interest

S_i = Fixed effect of i^{th} sex ($i=1$ to 2)

H_j = Fixed effect of j^{th} hormone ($j=1$ to 2)

L_k = Fixed effect of k^{th} level ($k=1$ to 3)

$(SH)_{ij}$ = Fixed effect of interaction of i^{th} sex and j^{th} hormone

$(SL)_{ik}$ = Fixed effect of interaction of i^{th} sex and k^{th} level

$(HL)_{jk}$ = Fixed effect of interaction of j^{th} hormone and k^{th} level

$(SHL)_{ijk}$ = Fixed effect of interaction of i^{th} sex, j^{th} hormone and k^{th} level

e_{ijk} = Error as independent and identically distributed as normal with mean zero

5.8 Results

The effects of hormone injection on the growth performance of male YEC (Table 5.1) showed that testosterone hormone significantly ($p<0.05$) influenced final weight, weight gain and feed intake such that their performances were higher than cocks on estradiol injection. Feed to gain ratio of cocks on estradiol administration was higher ($p<0.05$) than those on testosterone administration.

Cocks on testosterone injection had higher ($p<0.05$) feed intake, weight gain as well as final weight than cocks on estradiol injection.

Cocks on OIU hormone injection had higher ($p<0.05$) weight gain and final weight; and lowest feed to gain ratio than other levels of hormone injection. The feed intake of male YEC increased with the levels of administration of hormone

Table 5.1: Effect of hormone injection on growth performance of male Yoruba ecotype chicken

Parameters/treatment	Initial weight (g)	final weight(g)	Total weight gain (g)	Weight gain(g/b/d)	Feed intake (g/b/d)	Feed:Gain ratio
Hormone						
Estradiol	1566.00	1683.00 ^b	116.33 ^b	2.08 ^b	58.87 ^b	28.30 ^a
Testosterone	1574.00	1704.00 ^a	130.13 ^a	2.32 ^a	60.40 ^a	26.03 ^b
SEM	10.72	14.82	10.68	0.19	0.75	1.95
Level						
0IU	1566	1712 ^a	145.90 ^a	2.61 ^a	58.20 ^b	22.30 ^b
2IU	1568	1684 ^b	116.60 ^b	2.08 ^b	58.30 ^b	28.02 ^a
4IU	1576	1684 ^b	117.70 ^b	2.10 ^b	62.40 ^a	29.71 ^a
SEM	13.13	18.15	13.08	0.23	0.914	2.39
Hormone *Level	NS	NS	NS	NS	NS	NS

a,b: means with different superscript on the same column are significantly different (p<0.05). SEM=standard error of means and NS=not significant

The effects of hormone injection on growth performance of female YEC (Table 5.2) reflected that feed intake, change in weight, final weight, feed per dozen egg and egg weight of hens

on testosterone injection were significantly ($p<0.05$) higher than those on estradiol injection. Considering the levels of administration, final weight of hen, change in weight, feed intake, feed per dozen egg and egg weight increased ($p<0.05$) with increasing levels of administration.

The interaction between hormone and level of administration (Table 5.3) was significant with the feed intake of birds on testosterone significantly ($p<0.05$) increased with increasing levels of administration. The feed intake of birds on estradiol injection decreased ($p<0.05$) from 0IU to 2IU and increased at 4IU.

Table 5.2: Effect of hormone injection on growth performance of female Yoruba ecotype chicken

Parameter/ Treatment	Initial weight (g)	Final Weight (g)	Total weight gain (g)	Feed intake g/b/d	Feed per dozen egg	Egg weight(g)
Hormone						
Estradiol	1148	1238 ^b	90.65 ^b	81.40 ^b	1.92 ^b	37.80 ^b
Testosterone	1148	1261 ^a	113.52 ^a	88.53 ^a	2.54 ^a	39.82 ^a
SEM	7.61	14.77	12.79	0.49	0.17	0.31
Level						
0IU	1148	1232 ^b	84.37 ^b	78.85 ^c	1.82 ^b	37.71 ^b
2IU	1148	1247 ^b	99.51 ^b	81.46 ^b	2.40 ^{ab}	38.08 ^b
4IU	1147	1270 ^a	122.38 ^a	94.66 ^a	2.47 ^a	40.64 ^a
SEM	9.32	18.09	15.67	0.16	0.21	0.38
Hormone	NS	NS	NS	S	NS	NS
*level						

a,b,c: means with different superscript on the same column are significantly different ($p < 0.05$). SEM=standard of means error and NS=not significant

Table 5.3: Interaction of hormone and levels of administration on feed intake of Yoruba ecotype chicken

Hormone	Level	Feed intake (g/b/d)
Estradiol	0IU	78.90 ^c
	2IU	74.80 ^d
	4IU	90.50 ^b
Testosterone	0IU	78.80 ^c
	2IU	88.12 ^b
	4IU	98.82 ^a
SEM		1.57

a,b,c: means with different superscript on the same column are significantly different ($p < 0.05$), SEM=standard of means error

The effects of hormone administration on laying performance of YEC (Table 5.4) showed that the egg weight of birds injected with testosterone was significantly ($p < 0.05$) higher than those on estradiol. There was significant ($p < 0.05$) increase in egg weight with increased

levels of administration of hormones. The highest egg weight was recorded at 4IU while the lowest was at 0IU. The egg weight, hen day and hen house were not significantly ($p>0.05$) by the type of hormone injected.

Table 5.4: Effects of hormone injection on laying performance of Yoruba ecotype chicken

Hormone	Egg weight (g)	Hen day (%)	Hen house (%)
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Estradiol	37.83 ^b	54.53	54.53
Testosterone	39.97 ^a	46.34	46.34
SEM	0.54	3.04	3.04
Levels			
0IU	37.40 ^b	56.83	56.83
2IU	39.10 ^{ab}	46.10	46.09
4IU	40.20 ^a	48.38	48.38
SEM	0.66	3.73	3.73
Hormone*Level	NS	NS	NS

a, b means with different superscript on the same column are significantly different ($p < 0.05$). NS= non-significant, SEM=standard of means error

The effects of hormone injection on external egg quality of female YEC are shown in Table 5.5. The two hormones (testosterone and estradiol) did not significantly ($p > 0.05$) influence egg weight, egg width, egg shape, shell thickness and shell ratio. Hens on testosterone

hormone had higher ($p<0.05$) egg length than hens on estradiol hormone. Shell length, egg width and shell weight significantly ($p<0.05$) increased with increasing levels of administration. Egg weight increased by 3.62g ($39.13^b - 42.75^a$), egg length increased by 0.19 cm ($4.69^b - 4.88^a$), egg width increased by 0.09 cm ($3.43^b - 3.52^a$) and shell weight increased by 0.79g ($4.96^b - 5.75^a$) respectively with increasing levels of administration from 0IU to 4IU.

Table 5.5: Effects of hormone injection on external egg quality indices of Yoruba ecotype chicken

Parameter/tre	Egg	Egg	Egg	Egg	Shell	Shell	Shell ratio
		length			thickness		

atment	weight(g)	(cm)	width	shape	(mm)	weight(g)	
Hormone							
Estradiol	40.81	4.76 ^b	3.48	0.73	0.70	5.33	0.13
Testosterone	41.00	4.81 ^a	3.47	0.72	0.67	5.33	0.13
SEM	0.56	0.03	0.02	0.4x10 ⁻²	0.03	0.11	0.2x10 ⁻²
Levels							
0IU	39.13 ^b	4.69 ^b	3.43 ^b	0.73	0.64	4.96 ^b	0.13
2IU	40.83 ^{ab}	4.78 ^{ab}	3.47 ^{ab}	0.73	0.74	5.29 ^{ab}	0.13
4IU	42.75 ^a	4.88 ^a	3.52 ^a	0.72	0.68	5.75 ^a	0.14
SEM	0.68	0.04	0.02	0.01	0.04	0.13	0.3x10 ⁻²
Hormone	NS	NS	NS	NS	NS	NS	NS
*levels							

a,b,c: means with different superscript on the same column are significantly different (p<0.05). SEM=standard of means error

The effects of hormone injection on internal egg qualities of female YEC are presented in Table 5.6. Yolk width of hens on testosterone administration was higher (p<0.05) than hens on estradiol administration. Based on the levels of administration, yolk height increased (p<0.05) by 0.77cm (13.18^b – 13.95^a), yolk width increased by 0.27cm (3.52^c – 3.79^a), yolk

weight increased by 1.88g (12.54^b – 14.42^a), albumen weight increased by 2.08g (20.88^b – 22.96^a) and yolk ratio increased by 0.02 (0.32 – 0.34) respectively with increasing levels of administration from 0IU to 4IU.

Table 5.6: Effects of hormone injection on internal egg quality indices of Yoruba ecotype chicken

Parameter/treatment	Yolk height	Albumen height	Yolk width	Yolk weight	Albumen	Yolk colour	Albumen	Yolk ratio	Yolk index	Hughes unit
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nt	(Cm)	(Cm)	(cm)	(g)	weight	ratio				
					(g)					
Hormone										
estradiol	13.49	2.89	3.59 ^b	13.61	21.44	5.86	0.53	0.33	3.76	55.21
Testosterone	13.49	2.89	3.71 ^a	13.78	22.00	5.14	0.54	0.34	3.65	54.50
SEM	0.19	0.19	0.03	0.22	0.39	0.27	0.01	0.4X10 ⁻²	0.06	2.08
Level										
0IU	13.18 ^b	2.89 ^{ab}	3.52 ^c	12.54 ^b	20.88 ^b	4.67 ^b	0.54	0.32 ^b	3.76	56.14
2IU	13.35 ^a	2.50 ^b	3.64 ^b	14.13 ^a	21.33 ^b	8.08 ^a	0.52	0.35 ^a	3.67	50.89
4IU	13.95 ^a	3.28 ^a	3.79 ^a	14.42 ^a	22.96 ^a	3.75 ^b	0.54	0.34 ^a	3.69	57.89
SEM	0.24	0.23	0.04	0.27	0.48	0.33	0.01	0.4x10 ⁻²	0.07	2.55
Hormone*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Levels										

a,b,c: means with different superscript on the same column are significantly different (p<0.05). SEM=standard of means error

The effects of hormone injection on fertility, hatchability and sex ratio of YEC (Table 5.7) showed interaction between hormone injection and levels of administration on egg weight only while other parameters such as number of egg set, fertility, hatchability, percentage male and female had no significant interaction.

In consistency with Tables 5.3 egg weight of birds injected with testosterone were higher than those injected with estradiol. The levels of administration showed that egg weight was higher at 4IU (39.64g).

Table 5.7: Effects of hormone injection on fertility, hatchability and sex ratio of Yoruba ecotype chicken

Parameters	Egg	Egg	Fertility %	Hatchability %	Male %	Female %
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	number	weight (g)				
Hormone						
Estradiol	20.00	37.99 ^b	90.67	38.67 ^a	42.32	57.67
Testosterone	20.00	39.91 ^a	92.00	33.33 ^b	44.22	55.67
SEM	0.00	0.41	2.40	3.37	6.46	7.08
Level						
0IU	20.00	39.89 ^a	92.00	36.00	40.00	60.00
2IU	20.00	37.32 ^b	89.00	37.00	30.67	69.33
4IU	20.00	39.64 ^a	93.00	35.00	44.17	55.83
SEM	0.00	0.51	2.94	4.12	7.91	8.66
Hormone*	NS	S	NS	NS	NS	NS
level						

a, b means with different superscript on the same column are significantly different ($p < 0.05$). NS= non-significant and SEM=standard of means error

There was no significant ($p > 0.05$) difference in all the parameters of semen quality of YEC considered (Table 5.8). There was no interaction between hormone injection and levels of administration.

Table 5.8: Effects of hormone injection on semen quality of Yoruba ecotype chicken

Parameters	Volume (ml)	Total sperm Count (x10 ⁶ /ml)	Motility (%)	Sluggish (%)	Dead (%)	Abnormal (%)
Hormone						
Estradiol	0.79	1909.00	80.17	6.50	13.17	0.17

Testosterone	0.26	2587.00	83.17	7.67	8.17	0.92
S.E	0.20	311.79	4.47	1.98	3.08	0.36
Levels						
0IU	0.26	2475.00	85.88	6.63	7.38	0.13
2IU	0.57	2070.00	76.88	7.25	14.75	1.13
4IU	0.76	2199.00	82.38	7.38	9.88	0.38
SEM	0.25	381.86	5.48	2.42	3.77	0.44
Hormone*level	NS	NS	NS	NS	NS	NS

SEM=standard of means error

The effects of hormone injection on serum biochemical indices of YEC (Table 5.9) revealed no significant difference ($p>0.05$) in the sex, hormone and levels of administration on Aspartate Transaminase (AST), Alanine Transaminase (ALT), Total Protein (T. protein), Albumin (ALB), Total Cholesterol (TCHOL), Triglyceride (Trig), High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) except Alkaline Phosphatase (ALP) that significantly increased with level of administration

There was no interaction among sex and hormone injection, sex and levels of administration, hormone injection and levels of administration, and sex, hormone injection and levels of administration.

Table 5.9: Effects of sex and hormone injection on selected biochemical indices of Yoruba ecotype chicken

Parameters	ALT	AST	ALP	T.Chol.	LDL	HDL	Trig.	T. Prot.	ALB
Sex									
M	13.17	18.06	24.83	3.83	3.06	0.75	0.71	48.67	34.72
F	12.61	17.28	24.39	3.78	3.02	0.76	0.69	49.56	34.78

SEM	0.32	0.35	0.56	0.07	0.03	0.03	0.03	0.42	0.44
Hormone									
Estradiol	12.89	17.44	24.83	3.76	3.02	0.74	0.69	49.17	34.39
Testosterone	12.89	17.89	24.39	3.83	3.06	0.77	0.70	49.06	35.11
SEM	0.32	0.35	0.56	0.06	0.03	0.03	0.03	0.42	0.44
Levels									
0IU	12.75	17.83	22.58 ^b	3.86	3.08	0.78	0.66	49.25	35.08
2IU	12.83	17.75	26.08 ^a	3.81	3.05	0.76	0.74	48.92	34.75
4IU	13.08	17.42	25.17 ^a	3.73	3.00	0.73	0.69	49.17	34.42
SEM	0.39	0.42	0.68	0.07	0.03	0.04	0.04	0.52	0.54
Sex* hormone	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sex* level	NS	NS	NS	NS	NS	NS	NS	NS	NS
Hormone* level	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sex* hormone* level	NS	NS	NS	NS	NS	NS	NS	NS	NS

a, b means with different superscript on the same column are significantly different ($p < 0.05$). NS= non-significant, AST=Aspartate Transaminase (Iu/L), ALT= Alanine Transaminase (Iu/L), ALP= Alkaline Phosphatase (Iu/L), T.Pro=Total Protein (g/L), ALB=Albumin (Iu/L), T.Chol=Total Cholesterol (Mmol/L), Trig=Triglyceride (Mmol/L), HDL=High Density Lipoprotein (Mmol/L) and LDL=Low Density Lipoprotein (Mmol/L), M=male, F=female, SEM=standard of means error

The effects of hormone injection on steroid hormone of YEC are shown in Table 5.10. The female had significantly ($p < 0.05$) higher follicle stimulating hormone, oestrogen and progesterone than the males. The male on the other hand, had higher testosterone than the female.

Testosterone stimulated higher luteinising hormone level than estradiol injection. The luteinising hormone (LH) decreased with increased levels of administration irrespective of the hormone type.

There were significant interactions between hormone type and level of administration on luteinizing hormone. Interaction among sex and hormone injection, sex and levels of administration and sex, hormone and levels of administration were not significantly different.

Table 5.10: Effects of sex and hormone injection on selected steroids hormone of Yoruba ecotype chicken

Parameters	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (ng/ml)	Estradiol (ng/ml)	Progesterone (ng/ml)
Sex					
F	3.87	5.34 ^a	0.67 ^b	59.72 ^a	3.41 ^a
M	3.72	4.19 ^b	1.89 ^a	51.61 ^b	1.90 ^b

SEM	0.11	0.22	0.05	1.13	0.08
Hormone					
Estradiol	3.58 ^b	4.55	1.34	56.67	2.57
Testosterone	4.01 ^a	4.98	1.22	54.67	2.74
SEM	0.11	0.22	0.05	1.13	0.08
Levels					
0IU	4.18 ^a	5.22	1.22	54.92	2.53
2IU	3.45 ^b	4.33	1.26	56.83	2.76
4IU	3.76 ^b	4.76	1.37	55.25	2.68
SEM	0.14	0.27	0.05	1.38	0.10
Sex*hormones	NS	NS	NS	NS	NS
Sex* level	NS	NS	NS	NS	NS
Hormone* level	S	NS	NS	NS	NS
Sex*hormone*level	NS	NS	NS	NS	NS

Means with different superscript on the same column are significantly different ($p < 0.05$). NS= non-significant, S=significant, LH=luteinizing hormone, FSH=Follicle stimulating hormone, M= Male, F= Female, SEM=standard of means error

Table 5.11: Interaction between hormone injection and levels of administration on luteinizing hormone of Yoruba ecotype chicken

Hormone	Level	LH
Estradiol	0IU	3.300 ^b
	2IU	3.600 ^b
	4IU	3.833 ^b
Testosterone	0IU	5.050 ^a

	2IU	3.300 ^b
	4IU	3.683 ^b
SEM		0.190

Means with different superscript on the same column are significantly different ($P<0.05$). LH=luteinizing hormone, , SEM=standard of means error

The effects of hormone injection on Haematological indices of YEC are shown in Table 5.12. Male birds had higher ($p<0.05$) values of packed cell volume (PVC), Haemoglobin (Hb), Red blood cell (RBC), and platelet than female birds. The females had higher ($p<0.05$) mean corpuscular haemoglobin concentration (MCHC) than the males, both sexes had similar white blood cell (WBC) and its differentials.

Estradiol injection stimulated higher ($p<0.05$) monocytes, red blood cell, and haemoglobin in YEC than testosterone injection. On the other hand, injecting testosterone stimulated higher

mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelets in birds than estradiol injection.

Increase in the levels of administration significantly decreased ($p < 0.05$) WBC, MCV, MCH and MCHC and significant increased RBC and haemoglobin of birds.

Interaction between sex and hormone injection on white blood cell of YEC (Table 5.13) showed that estradiol increased WBC in male and decreased it (WBC) in females, While, testosterone injection acted inversely.

Interaction between sex and hormone injection on platelet (Table 5.13) showed that testosterone decrease platelet in male birds and increase platelet in female birds. Estradiol had the highest platelet count in males and the least value in females.

Interaction between sex and levels of administration on PCV of YEC (Table 5.14) showed that PCV increased with increased level of administration in male chicken while PCV decreased with level of administration in female chicken.

Interaction between sex and levels of administration on mean corpuscular haemoglobin concentration (MCHC) (Table 5.14) showed that MCHC increased with level of administration in male while it increased from 0iu to 2iu and later decreased at 4iu in female birds.

The interaction between hormone injection and level of administration on monocyte of YEC (Table 5.15) showed that monocyte increased from 0IU to 2IU and decreased slightly at 4IU with estradiol injection while decrease monocyte count with increased levels of administration was noticed with testosterone injection.

The interaction between hormone injection and levels of administration on mean corpuscular volume (MCV) of YEC (Table 5.15) showed that MCV decreased with levels of

administration on estradiol injection while MCV of birds on testosterone injection decreased from 0IU to 2IU and later increased at 4IU.

The interaction between hormone injection and levels of administration on means corpuscular hemoglobin (MCH) of YEC (Table 5.15) showed that MCH decreased with levels of administration for both estradiol and testosterone injection.

Table 5.12: Effect of sex and hormone Injection on Haematological Indices of Yoruba ecotype chicken

Parameters	WBC	Heterophil	Lymph	Mono	Eosin	Baso	RBC	PCV	Hb	MCV	MCH	MCHC	Platelet
SEX													
M	10.65	45.50	51.89	2.17	0.67	0.00	6.41 ^a	39.17 ^a	12.68 ^a	63.94	20.53	31.89 ^b	284.94 ^a
F	10.70	43.03	53.67	2.94	0.39	0.00	3.58 ^b	29.11 ^b	7.38 ^b	62.72	21.02	33.43 ^b	241.94 ^b
SEM	0.45	1.27	1.37	0.27	0.17	0.00	0.21	1.08	0.35	0.93	0.52	0.39	13.35
Hormone													
Estradiol	10.44	44.08	52.06	3.22 ^a	0.61	0.00	5.41 ^a	31.72	10.64 ^a	60.11 ^b	19.77 ^b	33.34 ^a	257.11 ^b
Testosterone	10.91	44.44	53.50	1.89 ^b	0.44	0.00	4.58 ^b	31.56	9.43 ^b	66.56 ^a	21.78 ^a	31.97 ^b	269.78 ^a
SEM	0.45	1.27	1.37	0.27	0.17	0.00	0.21	1.08	0.35	0.93	0.52	0.52	13.35
Levels													
0IU	12.44 ^a	45.75	51.83	2.25	0.58	0.00	4.15 ^b	31.58	9.18 ^b	73.08 ^a	23.48 ^a	31.50 ^a	269.50
2IU	9.89 ^b	42.75	53.71	3.17	0.42	0.00	5.22 ^a	29.92	9.88 ^b	58.42 ^b	19.30 ^b	33.03 ^b	256.50
4IU	9.70 ^b	44.29	52.79	2.25	0.58	0.00	5.62 ^a	33.42	11.05 ^a	58.50 ^b	19.55 ^b	33.46 ^b	237.33
SEM	0.55	1.56	1.68	0.33	0.20	0.00	0.25	1.32	0.43	1.14	0.64	0.47	16.35
Sex *Hormones	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S
Sex* Levels	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	S	NS
Hormones*Levels	NS	NS	NS	S	NS	NS	NS	NS	NS	S	S	NS	NS
Sex*Hormones* Levels	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S	NS	NS

Means with different superscript on the same column are significantly different ($P < 0.05$). Where WBC = White blood cell ($\times 10^6/\text{mm}^3$), Hete = Heterophil (%), Lymph = Lymphocyte (%), Mono = Monocyte (%), Eosin = Eosinophil (%), RBC = Red blood cell ($\times 10^6/\text{mm}^3$), Hb = Haemoglobin (g/mm^3), PCV = Pack cell volume (%), MCV = Mean corpuscular volume (f/mm^3), MCH = Mean corpuscular haemoglobin concentration (g/mm^3), Plt = Platelet ($\times 10^9/\text{mm}^3$), Baso = Basophil, SEM=standard of means error

Table 5.13: Interaction of Sex and Hormone Injection on MCHC, Platelet and WBC of Yoruba ecotype chicken

Sex	Hormone	MCHC _(g)	Platelet	WBC
M	Estradiol	33.64 ^a	320.00 ^a	11.89 ^a
	Testosterone	30.13 ^b	249.89 ^{bc}	9.42 ^b
F	Estradiol	33.04 ^a	194.22 ^c	8.99 ^b
	Testosterone	33.82 ^a	289.67 ^{ab}	12.40 ^a
SEM		0.413	12.876	0.439

a, b means with different superscript on the same column are significantly different ($p < 0.05$). MCHC= Mean Corpuscular Haemoglobin Concentration, WBC= White Blood cell, SEM=standard of means error

Table 5.14: Interaction of Sex and Levels of Administration on Packed Cell Volume of Yoruba ecotype chicken

Sex	Level	MCHC _(g)	PCV
M	0IU	29.70 ^b	35.83 ^b
	2IU	32.23 ^{ab}	39.67 ^{ab}
	4IU	33.73 ^a	42.00 ^a
F	0IU	33.30 ^a	27.33 ^c
	2IU	33.82 ^a	20.17 ^d
	4IU	33.18 ^a	24.82 ^{cd}
SEM		0.413	1.528

a, b,c means with different superscript on the same column are significantly different ($p < 0.05$). MCHC= on Mean Corpuscular Haemoglobin Concentration, PCV=Packed Cell Volume, M=Male, F=Female, SEM=standard of means error

Table 5.15: Interaction of Hormone Injection and Levels of Administration on Monocyte of Yoruba ecotype chicken

Sex	Level	MCV	MCH	Monocyte
Estradiol	0IU	62.67 ^b	20.48 ^b	2.17 ^{bc}
	2IU	60.00 ^b	19.58 ^b	4.17 ^a
	4IU	57.67 ^b	19.25 ^b	3.33 ^{ab}
Testosterone	0IU	83.50 ^a	26.48 ^a	2.33 ^{bc}
	2IU	56.83 ^b	19.02 ^b	2.17 ^{bc}
	4IU	59.33 ^b	19.84 ^b	1.17 ^c
SEM		1.840	0.568	0.250

a, b,c means with different superscript on the same column are significantly different ($p < 0.05$). MCV= Mean Corpuscular Volume MCH= Mean Corpuscular Haemoglobin, SEM=standard of means error

Table 5.16: Interaction of Sex, Hormone Injection and Levels of Interaction on MCH and MCV of Yoruba ecotype chicken

Sex	Hormone	Level	MCH	MCV
M	Estradiol	0IU	18.47 ^{cd}	57.00 ^d
		2IU	19.13 ^{cd}	57.00 ^d
		4IU	19.93 ^{cd}	58.33 ^d
	Testosterone	0IU	27.90 ^a	93.33 ^a
		2IU	17.93 ^d	58.00 ^d
		4IU	19.83 ^{cd}	58.67 ^d
F	Estradiol	0IU	22.50 ^{bc}	68.33 ^{bc}
		2IU	20.03 ^{cd}	63.00 ^{cd}
		4IU	18.57 ^{cd}	57.00 ^d
	Testosterone	0IU	25.07 ^{ab}	73.67 ^b
		2IU	20.10 ^{cd}	55.67 ^d
		4IU	19.85 ^{cd}	58.67 ^b
SEM			0.568	1.840

a, b,c means with different superscript on the same column are significantly different ($p < 0.05$). M=Male, F=Female, SEM=standard of means error, MCH= Mean Corpuscular Haemoglobin, MCV= Mean Corpuscular Volume

5.9 Discussion

The initial and final weights of both cocks and hens YEC on hormone injection revealed that male birds were heavier than their female counterparts. This may be attributed to the fact that hens convert their feed to egg while males convert their feed directly to flesh. This report is consistent with the earlier report of Sola-Ojo *et al.* (2008) who observed that the males of Fulani ecotype chicken were heavier than their female counterpart. The females consumed more feed than the males despite their smaller weight, this excess feed consumption by the females suggested that more energy is needed by hens for egg production. This observation is in agreement with the report of Kingori *et al.* (2014) who observed that egg producing type of indigenous chicken consumed higher energy feed than cockerels.

The increased feed intake with increasing levels of hormone administration suggests that high level of hormone injection could aid appetite in birds. The positive influences of testosterone and corticosterone have been reported and testosterone was indicated to increase leptin (Zou *et al.*, 2007) which is an appetite stimulating hormone (Niv-Spector *et al.*, 2005). Testosterone is the chief male sex hormone that promotes the development and maintenance of characteristics associated with manhood, virility, libido, muscular mass, excitement and aggressiveness in animal (Kiefer, 2008). It motivates the body for exercise and bone growth. All these functions of testosterone could be a reflection why feed intake of birds on testosterone injection increased with increasing levels of testosterone administration.

Testosterone injection decreased hen day production in laying hens. Observation during this study revealed that there was total egg cessation in the hens injected with 4IU testosterone on the third day after the administration of testosterone. Therefore, testosterone had innate ability to control ovulation and molting in YEC.

Estradiol on the other hand may possess ovulatory property that stimulates egg production hence, the reasons for increased hen day production of birds injected with estradiol hormone. This observation is consistent with the report of Abu *et al.* (2006) who observed that several natural and synthetic hormones have innate potentials to improve egg production and productivity in birds.

The increased egg weight and other egg qualities (yolk ratio and egg shape) could indicate the potentials of hormone injection in the production of high quality chicks. This finding concur with the report of Sklan *et al.* (2003) who observed positive relationship between improved egg weight and high quality day-old chick of indigenous chicken (Sklan *et al.*, 2003).

Hormone plays vital role in stimulating the granulosa cells of hen (Ansari-Pirsaraci, 2009) to activate folliculogenesis (Mohammadi and Ansari–Parsaraie, 2016) thereby resulting in pre-ovulatory follicles. The action of estradiol injection in this study indicated stimulation of ovulation while that of testosterone injection suppressed ovulation as reflected in their hen day production. This stimulatory effect on ovarian follicular development by estradiol agrees with the report of Kim *et al.* (2004) who observed that estradiol and progesterone exert regulatory effects on follicular development and have a positive association with egg production.

Testosterone was observed to function in mechanisms associated with follicular selection before entering ovulatory hierarchy (Mohammadi and Ansari Pirsaraie, 2016), the increased egg width, egg length and other egg qualities could relate to this function of testosterone in the ovary.

Testosterone injection was observed to increase percentage maleness in the flock and lower percentage hatchability and femaleness. It could be that maternal effect of testosterone on egg resulted in embryo mortality of female chicks. This is consistent with the findings of Riedstra

et al. (2013) who observed that testosterone increase maleness in mice. Maternal androgens in birds was observed by Eising *et al.* (2005) to have regulatory effect on chick's early development and offspring sex ratio.

Estradiol injection on the other hand, increased percentage female chicken in YEC. This could be the reason behind the use of estrogen-like substances in transgender procedures.

Testosterone and estradiol injection did not influence spermatogenesis adversely and fertility of egg was not affected.

Liver function tests are commonly used as clinical screening of liver disease and monitoring of hepatotoxic substances in the body (Ahamefule *et al.*, 2008). Aminotransferases such as Aspartate Transaminase (AST) and Alanine Transaminase (ALT) usually measure the concentration of intracellular hepatic enzymes that have leaked into the blood circulation and serve as an indicator of hepatocyte injury (Elizabeth, 2005). The observed values of AST and ALT did not indicate hepatocyte injury since they were not adversely influenced. Alkaline Phosphatase (ALP) acts as marker of biliary function and cholestasis (Wang *et al.*, 2014). The elevation of ALP with levels of administration in this study suggests marker for biliary dysfunction and cholestasis reported by Wang *et al.* (2014) who observed that growth hormone induced elevation of ALP in chicken. This might be part of the reason while hormone therapy is harmful to the body.

The action of hormone in elevating Alkaline Phosphatase (ALP) in this study could be due to inhibitory ability of hormone in preventing the bile from destruction (breaking down) of useful cholesterol which might serve as the template for steroid hormone formation.

Follicle stimulating hormone (FSH) and progesterone concentration affect egg laying potentials of birds (Adeyina *et al.*, 2015). Therefore, the high concentration of FSH and progesterone in female could indicate laying advantages of hen over cock.

Haematological parameters were paramount in the assessment of pathological and metabolic status of chicken (Afolabi *et al.*, 2012). The white blood cells (WBC) played prominent function in disease resistance with regard to the generation of antibodies and phagocytosis. The WBC obtained in this study (ranged $9.70 - 12.44 \times 10^6 \text{mm}^3$) corroborated the normal range of $6.95-18.65 \times 10^6 \text{mm}^3$ reported by Ikhimioya *et al.* (2000) for Nigerian Indigenous chicken. This could indicate that estradiol and testosterone injection up to 4IU posted no threat on the health status of the birds.

Injection of male with estradiol (female hormone) elevated WBC in male and injection of the females with testosterone hormone (male hormone) elevated WBC in female might reflect that administration of male hormone to female and vice versa, could appear as phagocyte in their body.

The differentials of WBC are heterophils, lymphocyte, monocyte and eosinophils (Bounous *et al.*, 2000). Among these, the lymphocytes are the most abundant in chicken and turkey (Bounous *et al.*, 2000) functioning in the production of antibodies (Afolabi *et al.*, 2012). The lymphocytes obtained in this result were within the normal range of 47.7-85.0% reported by Mitruka and Rawnsley (1977) for healthy chicken and this thought would guarantee the health status of the chicken in the face of treatment within the hormones.

Red Blood Cells (RBC), Packed Cell Volume (PCV), and Haemoglobin (Hb) values obtained in this study were within normal range of 27.38-40.70% PCV reported by Pampori and Igbal (2007) and haemoglobin of 7.06 g/dl-13.68g/dl reported by Ikhimioya *et al.*, (2000) for local chicken. Elevated RBC in birds reflects ability to endure respiratory stress. The RBC counts

of male birds in this study were more than females indicating the ability of male to survive stress better than females.

The platelets are responsible for haemostasis, trephocytosis and phagocytosis in birds. The male birds had more platelets than the females. The activation of more platelets by testosterone could indicate blood clotting potentials of testosterone in Yoruba ecotype chicken.

In conclusion, estradiol injection stimulated egg production and increased percentage femaleness in the flock. While testosterone administration increased egg weight and percentage male chicks in YEC.

CHAPTER SIX

6.0 EXPERIMENT 3

6.1 Effects of *in-ovo* hormonal administration on offspring sex ratio of Yoruba ecotype chicken

6.2 INTRODUCTION

Sex allocation theory in animals has derived benefit from the current advances in the studies of physiological mechanisms leading to sex ratio variation such as maternal glucose, stress, estradiol and testosterone levels (Helle *et al*, 2012). This physiological mechanisms has resulted in systematic changes in offspring sex ratios of vertebrate especially avian species (Komdeur and Pen, 2002; Engelhardt *et al.*, 2003). These changes indicated that some species of birds manipulate the male and female offspring number either during egg production time (Grant and Chamley, 2010) or by affecting sex- specific mortality during embryonic or post hatching development (Dijkstra *et al.*, 1998). The mechanisms of primary and secondary sex ratio biases in birds have been linked with steroid hormones resident inside the egg (Muller, *et al.*, 2002). In birds, the effect of high testosterone level is assumed to occur during embryo development and has been observed to bias offspring sex ratio towards males (Grant *et al.*, 2011). Maternal glucocorticoids and estradiol injection were also suggested to bias offspring sex ratio via sex-biased embryonic mortality towards females (Navara, 2010).

The offspring sex ratio in zebra finches was reported to vary in respect of variation in maternal hormones deposition of female zebra finches into eggs (Engelhardt *et al.*, 2003). The effect of maternal hormone transport on secondary sex ratio adjustment came from the study of Williams (1999) who injected female zebra finches before egg laying with 17- β -estradiol and discovered that the mother produced significantly more female than male fledglings. The increase in nestling mortality of birds was attributed to increase embryonic mortality of male estradiol injection (Williams, 1999). Estradiol injection lead to secondary

sex ratio biases by influencing mortality in a sex-specific way. Miyata *et al* (1999) reported that exogenous administration of estradiol reverse the sex of male reptile embryos to yield apparently normal females. Exogenous estradiol administration in bird also feminized genetic males (Akatsuka, *et al.*, 2004) and treatment of *Xenopus* tadpoles with estradiol was reported to transform genotypic males into functional females (Basu *et al.*, 2001).

The objective of this study was to investigate the effects of estradiol and testosterone *in ovo* administration on egg live germ, hatchability and offspring sex ratio of Yoruba ecotype chicken.

6.3 Materials and Methods

6.3.1 Estradiol and Testosterone hormones

Estradiol and Testosterone propionate injectable were purchased from Aromokeye Veterinary shop, opposite 'A' Division, Ilorin, Kwara state, Nigeria.

6.3.2 Experimental design

A total of eight hundred (800) Yoruba ecotype chicken's fertile eggs were collected from forty seven weeks old female YEC used in experimental 2. The eggs were weighed randomly and distributed into eight (8) treatment groups of five (5) replicates and twenty (20) eggs per replicate in a factorial (2x4) design. The four (4) treatment groups were injected independently with 0IU, 1IU, 2IU and 4IU levels of estradiol and testosterone propionate. Sterilized micro-niddles were used for the injection of eggs and the pores (site of injection) were immediately covered with liquid paraffin (to prevent infection) and set inside the incubators. Fertility and live germ tests were carried out at days 5 and 17 using candling machine. Chicks' weights were determined at hatching using sensitive electronic scale. Percentage survivability and offspring sex (% male and female) were determined at eight weeks of age.

6.3.3 Statistical analysis

Data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) procedure following a factorial (2x4) model (SAS 1999).

$$Y_{ij} = \mu + H_i + L_j + (HL)_{ij} + e_{ij}$$

Y_{ij} = The parameters of interest

μ = Over all mean for parameters of interest

H_i = Fixed effect of i^{th} hormone ($i=1$ to 2)

L_j = Fixed effect of j^{th} level ($j=1$ to 4)

$(HL)_{ij}$ = interaction of i^{th} hormone and j^{th} level

e_{ij} = Error as independent and identically distributed as normal with mean zero

6.4 Results

The effects of estradiol and testosterone propionate micro injection on egg fertility, hatchability and offspring sex ratio of YEC are presented in Table 26. Considering hormone injection only, there were significant ($p<0.05$) differences in percentage live germ, hatchability, survivability of chicks and percentage male and female in the flock. The percentage live germ and hatchability of eggs on estradiol injection were 12.57% and 46.43% respectively higher than those on testosterone injection. The percentage male chick on testosterone injection was 22.09% higher ($p<0.05$) than eggs injected with estradiol. Estradiol injection increased ($p<0.05$) percentage chick survivability and female percentage by 40.17% and 28.71%, respectively over testosterone injection.

The percentage live germ at 0IU, 2IU and 4IU were the same and significantly ($p<0.05$) higher than 1IU. The percentage survivability of chicks decreased with increasing levels of administration with 1IU and control (0IU) having the highest values.

The percentage maleness increased by 17.72% while percentage femaleness decreased by 14.45% from 0IU (control) to 1IU. This was accompanied with 8.15% decrease in maleness from 1IU to 2IU. The highest percentage maleness was at 4IU but survivability was lowest (58.00%) at this level of administration. Survivability decreased significantly ($p<0.05$) with increasing levels of administration of testosterone propionate micro injection while the levels of estradiol micro injection were not significantly ($P>0.05$) different from each other.

Table 6.1: Effects of estradiol and testosterone micro injection on live germ, hatchability and offspring sex ratio of Yoruba ecotype chicken

Parameters	Egg weight (%)	Live germ (%)	Hatchability (%)	Survivability (%)	Male (%)	Female (%)
Hormone						
Estradiol	39.81	76.45 ^a	62.05 ^a	97.50 ^a	41.55 ^b	58.45 ^a
Testosterone	38.96	66.84 ^b	33.24 ^b	58.33 ^b	53.33 ^a	41.67 ^b
S.E	0.36	2.41	3.70	4.79	7.65	7.01
Level						
0IU	39.69 ^b	74.36 ^a	52.61 ^a	93.50 ^a	41.17 ^a	58.83 ^a
1IU	42.20 ^a	62.00 ^b	46.92 ^b	91.17 ^a	49.67 ^a	50.33 ^a
2IU	38.37 ^{bc}	79.32 ^a	53.52 ^a	69.00 ^b	45.62 ^a	54.38 ^a
4IU	37.26 ^c	70.91 ^{ab}	37.53 ^c	58.00 ^c	53.31 ^b	36.69 ^b
S.E	0.51	3.41	5.24	6.77	10.82	9.91
Hormone*Level	NS	NS	NS	NS	NS	NS

Means with different superscript on the same column are significantly different (P<0.05). NS= non-significant, SEM=standard of means error

6.5 Discussion

The exogenous administration of estradiol hormone phenotypically resulted in sex ratio adjustment towards female. Estradiol is an active form of oestrogen; its presence inside the egg during embryo development may result to female emergency during hatching (Tran *et al.*, 2010). Detection of oestrogenic content of the allantois' fluid formed the basic principle in the application of Embrex's automated gender sorting device that sort out male from female embryonic eggs (Tran *et al.*, 2010). This observation is in tandem with the finding of Akatsuka *et al* (2004) who observed that estradiol hormone administration in *Xenopus* embryo regulate gene expression and bias offspring sex ratio toward female. The exposure of embryos in egg to pharmacological changes in estradiol concentration was reported by Thomas and Marion, (2013) to result in sex reversal of genetic males into phenotypic females at the levels of the gonad, gonaducts and external sex characteristics. The mechanism of action of estradiol injection in biasing sex ratio of YEC in this study may be similar to the aforementioned findings.

The *in ovo* administration of testosterone biased offspring sex ratio toward male while estradiol biased the offspring sex toward female. This could be as a result of changes in the hormonal content of the egg which serves as environment for embryo development. The steroids hormone composition of the egg affects developing embryo and determines the hatchling success (Sural, *et al.*, 2016). The skew in the sex ratio was reported by Muller *et al.* (2002) who observed that eggs hatched to male contain more testosterone than those that develop into female. This study also agrees with the findings of Grant *et al* (2011) who observed that high testosterone level during embryo development bias offspring sex ratio towards male.

Egg fertility, percentage live germ and hatchability are the essential consideration in reproductive performance of hen that can be affected by genetic components and environment (Kinggori, 2011). The higher percentage of live germ in eggs on estradiol *in ovo* administration compared with those on testosterone administration suggests that estradiol was not suicidal to embryonic development. Endogenously, estradiol has ability to regulates lipids synthesis, secretion and transportation in the liver of laying hens, while *in ovo* administration could regulates gene transcription through oestrogen receptors consisting of α -oestrogen receptor and β -oestrogen (Schneide, 2009). The live germ, hatchability and survivability of chicks in this study suggested that estradiol hormone favourably reacted with developing embryo and did not present any harmful effect on chick survivability.

The lower percentage live germ and hatchability observed on testosterone *in ovo* administration could be due to masculine effect of testosterone on egg yolk fatty acid that resulted in the death of developing embryo. This agrees with the observation of Ayidin and Cook (2004) that embryonic mortality occurs as a result of modification in the fatty acid profile of egg yolk. Also, Gam *et al* (2011) posited that avian species can adjust their sex ratio by sex specific mortality either postnatally through offspring protection or prenatally through embryo mortality and low fertility.

It can be concluded that estradiol injection increases percentage live germ, hatchability, survivability and female chick number while, testosterone injection increases maleness in the flock.

CHAPTER SEVEN

7.0 SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

The indigenous chicken stock in the world contributes significantly to animal protein intake particularly in the developing countries. The Yoruba ecotype chicken is one of the two prominent types of indigenous chicken in Nigeria that is reared by every household in the towns and villages in the west. The chicken serves as local assets, source of egg and occasional meat for the poor people living in the rural area and every family rear local birds in small flock of about 20-40 birds. The male and female chicken are usually kept together in the same flock because the farmer has difficulty in differentiating cockerel from pullet at early age. This practice favours the male at the expense of the female since the formal eat more than the later because of their aggressive feeding characteristics. The cocks therefore grow bigger, taller and heavier than the hen and more acceptable for meat purpose than female. Also, the meats of male are desirable for special dishes because of their toughness, pigmentation and sweetening flavour. The eggs from the hens are relatively scarce and expensive in the market since the limited one are mostly patronized by the ritualist.

In order to increase protein intake in the developing country like Nigeria, reproduction, breeding and multiplication of indigenous chicken must be targeted. To achieve this, all attempt should be made to increase cock production for meat purpose while egg production from local hens should be increased through dietary intake or administration of egg boosting (stimulating) drugs. Also conceited effort should be taken to separate male chick from female chick of indigenous chicken at early age so as to allow them to be raised separately and stop indiscriminate mating of the flock that make selection process difficult.

This study was conducted to investigate:

- (i) The effects of artificial insemination on fertility and chicks sexing methods in Yoruba ecotype chicken (YEC);
- (ii) The effects of graded levels of palm kernel oil dietary inclusion on serum biochemistry, haematology, hormone, reproductive response, egg characteristics and offspring sex ratio of the YEC;
- (iii) The effects of estradiol and testosterone hormones administration on serum biochemistry, haematology, hormone, reproductive response, egg characteristics and offspring sex ratio of the YEC;
- (iv) Effects of *in-ovo* hormones (estradiol and testosterone) administration on offspring sex ratio of YEC.

These four objectives were examined by carrying out four experiments.

- (i) Preliminary experiment investigated the effects of number of days post artificial insemination on fertility and hatchability in a completely randomized design, chicks sexing methods in percentile, weekly body weight and morphometric measurements of YEC in Student t-test.
- (ii) Experiment 2 examined the effects of dietary PKO (0, 1, 2, 3 and 4%) on serum biochemistry, haematology, hormonal status, reproductive responses and offspring sex ratio of YEC in a 2x5 factorial design.
- (iii) Experiment 3 evaluated the effects of estradiol and testosterone administration (IM) on serum biochemistry, haematology, hormonal status, reproductive responses, egg characteristics and offspring sex ratio of YEC in a 2x2x5 factorial design.

- (iv) Experiment 4 investigated the in-ovo estradiol and testosterone administration (0, 1, 2, and 4 IU) on hatchability and offspring sex ratio of YEC in a 2x4 factorial design. Data obtained were subjected to analysis of variance. Means were separated by the new Duncans multiple range test at $p < 0.05$.

The findings of this study revealed that:

- i. fertility was stable from day 1 to 12 except for a transient increase on day 6 and decreased from day 13 onwards;
- ii. Vent sexing is a potential tools for separating male from female NIC at day 3 after hatching;
- iii. male chicks had longer ($p < 0.05$) keel, shank and thigh length than female chicks at seventh and eighth weeks;
- iv. dietary PKO and estradiol and testosterone administration (IM) had no effect on serum biochemistry and haematology in YEC;
- v. estradiol administration (IM) resulted in lower feed per dozen egg ($p < 0.05$) and higher hatchability ($p < 0.05$) than testosterone administration;
- vi. dietary 4% PKO decreased ($p < 0.05$) feed intake, weight gain, feed per dozen egg, fertility and egg hatchability of YEC; and
- vii. *in-ovo* estradiol administration resulted in higher ($p < 0.05$) hatchability and percentage female chicks than testosterone administration.

7.2 RECOMMENDATIONS

Based on the findings of this study, fertile eggs can be collected within day 1 and 12 after artificial insemination in YEC. Male chicks can be separated from female YEC from 7 weeks and above using body weight and morphometric traits. *In-ovo* estradiol administration increased hatchability and the percentage of female chicks hatched. *In-ovo* estradiol administration of up to 2 IU is therefore recommended for increased female chicks production in YEC.

There is need for further research on:

- (i) Differentiation of the genetic male and female from phenotypic male and female produced in this study.
- (ii) Selection programme to develop indigenous chicken for efficient meat producing strain and egg producing strain.

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