EFFECT OF FREQUENCY OF EJACULATION ON SEMEN CHARACTERISTICS OF HEAVY ECOTYPE CHICKEN RAISED IN DERIVED SAVANNAH REGION OF NIGERIA

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CERTIFICATION

This is to certify that this work was carried out by Linus, Lawrencia Chineme (PG/M.Sc./14/67331) and approved by the Department of Animal Science, University of Nigeria, Nsukka.

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EXTERNAL EXAMINER	_____ Signature	_____ Date

DEDICATION

This Work is dedicated to God the Father, Son and Holy Spirit and to my late Parents Chief and Lolo Linus Nnopu

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I acknowledge our Lord Jesus Christ for the grace bestowed on me to be able to accomplish this work.

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ABSTRACT

Effect of frequency of ejaculation on semen characteristics of heavy ecotype chicken raised in the derived savannah region of Nigeria was studied using twelve heavy ecotype cocks. The cocks were randomly assigned to three treatments with four cocks in each treatment. Ejaculation frequencies once, twice and thrice per week, with T_1 representing once, T_2, twice and T_3, thrice were imposed on the birds. The experiment lasted for a period of eight weeks with a two-week pre-experimental period during which cocks were trained for semen collection and six weeks of semen collection schedule. Feed and water were supplied ad libitum during the experiment. Semen collected were analysed for volume, progressive sperm motility, percentage live and dead spermatozoa, percentage normal and abnormal sperm cells, sperm concentration and total sperm in the ejaculate. Result showed that ejaculate volume and total sperm in the ejaculate were not significantly ($p>0.05$) affected by the ejaculation frequencies while progressive sperm motility, percentage live and dead sperm cells, percentage normal and abnormal sperm cells and sperm concentration were all significantly ($p<0.01$) affected by the ejaculation frequencies. Cocks ejaculated twice weekly recorded the highest value sperm in motility 77.70 ± 0.99% , live 76.04 ± 0.90% and normal spermatozoa, 77.29 ± 0.85% while cocks ejaculated thrice weekly recorded the lowest values of 65.96 ± 1.87%, 66.38 ± 1.90%, 68.40 ± 1.92% for motility, live and normal spermatozoa respectively. Also the cocks ejaculated thrice recorded the highest value for dead (33.44 ± 1.90%) and abnormal spermatozoa (31.59 ± 1.92%) while cocks ejaculated twice recorded the lowest values of 23.95 ± 0.90%, 22.70 ± 0.85% for dead and abnormal spermatozoa, respectively. It was concluded that twice weekly ejaculation gave better semen quality. Therefore, it is recommended that twice per week ejaculating frequency should be used for cocks for optimum semen quality and for high semen output.

CHAPTER ONE
INTRODUCTION

1.0 Background of the study

Rapid human population growth and low protein intake are some of the major problems facing developing countries like Nigeria. Poultry production is one of the surest and fastest means of bridging the animal protein supply needs of any nation, especially for countries like Nigeria where it has been reported that an average individual consumes about 7.5g of animal protein as against 28g consumed by an average Briton (Mmereole *et al*., 2001).

The local chickens constitute the majority of poultry types in Nigeria, being about 106 million in population (Ajayi, 2010) with more than 80% in the rural areas where they contribute substantially to annual meat and egg production. There are various ecotypes of local chicken in the different agro ecological zones of the country and they vary in shape, size and plumage colours (Oluyemi *et al*., 1982). The different ecotypes can be grouped into two major categories on the basis of body size and body weight as ólightô ecotype and óheavyô ecotype (Momoh and Nwosu, 2008). Light ecotype refers to those types belonging to the swamp, rainforest and derived savannah agro-ecological zones whose mature body weights range between 0.68-1.5kg. The heavy ecotype represents the types from the dry savannah (Guinea and Sahel savannah), montane regions and cattle kraals of the north, whose mature body weights range from 0.9-2.5kg (Atteh, 1990).

The importance of semen evaluation in poultry breeding (natural and artificial breeding), for selecting breeding males or for routine monitoring of their reproductive performance cannot be over emphasized. Semen of breeding cocks is usually evaluated to ascertain the quality before it is employed for breeding programmes.

The assessment of semen quality characteristics of Nigeria local chicken gives excellent indices of its reproductive potential (Zahradden *et al*., 2005) and has been reported to be a

major determinant of fertility and subsequent hatchability of eggs (Peters *et al*., 2004). For efficient breeding programme, artificial insemination (AI) is preferred to natural mating. One of the advantages of AI over natural mating is the efficient use of male (Koophar *et al*., 2010). Successful application of artificial insemination to commercial hatchery operations depends on the quantity and quality of sperm obtained from breeder males (Zhaug, 2006). Higher semen output in terms of semen volume and sperm concentration determines the number of females that can be inseminated with it. While increased semen output can be achieved from breeder male through more frequent ejaculations (Fan *et al.,* 2004; Klimowicz *et al*., 2005, Zhaug 2006), evidence exists that some semen quality parameters decrease progressively with increase in the frequency of ejaculation (Nwachukwu *et al*., 2006; Onukwufor and Ezekwe 2006). Most of the research findings on the local chickens of Nigeria (Nwosu, 1979) were obtained from research work on the chicken types described as light ecotype found in the southern part of the country. The chicken type from savannah, montane regions and cattle kraals of the north described as heavy ecotype have not been so much evaluated. Therefore, this study was aimed at evaluating the effect of frequency of ejaculation on semen characteristics of heavy ecotype chicken raised in Derived Savannah region of Nigeria.

1.1 Objective

The broad objective of this study was to evaluate the effect of frequency of ejaculation on semen characteristics of heavy ecotype raised in Nsukka, Enugu state.

Specific objectives were to evaluate the effect of ejaculation frequencies on:

i. Semen Colour

ii. Semen Volume

iii. Semen Motility

iv. Live and dead spermatozoa

v. Normal and abnormal spermatozoa and

vi. Sperm concentration

1.3 Justification

High semen output is achieved through frequent ejaculation and the volume and concentration determine the number of females to be inseminated. Semen needs to be evaluated so as to ascertain its quality for use in artificial insemination (AI). Artificial insemination is one of the most effective and widely used techniques for the genetic improvement of farm animals. It is employed on breeder farms to maintain the maximum use of males, as well as to ensure disease prevention, high fertility rates and for economic reason. To obtain the maximum number of spermatozoa and maintain high rates of sperm production, the semen needs to be collected at a frequency that maintains output over time. Therefore, the result of this study will help in determining the frequency that gives better semen quality for use in the genetic improvement programme of local chicken ecotypes in Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2.0 Evolution of Domestic Fowl

There is conflicting information about the actual centre and time of domestication as well as ancestors of domestic chicken. Horst (1989) reported that all domestic fowls originated from small jungle fowl (*Gallus gallus*) of South-East-Asia. Crawford (1990) reported that chickens were domesticated from the red jungle fowl in the Indus valley about 2000 B.C. These authors concluded that the red jungle fowl was a convincing ancestral wild form of the domestic fowl, commonly called chicken, unlike the other two species *Gallus sonnerati* and *Gallus laafayetti* that other authors previously reported on. Evidences for these observations were derived from archeological, vegetation, climatic and geographical information. Through DNA fingerprinting, Siegal *et al*., (1992) further verified the red jungle fowl as an ancestral form of the domestic chicken.

2.1 Anatomy of Cocks

The male birdôs reproductive tract consists of the paired testes, epididymides, ductus deferens (sperm duct) and the cloaca. The primary sex organs of the cock are the testes, with their main function being the production of sperm and male sex hormone, testosterone. Both testes are functional in the male when puberty is attained. The size of the two testes may differ, the left testis usually being 0.5-3g heavier than the right testis (Etches, 1996). The gross weight of the paired testes is on average 25g, and the sperm produced per gram of testicular parenchyma is approximately 100×10^6 with a daily sperm production of 2.5×10^9 sperm/ml. An accurate method of determining the quantity of sperm a bird could produce is generally by measuring the circumference of the testes, meaning the larger the size of testes, the greater the sperm production (Senger, 2003). The testes in the cock are located in the centre of the body cavity and spermatogenesis occurs at body temperature (41^oC) as opposed to the mammalian

scrotal temperature of 24 to 26^{o}C (Tuncer *et al*., 2006). Accessory sex glands (prostate gland, seminal vesicle, bulbourethral gland) which are present in most mammals, are absent in birds (Hafez and Hafez, 2000).

In birds with distinct breeding cycles, the testes atrophy after a period of active sexual stimulation. However, the testes never become as small as they were in the prenuptial stage. Microscopically, the testis consists almost entirely of tubular structures known as seminiferous tubules (Bearden *et al*., 2004). Two types of cells line these tubules, spermatogonia cells and Sertoli cells. The spermatogonia cells proliferate and differentiate through definite stages of development to form sperm (Bearden *et al*., 2004). Spermatogonia initially multiply and grow to form considerably enlarged cells called primary spermatocytes. These cells then enter a period of maturation in which the first maturation division forms secondary spermatocytes and the second maturation division forms the spermatids. Each spermatid develops into a spermatozoon (Bearden *et al*., 2004). Spermatids are produced by meiotic division, that is, without replication of chromosomes, merely a division of those already present. Therefore, each spermatid has half of the normal complement of chromosomes, none of them paired (Bearden *et al*., 2004). Sertoli cells are large cells interspaced between spermatogonia which extend from the base of the seminiferous epithelium to the interior of the tubules. Spermatids attach themselves to the Sertoli cells and some specific relationship seems to exist between the two cell types which cause the spermatids to change into active sperm (Bearden *et al*., 2004). Seminiferous tubules of immature males are small and lined by a single layer of cells.

The mature testis has large irregular-shaped tubules with a multi-layered germinal epithelium consisting of cells representing all stages of spermatogenesis. This is what causes the testis to swell in size during the breeding season. Testosterone is produced by cells known as interstitial cells of Leydig. These cells are located in the spaces between seminiferous tubules.

Embedded within connective tissue at the attachment of the testis is the epididymis. It consists of a chord-like system of ductules. It is very short by comparison with that present in mammals. The networks of seminiferous tubules (from the testis) unite in the epididymis and the contents flow into and through the ductules, ultimately emptying into the ductus deferens. In mammals, sperm is stored in the epididymis. Sperm also undergoes a maturation process in these ductules in which they develop mobility (Bearden *et al*., 2004). Leading from the epididymis is a very extensive, convoluted tube which runs posteriorly and ends in the cloaca by an erectile papilla (ejaculatory duct) which projects into the latero-ventral urodeum. The urodeum is the mid-region of the cloaca. In birds, the ductus deferens is the major storage organ for sperm. Sperm undergo maturation in the ductus deferens of birds, not in the epididymis (Beraden *et al*., 2004). Sperm taken directly from the epididymis lacks fertilizing capacity, whereas those taken from the ductus deferens can fertilize. About one-half to two-thirds of the contents of both ducti are expelled during ejaculation. In sexually active birds, it takes one to four days for sperm to be formed and reach the ductus. Finally, the copulatory apparatus consists of two papillae and the rudimentary copulatory organ. The papillae are equipped with luminar through which semen are discharged and are located in the ventral floor of the cloaca.

2.2 Physiology of sperm production

Production of sperm is initiated by adequate secretion of gonadotropin releasing hormone from the hypothalamus, the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior lobe of the pituitary and the secretion of the gonadal steroids (testosterone and estrogen). LH acts on the Leydig cells within the testes to stimulate the production of the male sex hormone testosterone. Testosterone within the semniferous tubules is essential for spermatogenesis while the Leydig cells sustain high levels of LH (Senger, 2003). Testosterone is responsible for a variety of secondary sex characteristics such as male

sexual behavior (including song), feather form and colour (different from that of the female), and the development of a comb and wattles in some species. Testosterone may also help maintain spermatogenesis once it has been established under the influence of the gonadotropic hormone from the anterior pituitary gland. This hormone aids in spermatocytogenesis, the transport and deposition of sperm in the female reproductive tract (Bearden *et al*., 2004). As the cock reaches maturity, the production of testosterone is stimulated by the increasing concentration of circulating gonadotropins (Etches, 1996), The major gonadotropins involved are FSH and LH (also called interstitial cell stimulating hormone in the male). FSH acts on the germ cells in the semniferous tubules to stimulate spermatogenesis up to the secondary spermatocyte. FSH also stimulates the Sertoli cells to secrete androgen binding protein (Hafez and Hafez, 2000).

2.3 Functions of the accessory sex glands in the cockerel

The prostate, vesicular and bulbo-urethral glands are the accessory glands present in most domestic animals, with their main function being the production of secretions that aid in the sperm transport. For example, fructose and citric acid are components of the seminal vesicle secretions in domestic animals, with citric acid being found only in the stallionôs seminal vesicles. The prostate is the only accessory gland common to all mammals, while the epididymis and the vas deferens are the only accessory organs present in poultry (Hafez & Hafez, 2000). Cockerels have no secondary sex glands. Therefore, the seminal fluid is derived entirely from the testes and the underlying ducts. Additionally, when semen is collected from the cockerel by abdominal massage, there are lymphatic exudates from the phallic folds which contribute to the ejaculate.

2.4 Cockerel semen composition

In the male, semen is composed of sperm and seminal plasma secreted by the reproductive ducts, the epididymis and vas deferens (Fujihara, 1992) and in the case of the avian species,

the seminal fluid is also produced in the testes. Avian seminal fluids are often similarly rich in various peptides, proteins and other substances (Al-Aghabari *et al*., 1992; Cerolini *et al*., 1997). All these secretions in the testes are controlled by the endocrine hormones carried to them through the blood stream. The pituitary FSH and LH regulates the testes which in turn produce testosterone, that controls the testicular development and secretions (Hafez, 1974).

Table 1: Chemical components of semen in the domestic cockerel.

Components	**Composition**
Protein (g/100 ml)	1.8-2.8
pH	7.2-7.6
Fructose (mg/100ml)	4
Sorbitol (mg/100ml)	0-10
Inositol (mg/100ml)	16-20
Glycerylphosphoryl choline (GPC) (mg/100ml)	0-40
Ergothioneine (mg/100ml)	0-2
Sodium (mg/100ml)	352
Potassium (mg/100ml)	61
Calcium (mg/100ml)	10
Magnesium (mg/100ml)	14
Chloride (mg/100ml)	147

Source: Hafez and Hafez, 2000

2.5 Semen quality

Saacke (1983) stated that semen quality traits are classified according to the sperm viability or morphology. The morphology of sperm is also considered to reflect the physiological status of the male for sperm production and reflects the viability of storage in the gonadal ducts. Viability, as other semen traits, can reflect the fertility status, but it also measures the humanôs interaction with semen as it is collected, processed and inseminated. Semen traits related to fertility (sperm motility, velocity and acrosome morphology) may affect the penetration of the cervical mucus and are thus important for semen preservation and ultimately fertilizing capacity. Parker and McDaniel (2002) reported that in poultry there is no

real breeding soundness evaluation. Roosters are selected based on physical characteristics that are associated with the mature males for example comb, wattle size and colour, body size and shank length. However, it is very important to evaluate the semen quality, as it predicts the fertility of an individual male. Fertility and hatchability according to Tabatabaei *et al*. (2009) depends on genetic, physiological, social and environmental factors. These are interrelated heritable traits, which may vary among breeds. Sperm numbers, type of cocks and age may affect the *in vivo* storage of sperm, and subsequently the fertility of the eggs. The conditions which cause low sperm numbers or single sperm activity at the site of fertilization can cause a reduction in the actual number of chicks being hatched. Low fertility and early embryonic mortality may be the result of low sperm activity (Bearden *et al*., 2004). This condition is normally associated with older breeder hens or any flock experiencing infrequent mating activity (Bramwell, 2002).

2.6 Factors affecting semen production

According to Anderson (2001), there are many factors that may influence the production of semen and a thorough knowledge of the physiology of cockerel reproduction is essential to enable an understanding of male fertility. There are also many external and internal factors that may affect the male, and may influence the production of semen. The reproductive functions in the male are endocrine, controlled by the pituitary, testes and to a certain extent external factors. These factors are listed below.

2.6.1 Season

Seasonal factors exert significant influence on domestic birds reared in the tropics. Direct climatic factors acting on the birds include high ambient temperature and relative humidity, resulting in severe heat stress and indirect on the diet and management. In the northern guinea savannah zone of Nigeria, three seasons-the cold-dry (harmattan), hot-dry and rainy seasons are recognized (Igono *et al*., 1982). Each season in the zone has its positive or negative

effects on livestock production. For example, the harmattan season has been described as thermally stressful to goats (Ayo *et al.,* 1999), pigs (Adenkola *et al*., 2009), and poultry (Ayo *et al*., 2007), while the negative impact of environmental stress on poultry is minimum during the rainy season (Obidi *et al*., 2008). Machebe and Ezekwe (2005) showed seasonal variations in the ejaculate quality of Nigerian local cocks in the warm-wet, rainforest zone of Nigeria.

2.6.2 Ambient Temperature

It has been established that the high ambient temperature in tropical environment and relative humidity have adverse effects on livestock and poultry species (McDonald *et al.,* 1995; McDaniel *et al.,* 1996; Bah *et al.,* 2001; Ayo *et al.,* 2007; Ayo and Sinkalu, 2007).

Direct climatic factors acting on the birds include high ambient temperature and relative humidity, resulting in severe heat stress. Heat stress can be one of the main limitations in poultry production and reproduction, more especially in hot areas. Elevated environmental temperatures pose a threat to the general wellbeing of the cockerels. Such detrimental effects limit reproduction characteristics of the males thus inhibiting spermatogenesis and a decrease in the secretion of gonadotrophins (Bah *et al*., 2001; Ayo and Sinkalu, 2007; Obidi *et al*., 2008; Oguntunji *et al.,* 2008). The report of Obidi *et al.* (2008) also revealed that a significant proportion of ejaculated spermatozoa were morphologically deformed during the hot-dry season. Such sperm abnormalities include microcephalic, bent head, broken mid-piece, and cytoplasmic droplets. Sperm abnormalities have been reported to be important contributory factors to heat-stress-induced infertility in avian species (Penfold *et al*., 2000). The optimum ambient temperature range for poultry is 12ï 26°C (Plyaschenko and Sidorov, 1987). Body temperature increase sperm metabolism, sperm motility and sperm quality are generally lower in heat-stressed cockerels (McDaniel *et al*., 1999). Although research concerning hyperthermia on semen characteristics is lacking, several researchers have found that sperm can function at normal body temperature (Karaca *et al.,* 2002). Froman and Feltmann (2005)

found sperm to be motile at a body temperature of 41°C, and decline with time after ejaculation. Heat stress may be evaluated by measuring the rectal temperature which is the true reflection of the internal body temperature (Ayo and Sinkalu, 2007).

2.6.3 Photoperiod or daylight length

Most domestic birds in the temperate climate are seasonal breeders and in most birds, photoperiod stimulates spermatogenesis, especially semen production in the White Leghorn (Mosenene, 2009) for example. The duration and intensity of photoperiod may have an effect on the conditioning of the chickens for reproduction (Anderson, 2001). Short days do not stimulate gonadotrophin secretion, as they do not illuminate the photosensitive phase. However, long days illuminate the photosensitive phase and therefore the gonadotrophin LH is secreted. Photo-schedules in poultry production are currently being practiced, and designed to maximize the yield of semen for a prolonged period, by delaying the onset of photo refractoriness which is a machanism in seasonal birds that limits reproductive activity to times in the year, that optimise successful rearing of offspring. In addition, the generation interval can be reduced when photo stimulation is practiced at an earlier age (Etches, 1996). Time of the day for the collection of semen also affects the quality and quantity of cockerel semen (Machebe and Ezekwe, 2005) because semen production has been noted to be higher when collected in the morning and evening when the environment is cooler (Peters *et al*., 2008).

2.6.4 Nutrition

Feed restriction causes stress in cockerels, while low water intake induces the males to lose body weight. This disruption can lead to a permanent nonfunctional testis and a reduced reproductive performance in the mature cockerel. The nutrient requirements of males have generally received less attention, and it is a common practice that cockerels are given the same diets that have been formulated for the hens. This affects the males in that they often

suffer from chronic gout due to high amounts of calcium and protein intake that exceeds their metabolic requirements. The diet also affects the production of semen, in that the production of semen is decreased, although the semen characteristics are not affected. Males consuming less quantities of protein in the diet ejaculate more frequently, however, their lifetime sperm output far exceeds that of males consuming higher amounts of protein (Etches, 1996). According to Ezekwe *et al*. (2003), the physical quality traits began to fall as the level of underfeeding increased but this trend was not observed in the biochemical constituents. This implies that the spermatogenic functions of the testis can be said to be more responsive to underfeeding than the secretory activity of the reproductive tract. This supports the findings of Davies *et al*. (1957) that underfeeding diminishes the ability of the gonads to respond to gonadotropin stimulation.

2.6.5. Disease

The importance of good health can not be over emphasized. Disease affects animalôs fertility in so many ways. Pathogenic infections from bacteria and fungi should be viewed with seriousness since it could lead to functional and anatomical changes which might result in varying degrees of impotency (e.g. poor semen quality, infertility, sterility etc) (Corbel *et al.,* 1978). Chronic disease such as aspergillosis or avian tuberculosis may cause reduced fertility before clinical signs become noticeable (Dzoma, 2010). Internal parasites may result in decreased fertility directly or via a decrease in the viability of essential nutrients to the animal (Shanawanyi, 1999). External parasites may cause irritation, general disturbance and sometimes blood loss

2.6.6 Body weight of the cockerel

The body weight of the cockerel is important when selecting for breeding flock performance. A significant negative correlation has been established between the growth rate or body weight and the reproductive performance in males (Lukaszewicz and Kruszynski, 2003). It is

crucial for the males to reach a minimum body weight typical for a given breed, strain or type, before being used for breeding (Lisowski and Bednarczyk, 2005). The number of sperm per ejaculate and body weight has been positively correlated and it may be concluded that body weight and length of the shank, comb and wattle are good predictors of semen attributes in cockerels. This is contrary to the reported negative effect of bodyweight on semen production (Galal, 2007).

2.7 Semen Collection

The goal of the semen collector is to obtain the maximum volume of clean, high quality semen from a good donor bird with minimal amount of handling. Development of a simple and effective method of semen collection as well as insemination is essentially helpful in both the areas of research and production (Chelmonska *et al*., 2008). Attainment of this requires that good quality semen devoid of environmental contamination should be collected from a good donor. To obtain maximum amount of semen from more birds, the animals should be housed individually (in a space approximately 2 m^2). To ensure that clean semen is obtained, feed should be removed at least 12 hours before collection; and birds should be trained once or twice a few days before the onset of semen collection. To ascertain the volume and colour from a bird, milking can be done three or four times (Dhama *et al*., 2014). Unlike the domestic mammals, the avian species cannot be easily trained to mount a dummy and ejaculate due to their tricky nature and their disposition to usually fly (as in the wild birds). Methods for collection of semen as outlined by Dhama *et al*. (2014) are as follows:

2.7.1 Co-operative Approach

Obtaining semen from birds was originally developed by Falcon and it involves the use of male imprinted onto their handler (Samour, 2004). This requires cooperation from the donor birds (usually hand-reared without the presence of their conspecifics) which can be achieved by an external stimulus which are usually behavioral example include voice, nest, and food

(Hamerstrom, 1970). The handler constantly interacts with the bird through the simulation of their different calls, food presentation, the mimicking of their displays. This interaction is intensified at the onset of their breeding season and directed toward soliciting copulation. This involves simulating their courtship ritual consisting of a complex series of displays and vocalizations. On the attainment of the necessary level of conditioning, the bird is enticed to copulate on a special hat won by the handler while the semen is collected through a rubber ring fitted around the hat (Boyd and Schwartz, 1983). The lead point of this technique is that there is no stress and it is injury free. Also no handling of the birds is involved. The major advantage of this technique is that the quality of semen is good without contaminants like faeces and urine. However, the quantity of semen obtained will be less which is the drawback of this technique. The co-operative approach technique showed promising results with artificial vagina in case of Muscovy ducks (Gvaryahu *et al*., 1994), Emu (Malecki *et al*., 1997), quail (Chelmonska *et al*., 2008) and Ostrich.

2.7.2 Abdominal massage technique

This is the most widely accepted and used technique for semen collection (Dhama *et al*., 2014), and is a non-invasive method originally developed and used by Burrows and Quinn in 1935 for collection of semen from domestic chickens (Donoghue and Wishart, 2000). Using this technique, slight adjustments have been made for application in other breeding programs (Dhama *et al.,* 2014). Semen collection in birds of prey has been demonstrated by (Weaver, 1983). The animal is wrapped with a soft towel and placed on its chest on a padded table or is held upside down while an operator holds the legs firmly. Series of strokes at the lower back and abdomen are done for approximately 1 or 2 minutes. Subsequent strokes are directed laterally to the cloaca until the papillae of the ductus-deferens is exposed by the handler. At this point, milking stoke are applied to the bird until semen appears at the tip of the papillae. In chickens and turkeys, the abdominal massage technique involves massaging the cloacal

region to achieve phallic tumescence (Bakst and Long, 2010). Properly restrained donors are stroked gently in the back region behind the wings which stimulates most males with phallic engorgement at which state the cloaca can be squeezed to collect the semen (Cooper, 1977). This technique is referred to as milking the animal. Little additional semen can be expressed after two cloacal strokes; additional cloacal strokes may cause damage to the phallic and cloacal regions and contribute to semen contamination (Malecki *et al.,* 2008). Attempts have been made in collecting and investigating the physical as well as the biochemical characteristics of the semen of broiler chicken (Dhama *et al.,* 2014).

2.7.3 Electro-Ejaculation

Electro-ejaculation was originally developed for semen collection in ducks, geese, psittacines and pigeons (Dhama *et al*., 2014), to increase efficient use of outstanding males in commercial waterfowl production programs, by using artificial insemination techniques (Jaime and Samour, 2004). The most commonly used technique involves the use of 2 poles, one attached to a hypodermic needle inserted on the synsacral region and the second inserted into the cloaca (Samour *et al.,* 1985). Thirty volts are applied at a current of 0.06-0.08 A for 3 seconds repeated 3-5 times at intervals of 5 seconds (Watanabe, 1957). In this study, semen collected through electro-ejaculation was better than that from massage. The use of electro-ejaculation was first reported in nondomestic species using psittacine species (Harrison *et al*., 1983). Electro-ejaculation also has been carried out in ducks and geese by using a single probe inserted into the cloaca (Samour *et al*., 1985). In this study, males were lightly anesthetized with a combination of ketamine at 25 mg/kg and xylazine hydrochloride at 5 mg/kg administered intramuscularly. Birds were wrapped in a soft towel and a single probe fitted with 2 bronze rings (to act as electrodes) was inserted into the cloaca. The probe was connected to a potentiometer and a voltmeter with a range of 0-30 V. Stimulation starts with pulses of 5 V, each lasting 3 seconds at 5-second intervals. Pulses were given 5-9 times, after

which the voltage was increased in 5-V stages up to 30 V (Samour *et al.*, 1985). The use of this technique in non domestic avian species has been very limited. The electro-ejaculation equipment used must be properly designed and adequate protocols must be instituted before attempting this procedure. Animal welfare issues should also be taken into consideration, including the use of general anesthesia or adequate restraint and handling carried out by experienced handlers (Jaime and Samour, 2004). The advantage of electro ejaculation over cooperative and massage method of semen collection is that, it does not require training while cooperative and massage require training to obtain good quality semen which takes time. Duck semen collected by electro ejaculation contained a greater number of spermatozoa and was released in larger quantities than that collected by conventional massage technique.

2.8 General Evaluation

The evaluation of poultry semen is important for AI as it does not only involve the collection of semen, but also the quality and many other aspects are to be considered prior to use, for example, level of contamination of the ejaculate and the viability (motility) of the sperm. There are many parameters that can be used to evaluate the overall quality of cockerel semen and consequently estimate the extent to which semen can be extended. These include ejaculate volume, semen concentration, and total number of sperm, sperm motility and morphology.

2.8.1 Semen Colour

The colour of semen is generally an indication of the density of the ejaculate. The semen of the domestic fowl varies from a dense opaque suspension to a watery fluid secreted by various reproductive glands, from a relatively high sperm density or degrees of clear to milky white, with declining sperm numbers (Peters *et al*., 2008). Moreover, colour of semen sample is also evaluated to determine the presence of any lession or infection in the genital tract. The semen collector can affect semen quality by contamination of semen with faeces, urine and

blood which can be detected by evaluation of colour of semen (Alkan *et al*., 2001). Sometimes flakes of blood may be present, which may be a result of excessive force being used during the collection process or injury. Semen samples that are contaminated by faeces do not have to be discarded, but diluted with antibiotics e.g. penicillin and dihydrostreptomycin or neomycin to reduce the loss of sperm. This however is not recommended. Antibiotics can also increase fertility when used as a diluent in semen (Bearden *et al*., 2004).

2.8.2 Ejaculate Volume

The cockerel produces between 0.1 ml and 1.5 ml of semen per ejaculation, with 0.6 ml being the average ejaculate volume recorded. Different cockerels of the same species often produce different volumes of semen at different times (Anderson, 2001). The average volume ejaculated using the abdominal massage technique is approximately 0.25ml and contains on average 5000 $x10^6$sperm/ml (Gordon, 2005). Onukwufor and Ezekwe (2007) reported mean values of 0.378± 0.009, 0.353±0.007 and 0.339±0.004 ml at twice weekly, four consecutive times weekly and two times daily twice per week, respectively for three phenotypes of local chickens. Nwachukwu *et al*. (2006) got a mean of 0.11±0.009 and 0.12±0.009 ml for three genotype of local chicken at once and twice frequency per week. Similarly, Nwoga *et al*. (2013) working with local turkey, reported 0.37±0.01, 0.28±0.02, 0.30±0.02 and 0.34±0.01 ml for once biweekly, once, twice and thrice weekly collections, respectively. Zahraddeen *et al*. (2005) also reported 0.11±0.34, 0.12±0.02, 0.22±0.03 ml for local turkeys at once twice and thrice frequencies per week, respectively. The reasons for the variations in values were attributed to frequencies of collections and different genetic background of the birds.

2.8.2.0 Factors That Affect Cockerel Ejaculate Volume

The quantity of semen collected by the massage procedure is dependent upon the male species, breed, season, temperature, age, nutrition, frequency and technique of semen collection.

2.8.2.1 Species and Breed

Reports have shown that semen quality trait varies among genotype like breed (Machebe and Ezekwe, 2000, Peters *et al*., 2008), strains (Murugesan *et al*., 2013) and line (Tarif *et al*., 2013). The active reproductive period influences the volume of semen produced. It has been reported that ejaculate volume and sperm concentration are dependent on the strain and breed of the cockerel e.g. the Naked Neck and Frizzle genotypes produce higher ejaculates, compared to the normal feathered breeds of cockerels (Machebe and Ezekwe, 2005). Researchers have recommended the use of comb length and wattle length as good indicators for the selection of quantitative traits in cockerels. Larger combs may reliably indicate cockerels with greater semen production, higher androgens levels or increased mating activity (Kotlowska, 2005; Zahraddeen *et al.,* 2005; Nwachukwu *et al.,* 2006; Galal *et al.,* 2007).

2.8.2.2 Nutrition

According to Kabir *et al*. (2007) nutrition affects semen quality. Ejaculate volume, sperm density, and fertilizing capacity of cockerel semen can be reduced by restricted feed intake. However, a reduced protein level of as low as 6.9% has no adverse effect on fertility in males (Hocking and Bernard, 1997). The body weights of breeder cockerels have to be well managed in the attainment of sexual maturity of the cockerels to coincide with that of the hens. Too heavy males tend to be over-fleshed and generally have reduced persistency in semen production. Overfed males also show reduced fertility, compared to cockerels fed to

meet a target body weight. However, underfed cockerels recorded a reduced semen volume and low fertility (Das, 2002; Renema *et al*., 2007).

2.8.2.3 Age, Frequency and Technique of Semen Collection

In poultry species, semen quality is affected by age (Longe *et al*., 2010 , Kotloska *et al*., 2005, Kelso *et al*., 1996), time of collection (Egbunike and Nkanga, 1999, Machebe and Ezekwe, 2002), technique of collection frequency of collection (Riaz, *et al.*, 2004). For example, semen volume, semen concentration and sperm motility changes with the age of the male, leading to a progressive decline in fertility. Semen concentration appears to be the seminal trait that is commonly affected by frequency of ejaculation because semen concentration declines progressively with an increase in the ejaculate frequency (Santayana, 1985). A report has shown the frequency of collection of semen and age of the cockerels and the biochemical parameters of the semen to be more affected by age than the ejaculate volume. The changes in semen quantity and quality may be related to an increasing age of the cockerel. It has also been reported that in boars, too frequent collection of semen cause temporary loss of fertility (Hafez and Hafez, 2000, Kotlowska *et al*., 2005; Tuncer *et al.,* 2006).

2.8.2.4. Season and temperature

Hot-dry season, characterized by elevated ambient temperature and high relative humidity causes a temporary decrease in sperm production and fertility (Nayak and Misra .1991). Semen volume was reported by Machebe and Ezekwe (2005) to be lowest during the hot-dry season relative to the rainy and harmattan seasons in local breeder cocks in South-eastern rain forest region of Nigeria.

2.8.3 Sperm Motility

Sperm motility assessment is indicative of the viability of sperm and the quality of the semen sample. Evaluation of raw semen gives the performance of the sperm in its own accessory gland fluid, which is often hindered when higher sperm concentrations make it difficult to distinguish individual sperm motility patterns. Hence an aliquot of semen is usually extended prior to evaluation (Hafez and Hafez, 2000). Percentage motility of 60% and above is recommended while semen with less than 40% motility is not suitable for use in artificial insemination unless the ejaculate is from an exceptionally superior sire (Bearden *et al*., 2004). Donaghue and Walker, (1999) have shown that positive correlations exist between sperm motility (an index of live sperm) and fertility in chicken. The sperm motility and fertilizing ability of cockerel sperm generally deteriorates within 1 hour after collection, if stored *in vitro* (Dumpala *et al*., 2006). There are however, several factors affecting sperm motility following semen dilution. Generally microscopic sperm motility assessment is subjective. Onukwufor and Ezekwe (2007) gave mean values of 72.034±0.69%, 73.29±0.81%, 71.38±0.48% for 3 phenotypes of local cock at twice weekly, four consecutive days weekly and two times daily twice per week ejaculation frequencies of collection respectively. Nwachukwu *et al.* (2006) reported progressive sperm motility of 64.15±2.014% and 67.75±2.027% for cocks on once and twice per week collection schedule. Nwoga *et al*. (2013) obtained 84.00±1.24%, 23.75±6.17%, 76.25±1.25%, 84.00±1.24% in local turkeys for once biweekly, once, twice and thrice per week semen collection frequencies respectively. Also Zahraddeen *et al*. (2005) working with local turkeys gave values of 84.25±2.23, 83.47±2.36, 80.17±2.64% at once, twice and thrice collection frequencies per week, respectively.

Parameters used to evaluate sperm motility include the following (Hafez and Hafez, 2000):

- Percentage of sperm which are motile (normal is 70 to 90% motile sperm),
- Percentage of progressively motile sperm,

- Sperm velocity (based on an arbitrary scale of 0-4 with 0 being stationary and 4 fast moving).
- Longevity of sperm motility in raw semen at room temperature (20 to 25°C) and in extended semen at room temperature or a refrigerated temperature of 4 to 6°C.

Research has shown avian sperm motility to be dependent on the amount of oxygen and Ca^{++} ions present in the semen (Parker and McDaniel, 2006). In poultry, motility is affected by temperature (McDaniel *et al*., 1996), Age (Tabatabaei *et al*., 2010, Cerolini *et al*., 1997), strains (Peters *et al*., 2008, Machebe and Ezekwe 2005) and season (Machebe and Ezekwe, 2005). Semen from avian species that has been studied. included turkeys, ganders, pheasants, drakes, and cocks (Wishart, 2009). It was found that semen of older birds had significantly lower motility, viability and mass movement than younger birds. McDaniel *et al*. (1996) reported that an ambient temperature of > 31oC depressed rooster sperm motility. It has been reported by Machebe and Ezekwe (2005) that sperm motility is dependent on strain e.g naked neck produce more motile sperm when compared to normal feathered cockerels. Also research has shown that sperm cell is more motile during the rainy than hot dry season (Machebe and Ezekwe. 2005).

2.8.4 Sperm concentration

Sperm cell concentration is defined as the number of cells per ml of ejaculate and normally predicts the number of breeding units that can be inseminated (Cole and Cupps, 1977). The density of the semen samples in the cockerel ranges from less than 800 x 10^3 to over 6 x 10^6sperm/ml (Anderson, 2001). It is therefore important to record the concentration of each ejaculate of the male. Lower sperm concentrations could be indicative of a serious problem in the fertility of the cockerel. This problem may be due to disease or insufficient stimulation of the animal before collection. Normally semen concentrations are not related to infertility;

however ejaculates containing less than 500 million cells per ml have been associated with low fertility rates (Bearden *et al*., 2004). Onukwufor and Ezekwe (2007) gave mean values of 5.526±0.027, 3.978±0.175 and 4.039±0.052 $x10^9$ for twice, four consecutive days two times daily twice per week semen collection schedules respectively while working with cocks. Nwoga *et al.* (2013) reported 4.50±0.78, 3.94±2.44, 13.00±4.47 and 4.50±0.78 $x10^9$ for once biweekly, once, twice and thrice per week on local turkeys. Zahraddeen *et al*. (2005) reported 2.80±166.03, 2.87±117.40 and 2.75±97.86 x 10^9 for once, twice and thrice per week respectively on local turkeys. Factors that affect concentration include Season (Bah et al., 2001), temperature (Elagib 2012), age (Gabriel et al., 2009) and frequency of collection (Onukwufor and Ezekwe. 2007).

2.8.5 Sperm Morphology

The morphology of sperm is also considered to reflect the physiological status of the male for sperm production and reflects the viability of storage in the gonadal ducts.

Normally the sperm cell consists of a head, mid-piece and tail portion. The head contains the nucleus, containing the genetic material, which is the sireôs genetic contribution to the offspring. The post nuclear cap which covers the posterior part of the nucleus and acrosome which covers the anterior part of the nucleus both protect the nucleus. If the acrosome is malformed or damaged the sperm cell will not be able to fertilize the ova by penetrating the zona pellucida. The total abnormal spermatozoa within the range of 8% to 10% has no adverse effect on fertility, but if the accumulated total abnormal spermatozoa exceeds 25% in the ejaculate, reduced fertility may be anticipated (Bearden *et al*., 2004). Blesbois (2007) described an eosin-nigrosin stain technique to assess the morphology of cockerel semen. Sperm morphology can serve as an indicator of semen quality and short-comings in the male. Onukwufor and Ezekwe (2007) had mean of 17.24±0.69%, 17.66±0.41% and 16.71±0.35% for abnormal sperm on three phenotypes of local cock when semen was collected at twice,

four consecutive days, two times daily twice per week respectively. Also Zahraddeen *et al*. (2005) obtained 14.33±1.56%, 12.58±1.10% and 13.92±1.10% for abnormal sperm on local turkeys at once, twice and thrice frequencies per week while Nwoga *et al*. (2013) reported 96.13±0.23%, 41.00±10.59%, 91.06±0.29% and 94.45±0.41% for normal sperm while working with local turkeys ejaculated once biweekly, once, twice and thrice per week, respectively. Seasonal impact on sperm abnormality has been reported in Northern Pintail duck (Penfold *et al.*, 2000). Obidi *et al*. (2008) observed that the ejaculates of two breeder cocks had the highest and lowest abnormality during the harmattan and rainy seasons, respectively. Bah *et al*. (2001) working with Sahelian cocks and Rekwot *et al.* (2005) working with Red strain of Shika brown breeder cocks showed that the raining season is favourable to spermatogenesis. Age also affect sperm abnormality according to Obidi *et al.* (2008). They observed higher percentage of abnormal sperms in older cocks than in the younger cocks.

2.8.6 Live and dead sperm

The proportion of live and dead cells can be estimated by supravital staining with a stain mixture such as negrosin-eosine. The cells that were alive when the stain was applied exclude the stain and the dead cells stain red with eosine against the dark nigrosine background. The results of live sperm counts are highly correlated with visual estimates of progressively motile sperm cells but, the later averages are lower than the percentage of unstained spermatozoa (Bearden *et al.*, 2004). Nwoga *et al*. (2013) reported 97.00±0.38%, 47.00±12.14%, 96.50 ±0.18% and 97.13±0.22% for live sperm on local turkeys at once biweekly, once twice and thrice, respectively. Also, Zahraddeen *et al*. (2005) obtained 83.50±2.82%, 81.42±1.99% and 77.08±1.99% while working with local turkey ejaculated once, twice and thrice frequencies of collection respectively. Obidi *et al*. (2008) demonstrated seasonal variations in percentage dead sperm obtained in two strains of breeder cocks. Bah *et al*. (2001) and Rekwot *et al*.

(2005) observed that the hot-dry season adversely affect semen quality, leading to a high percent of dead sperm.

2.9 Factors Affecting Cockerel Semen Quality Post Ejaculation

2.9.1 Ambient Temperature

A decrease in ambient temperature after collection of semen decreases the activity (motility) of the sperm and semen should also not be exposed to the sun (Anderson, 2001). The sperm membrane is susceptible to changes in temperature, and this may affect the movement of the sperm, causing deterioration in quality and fertilizing capacity. Therefore care must be taken to maintain the required temperatures (Senger, 2003). The ambient temperature is never constant and higher temperatures can increase the metabolism of the sperm cell, while cooler temperatures are less of a problem as it reduces the metabolic rate and slows down the sperm movement (Hafez and Hafez, 2000).

2.9.2 Semen Osmotic Pressure in Poultry

Latif *et al.* (2005) stated that an increase in the osmotic pressure can be ascribed to the contamination of broiler semen with urine and bacteria, which in turn results in the clumping of sperm. A 375mmHg osmotic pressure is optimum for the short term storage of semen. However the recommended osmolarity of the Blom stain technique is lower and was quantified as 220mOsmol/kg dissolved in diluents, with composition similar to that of seminal plasma. However these hypo-osmotic conditions resulted in the swelling of the sperm head (Lukaszewicz *et al.,* 2008). The semen diluents must be isotonic, as the osmotic pressure created by the solution may be detrimental to the sperm cell (Senger, 2003).

2.9.3 Concentration of Sperm per Ejaculate

The sperm cell contains potassium (K^{+}) as a major cation, whereas sodium (Na^{++}) is the principal cation in the seminal plasma. Potassium is a natural metabolic inhibitor and by increasing the cellular concentration, it increases the ratio of potassium to sodium which

again reduces the metabolic activity in the sperm. The addition of fructose will not greatly change the metabolic rate, but will extend the life span of the sperm. Excessive dilutions suppress sperm motility and the metabolic rate of the sperm (Bearden *et al.,* 2004).

2.9.4 Antimicrobial Agents

There are varieties of bacteria that could contaminate semen after collection and this can be minimized by cleaning the area around the sex organs and using sterile equipment. Gentamicin, Tylosin and Linco-Spectin are generally added to the semen during processing and storage to limit bacterial growth (Bearden *et al.,* 2004). These agents have not demonstrated any direct effect on metabolic rate of the sperm. Some organisms are not pathogens, but do compete with semen for nutrients and produce metabolic by-products that may have an adverse effect on the viability of the sperm. The antibacterial agents may extend the fertile life of the semen by controlling bacterial growth, thus saving energy substrates for sperm metabolism and maintenance (Etches, 1996; Bearden *et al.,* 2004).

2.9.5 Cockerel semen pH

The accessory fluids are of blood origin and contain aldose (presumably blood glucose). Cockerel semen lacks aldose and has a lower pH than the seminal fluid. If a large amount of seminal fluid is ejaculated, it will increase the semen volume, pH and glucose content and cause a decrease in the density of the semen. The pH of semen is slightly (pH 6.8) below neutral (pH 7) immediately after ejaculation in most poultry species. The loss in Co_2 may result in an increase of the pH, which may again be neutralized by the production of lactic acid during sperm metabolism (Cole & Cupps, 1977). The semen extenders used must provide the necessary buffers to prevent changes in pH, produced by the sperm metabolism.

The most common buffers used in semen diluents are Tris, sodium citrate and sodium phosphate. A change in semen pH generally affects the sperm motility negatively and an

increase in pH has been associated with poor buffering capacity. The pH should be measured in the fresh sample as semen pH will deteriorate within a short period of time.

2.9.6 Gonadotrophic hormones

Bearden *et al*. (2004) reported testosterone and other androgens to suppress the sperm metabolic rate, but the concentration of the androgens found in a male system apparently has no permanent effect on sperm viability. There are fluids produced in the female tract which also increase the metabolic activity and hence motility of the sperm and it is thought that estrogen is involved in the development of the female tract, but other unidentified factors may also be involved. The increased metabolic activity of the sperm cell in the female tract is likely to increase sperm motility, which increases the frequency of collusions between the sperm cell and the oocyte in the oviduct.

2.9.7 Gasses

A low concentration of Co_2 stimulates aerobic metabolism of the sperm. The metabolic rate is suppressed if the partial pressure exceeds 5%. O_2 is necessary for aerobic metabolism, but higher levels of O_2 may be toxic, and depress the metabolic rate. This factor is not likely to occur in the laboratory, unless the O_2 or Co_2 is bubbled through the semen. Anaerobic sperm metabolism can occur under nitrogen, hydrogen, or helium gasses with no effect on the metabolic rate (Bearden *et al.,* 2004).

2.9.8 Photoperiod

According to Bearden *et al.* (2004), the lighting in the laboratory can suppress the metabolic rate, motility, and fertilizing capacity of the sperm. A greater effect was observed when semen was in contact with O_2. The enzyme catalase will prevent the harmful effect of light or photoperiod. This demonstrates that light causes a photo-chemical reaction in the semen that result in the production of hydrogen peroxide, which is detrimental to the sperm. Semen should be protected from light and also against direct sunlight.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Location of the Experiment

The experiment was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Nsukka lies in the derived savannah region and is located on latitude $5^{o}22^{1}$N and longitude $7^{o}24^{1}$E (Asadu, 2002). The climate of Nsukka is humid tropical with a relative humidity range of 56.01 to 103.83%. The mean daily ambient temperature is between 26-32.8^{o}c (Uzodinma and Ofoefule, 2009). Annual rainfall ranges from 1567.05mm to 1846.98mm (Meteorological centre, crop Science Department of Nigeria Nsukka, 2009 unpublished).

3.1 Management of Experimental birds

A total of twelve local heavy ecotype chickens aged between 48 - 50 weeks with an average weight of 1.50 - 2.50 kg were selected from the breeder stock at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Battery cage system was used to rear the birds and the birds were fed with breeder diet. The experimental cocks were fed *ad-libitum* with commercial growers mash ration feed from Top feeds Nigerian Limited ® containing 16.5% Crude Protein, 2400 kcal/kg Metabolizable Energy and Water offered throughout the experimental period. All necessary vaccinations were administered to the birds in line with the vaccination schedules for the University poultry farm. The experimental cocks were also de-wormed with Levamisol (Levaget®) before collection of semen commenced.

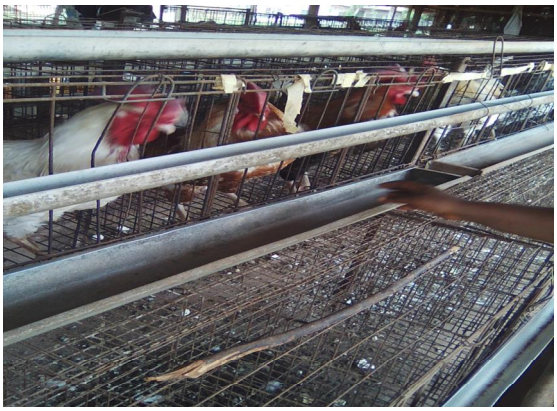

Fig 1: Experimental Birds

3.2 Experimental design

The experiment was conducted in a completely Randomised Design (CRD). The 12 cocks were randomly assigned to three semen collection frequencies of once, twice and thrice (T_1, T_2 and T_3) with four cocks each.

Table 2: Semen Collection Schedule

Treatment	**T_1**	**T_2**	**T_3**
Days	Monday	Monday, Wednesday	Monday, Wednesday and Saturday
Frequency of collocation	Once	Twice	Thrice

3.3 Data collection

The experiment lasted for a period of eight weeks. The cocks were given preliminary training for two weeks before the actual semen collection exercise of the experiment. The cocks were trained daily for semen collection by the massage technique (back lumbar / abdominal massage method) which was by massaging the area around the cloaca before milking the semen as described by Burrows and Quinn (1935). This was considered necessary not only for effective semen collection but also to make the cocks familiar with the semen collector.

The technique involves restraining the male and gently stroking the back of the bird (cock) from behind the wings towards the tail with firm rapid strokes. The male responds with tumescence (erection) of the phallus, at which time the handler gently squeezes the cloaca expressing semen through the external papillae of the ducts deferens collecting the semen into the centrifuge. Calibrated centrifuge tubes used for the collection were initially rubbed in between the palms to pre-heat them in order to prevent cold shock to the sperm cells. Each tube was held in such a way that the palm covered the lower part containing the ejaculate, so as not to expose it to sunlight which is another detrimental factor. During each collection, semen was gently collected according to treatments. Semen samples collected from each treatment was evaluated immediately after collection.

Massaging process

Collection of semen with tube

Fig 2: Massaging process and semen collection with centrifuge tube

3.4 Data evaluation

3.4.1 Semen colour

The colour of the semen was determined as described by Bearden *et al*. (2004). Only the creamy white samples were analysed. Semen colour ranged from creamy to colourless.

3.4.2 Semen volume

Ejaculate was collected using a calibrated centrifuge tube and the volume read off directly and recorded in mililiter.

3.4.3 Progressive Motility

The progressive sperm motility was determined using the method described by Bearden *et al*., (2004). A drop of semen was placed on a clean pre-warmed microscope slide and a drop of 0.9% physiological saline was added using a plastic dropper. The semen and the diluent were gently and thoroughly mixed and a cover slip placed on the mixture which was then clipped on the microscope stage and viewed using x400 magnification. The percentage motile sperm was determined by scoring from a range of 0-100.

3.4.4 Percentage Live and Dead, Normal and Abnormal Spermatozoa

The percentage live and dead, normal and abnormal spermatozoa were determined by staining technique as described by Bearden *et al*. (2004). A drop of semen sample was placed on a clean dry slide and two drops of eosine-negrosine mixture (blue dye) added to it using a dropper. The semen and the dye were mixed thoroughly. A smear was made by placing another warm slide over the first, spreading the mixture evenly between the two slides. The slides were separated by pulling the ends in opposite directions with a smooth motion and air dried. After, the dried slides were clipped to the microscope and observed at x1000 magnification, with oil immersion for the number of live and dead, 100 spermatozoa were counted per slide and the average of each count determined. The spermatozoa which picked up the stains were considered dead while those that did not were considered alive. From the

same slides, the morphologically normal and abnormal sperm cells were determined and all expressed in percentage.

3.4.5 Sperm concentration and total sperm per ejaculate

The sperm concentration was determined using a haemocytometer as described by Bearden *et al.*, (2004). The haemocytometer was cleaned with a cotton wool before use. About 0.05ml semen, 0.2ml sodium citrate and 0.75ml ethyl alcohol were placed in a 10ml beaker using insulin syringe and mixed thoroughly and then filtered with filtring paper. The Neubauer chambers of the haemocytometer were loaded using the capillary tube. Both chambers of the haemocytometer were each loaded with the titrated mixture with a cover slip placed over the chamber and allowed to settle for two minutes. The loaded haemocytometer was clipped to the microscope and the numbers of spermatozoa in the four diagonal small squares inside a larger square were counted. The same counting was done for the second chamber and the average taken and used for calculation of sperm concentration as follows.

$$\text{Sperm concentration} = \frac{N \times DF \times 10^6}{A \times D}$$

Where N= Number of cell counted

$$\text{Dilution factor} = \frac{\text{Semen+ Sodium citrate+ Ethyl alcohol}}{\text{Semen}} = \frac{0.05+0.2+0.75}{0.05} = \frac{1}{0.05} = 20$$

A = Area of the haemocytometer ($1/400mm^2$)

D = Depth of the haemocytometer (0.1mm)

Total sperm count = Sperm concentration X total volume of ejaculate (Hafez, 1985).

3.5 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD), using SPSS 2001. Significantly different means were separated using Duncanôs New Multiple Range Test (Duncan, 1955).

3.6 Statistical model

X_{ij} = U+ Ti+ e_{ij}

where X_{ij} = any observation or measurement

U = Population mean

Ti = Frequency of collection

eji = Experimental Error

CHAPTER FOUR

RESULTS AND DISCUSSION

The result of semen characteristics of cocks subjected to different ejaculation frequencies is presented in table 3.

Table 3: Semen characteristics of cocks subjected to different ejaculation frequencies

Parameters	**Once**	**Twice**	**Thrice**	**Sig level**
Volume (ml)	0.46±0.04	0.61±0.52	0.62±0.08	NS
Motility (%)	74.16±1.19^{a}	77.70±0.99^{a}	65.96±1.87^{b}	**
Live (%)	73.75±1.32^{a}	76.04±0.90^{a}	66.38±1.90^{b}	**
Dead (%)	26.25±1.32^{a}	23.95±0.90^{b}	33.44±1.90^{a}	**
Normal (%)	74.16±1.19^{a}	77.29±0.85^{a}	68.40±1.92^{b}	**
Abnormal (%)	25.83±1.19^{b}	22.70±0.85^{b}	31.59±1.92^{a}	**
Concentration (x10^{9}/ml)	5.65±0.06^{a}	5.09±0.11^{b}	4.73±0.12^{c}	**
Total Sperm (x10^{9})	3.35±0.31	2.65±0.24	3.01±0.33	NS

a,b,c -Row means with different superscripts are highly significant**-P<0.01, NS-Not significant

Semen volume

The result indicates that the volume of semen was not significantly (p>0.05) affected by collection frequencies. Similar result had been reported by Fan *et al*. (2004) with Taiwan country chicken, Nwachukwu *et al*. (2006) with Nigerian local chicken and Khongsen *et al*. (2015) with Betong and Dang cocks.

Progressive motility

The percentage progressive sperm motility was significantly ($p<0.01$) affected by frequency of ejaculation. The highest sperm motility 77.70±0.99% was obtained from ejaculates collected two times weekly and the lowest (65.96±1.87%) from ejaculates collected three times weekly. This is in conformity with the findings of Fan *et al*. (2004) who obtained a significant difference of 54.5%, 69.9%, 73.4% and 68.1% when different ejaculation frequencies of 1, 2, 3, and 6 were used, respectively. Also, Onukwufor and Ezekwe (2007) reported a significant difference of 72.03±0.69, 73.29±0.81 and 71.38±0.48% when different collection frequencies of two times, four consecutive days and two times daily twice per week were used respectively. Nwachukwu *et al*. (2006) however observed a non significant difference when different ejaculation frequencies of once and twice were used. Ashizawa and Sano (1990) reported that sperm cells gain motility at the vas deferens. The difference in the result might be attributed to high frequency of collection which could lead to the collection of immotile sperm cells as frequency of collection increased.

Percentage Live Sperm and Dead Spermatozoa

Percentage live and dead sperm was significantly ($P < 0.01$) affected by ejaculation frequency. The highest live sperm of 76.04±0.90 and 33.44 ±1.90% was obtained from ejaculate collected two times per week and the lowest (66.38 ±1.90% and 23.95 ±0.90%)) from ejaculates collected three times per week respectively. This result agrees with those of Nwoga *et al*. (2013) who reported significant difference of 97.00 ±0.3890, 47.00 ±12.14%, 96.50 ±0.18%, 97.13 ±0.22% when different ejaculation frequencies of once bi weekly, once, twice and thrice weekly were used, respectively on the other hand, Nwachukwu *et al*. (2006) reported a non significant difference of 72.42 ± 0.71% and 78.47 ± 0.68% when once and twice ejaculation frequencies were used for live sperm. Ghonim *et al*. (2009) had reported a significant difference of 28.91 and 26.30% when different ejaculation frequencies of once and

twice were used for abnormal sperm. This observation may be attributed to the high ejaculation frequency which leads to collection of immature sperm cells that will not be able to withstand the stress of processing which will invariably affect percentage live sperm.

Normal Sperm and Abnormal Spermatozoa

Percentage normal and abnormal spermatozoa was significantly ($P<0.001$) affected by frequency of collection. The highest of 77.29 ±0.85% and 31.59 ±1.92% was obtained from ejaculates collected twice weekly and the lowest (68.40 ± 1.92% and 22.70 ±0.85%) from ejaculates collected thrice weekly respectively. This result agrees with those of Nwoga *et al*. (2013) who reported a significant difference of 96.13±0.23%, 41.00±10.59%, and 91.06±0.29% for once bi weekly, twice and thrice weekly and disagrees with those of Zahraddeen *et al*. (2006) who reported a non significant difference in local toms for normal sperm. However, Onukwufor and Ezekwe (2007) reported a non significant difference of 17.24 ± 0.69%, 17.66 ±9.41% and 16,71 ±9.35% when ejaculation frequencies of 2 times, 4 consecutive days and 2 times daily twice weekly were used, respectively for abnormal sperm. High frequency of ejaculation could lead to the depletion of sperm reserves in the epididymis/vas deferens thereby resulting in the rapid transit time of sperm cells many of which might be immature to fill the vacuum created.

Sperm concentration

Sperm concentration of cocks were significantly ($p<0.01$) affected by collection frequencies. Once per week collection yielded ejaculates with the highest concentration of spermatozoa followed by two times per week collection while three times per week gave the lowest concentration of spermatozoa in the ejaculates. This is consistent with the findings of Santayana, (1985), Zahraddeen *et al*. (2006) who reported that sperm concentration decreased with increasing frequency of semen collection. However, Riaz *et al*. (2013), Nwoga *et al*. (2013), Noirault and Billard (1999), reported a non significant difference in sperm

concentration. The high value obtained for once per week collection could be attributed to the length of the rest period that the cocks had which could confer better physiological condition to them than those that had higher frequencies of collection. Onukwufor and Ezekwe (2007) had earlier reported similar observations

Total sperm

Total sperm in ejaculate was not significantly ($p>0.05$) affected by frequency of semen collection. Similar result had been reported by Zahraddeen *et al*. (2006) in local toms

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Conclusion

This study was carried out to determine the effect of ejaculation frequencies on semen characteristics of heavy ecotype cocks. The results showed that ejaculation frequency significantly affected all semen quality parameters measured except semen volume and total sperm. Cocks under once and twice collection frequencies gave better semen characteristics than thrice collection frequency.

Recommendation

Based on the result of this study, it is therefore recommended that, in breeding programme for genetic improvement of cocks through artificial insemination, semen should be collected twice per week for optimum semen quality and high semen output.

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