EFFECT OF VARYING LEVELS OF ESTRADIOL BENZOATE ON OVARIAN CHANGES, LAYING PERFORMANCE AND EGG QUALITY PARAMETERS OF LOHMANN BROWN HENS

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Dr. Abdussamad Muhammad Abdussamad and has not been presented any where for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write-up (Suleiman Ahmadu SPS/14/MAS/00012) were carried out under my supervision.

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DEDICATION

This research work is dedicated to my family for their encouragement and support.

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ABSTRACT

The current experiment studied ovarian changes, egg laying and egg quality parameters in eighteen Lohmann Brown hens treated intramuscularly with 0, 0.2, 0.4, 0.6, 0.8, and 1 mg/bird Estradiol Benzoate administered through the keel muscle. The treatment was given twice a week for a total period of six weeks in a completely randomized design. Data obtained were analyzed using GraphPadInStat® statistical package. Estradiol Benzoate treatments showed no significant effect on the median weights of oviduct and ovarian stroma, median counts of large yellow, small yellow, large white and medium white follicles, median ovulation rate, median pause days and intersequence pause. Similarly, there were no significant changes in median number of eggs laid, egg weight and quality parameters (Egg length and width, yolk and albumen volumes, and egg shell thickness and weight). Strong, positive and highly significant correlations were recorded between egg weight and egg quality parameters. There was moderate, positive correlation between egg length and shell weight. The multiple regression equation "Egg Weight = -2.599 + 1.182*(Albumen Volume) + 45.425*(Shell Thickness)" explained 93.7% of the variation in egg weight and was the best among other simple, multiple and allometric regression equations. The limits of agreement between actual and predicted egg weights were smaller in magnitude. In conclusion, ovarian changes, oviposition pattern, egg laying and quality parameters were similar among Estradiol Benzoate treatment levels. Egg weight increased with increase in egg quality parameters. Also, shell weight increased with increase in egg length. There was agreement between actual and predicted egg weights. It was observed that albumen volume and shell thickness can be employed in the prediction of egg weight where a weighing scale is not readily available.

CHAPTER ONE

1.0INTRODUCTION

1.1 BACKGROUND INFORMATION

Poultry plays an important role in human nutrition, employment and income generation (FAO, 2013). Poultry account for more than 30% of all animal protein consumption (Permin& Pedersen, 2000). Among the numerous animals found in Nigeria, poultry birds such as chickens, turkeys, ducks, guinea fowls and pigeons provide economic means of sustenance to the populace (Crawford, 1984; Dafwang, Musa, Abdu &Umoh,2010). Chickens are very important and have been recognized as an important animal resource among the avian species (Olowofeso,2005).

Poultry production in Nigeria is not specific to any part of the country because of climatic constraints or pest and diseases as in the case with other livestock (Olumu, 1995). Poultry can be kept in small space with intense management to obtain an optimum production (Olumu, 1995). Chicken is among the affordable protein sources which provide eggs for Nigeria's dense population (Obioha, 1992). Egg is the most versatile and near perfect food in nature and is rich in protein, vitamins and minerals (Olowofeso,2005). Egg is a biological structure for reproduction, which protects and provides complete diet for the developing embryo and its yolk serves as the principal source of food for the first few days of the chick's life (Abanikannda& Leigh, 2007).

Depending on breed and season the laying cycle of the chicken flock usually covers a span of 12 month of egg production beginning when the birds reach 18-22 weeks of age (Jacob, Wilson, Miles, Butcher & Mather, 2014). Egg laying rises sharply and reaches a peak of about 90% in 6-8 weeks later and begins to decline after 12 month of oviposition (Jacob, Wilson, Miles, Butcher & Mather, 2014). Lohmann Brown is an egg-laying strain of chicken selectively

bred from New Hampshire and other brown egg-laying breeds. They lay at about 18 weeks, laying 1 egg per day and up to 300 eggs a year (Feltwell, 2010).

Hormones are essential for reproductive function in poultry (Forgó, Péczely, Do Thi&Hargitai, 1996). The hormonal control of spermatogenesis and mating involves luteinizing hormone-releasing hormone (LH-RH), gonadotropins and androgens (Turner, Riggs &Spelsberg, 1994). The situation in the egg producing female is more complex and is concerned with the interaction of two asynchronous rhythms namely the LH release mechanism and the process of follicular maturation (Forgó et al., 1996).

Estrogen is an intra-ovarian factor which has effect on the hypothalamus, pituitary, liver, skeleton and calcium homeostasis (Turner, Riggs &Spelsberg, 1994). It is also the main reproductive hormone affecting growth, development, maturation and functioning of the reproductive tract as well as sexual differentiation and behavior (Balthazart, Cornil, Charlier, Taziaux& Ball, 2009). Estrogens (estradiol-17ß), which are synthesized and secreted by the gonads during avian embryonic development, regulate growth and differentiation of the sex accessory structures (Johnson, 1990).

Estradiol Benzoate alters the activity of endogenous secretion of follicle stimulating hormone (FSH) and anterior pituitary gland; hence, lower the endocrine FSH stimulation of follicular growth (Follet&Redshaw, 1974).It was hypothesized that exogenous estrogen enhance growth hormone (GH) secretion and promote somatic growth (Eden, 1979).

Ovarian follicular hierarchy is a series of follicles with different diameters. Ovulation is achieved after follicles mature (Etches, 1996). Most of the follicles are not ovulated, but many participate in producing steroid hormones. In the early stages of follicular development, small follicles begin to produce estrogen and androgen (Etches, 1996). When follicles start to form

yolk, estrogen production in this follicle begins to decline. For several hours before ovulation, only the largest follicle produces progesterone (Miller & Wu, 1981). Ovulation is the major controlling factor influencing the subsequent steps in the formation and laying of the egg (Miller & Wu, 1981).

1.2 PROBLEM STATEMENT

Egg production tends to be affected by excessive follicular recruitment which often leads to internal or double (nonviable) ovulations (Etches, 1995). In laying birds, normal levels of reproductive hormones at the hypothalamus and ovary are interrupted by heat stress, leading to reduction in levels and functions. Low level of gonadal hormones affects the development of the oviduct which occurs before and during sexual maturation (Donoghue, Krueger, Hargis, Miller & El-Halawani, 1989). The reduction in laying performance associated with heat stress is a well-known phenomenon in domestic birds (Etches, John &Verrinder, 1995). The activity of neuroendocrine system of poultry is altered by high ambient temperature resulting in activation of the hypothalamic-pituitary-adrenal (AHP) axis (Quinteiro-Filho et al., 2012).

Estradiol Benzoate may lower the endogenous secretion of FSH and LH by the anterior pituitary gland, thereby reducing the endogenous FSH stimulation of oocyte growth. It is also hypothesized that exogenous estradiol benzoate would reduce hepatic production of the yolk precursors and so lead to hypertrophy of liver (Follet&Redshaw, 1974). Follet and Redshaw (1974) also reported that exogenous estradiol decrease accumulation of radioactive vitellogenin by the ovaries of *Xenopus,* whereas exogenous estradiol together with FSH increase precursor uptake.

The administration of estradiol results in dose-related decrease in FSH accompanied by a less pronounced decrease in inhibin (Miller & Wu, 1981). Indiscriminate administration of

Estradiol Benzoate in laying hens leads to spontaneous development of ovarian cancer (Wojtysiak&Kapkowska, 2005). Estradiol concentration higher than 30% by sexual maturity alters stages of ovarian development and lead to high liver weight in female chicken (Robinson &Renema, 1995).

Cracked shells due to age-related inclinations are serious problems in poultry production, and the effect of Estradiol Benzoate given in low continuous dose improve shell strength not bone strength in laying hens (Stadelman, 1977). Stadelman (1977) also reported that estradiol-treated females never had increased in egg mass and hence, Estradiol Benzoate decreases the mass of yolk protein and lipid in eggs. This suggests that estradiol may have actually decreased the uptake of yolk precursors by the growing follicles.

1.3 JUSTIFICATION FOR THE STUDY

Estradiol plays a central role in the determination of egg mass and quality (Wallace, 1985). Steroid hormones have been implicated in the regulation of calcium metabolism in laying hens through several modes of action (Johnson, 1990). Shortly before sexual maturity, the formation of medullary bone and a parallel increase in calcium retention are induced by the action of estrogens (Nys, Mayel-Afshar, Bouillon, Van Baelen& Lawson, 1989).

Estrogen stimulates vitellogenesis, via its action on the liver, feed intake and the deposition of calcium within the medullary portion of long bones (Bacon, Brown & Musser, 1980; Johnson, 1986).It is also increases eggshell thickness (Wistdedt, Ridderstrale, Wall & Holm, 2014). In addition, gonadal hormones regulate the rapid development of the oviduct, which occurs before and during sexual maturation (Forgó et al., 1996).Treatment of immature Japanese quails and young female chickens with estradiol enhances growth of the oviduct and formation of tubular secretory glands and epithelial differentiation (Forgó et al., 1996).

External and internal quality traits of the eggs are significant in poultry breeding due to their influence on reproductive performance of future generations, and quality growth of chicks (McDaniel, Roland & Coleman, 1979).Eggshell quality is significant in protection of the embryo from the detrimental effects of various environmental elements, regulation of gas and moisture exchange, and provision of calcium for embryonic development which are the most important functions of the eggshell (Narushin& Romanov, 2002). Poor eggshell quality, i.e. low breaking strength or the presence of shell defects, negatively affects the profitability of egg production as well as decreasing the hatchability of eggs (Narushin& Romanov, 2002). Moreover, quality egg shell is necessary in order to protect the egg against the penetration of pathogenic bacteria.

Estradiol Benzoate indirectly affects gonadotropin secretion by modifying the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, or its direct effect on anterior pituitary gland (Davies, Massa & James, 1980). Low dosage of Estradiol Benzoate decreases the secretion of gonadotropins in young photo-stimulated quails (Davies et al., 1980). Depending on the species, addition of estradiol to pituitary cell culture inhibits spontaneous secretion of FSH (Miller & Wu, 1981).

1.4 OBJECTIVES

The broad objective of the study was to determine the effect of different doses of Estradiol Benzoate on ovarian changes, laying performance and egg quality parameters of Lohmann Brown hens. The specific objectives are as follows:

1. To determine the effect of Estradiol Benzoate on ovarian changes, number of eggs laid and egg quality parameters in Lohmann Brown hens.
2. To establish in Estradiol Benzoate-treated Lohmann Brown hens, relationships among egg quality parameters.
3. To predict egg weight from egg quality parameters in Lohmann Brown hens treated with different concentrations of Estradiol Benzoate.
4. To determine agreement between predicted and actual egg weights.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 POULTRY

Poultry is a general term for the species of birds domestically raised primarily for economic purpose of producing eggs and meat. Poultry account for more than 30% of all animal protein consumption (Permin& Pedersen, 2000). Poultry plays an important role in human nutrition, employment and income generation (FAO, 2013). Poultry are adapted to almost all environments that humans have explored, without restrictionby climatic constraints or pest and diseases as in the case with other livestock. Poultry can be kept in small space with intense management to obtain an optimum production (Olumu, 1995).

Poultry make use of different systematic plan of action that let them to reproduce under a diverse environmental condition (Johnson, 2000). Stimulation of reproductive activity in birds is under the influence of external stimuli and internal control mechanisms that have developed over the course of evolution to maximize the probability of survival of the young. Despite the wide variety of reproductive patterns among birds, it is clear that all control systems have certain elements in common (Robinson &Renema, 1995).

2.1.1Lohmann Brown Layers

Lohmann brown layers are egg-laying strains of chicken of hybrid origin, selectively bred from New Hampshire and other brown egg-laying breeds of chicken (Feltwell, 2010). They start laying at about 18 weeks, laying 1 egg per day and up to 300 eggs in a year (Feltwell, 2010). Research have shown that Lohmann Brown strain has a higher reproductive performance and feed conversion rate compared to other commercial poultry strains available in Nigeria and also gives higher financial gain in terms of production (Olawumi&Adeoti, 2009).

2.1.2Reproduction in Poultry

Avian species utilize a variety of reproductive strategies that allow them to reproduce under a diversity of conditions and environments. Endocrine and behavioral components of reproduction are directed by the hypothalamus in response to environmental triggers, such as photoperiod. Superimposed on these triggers are internal factors such as stage of the life cycle and general health (Ottinger& Bakst, 1995).

The primary components of the "integrated" reproductive system are the hypothalamus, pituitary gland, and gonads. Accessory organs, which include the oviduct, excurrent duct system, and associated cloacal structures, are also critical to reproductive success. Moreover, endocrine and behavioral components of reproduction must function synchronously for successful reproduction (Ottinger& Bakst, 1995).

The reproductive system is driven by the action of hormones. Hormones are chemicals substances that are produced and released by the gland, which are carried in the blood stream to affect the particular organ (Robinson &Renema, 1995). Similarly, for a hormone to take effect in a specific cell or tissue, that cell or tissue must necessary have the hormone receptor (Robinson &Renema, 1995).

The hypothalamus occupies a central position in the mechanism that controls reproduction and to release gonadotrophin releasing hormone (GnRH), that stimulate the release of gonadotrophin hormones from the anterior pituitary (Bearden, Fuquay & Willard, 2004). The hypothalamus is located within the brain and it controls the autonomic nervous system, those activities over which an animal has no conscious control (Bearden et al., 2004).

There are special cells in the hypothalamus that are thought to receive light energy by photostimulation in response to longer days. The light response involves the stimulation of

specialized cells within the brain. That means the light intensity must be adequate to have the light energy that penetrate the feathers, skin, skull and the brain (Anderson, 2001).

There is a network of blood vessels that link the hypothalamus and the anterior pituitary gland. This means that the message from the hypothalamus (GnRH) is sent directly to the anterior pituitary gland in a rapid manner (Bearden et al., 2004). The hypothalamus produces a gonadotropin-releasing hormone (GnRH) that stimulates pituitary gland production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn regulate ovarian and testicular function. Gonadal steroids, primarily testosterone, estradiol, and progesterone return to the central nervous system through the bloodstream and provide a feedback regulation of hypothalamic GnRH production and release (Bearden et al., 2004).

The anterior pituitary gland is an endocrine gland, which realizes gonadotropin hormones which affect the gonads. The two major ones are follicle Stimulating Hormone and luteinizing Hormone (Wojtysiak&Kapkowska, 2005).

The hypothalamus which is inactive gonadatrophically except when the system receives specific environmental cues which are stimulatory in nature and the most common examples are photoperiodic species from mid to high latitudes. The reproductive cycle of photoperiodic species is rigidly controlled by day length (photoperiodism) (Anderson, 2001).

However, additional environmental conditions (e.g. singing male, certain types of foods, nest sites) may be needed to achieve complete reproductive activity. Many periodically breeding species are not highly photoperiodic, but rather use other sources of environmental information. This clearly in the case of periodic breeders of equatorial regions (Anderson, 2001).

In some species, reproductive activity is based on an endogenous circannual (yearly) cycle that is entrained into an annual cycle by some reoccurring event, be it the annual photo cycle or some other environmental factors (Anderson, 2001).

2.1.3Physiology of Egg Formation

The oviduct is highly convoluted, formed like a tube and suspended in the abdominal cavity attached dorsally by a ligament of peritoneal membrane. On the ventral side of the oviduct a similar ligament, not connected to the body wall with a muscular band along the oviduct (Etches, 1996). Ovulation is the major controlling factor influencing the subsequent steps in the formation and laying of the egg. In the female reproductive tract, ovum in the oviduct will receive 15 min after ovulation by the infundibulum and provide the right environment for fertilization and albumin secretion(Etches, 1996).

The infundibulum is the first part of the oviduct, captures the ovum within 15 minutes after ovulation (Etches, 1996). Sperm storage sites that called glandular groves are located in the infundibulum, which is the site of fertilization. The short period during which fertilization can take place, before the first layer of albumen is deposited is called “window of fertilization” and is only about 15 minutes long (Wishart&Horrocks, 2000). Magnum, which is the second and longest part of the oviduct, the majority of the albumen is secreted during approximately 3 hours (Wyburn, Johnston & Draper, 1970).

The two shell membranes are formed in the isthmus. The inner shell membrane surrounds the albumen and the outer shell membrane functions as a support for the eggshell. In the last part of isthmus the mammillary cores are formed which will function as crystallization sites and anchor the shell to the outer membrane (Solomon, 2010). The egg stays in this part of the oviduct for approximately 1.5 hour (Draper, Davidson, Wyburn& Johnston, 1972). It then enters the shell

gland where the albumen is diluted in a process called plumping and the hard shell is deposited during the next 18-22 hours (Etches, 1996).

Finally, the cuticle, a waxy layer covering the pores in the shell is formed (Baker & Balch, 1962). The egg leaves the shell gland and passes the utero-vaginal sperm storage tubules, where the first storage of sperm occurs and then enters the vagina where it remains for a few minutes before oviposition through the cloaca (Baker & Balch, 1962). Oviposition occurs afteregg calcified and requires coordination with muscular activity (Etches, 1996).

Ovary

In the normal female bird only the left ovary and oviduct develop during sexual maturation. The ovary is situated on the left side of the abdominal cavity close to the median line, attached to the dorsal wall by a fold of peritoneum, the mesovarium (Hodges, 1974). The ovary consists of follicles, of which a small number constantly grow in diameter as yolk transported from the liver is incorporated (Hodges, 1974). The five largest oocytes are arranged in a hierarchy and at ovulation the largest ovum is ovulated (Johnson, 2000).

Stroma

The ovary is made up of the stroma, which is the base supporting structure. It is also composed of the vast pool of small undifferentiated follicles. The pool of follicles is established prior to the chick hatching (Robinson &Renema, 1995).

Ovarian follicles

As the follicles increase in size, they begin to produce estrogen and progesterone. These hormones in turn affect the secretion of gonadatrophins which regulate ovarian function (Johnson, 2000). Progesterone is the most important ovarian hormone regulating pituitary activity; its action is mainly on LH and is clearly involved in hormonal control of ovulation of

the mature follicle (Bedecarrats, Mcfarlane, Maddineni&Ramachandran, 2009). The maximal amount of LH released into the blood occurs 6-8 hours before ovulation. An increased amount of circulating LH apparently increases progesterone secretion from the follicle before ovulation. Therefore progesterone is now inhibitory to LH secretion which helps prevent more than one follicle from ovulating at the same time (Bedecarratset al., 2009).

In birds, progesterone secretion decreases rapidly in the post ovulatory follicle and is negligible after 24 hours. This helps decrease progesterone to a low level again which is stimulatory for additional LH secretion which promotes ovulation of the next mature follicle (Bearden et al., 2004).

In the male FSH causes the growth of seminiferous tubules (spermatogenesis), while LH stimulates the development of Leydig cells which in turn produce testosterone. Testosterone initiates male sexual behavior and also inhibits the hypothalamus to produce GnRH, which result gonadatrophins secretion inhibition by the anterior pituitary gland (Bedecarratset al., 2009). Gonadotropin releasing hormone inhibitiondecreases testosterone production. This inhibitory effect of testosterone provides a feedback control system for maintaining testosterone secretions at a constant level (Bedecarratset al., 2009).

Follicular development

During maturation follicles are generally categorized according to their size and color of the yolk. In Galliformes, the smallest follicles are visible on the surface of the stroma (about 0.2-1.0 mm in diameter) are termed small white follicles. As the small white follicle increases in diameter, it reached a stage when yellow yolk becomes visible. These events proceed without significant gonadotropin support (Johnson, 1990).

At the time of egg production, follicular oocytes will be developed into a

hierarchialarrangement, with the Fl follicle destined to ovulate and the F2 follicle second in the succession of daily ovulations. Once the follicle has been recruited into hierarchy, the gonadotropin support becomes critical, with FSH and LH providing stimulation of steroid production and regulation of factors that modulate growth, development, and finally ovulation of the follicle (Li & Johnson, 1993).

Morphologically, the yellow follicle in the rapid growth stage consists of a number of cellular and acellular layers surrounding the yolk. The perivitelline layer is an acellular fibrous layer homologous to the mammalian zonapellucida. The granulose cell layer, which is a monolayer of cuboidal cells, surrounds the oocyte with cytoplasmic projections that interconnect adjacent granulosa cells as well as form junctional complexes with the surface of the oocyte (Tilly & Johnson, 1991).

The defined granulosa cell basement membrane is nearly 1 μm thick. The theca interna, theca externa, and the germinal epithelium constitute the remaining cell layers. The thecal and granulose cells are the primary sources of the steroids produced by the ovary; small follicles that are in initial stages of development (primary follicles) produce proportionally greater amounts of estradiol, and large follicles (F1-F3) produce high concentrations of progesterone. Progesterone secreted by the large follicles stimulates the preovulatory surge in gonadotropins (Tilly & Johnson, 1991).

Egg

Egg is a biological structure for reproduction, which protects and provides complete diet for the developing embryo and its yolk serves as the principal source of food for the first few days of the chick's life (Abanikannda& Leigh, 2007).External and internal quality traits of the

eggs are significant in poultry breeding due to their influence on reproductive performance of future generations, and quality growth of chicks (McDaniel, Roland & Coleman, 1979).Egg is the most versatile and near perfect food in nature and is rich in protein, vitamins and minerals (Olowofeso,2005).

Ovulatory cycle

Generally, only the Fl follicle ovulates with each daily ovulatory cycle, which in Galliformes is about 26 hours. This cycle of oviposition is followed by ovulation within 60 minutes and oviposition again 26 hours later. The cycle continues until the hen rests for a day (pause day) at the end of a clutch or set of eggs laid sequentially (Etches, 1990).

Photoperiod appears to be the major factor in determining the end of the clutch and in setting the timing for the first egg of the next clutch. The length of the clutch varies individually and with the strain of chicken. For example, heavy strains of chickens tend to have shorter clutches than the leghorn hen, which is a light strain selected for egg production (Etches, 1990).

Ovulation in domestic hen occurs coincident with an increase in FSH binding to ovarian tissue (Etches & Cheng, 1981). Other researchers find less pronounced cycle related to fluctuations in circulating FSH (Krishnan, Proudman, Bolt & Bahr, 1993).Endocrine mechanisms regulating pituitary release of avian FSH is not clear (Krishnan et al., 1993).

Hattori, Ishii and Wada(1986) have reported that LHRH-I induces FSH secretion *in vivo* and *in vitro* in the quail, while Krishnan et al.(1993) failed to detect any stimulatory effect of LHRH-I. The reasons for this discrepancy are not apparent. A primary role for FSH is related to granulosa cell differentiation and the induction of steroidogenesis in prehierarchical follicle granulosa cells (Etches & Cheng, 1981).

2.1.4 Egg Quality Parameters

In general, the characteristics of egg quality have genetic basis. Egg quality is a factor which contributes for better economy price of fertile and table eggs (Buss, 1982). Economic success for a productionflock is measured with total number of eggsproduced (Moniraet al., 2003).

Egg weight and shell thickness

Egg quality is presented by its weight, percentage of eggshell thickness and strength of eggshell (Stadelman, 1977). Genetic differences in eggshell quality characteristics exist between species, and between breeds, strainsand families within the lines (Buss, 1982).Egg weight and eggshell thickness differ between various lines; genotype has direct influence on egg weight and eggshell characteristics (Halaj&Golian, 2011). Many studies showed that hens with coloured feathers lay bigger eggs than hens with white feathers (Halaj&Golian, 2011). Egg quality is composed of those characteristics of an egg that affect its acceptability by consumers; it is therefore important that attention is paid to the problems of preservation and marketing of eggs to maintain the quality.From the consumer's point of view, egg weight is the most important quality trait (Dudusola, 2010).

Shell weight

Among many quality characteristics, external factors including egg weight and shell weight are important in consumer's acceptability (Dudusola, 2010). Internal quality of the egg begins to decline as soon as the egg is laid (Gerber, 2012). The management and nutrition of the hen do play a role in internal egg quality, egg handling and storage practices have a significant impact on the quality of the egg reaching the consumer (Gerber, 2012).

2.1.5 Hormonal Action on Egg Production

Steroid production

The main sources of sex steroids in blood circulation of the laying hen are granulose and theca layers of ovarian follicles. The cells of the theca layer of white non-hierarchical follicles produce mainly estradiol (Johnson & Wood, 2009).During follicular growth and transition to the preovulatory hierarchy, the cells of the granulosa layer is stimulate mainly by follicle stimulating hormone (FSH) and gradually increase the synthesis and secretion of progesterone (Kato, Shimada, Saito, Noda &Ohta, 1995).

The yellow hierarchical follicles are stimulated by LH and gradually potentiate the progesterone production by the granulosa cells so that the preovulatory follicle (F1) becomes the major source of progesterone in circulation (Nitta, Mason & Bahr, 1993).

Estradiol

Estradiol is a steroid hormone strongly associated with female reproduction and female reproductive behavior. The laying hen is not in exception; in the adult bird, oviduct function is maintained by estradiol together with progesterone and androgen (Etches, 1996).

During avian embryonic development, estradiol are synthesized and secreted by the gonads regulate growth and differentiation of the accessory sex organs (Johnson, 1990). Steroid hormones have been implicated in the regulation of calcium metabolism in laying hens, throughout several modes of action. Shortly before sexual maturity the formation of medullary bone and a parallel increase in calcium retention are induced by the action of estradiol(Nys, Mayel-Afshar, Bouillon, Van Baelen& Lawson, 1989).Estradiol stimulates vitellogenesis, via its action on the liver, feed intake and the deposition of calcium within the medullary portion of long bones (Johnson, 1986).

However, gonadal hormones regulate the rapid development of the oviduct, which occurs before and during sexual maturation. Treatment of immature Japanese quail and young female chickens with estradiol enhances growth of the oviduct and formation of tubular secretory glands and epithelial differentiation (Forgó, Péczely, Do Thi&Hargitai, 1996). In birds, as in other vertebrates, reproduction is controlled by the hypothalamic-pituitary-gonadal axis with each component secreting specific neuropeptides or hormone (Bedecarratset al., 2009). Estradiol synthesis occurs in the cells of theca and granulosa cells of the ovary (Wojtysiak&Kapkowska, 2005). Cholesterol is a steroid hormone precursor; it forms through a series of enzymatic reactions (Levi et al., 2009). Estradiol is produce in the small follicles; it associated with the enzymes in the theca layer that plays a role in the biosynthesis of steroid as P450 aromatase converts testosterone into estrogen (Wojtysiak&Kapkowska, 2005).

The small follicles and ovarian stroma containing more than 50% of aromatase that produces 85% of estrogen (Amstrong, 1984). Ovarian follicular hierarchy is a series of follicles with a different diameter; ovulation is reached after follicles mature (Buchanan, Robertson & Hocking, 2002). Many follicles participate in producing steroid hormones from ovaries, in the early stages of follicular development; small follicles begin to produce estradiol and androgen (Etches, 1996).

When follicles start to form yolk, estradiol production in this follicle begins to decline and several hours before ovulation, only the largest follicle produces progesterone (Amstrong, 1984). Increase concentrations of progesterone will stimulate secretion of GnRH by the hypothalamus to stimulate LH secretion; an increase of LH in the blood stimulates the secretion of progesterone. Positive feedback loop between progesterone and LH will result

preovulationyolk that causes tearing of the follicle. Release of yolk from the follicle at ovulation occurs when stigma ripped (Etches, 1996).

Ovarian response to gonadotropin concentrations enhance by increasing estradiol production, estradiol circulating in the liver enter by diffusion and stimulates the synthesis vitellogenin (Levi et al., 2009). Vitellogenin circulate to the surface layer of the growing oocytes and will be captured by the receptor. The process of endocytosis occur by cytoplasmic translocation forming yolk bodies together with the proteolytic cleavage of a sub unit vitellogenin, yolk lipoprotein, lipovitellin and fosvitin (Levi et al., 2009).

The existence vitellogenin shows yolk lipoprotein accumulation in the oocyte, continuous activity of the liver in the synthesis of vitellogenin can induce liver degeneration resulting in liver damage (Saraswati, Manalu, Ekastuti&Kusumorini, 2013). Vitellogenin carried by the blood stream to the ovaries for follicles growth (Elnagar&Abd-Elhady, 2009).

Estradiol is involved in the induction of numerous female sexual characteristics such as the development of an incubation patch, plumage color, and development of the oviduct, nest building behavior, and the mobilization of calcium for egg shell production (Wojtysiak&Kapkowska, 2005). Estradiol probably works synergistically with other hormones (e.g. progesterone, prolactin) to initiate these activities. Apart from its role in the ovulatory cycle, progesterone acts with estradiol in the development of the oviduct and probably promotes incubation behavior (Wojtysiak&Kapkowska, 2005).

Exogenous estradiol

Exogenous estradiol stimulates synthesis of 1, 24 dihydroxycholicalciferol which save calcium required for egg shell deposition by increasing calcium absorption (Soares, 1984).

Exogenous estradiol at a dose of10 µg/kg body weight increases eggshell weight and thicknessin Tegel pullets (Saki,Iji&Tivey, 2002).

Intramuscular injection of 3-5 weeksold female Japanese quails with100µg/bird per day Estradiol Benzoate causeda significant increase in egg weight (Elnagar&Abd-Elhady, 2009).Estradiol stimulates the production of yolk proteins vitellogenin II and apolipoprotein by the liver and supports the oviductal epithelial cells (Koch, Moritz, Lay & Wilson, 2007).In female birds, estradiol triggers egg formation, both in terms of yolk lipiddeposition and albumen synthesis (Johnson, 2000).

Estradioladministration to females induces an increase in yolk precursors (vitellogenin), and low-density lipoprotein, that arethe primary sources of yolk proteins (Johnson, 2000). The synthesis andsecretion of egg albumen by the oviduct is underestrogenic control (Yu, Campbell& Marquardt, 1971). Yolk lipids aresynthesized in the liver under the influence of estradiol (Walzem, 1996).

2.1.6Pause Days in the Egg Production Cycle

Pause day is typically of 40 to 44 hours duration (Robinson &Renema, 1995). The delay in time of lay from one day to another is called “lag”. Hens that lay long sequences must lay them at close to 24 hour intervals so that there is minimal lag (Robinson &Renema, 1995). Hen lays an egg a day and continues to lay for certain period of time and takes a gap of one or few days (pause days). The exact physiological mechanism involved in taking pause between the sequences is not fully known (Robinson &Renema, 1995).

At about 6 hours before a hen ovulates, it experiences a surge of GnRH which results in LH surge, this hormone activity is limited to a finite period of the day. This is called the “open period for LH release”. If there is a mature pre-ovulatory follicle it will respond to this burst of

LH release and will produce progesterone (Robinson &Renema, 1995). The progesterone stimulates further LH release and is called “positive feedback” where the release of one hormone triggers further release of another hormone (Robinson &Renema, 1995).

Normally ovulation follows oviposition by a period of about 15-45 minutes and this is not the case when a hen lays an egg late in the afternoon. In this case, the hen does not ovulate soon after oviposition because it is too late in the day (has exceeded the time limit of her open period for LH release). She will not lay an egg the next day (pause day). The hen will hold the mature F1 follicle overnight and ovulate at the very early in the following open period for LH release. This means she will lay early in the day and a new sequence will begin (Robinson &Renema, 1995).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION AND DURATION OF STUDY

The study was conducted at Poultry Unit of Faculty of Agriculture Teaching and Research Farm, Bayero University Kano (GPS Coordinates: 11.97643^{o}N, 008.42995^{o}E, 469 m above sea level). The duration of the experiment was six weeks.

3.2 SAMPLE SIZE DETERMINATION AND EXPERIMENTAL DESIGN

Resource equation of Mead, Gilmour and Mead (2012) was used to determine the sample size. The equation is given as follows:

$$N - 1 = T + E$$

Where,

N = Number of experimental units

T = Number of test groups - 1

E = Number that estimates the error (the remainder)

Mead and Colleagues stated that E should be between 10 and 20. Within this range, the analysis has the most statistical power for experiment. Therefore, a sample size of 18 was gave an estimate of error (E) of 12 that is within the range of 10 and 20 as proposed by Mead et al. (2012). Hence eighteen birds were used in the experiment.

Eighteen Lohmann Brown layers were completely randomized into six treatment groups; each group comprised three replicates. The groups were given different levels of Estradiol Benzoate at 0, 0.2, 0.4, 0.6, 0.8 and 1 mg/bird designated as treatment A, B, C, D, E and F, respectively.

3.3 ESTRADIOL BENZOATE AND ITS ADMINISTRATION

The brand ofEstradiol Benzoate used in this study was Super Estradiol® (New Century Pharmaceutical Company Limited, China). It came in 10 ml glass bottle, with each ml containing 2 mg Estradiol Benzoate. The pack of the Estradiol for this experiment was kept in a refrigerator and protected from direct sunlight throughout the study period. The hormone formulation transported early in the morning on ice pack to the experimental site.

Estradiol Benzoate injection of 0.2% was administered twice weekly (Monday and Thursday) in the morning between 10:00 am and 11:00 am for a total period of six weeks. It was administered through deep intramuscular injection into the keel muscle at a dose level of 0.1, 0.2, 0.3, 0.4 and 0.5 ml/ bird which translates into 0.2, 0.4, 0.6, 0.8 and 1 mg/bird, respectively. Control birds were given 1 ml normal saline/bird. Each syringe and needle used was disposed by incineration after the injection.

3.4 MANAGEMENT OF HENS

Eighteen Lohmann Brown hens at Twenty four weeks old were purchased from SOVET International Nigeria Ltd., Tarauni, Kano. All the eighteen Lohmann Brown hens were managed intensively in a battery cage system, and each was housed in a separate cage measuring 60× 50 × 75 (i.e. Length × Breadth × Height in centimeters). Birds were allowed to adapt to the environment for two weeks. During the experimental period, birds were fed with SOVET super layer mash® *ad libitum*, (16% Crude protein, 5.0% Fat, 6.0% Fiber, 3.5% Calcium, 0.40% Phosphorus, and 2600 kcal/kg Metabolizable energy) with water available throughout the study period. They were sprayed with cypermetherinbutycabityl 6-propyperonol (zee-on®, Dappo Limited, Farm Center Kano) against external parasites. Piperazinedihydrochloride (Piper Dewormer®, Kepro Pharmaceutical, Devender-Holland) was administered in drinking water

(1g/liter) against internal parasites. Glucose, multivitamins (Anupco®, Anglia Nutrition Products Company, UK) and Oxytetracycline HCL (Oxywin®, SellwellPharceuticals Ltd., India) was given (1 g/liter) in drinking water. It was ensured that the hens undergone all vaccination schedules at the farm before purchased.

3.5 DATA COLLECTION

3.5.1 Laying Performance

Egg produced by individual bird were collected, labeled and recorded on daily basis between 3:00 pm and 4:00 pm for a period of six weeks. Individual daily egg production was used to determine hen-day egg production (HDEP) by using the formulae below described by North (1984).

$$\text{HDEP} = \frac{\text{Number of eggs produced on daily basis}}{\text{Number of birds available in the flock on that day}} \times 100$$

The hen-day egg production (HDEP) was considered as a measure of ovulation rate.

3.5.2 Oviposition Pattern

Number of egg sequences

This is the number of times the hen laid eggs in sequence during the experimental period. The number of sequences was determined from the oviposition record following the procedure of Blake and Ringer (1987).

Egg sequence length

This is the number of eggs laid by individual hen on successive days without pause. The egg sequence length was determined from oviposition record following the procedure of Blake and Ringer (1987).

Pause days

This was determined as the number of days when no oviposition was observed during the experimental period.

Intersequence pause days

These are the number of pause days between egg sequence length and another. Intersequence pause days were determined from oviposition record during the experimental period.

3.5.3 Egg Weight (g)

Egg weight (g) was measured by placing the egg on a sensitive digital scale (Digital Scale®, China) and the egg weight was recorded.

3.5.4 Egg Quality Parameters

Egg shell weight (g)

Egg shell weight was determined by placing the shell on a digital weighing scale after thorough cleaning with cotton wool and the weight was recorded.

Egg shell thickness (mm)

Egg shell thickness was measured using digital Vernier caliper at three spots (broad, narrow and mid section) and the average thickness was recorded.

Egg length and egg width (mm)

The egg length and egg width was determined using digital vernier caliper and recorded.

Albumen volume (ml)

Albumen volume was determined by carefully broken the egg around the equator, and the albumen was separated from the yolkby the used of table spoon and poured into a measuring cylinder.

Yolk volume (ml)

Yolk was poured into a measuring cylinder and the volume in ml was recorded. After each measurement, the measuring cylinder was washed and rinsed thoroughly before the next measurement is taken.

3.5.5 Ovarian and Oviduct Changes

The birds were slaughtered at the end of experiment, the abdominal cavity was cut open using scalpel blade and the ovary was harvested. The ovaries was then stored in 10% Neutral Buffered Formalin. They were removed from the fixative three days after storage. Follicles were classified based on the modification of procedure described by Renema et al. (1995) as follows:

- Large yellow follicles(>10 mm diameter)
- Small yellow follicles (5–10 mm diameter)
- Large white follicles (3–5 mm diameter)
- Medium white follicles (1–3 mm diameter).

The number of normal large yellow follicles (LYF), small yellow follicles (SYF), large white follicles (LWF) and medium white follicles (MWF) was recorded.

Oviduct and the stroma (the ovarian tissue remaining after removal of the follicles) were weighed using sensitive digital scale(Digital Scale®, China).

3.5.6 Data Analyses

All statistical analyses were carried out using GraphpadInstat package (GraphPadInStat, version 3.05,32 bit for Win 95/NT, GraphPad Software Inc., 2000) The effect of Estradiol Benzoate on ovarian changes, laying performance and egg quality parameters was analyzed using Kruskal-Wallis Test with mean rank differences (Kruskal-Wallis Test) compared using Dunn's Multiple Comparisons Test. Relationships among egg quality parameters was determined using Pearson Moment Correlation or Spearman Rank Correlation as the case may be. Prediction of egg weight from egg quality parameters in Lohmann Brown hens treated with different concentrations of estradiol benzoate was carried out using simple linear, multiple and allometric regression equations. The Bland-Altman plot as implemented inMedCalc Statistical Software version 48.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) was used to determine the agreement between predicted and actual egg weights.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Ovarian Changes

Oviduct weight

The effect of varying levels of Estradiol Benzoate onoviduct weight in Lohmann Brown hens is presented in Table 1. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 11.066) difference in median oviduct weight of 56.80 g, 60.85 g, 36.70 g, 36.30 g, 45.40 g and 32.50 g across respective Estradiol Benzoate treatments levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum oviduct weight recorded was 54.5 g, 57.2 g, 36.3 g, 27.1 g, 44.8 g and 31.7 g across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum oviduct weight of 58.2 g, 64.5 g, 43.3 g, 45.5 g, 65.2 g and 36.0 g, respectively.

Large yellow follicle count

The effect of varying levels of Estradiol Benzoate on count of large yellow follicles in Lohmann Brown hens is presented in Table 2. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 2.348) difference in median count (5, 6, 6, 5, 5 and 6) of large yellow follicles across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs.* maximum count of large yellow follicles were 4 *vs*. 6, 6 *vs*.6, 5 *vs*.6, 4 *vs*.6, 4 *vs*. 7 and 5 *vs*.6 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 1: Summary Statistics (including KW Statistic) of Oviduct Weight across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	56.80	54.5	58.2	37	12.333	
0.2	2	60.85	57.2	64.5	28	14.000	
0.4	3	36.70	36.3	43.3	18	6.000	
0.6	2	36.30	27.1	45.5	11	5.500	11.066^{ns}
0.8	3	45.40	44.8	65.2	33	11.000	
1	3	32.50	31.7	36.0	9	3.000	

ns = not significant, P>0.05

Table 2: Summary Statistics (including KW Statistic) of Large Yellow Follicles across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	5	4	6	19.0	6.333	
0.2	2	6	6	6	23.0	11.500	
0.4	3	6	5	6	28.5	9.500	
0.6	2	5	4	6	13.5	6.750	2.348^{ns}
0.8	3	5	4	7	23.5	7.833	
1	3	6	5	6	28.5	9.500	

ns = not significant, P>0.05

Small yellow follicle count

The effect ofvarying levels of Estradiol Benzoate on count of small yellow follicles in Lohmann Brown hens is presented in Table 3. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 7.517) difference in median count (7.0, 6.0, 12.0, 6.5, 10.0 and 12.0)of small yellow follicles across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum count of small yellow follicles recorded was 6, 5, 6, 4, 8 and 9 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum count of small yellow follicles of 9, 7, 12, 9, 11 and 17, respectively.

Large white follicle count

The effect ofvarying levels of Estradiol Benzoate on large white follicle counts in Lohmann Brown hens is presented in Table 4. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 6.186) difference in median count of large white follicles (12.0, 8.5, 8.0, 13.0, 12.0 and 13.0) across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum count of large white follicles were 6 *vs*. 17, 8 *vs*.9, 8 *vs*.11, 12 *vs*.14, 8 *vs*. 15 and 12 *vs*.19 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 3: Summary Statistics (including KW Statistic) of Small Yellow Follicles across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	7.0	6	9	18.0	6.00	
0.2	2	6.0	5	7	7.5	3.75	
0.4	3	12.0	6	12	31.5	10.50	
0.6	2	6.5	4	9	10.0	5.00	7.517^{ns}
0.8	3	10.0	8	11	30.0	10.00	
1	3	12.0	9	17	39.0	13.00	

ns = not significant, P>0.05

Table 4: Summary Statistics (including KW Statistic) of Large White Follicles across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	12.0	6	17	25.5	8.500	
0.2	2	8.5	8	9	9.5	4.750	
0.4	3	8.0	8	11	14.0	4.667	
0.6	2	13.0	12	14	22.5	11.250	6.186^{ns}
0.8	3	12.0	8	15	27.0	9.000	
1	3	13.0	12	19	37.5	12.500	

ns = not significant, P>0.05

Medium white follicle count

The effect ofvarying levels of Estradiol Benzoate on count of medium white follicles in Lohmann Brown hens is presented in Table 5. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 10.401) difference in median count of medium white follicles of 38.0, 48.5, 25.0, 36.0, 58.0 and 49.0 across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum medium white follicle count recorded was 31, 40, 10, 34, 47 and 33 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum count of medium white follicles of 50, 57, 25, 38, 68 and 50, respectively.

Ovarian stroma weight

The effect ofvarying levels of Estradiol Benzoate onovarian stroma weight in Lohmann Brown hens is presented in Table 6. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 5.839) difference in median ovarian stroma weight (5.0, 5.0, 6.0, 5.5, 7.0 and 5.0 g) across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum ovarian stroma weight were 5 g*vs*.5 g, 4 g*vs*.6 g, 4 g*vs*.7 g, 5 g*vs*.6 g, 6 g*vs*. 7 g and 4 g*vs*. 6 g across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 5: Summary Statistics (including KW Statistic) of Medium White Follicles across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	38.0	31	50	24.0	8.000	
0.2	2	48.5	40	57	23.0	11.500	
0.4	3	25.0	10	25	6.0	2.000	
0.6	2	36.0	34	38	13.5	6.750	10.401^{ns}
0.8	3	58.0	47	68	41.0	13.667	
1	3	49.0	33	50	28.5	9.500	

ns = not significant, P>0.05

Table 6: Summary Statistics (including KW Statistic) of Ovarian Stroma Weight across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	5.000	5.000	5.000	18	6.000	
0.2	2	5.000	4.000	6.000	13	6.500	
0.4	3	6.000	4.000	7.000	28	9.333	
0.6	2	5.500	5.000	6.000	17	8.500	5.839^{ns}
0.8	3	7.000	6.000	7.000	41	13.667	
1	3	5.000	4.000	6.000	19	6.333	

ns = not significant, P>0.05

4.1.2 Oviposition

Ovulation rate

The effect ofvarying levels of Estradiol Benzoate onovulation rate in Lohmann Brown hens is presented in Table 7. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 9.907) difference in median ovulation rate (66.7, 100.0, 100.0, 100.0, 66.7 and 100 %) across respective Estradiol Benzoate treatments (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum ovulation rates were 0.0 %*vs*.100.0 %, 33.3 %*vs*.100.0 %, 33.3 %*vs*.100.0 %, 0.0 %*vs*.100.0 %, 33.3 %*vs*. 100.0 % and 33.3 %*vs*. 100.0 % across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Laying pause days

The effect ofvarying levels of Estradiol Benzoate on laying pause days in Lohmann Brown hens is presented in Table 8. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 4.929) difference in median laying pause days (14, 5, 4, 8, 7 and 5 days)across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum laying pause days were 6 *vs*.15, 4 *vs*.6, 1 *vs*.11, 7 *vs*.9, 5 *vs*. 10 and 4 *vs*. 10 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 7: Summary Statistics (including KW Statistic) of Ovulation Rate across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	38	66.7	0.0	100	3458.5	91.013	
0.2	38	100.0	33.3	100	4272.5	112.430	
0.4	38	100.0	33.3	100	5006.0	131.740	
0.6	38	100.0	0.0	100	4252.5	111.910	9.907^{ns}
0.8	38	66.7	33.3	100	4430.0	116.580	
1	38	100.0	33.3	100	4686.5	123.330	

ns = not significant, P>0.05

Table 8: Summary Statistics (including KW Statistic) of Number of Laying Pause across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	14	6	15	38.5	12.833	
0.2	2	5	4	6	10.5	5.250	
0.4	3	4	1	11	18.0	6.000	
0.6	2	8	7	9	20.5	10.250	4.929^{ns}
0.8	3	7	5	10	27.5	9.167	
1	3	5	4	10	21.0	7.000	

ns = not significant, P>0.05

Intersequence pause days

The effect ofvarying levels of Estradiol Benzoate onintersequence pause days in Lohmann Brown hens is presented in Table 9. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 2.459) difference in median intersequence pause days of 4.0, 2.5, 2.0, 5.5, 2.0 and 3.0 days across respective Estradiol Benzoate treatments levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum number of intersequence pause days recorded was 2, 1, 0, 4, 1 and 0 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum intersequence pause days of 5, 4, 6, 7, 5 and 7, respectively.

Number of egg sequence

The effect ofvarying levels of Estradiol Benzoate on number of egg sequence in Lohmann Brown hens is presented in Table 10. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 2.868) difference in median number of egg sequence (5.0, 3.0, 3.0, 5.5, 3.0 and 4.0) across respective Estradiol Benzoate treatments levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum number of egg sequence were 4 *vs*.5, 2 *vs*.4, 0 *vs*.6, 4 *vs*.7, 2 *vs*. 6 and 1 *vs*. 6 across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 9: Summary Statistics (including KW Statistic) ofIntersequence Pause across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	4.0	2	5	28.5	9.500	
0.2	2	2.5	1	4	13.5	6.750	
0.4	3	2.0	0	6	21.5	7.167	
0.6	2	5.5	4	7	25.5	12.750	2.459^{ns}
0.8	3	2.0	1	5	22.0	7.333	
1	3	3.0	0	7	25.0	8.333	

ns = not significant, P>0.05

Table 10: Summary Statistics (including KW Statistic) of Number of Egg Sequence across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	5.0	4	5	31.50	10.500	
0.2	2	3.0	2	4	12.00	6.000	
0.4	3	3.0	0	6	20.50	6.833	
0.6	2	5.5	4	7	24.50	12.250	2.868^{ns}
0.8	3	3.0	2	6	23.00	7.667	
1	3	4.0	1	6	24.50	8.167	

ns = not significant, P>0.05

Egg sequence length

The effect ofvarying levels of Estradiol Benzoate onegg sequence length in Lohmann Brown hens is presented in Table 11. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 4.436) difference in median egg sequence length of 19.0, 31.0, 22.0, 24.5, 22.0 and 23.0 eggs across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum egg sequence length recorded was 16, 28, 0, 21, 16 and 21 eggs across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum egg sequence length of 30, 34, 23, 28, 28 and 27 eggs, respectively.

Number of eggs laid

The effect ofvarying levels of Estradiol Benzoate on number of eggs laid in Lohmann Brown hens is presented in Table 12. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 4.929) difference in median number of eggs laid(24, 33, 34, 30, 31 and 33 eggs) across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum number of eggs laid was 23, 32, 27, 29, 28 and 28 eggs across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum number of eggs laid of 32, 34, 37, 31, 33 and 34 eggs, respectively.

Table 11: Summary Statistics (including KW Statistic) of Egg Sequence Length across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	19.0	16	30	21.5	7.167	
0.2	2	31.0	28	34	29.0	14.500	
0.4	3	22.0	0	23	18.0	6.000	
0.6	2	24.5	21	28	18.5	9.250	4.436^{ns}
0.8	3	22.0	16	28	23.0	7.667	
1	3	23.0	21	27	26.0	8.667	

ns = not significant, P>0.05

Table 12: Summary Statistics (including KW Statistic) of Number of Eggs Laid across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	24.0	23	32	12.5	4.167	
0.2	2	33.0	32	34	23.5	11.750	
0.4	3	34.0	27	37	33.0	11.000	
0.6	2	30.5	29	31	13.5	6.750	4.929^{ns}
0.8	3	31.0	28	33	23.5	7.833	
1	3	33.0	28	34	30.0	10.000	

ns = not significant, P>0.05

4.1.3Egg Quality Parameters

Egg weight

The effect ofvarying levels of Estradiol Benzoate on egg weight in Lohmann Brown hens is presented in Table 13. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 5.184) difference in median egg weight of 54.150, 58.125, 48.890, 54.835, 58.080 and 58.020 g across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum egg weight recorded was 45.05, 57.27, 47.65, 54.06, 53.35 and 57.54 g across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum egg weights of 54.74, 58.98, 61.78, 55.61, 58.13 and 58.47 g, respectively.

Egg length

The effect ofvarying levels of Estradiol Benzoate on egg length in Lohmann Brown hens is presented in Table 14. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 5.804) difference in median egg length (51.80, 53.71, 52.03, 54.27, 53.92 and 54.58 mm) across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum egg length were 49.09 mm*vs*. 53.33 mm, 52.73 mm*vs*. 54.69 mm, 49.88 mm*vs*.55.04 mm, 53.77 mm*vs*.54.77 mm, 52.89 mm*vs*.77.86 mm and 53.77 mm*vs*.55.68 mm across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 13: Summary Statistics (including KW Statistic) of Egg Weight across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	54.150	45.05	54.74	14	4.667	
0.2	2	58.125	57.27	58.98	24	12.000	
0.4	3	48.890	47.65	61.78	21	7.000	
0.6	2	54.835	54.06	55.61	13	6.500	5.184^{ns}
0.8	3	58.080	53.35	58.13	29	9.667	
1	3	58.020	57.54	58.47	35	11.667	

ns = not significant, P>0.05

Table 14: Summary Statistics (including KW Statistic) of Egg Length across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	51.80	49.09	53.33	11.0	3.667	
0.2	2	53.71	52.73	54.69	17.0	8.500	
0.4	3	52.03	49.88	55.04	20.0	6.667	
0.6	2	54.27	53.77	54.77	21.5	10.750	5.804^{ns}
0.8	3	53.92	52.89	77.86	32.0	10.667	
1	3	54.58	53.77	55.68	34.5	11.500	

ns = not significant, P>0.05

Egg width

The effect ofvarying levels of Estradiol Benzoate on egg width in Lohmann Brown hens is presented in Table 15. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 5.507) difference in median egg width of 41.820, 43.565, 41.020, 42.340, 42.320 and 42.400 mm across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum egg width recorded was 40.58, 43.29, 39.62, 42.17, 42.28 and 41.32 mm across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum egg width of 42.49, 43.84, 43.59, 42.51, 43.14 and 43.06 mm, respectively.

Yolk volume

The effect ofvarying levels of Estradiol Benzoate on yolk volume in Lohmann Brown hens is presented in Table 16. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 7.853) difference in median yolk volume (13.380, 14.855, 13.810, 14.090, 13.500 and 15.000 ml) across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum yolk volume were 12.63ml*vs*.13.70ml, 14.56ml*vs*.15.15ml, 12.59ml*vs*.16.56ml, 13.76ml*vs*.14.42ml, 12.90ml*vs*. 14.12ml and 14.29ml*vs*. 15.18 ml across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 15: Summary Statistics (including KW Statistic) of Egg Width across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	41.820	40.58	42.49	17	5.667	
0.2	2	43.565	43.29	43.84	30	15.000	
0.4	3	41.020	39.62	43.59	19	6.333	5.507^{ns}
0.6	2	42.340	42.17	42.51	17	8.500	
0.8	3	42.320	42.28	43.14	28	9.333	
1	3	42.400	41.32	43.06	25	8.333	

n = not significant, P>0.05

Table 16: Summary Statistics (including KW Statistic) of Yolk Volume across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	13.380	12.63	13.70	12	4.000	
0.2	2	14.855	14.56	15.15	26	13.000	
0.4	3	13.810	12.59	16.56	25	8.333	7.853^{ns}
0.6	2	14.090	13.76	14.42	18	9.000	
0.8	3	13.500	12.90	14.12	17	5.667	
1	3	15.000	14.29	15.18	38	12.667	

n = not significant, P>0.05

Albumen volume

The effect ofvarying levels of Estradiol Benzoate on albumen volume in Lohmann Brown hens is presented in Table 17. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 5.625) difference in median albumen volume of 34.160, 37.960, 31.320, 34.855, 38.090 and 37.880 ml across respective Estradiol Benzoate treatments (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum albumen volume recorded was 31.25, 37.29, 29.24, 34.10, 35.48 and 37.18 ml across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatments, respectively. Estradiol Benzoate treatments (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum albumen volume of 35.65, 38.63, 42.52, 35.61, 38.68 and 37.94 ml.

Egg shell thickness

The effect ofvarying levels of Estradiol Benzoate onegg shell thickness in Lohmann Brown hens is presented in Table 18. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 1.364) difference in median egg shell thickness (0.330, 0.335, 0.350, 0.340, 0.340 and 0.330 mm) across respective Estradiol Benzoate treatments levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). Minimum *vs*. maximum egg shell thickness were 0.27mm*vs*.0.36 mm, 0.33mm*vs*.0.34 mm, 0.33mm*vs*.0.35 mm, 0.34mm*vs*.0.34 mm, 0.33mm*vs*. 0.34 mm and 0.32mm*vs*. 0.36 mm across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively.

Table 17: Summary Statistics (including KW Statistic) of Albumen Volume across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	34.160	31.25	35.65	15	5.000	
0.2	2	37.960	37.29	38.63	24	12.000	
0.4	3	31.320	29.24	42.52	20	6.667	5.625^{ns}
0.6	2	34.855	34.10	35.61	11	5.500	
0.8	3	38.090	35.48	38.68	34	11.333	
1	3	37.880	37.18	37.94	32	10.667	

n = not significant, P>0.05

Table 18: Summary Statistics (including KW Statistic) of Egg Shell Thickness across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	0.330	0.27	0.36	21.5	7.167	
0.2	2	0.335	0.33	0.34	15.0	7.500	
0.4	3	0.350	0.33	0.35	32.0	10.667	1.364^{ns}
0.6	2	0.340	0.34	0.34	20.0	10.000	
0.8	3	0.340	0.33	0.34	25.0	8.333	
1	3	0.330	0.32	0.36	22.5	7.500	

n = not significant, P>0.05

Egg shell weight

The effect ofvarying levels of Estradiol Benzoate on egg shell weight in Lohmann Brown hens is presented in Table 19. There was no statistically significant ($P>0.05$, Kruskal-Wallis statistic = 10.758) difference in median egg shell weight of 6.310, 7.270, 6.990, 8.535, 7.220 and 6.760 g across respective Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The minimum egg shell weight recorded was 5.20, 7.14, 5.82, 7.20, 7.18 and 6.48 g across 0, 0.2, 0.4, 0.6, 0.8 and 1 mg Estradiol Benzoate treatment levels, respectively. Estradiol Benzoate treatment levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg) gave respective maximum egg shell weight of 6.39, 7.40, 7.04, 9.87, 7.57 and 7.22 g, respectively.

Table 19: Summary Statistics (including KW Statistic) ofEgg Shell Weight across Estradiol Benzoate Treatments in Lohmann Brown Hens

Estradiol Benzoate (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	6.310	5.20	6.39	8.0	2.667	
0.2	2	7.270	7.14	7.40	23.0	11.500	
0.4	3	6.990	5.82	7.04	17.0	5.667	10.758^{ns}
0.6	2	8.535	7.20	9.87	27.0	13.500	
0.8	3	7.220	7.18	7.57	37.5	12.500	
1	3	6.760	6.48	7.22	23.5	7.833	

n = not significant, P>0.05

4.1.4Relationship among Egg Quality Parameters

The relationship among egg quality parameters in Lohmann Brown hens treated with estradiol benzoate (pooled data) is presented in Table 1. Strong, positive and highly significant ($P<0.01$) correlations were observed between egg weight and other egg quality parameters ($r = 0.790$-0.948). Egg width was highly and significantly ($P<0.01$) correlated with yolk ($r = 0.694$) and albumen ($r = 0.834$) volumes. The relationship between yolk and albumen volumes was also significantly ($P<0.01$) strong ($r = 0.797$). Egg length was significantly ($P<0.05$) correlated ($r = 0.542$) with shell weight.

4.1.5Prediction Equations and Bland-Altman Plot

Prediction equations for egg weight involving selected egg quality parameters are shown in Table 21. Egg weight was predictable from egg width and albumen volume singly with high reliability (R^2 = 70.3 and 89.9%, respectively; $P<0.001$). However, better and more reliable estimates (R^2= 90.0, 90.3, 90.7, 92.4 and 93.7%, respectively; $P<0.001$) were obtained when egg length *vs*. albumen volume, yolk volume *vs*. albumen volume, egg width *vs*. albumen volume, albumen volume *vs*. shell weight, and albumen volume *vs*. shell thickness were fitted into the model. Other combinations such as egg length *vs*. yolk volume, yolk volume *vs*. shell thickness, yolk volume *vs*. shell weight, egg width *vs*. shell weight, egg length *vs*.egg width, egg width *vs*. shell thickness, and egg width *vs*. yolk volume gave significantly ($P<0.001$) good reliability of 66.0, 67.9, 68.1, 70.5, 70.7, 78.2 and 78.7%, respectively. The use of allometry did not further improve the accuracy of predictions (R^2 = 30.1-89.1 %; $P<0.05$-$P<0.001$).

Table 20: Relationship among Egg Quality Parameters in Lohmann Brown Hens Treated with varying levels of Estradiol Benzoate

Pearson Correlation Coefficients among Egg Weight, Width, Yolk Volume and Albumen Volume			
	EW	YV	AV
EWT	0.839**	0.790**	0.948**
EW		0.694**	0.834**
YV			0.797**

Spearman Rank Correlation Coefficients among Egg Length, Shell Thickness and Shell Weight		
	STH	SWT
EL	0.097	0.542*
STH		0.305

**P<0.01; EWT = Egg Weight, EW = Egg Width, YV = Yolk Volume, AV = Albumen Volume
*P<0.05; EL = Egg Length, STH = Shell Thickness, SWT = Shell Weight

Table 21: Prediction Equations for Egg Weight using Egg Quality Parameters as Regressors

Function	R^2 (%)	Level of Significance
$Y = -86.626 + 3.357X_2$	70.3	***
$Y = 7.062 + 3.408X_3$	62.5	***
$Y = 9.040 + 1.282X_4$	89.9	***
$Y = 16.976 + 113.84X_5$	26.9	*
$Y = -86.612 + 0.042X_1 + 3.411X_2$	70.7	***
$Y = -1.049 + 0.134X_1 + 3.464X_3$	66.0	***
$Y = 10.181 + 0.027X_1 + 1.291X_4$	90.0	***
$Y = -63.907 + 2.241X_2 + 1.732X_3$	78.7	***
$Y = -10.997 + 0.624X_2 + 1.106X_4$	90.7	***
$Y = -93.403 + 3.006X_2 + 64.546X_5$	78.2	***
$Y = -84.865 + 3.280X_2 + 0.217X_6$	70.5	***
$Y = 6.934 + 0.411X_3 + 1.179X_4$	90.3	***
$Y = -5.689 + 2.991X_3 + 55.626X_5$	67.9	***
$Y = 2.088 + 3.211X_3 + 1.110X_6$	68.1	***
$Y = -2.599 + 1.182X_4 + 45.425X_5$	93.7	***
$Y = 5.703 + 1.228X_4 + 0.753X_6$	92.4	***
$Y = 0.003X_2^{2.657}$	70.4	***
$Y = 4.840X_3^{0.919}$	62.1	***
$Y = 2.641X_4^{0.848}$	89.1	***
$Y = 120.301X_5^{0.715}$	30.1	*

Y = Egg Weight; X_1 = Egg Length, X_2 = Egg Width, X_3 = Yolk Volume, X_4 = Albumen Volume, X_5 = Shell Thickness, X_6 = Shell Weight; *P<0.05, ***P<0.001

The Bland-Altman Plot of agreement between actual and predicted egg weights in Estradiol-treated Lohmann Brown hens is shown in Figure 20. The ranges of limits of agreement were small. The lower limit of agreement was plotted at -2.0790 g (95 % Confidence Interval = -3.0159 to -1.1421 g), whereas the upper limit was around 2.1612 g (95 % Confidence Interval = 1.2243 to 3.0981 g).

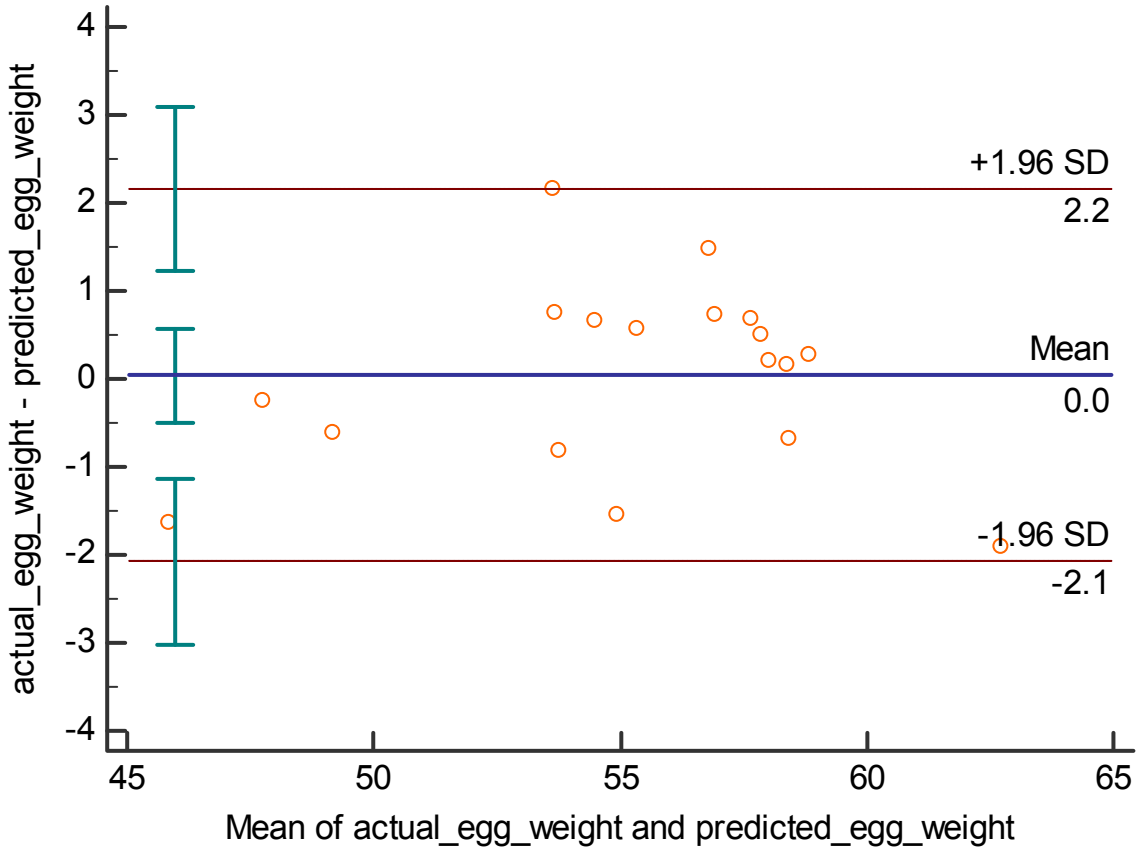

Figure 1.4 Bland-Altman plot of the agreement between Actual and Predicted Egg Weights in Estradiol-Treated Lohmann Brown Hens

4.2 DISCUSSION

Estradiol has been found to have dose-dependent effects on oviduct mass in non-breeders (Williams &Martyniuk, 1999). During breeding, negative feedback systems may operate to reduce dose-dependent effects, so that estradiol levels above some threshold stimulate complete growth of the oviduct (Christian & Williams, 1999). Such regulation may explain why significant changes in median ovarian parameters were not observed in the present study. Also, birds were treated with Estradiol Benzoate during the active laying phase when circulating levels of endogenous estradiol are naturally high (Dawson, 1983). This is capable of triggering a negative feedback response that could mask any dose-dependent effect of exogenous estradiol.

Large yellow follicles are recruited from the pool of small yellow and large white follicles in the ovary (Gilbert, Perry, Waddington &Hardie, 1983). It is, therefore, not surprising that non-significant changes in counts of small yellow and large white follicles across Estradiol Benzoate treatments as recorded in the current study gave rise to a non-significant change in the count of large yellow follicles. Increase in intersequence pause length of more than 3-4 days duration may be the consequence of reduced rate of follicular maturation and its subsequent recruitment into hierarchy following ovulation as the bird ages which is partly regulated by FSH (Etches & Cheng, 1981).

Miller and Wu (1981) used pituitary cell cultures to investigate factors, such as species, sex, and age, which might be associated with differences in FSH regulation by estrogens. They observed no effect of Estradiol-17β treatment on rabbit pituitary cell FSH secretion with a spontaneous secretion of FSH in rat pituitary cell cultures. This phenomenon could be explained by the possibility that different species and types within species respond differently to Estradiol-17β administration (Takahashi & Jensen, 1985). In addition, different estrogens at different doses administered by different routes do not have the same pharmacodynamic effects (de

Lignières&Silberstein, 2000). The non-significant changes in overall response parameters in the current study could be explained by the afore-mentioned postulations. For example, a commercial strain (Lohmann Brown) of chicken was used as the experimental subject and Estradiol Benzoate was administered as an absolute dose. Also, some parameters such as ovarian changes were determined at the conclusion of the experiment not during the experiment. However, parameters of oviposition and egg quality were recorded during the experiment. It is, therefore, possible that effects observed were not fully representative of the intervention given.

Prediction equations for egg weight involved the selection of egg quality parameters in which egg weight was predictable with high reliability. Best prediction equation explained 93.7% of the variation in egg weight. This provides an indication for better prediction of egg weight from egg quality parameters. Farooq et al. (2001) reported that egg weight was easily predictable from egg length and width as positive association among these traits existed. The use of allometry did not further improve the accuracy of egg weight predictions. Dzialowski and Sotherland (2004) reported that intraspecific variation in egg composition and its allometric relationship with egg weight as a result of differences in sizes of eggs has been well studied. The intraspecific variation in egg has been attributed to several factors such as heritability, laying sequence and some other factors (Alisauskas, 1986).

The agreement between the two methods of egg weight measurement; that is, the sensitive weighing scale and the prediction equation with the highest R^2, indicates that the two methods are mutually exclusive in terms of usage. The use of the prediction equation in the determination of egg weight is, therefore, reliable.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 SUMMARY

The experiment was carried out to determine the effect of exogenous estradiol administration on ovarian changes, ovipositionpattern, number of eggs laid and egg quality parameters in Lohmann Brown hens. During the six weeks of experimental period, the eggs produced by individual birds were recorded on daily basis and used for determination of egg quality parameters. Records of egg production were used to determine parameters of oviposition pattern and number of eggs laid. After slaughtering the birds at the end of the experiment, the ovaries were harvested and fixed in 10% neutral buffered formalin for three days. On removal from the fixative, follicles were classified into large yellow follicles (>10 mm in diameter), small yellow follicles (5-10 mm in diameter), large white follicles (3-5 mm in diameter) and medium white follicles (1-3 mm in diameter). The weights of the oviduct and stroma (the ovarian tissue remaining after removal of the follicles) were recorded.

Data was analyzed using GraphPadInStat statistical package and results showed non-significant difference in ovarian changes and oviposition pattern following treatment with varying levels of Estradiol Benzoate. Similarly, no significant effects of Estradiol Benzoate on number of egg laid and egg quality parameters were recorded.

Strong, positive and highly significant ($P<0.01$) correlations were observed between egg weight and other egg quality parameters ($r = 0.790$-0.948). Egg width was highly significantly correlated with yolk and albumen volumes. Relationship between yolk and albumen volumes was also significant. Egg length was also significantly correlated with egg weight. Egg weight was predictable from albumen volume and shell thickness with high reliability. Significant

positive correlations were observed between egg weight and other egg equality parameters, egg width and yolk volume, yolk and albumen volumes, and egg length and shell weight.

5.2 CONCLUSION

In conclusion, administration of Estradiol Benzoate had no effect on ovarian changes and oviposition pattern. Similarly, number of eggs laid and egg quality parameters were not affected by Estradiol Benzoate administration. Egg weight was highly predictable from albumen volume and shell thickness. There was relationship between actual egg weight and its predicted counterpart.

5.3 RECOMMENDATIONS

Based on the research conducted and the results obtained, the following recommendations were made:

1. Albumen volume and shell thickness should be employed in the prediction of egg weight.
2. The best egg weight prediction equation should be used for egg weight determination in the event where a weighing scale is not available.

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