EFFECTS OF SEASON ON SEMEN CHARACTERISTICS AND FERTILITY OF SHIKABROWN BREEDER COCKS

BY

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DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria under the supervision of Professors P.I. Rekwot and J.O. Ayo and Dr. B.I. Onyeanusi.

The work of other investigators referred to in this study is duly acknowledged. No part of this thesis has been previously submitted for a degree or diploma.

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CERTIFICATION

This thesis entitled “Effects of season on semen characteristics and fertility of Shikabrown breeder cocks” by **OBIDI, Joseph Ayodele** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Divinity and supremacy of the GODHEAD, in whom we live and move, and in whom we have our being. (Acts 17:28)

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ABSTRACT

The seasonal influence on the reproductive cycle of Shikabrown breeder cocks was characterized by evaluating seminal traits, weekly for the three seasons associated with the Northern Guinea Savannah zone. Semen samples were analyzed by gross and routine laboratory methods for volume, motility, concentration, colour, percent dead, and percent total abnormality.

The highest volume and concentration values of 0.50 $\pm$ 0.03 ml and 3.60 $\pm$ 0.16x10^9/ml, respectively were obtained from Shikabrown White breeder cocks during the rainy season, while the lowest volume and concentration values of 0.39 $\pm$ 0.01ml and 2.90 $\pm$ 0.04 x10^9/ml, respectively were obtained for the same strain during the hot-dry season. Spermatozoa motility of 80.5 $\pm$ 1.1% and 82.3 $\pm$ 0.5% were obtained during the harmattan and rainy season respectively, for the Shikabrown White breeder cocks. Percent dead sperm value of 4.6 $\pm$ 8.3% and 6.40 $\pm$ 0.5% were recorded for the rainy and hot-dry seasons respectively; which were statistically different from the value of 9.0 $\pm$ 1.0% obtained during the harmattan season for the Shikabrown White breeder cocks.

For the Shikabrown Red breeder cocks spermatozoa volume and concentration was 0.44 $\pm$ 0.02ml and 0.45 $\pm$ 0.05ml; and 3.44 $\pm$ 0.05 x 10^9/ml and 3.53 $\pm$ 0.09 x10^9/ml were recorded for the harmattan and rainy seasons respectively. These values were significantly ($P < 0.01$) different from the values of 0.30 $\pm$ 0.02ml and 2.50 $\pm$ 0.08x10^9/ml obtained during the hot-dry season for volume and concentration, respectively during the hot-dry season for the Shikabrown Red breeder cocks. Per cent dead sperm in the Shikabrown Red cocks for the rainy and hot-dry seasons (5.50 $\pm$ 1.0% and 6.20 $\pm$ 1.2%, respectively) were not statistically ($P < 0.05$) significant, but were significantly ($P < 0.01$) different from the value of 8.40 $\pm$ 0.5% obtained during the harmattan season.

The ejaculate trait for per cent abnormality in Shikabrown Red cocks were not significantly different from that of the Shikabrown White strains. In summary, although spermatozoa were recovered throughout the year, optimal gamete quality was observed during the rainy and harmattan seasons. Previous studies in cocks have demonstrated seasonal effects on seminal traits, this is the first study to demonstrate a distinct effect of season on fertility and testicular functional integrity.

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CHAPTER ONE

1.0 Introduction

In the animal kingdom, birds (a*ves*) just like the fish (p*isces)* and frogs (a*mphibian*) are oviparous; that is, they lay many eggs with abundant amount of yolk and they have poorly developed external genitalia (Hafez, 1990). Embryonic and foetal developments take place outside the body of the birds. In cocks, the paired testes are attached to the dorsal wall of the abdomen on either side of the midline adjacent to the anterior parts of the kidney and just posterior to the lungs (Lake, 1978). Semen is produced in the testes and stored in the *ductus deferens*, which extends tortuously from the testes and ends in two papillae or wart-like projections (phallus or penis) into the cloaca or vent (Lake, 1978).

Little attention has been paid to the role of males in improving egg production of domestic fowl, with the exception of progeny testing and selection of males on the basis of sib performance. Semen and egg productions appear to be related (Marks, 1981). A very effective selection method used to improve egg production in chicken is the Osborne index, which involves the use of egg production of full and half sisters of males selected for egg characteristics (Johari *et al.*,1991).

There is scanty information between Osborne index and semen quality traits. Information on the relationship between semen quality traits and egg production of full sisters of males has been documented (Thiyagasundaran *et al*., 1985).

It is imperative that male animals should be in good physical and sexual health conditions either for natural mating or collection of good quality semen for artificial insemination (Zemjanis, 1970; Roberts, 1971). As a powerful tool for genetic

manipulation, artificial insemination has been reported to increase fertility and hatchability rates in turkeys and chickens (Mahanta *et al*., 1991).

Breed and seasonal differences in semen production of cocks have been reported (Egbunike and Oluyemi, 1979; Rekwot *et al*., 2005). The effects of season on semen quality with concomitant effects on fertility in birds have also been reported (Nayak and Misra, 1991; Rekwot *et al*., 2005). Onuora (1982) showed the significant effects of low relative humidity, high temperatures, low rainfall and peak amount of sunshine on seminal characteristics of cocks, resulting in poor semen quality. Genotypic differences affect body size and semen characteristics of cocks, while age differences significantly affect variation in body size, semen volume and pH. Nwagu *et al*. (1996) showed that colour of semen was positively correlated with semen concentrations.

It is desirable to incorporate the techniques of semen evaluation and artificial insemination in poultry breeding programmes in Nigeria in order to increase the rate of genetic progress. Breeding soundness examination in the area of semen collection and evaluation of the breeding stock cocks are important in identifying and selecting sires of superior genetic quality. This present study was aimed at examining the influence of season on ejaculate characteristics and reproductive function of Shikabrown White and Shikabrown Red breeder cocks in National Animal Production Research Institute (NAPRI), Shika, located in the Northern Guinea Savannah zone of Nigeria.

1.1 The major objectives of this work were:

i. To determine the effect of season on semen characteristics of Shikabrown White and Shikabrown Red breeder cocks.

ii. To determine the effect of time of insemination on fertility rates and hatchability of breeder hens.

iii. To determine the gonadal sperm and spermatid reserves of Shikabrown White and Shikabrown Red breeder cocks.

1.2 Research Justification

With the recent ban on the importation of poultry and their products into Nigeria, there is a huge challenge for indigenous poultry production to meet the immediate and future protein needs of our increasing population. This challenge calls for improvement and even a change in our current extensive traditional methods of livestock production to the scientifically based, and highly productive intensive management system. Except for the recent attempt by National Animal Production Research Institute, Shika– Zaria, there is no established practical national poultry-breeding programme in Nigeria. At the present time, chicken-breeding flocks used for the production of hatching eggs are normally under natural mating conditions and maintained in floor pens. Cock semen is hardly evaluated. The evaluation of semen is of value in the detection of all morphological deformities, which can lead to low fertility. Often, fertility is assessed in the physical appearance of breeder cocks. In breeding, it has been established that semen quality is more important than physical appearance (Lake 1978; Sexton, 1979).

Selection for genetic improvement is more rapid and extensive through semen evaluation, and in artificially inseminated birds than in flock-mated birds (Wishart *et*

al., 2001). The basis for this is that only high-performing birds are retained for breeding and cocks with exceptional reproductive traits are used for artificial insemination (Jean, 1983). Semen evaluation of cocks for this rapid genetic improvement is the foundation, since semen from good cocks can be diluted for inseminating more hens (Jean, 1983).

The adoption of this scientific approach of semen evaluation in chicken in Nigeria may stimulate further research into multiple factors affecting semen production in poultry, which include genetics, nutrition, diseases, ambient temperature and age of cocks. Semen preservation and storage, and consequently, fertility are required and almost unavoidable in breeders housed in cages. With artificial insemination broiler breeders produce eggs at a higher rate and have better-feed conversion ratio than breeders maintained in conventional manner (McDaniel and Sexton, 1977). Semen production by cocks housed in wire cages has been reported to be equal to that of males maintained on litter floors (Wishart *et al*., 2001); and egg production of caged chicken hens on artificial insemination is reported to be higher than that of floor housed chicken (Wishart *et al*., 2001). Wishart *et al*. (2001) also reported that caged breeder hens produced large eggs and required less feed per dozen eggs than floor housed hens.

Rozenboim *et al.* (2003) observed that artificial insemination improves fertility and, hence, hatchability by eliminating factors such as sexual adjustment and non-synchronized breeders in ostriches. In addition, it reduces drastically the maintenance costs for breeding flocks by reducing the number of male birds. This observation was in agreement with the findings of Jean, (1983) who demonstrated that fewer cocks were required with artificial insemination than with natural mating. With artificial insemination, housing breeders in cage facilities similar to commercial egg production

units would allow for mechanical egg collection and, therefore, eliminate much of the manual labour associated with floor type pens, and possibly reduce the cost of producing hatching eggs (Rozenboim *et al.*, 2003).

Finally, the adoption of semen evaluation and artificial insemination will be a useful tool in disease control programmes in the poultry industry in Nigeria. Therefore, the information obtained from this research may be of great value to the Nigerian poultry industry. Performance test on breeder cocks through their semen evaluation will enable selection for the best males, which could be extensively and rapidly used for artificial insemination.

The main disadvantage of semen evaluation and artificial insemination in chicken breeders is the labour, that is, the team of inseminators required for carrying out the operation. Technical know-how is essential for milking cocks, the operation of semen evaluation, and especially for the insemination. However, the above- mentioned advantages of semen evaluation and artificial insemination in breeder birds over our conventional floor birds may more than offset the cost of labour involved in artificial insemination.

1.3 Statements of Hypotheses

The statement of hypotheses for this research are as follows:-

1. Ho = That semen characteristics vary with seasons in Shikabrown breeder cocks.
   H_1 = That semen characteristics are not affected by seasons in the Shikabrown breeder cocks.
2. Ho = That fertility and hatchability are affected by timing of artificial insemination in Shikabrown breeder hens.
3. H_1 = That timing of artificial insemination has no effect on fertility and hatchability of Shikabrown breeder hens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Domestic Chicken

The indigenous fowls of Nigeria are generally small, late maturing and of rather nervous disposition. They produce white shelled and fewer eggs than their exotic counterparts (Kekeocha, 1995). These fowls are beautifully plumed with the feather colour varying from yellow, black, greenish-black, brown, gold, red and sometimes a combination of these colours (Ikpi and Akinwumi, 1981).

Under the extensive management system where such birds thrive, they are very economical; they are fed occasionally and irregularly on grains, mostly maize in the South and millet in the northern part of Nigeria. They have great foraging ability, and are assumed to be more resistant to local diseases. Local chickens have the good qualities of being good "mothers" and can rear three to four brood annually and reproductive qualities are uncontrolled (Kekeocha, 1995).

2.2 Effects of Meteorological Elements on Poultry Production

The meteorological elements constitute a complex system, which act upon the body of birds. Jointly, they may be expressed as climate, that is, as long- term average conditions, or as weather, i.e; as short-term fluctuations. They may affect birds outside or in their pens. Their impact on birds could either be beneficial or detrimental. The meteorological elements can influence the birds singly, or in combinations, for example, low ambient temperature plus high air movement (cold), or high ambient temperature plus high relative humidity plus solar radiation (heat) (Bianca, 1976). Reason for the concern for and emphasis on the meteorological elements relates to the fact that they are not constant, but continuously changing. Such changes also affect the

internal environment, including the blood of the animal through the nervous and the endocrine systems (Bianca, 1976; Mearns, 1997).

In the Northern Guinea Savannah Zone of Nigeria, three seasons, the cold-dry (harmattan), hot-dry and cold-wet (rainy) seasons are recognized (Igono *et al*., 1982). Each of these seasons has its positive or negative effects on livestock production. For example, the harmattan season has been described as thermally stressful to cattle, sheep and goats (Ayo *et al*., 1998), while the effect of heat stress on poultry is minimal during the rainy season (Onuora, 1984; Bannor and Ogunsan, 1987).

Climatic 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. In temperate countries, where animals are kept predominantly indoors, the adverse effects of heat stress are minimum because ambient temperature is monitored and regulated constantly. However, in Nigeria indigenous avian species are predominantly subjected to heat stress because they are reared mainly under the free-range management system, involving their free ranging in search of feeds and lack of adequate protection against high ambient temperatures. Even when protection is provided against heat, it is usually in form of temporary light shade and radiation shield (Bannor and Ogunsan, 1987; Ayo *et al*., 1996).

The effect of hot environment on the body of birds depends not only on the nature of the climatic factors involved, but also on their intensity. A hot environment affects the entire bird (Egbunike, 1979). This means that a greater number of physiological activities undergo specific changes in birds and mammals (Donald, 1998). In general, a moderately high and short-term ambient temperature evokes

neuro-endocrine, cardio-respiratory and behavioural responses, which allow the animals to adjust to the altered environment (Becker *et al.*, 1992).

In modern poultry production, stress phenomenon is not only common but on the increase. It causes heavy economic loss on this important branch of livestock production as a result of stunted growth, decrease in laying, increased cost of production, high mortality and reproductive failure (Morsy, 1998). In Nigeria, the combination of heat and high humidity reaching climax at the onset of the rainy season constitutes extreme thermal stress and depress the production of layers, whereas low ambient temperature and relative humidity prevailing from November to February during the harmattan season increase egg production (Onuora, 1984; Bannor and Ogunsan, 1987).

The normal functions of a bird are efficient if its body temperature is kept constant or at least within narrow limits. If ambient temperature changes gradually, the bird succeeds in adapting to the changes, but if the changes are rapid, they induce stress (Mearns, 1997). The optimal ambient temperatures range for poultry is 12-26^{0}C. At low AT (7^{0}C and below) birds become depressed, drowsy and their production level falls. The negative effects of high ambient temperatures observed during the hot season are even more pronounced than the low temperatures. At high ambient, the physiological potentials of birds are sharply reduced (Bianca, 1976; McDaniel *et al.*, 1996).

Thermal stress, which is more pronounced during the hot season, affects all phases of semen production in cocks. Although high temperature stimulates testicular growth in the early phase and promotes increased semen volume as well as

concentration, it subsequently suppresses reproductive capacity manifested in decreased semen quantity and quality with time (Edens, 1983; McDaniel *et al*., 1995).

If poultry production is to be improved and successfully industrialized in northern Nigeria, further research is required on mechanisms of adaptation of poultry breeds to the three seasons in this part of the country (Ayo and Oladele, 1998).

2.3 Reproduction in Male Avian Species

Avian, *(aves*), fish (*pisces*) and amphibian species are oviparous, and in some cases the eggs are fertilized outside the body of the female, whose external genitalia are poorly developed. Their eggs are large and are produced in abundance, surrounded by abundant yolk (Hafez, 1986).

There are about 6,000 or more species of birds and only a few have been domesticated. Those that have provided food for mankind are distinguished by their high rate of reproduction (Etches, 1996). The function of the male is to produce semen and in the case of natural mating to copulate and ejaculate the semen into the everted cloaca of the female. A sexually active rooter produces about 3 billion spermatozoa daily, and semen production is carried out by the testes, which have both spermatogenic and endocrine functions (Etches, 1996).

The testes are suspended from the dorsal body wall in the midline adjacent to the anterior ends of the kidneys just posterior to the lungs. The left testis is sometimes larger than the right (Lake, 1971). Spermatogenesis proceeds at the internal body temperature of 41^{o}C as opposed to scrotal temperatures of 24 – 26^{o}C in mammals (Etches, 1996). The post-abdominal air sacs are close to the testes and it was thought that this might reduce the temperature within these organs to be about 3 or 4^{o}C lower than body temperature due to air movements in inspiration and expiration (Lake, 1971).

It was later suggested that spermatogenesis is more intense during the night, when the activity of the fowl is lowest and the body temperature may be reduced. Raised temperatures interfere with spermatogenesis in birds (Rekwot *et al.*, 2005). The supply of blood to and the arrangement of blood vessels within the fowl testes are simpler than in mammals, with no pampiniform plexus of blood vessels as seen in mammals. The testicular artery is extremely short and branches inside the testes to form many arterial loops amongst the seminiferous tubules. Venous drainage runs to superficial veins, which unite and run directly into the posterior vena cava, the common vein is extremely short. The *tunica albuginea* of the testes is extremely thin. Intestitial tissue containing blood vessels and Leydig cells, which secrete androgens is not readily visible in histological sections of the testes of a mature cock because of the great expansion of the tubules (Lake, 1971). In the female only the left ovary is functional, but in the male poultry both testes are functional. As sexual maturity is attained, the weight of the paired testes increases from 2-4 g to 25-35 g. The left testis is usually larger than the right (Etches, 1996). Semen is stored in the *ducti deferentes*, which end in two papillae projecting into the cloaca (vent) in the fowl, guinea fowl and turkey (Lake, 1971). The *ducti deferentes* in the duck and goose open at the base of the penis in the cloacae, and semen, which is exuded from them, flows down the grooves spirally arranged down the length of the projecting penis (phallus).

2.4 Factors affecting Reproduction in Male Poultry Species

2.4.1 Age and Breed

Semen production starts at about 20 weeks of age and stabilizes after 35 to 36 weeks of age (Culbert *et al.*, 1977), with noted increase in testosterone levels as the male chicken approaches maturity. Libido in the male is not necessarily correlated with

high fertility; the most frequent copulation produces many aspermic mating. The reproductive capacity of the testes and genital organs to produce semen is influenced by age and breed (McDaniel *et al.,* 1995). Munro (1938) noted that survival of spermatozoa in the *ductus deferens* is not androgen dependent, but the phallus and vascular body structures associated with the transparent fluid formation appear to be androgen dependent (Nishiyama, 1955).

Commercial egg-laying male strains of fowl produce small amounts of semen between 16 to 20 weeks of age, but good semen for artificial insemination purposes may not be obtained until when cocks reared under good management are about 25 to 32 weeks of age. (Nwagu *et al.,* 1996). The age at onset of semen production and the maximum volume of semen obtainable from a male bird depends to some extent upon the breed and strain, age of sexual maturity, the season and the lighting schedules during rearing (Etches, 1996). Medium weight laying strains and broiler male generally matures latest. These yield semen at between 26-28 week of age on a constant 14 hours day length (Brillard, 2003).

Turkey toms begin to produce semen when 21 weeks old but sufficient semen of good quality is obtained at 32-36 weeks of age. Drakes will give semen when 6 to 7 months of age (Lake and Stewart, 1978). With exceptionally good management, the male guinea fowl can start producing semen at 5 to 5.5 months of age (Lake and Stewart, 1978).

2.4.2 Temperature

Ambient temperatures may influence reproductive ability by altering feed intake of the bird. There may be breed differences in adaptation to temperature, thus varying the responses to ambient temperature of about 8^{o}C were found to retain both testicular

growth and spermatogenesis in growing White Plymouth Rock males. Aerobic respiration is lower in males kept in cooler environments (John *et al.,* 1995).

The reproductive performance of the rooster can be greatly depressed during environmental stress. Whereas some information has been obtained on heat-stress infertility of exotic and small breeds of chickens, very little research has been conducted on the fertility of modern day breeders that have been exposed to elevated ambient temperatures (McDaniel *et al.,* 1996).

McDaniel *et al.* (1995) recently subjected sexually mature birds to high temperatures and determined that the male broiler breeder contributes more to heat-induced infertility than the female. When the male broiler breeder was exposed to 32^{o}C, fertility declined to 42% and *in vivo* sperm-egg penetration declined to 52% as compared to results obtained from males maintained at 21^{o}C (McDaniel *et al.*, 1996). These differences found when the male bird was maintained at high temperatures, were not evident when the female bird alone was exposed to elevated ambient temperatures.

Significant effects of ambient temperature on male fertility were also evident within 12 hours of challenge at a typical summer temperature of 29^{o}C. Semen characteristics such as semen volume, sperm concentration, and percentage of dead sperm were unaffected by heat treatment (Edens, 1983). Although a reduction in *in vivo* sperm-egg penetration and fertility had been demonstrated when the male broiler breeder was subjected to high temperature in a previous study (McDaniel *et al.*, 1995), the mechanisms responsible for the in *in vivo* sperm-egg penetration may have been due to a decrease in the number of sperm being stored in the hen's oviduct, when the male bird is exposed to elevated environmental temperatures (McDaniel *et al.*, 1996). Sperms are stored within the utero-vaginal junction and infundibulum sections of the

hen's oviduct (Bakst *et al*., 1994). A decrease in oviductal sperm storage would result in fewer sperm being available to bind to, penetrate and fertilize the egg. On the other hand, it is just likely that sperm from heat-exposed males are being stored properly in the hen's oviduct but is unable to bind to and penetrate the ovum (McDaniel *et al.*, 1995).

2.4.3 Light or Photoperiod

Light is an extremely important factor stimulating gonadal activity in birds and it involves a retino-hypothalamo-hypophyseal pathway. The body and gonadal growth is invariably influenced by light of different wavelength. Body growth has been found to be stimulated by blue light. Pituitary and testes weights tended to be greater in male chicks, reared under violet-blue or red light for about 21 days. Manipulating the daily photoperiod light quality and intensity can produce various effects on growth and reproductive performance of poultry. Light treatments can influence activity and food intake in the bird, thereby affecting growth and reproduction (Umeda *et al*., 1992). Exposure of birds to 14 hours of light daily hastened the onset of semen production (Lake, 1971). Most hens will commence egg production by 24 weeks of age, when exposed to 8 hours of light and 16 hours of darkness throughout their life (Etches, 1996). The age of sexual maturity for these hens can be advanced to 22 weeks by providing photo-stimulation. Exposure of castrates to short days produces a slow rise in plasma luteinizing hormone (LH), and a maximum LH level that is much lower than that observed when castrates are transferred to long days. The rate of increase in plasma LH following castration is greater in capons exposed to long days (Etches, 1996). When birds have been exposed to long days for an extensive period, exposure to

short days intensifies inhibition of gonadotrophin-releasing- hormone (GnRH) and reproduction ceases (Etches, 1993).

2.4.4 Diurnal rhythm

Greater numbers of spermatozoa in guinea fowls were obtained in collections made at 6 - 7 pm than in the morning or mid-day (Onuora, 1982). It is not known whether this phenomenon is associated with rhythmic changes in the metabolic rate. Spermatogenesis varies with possible diurnal changes in body temperature, and the period of highest mating activities are found to coincide with that of greatest semen yield (Lake, 1971).

2.4.5 Nutrition

Adequate feed is essential to maintain males in good reproductive condition and starvation leads to a marked reduction in semen production, and this can be almost completely reversed about 14 days after resumption of normal feeding (Boone and Hughes, 1969). Cocks are often obliged to consume feed intended for laying hens and studies on specific nutrient requirements for semen production are rear (Boone and Hughes, 1969).

The effect of dietary calcium levels on semen characteristics of White Leghorn males were studied; semen characteristics and fertility differed insignificantly (Surai, 2002). Dietary protein during growth of males influences the onset of sexual maturity, 9% crude protein or less delays sexual maturity (Surai, 2002). Adult White Leghorn males fed 9% dietary protein produced normal quantities of semen, this diet contained 1% calcium and had an energy value of 2853 Kcal/kg (Lake, 1971).

Pana *et al.* (2000) showed that Cornish broiler cocks, whose daily feed consumption was limited to 130 g/cock/day produced ejaculates whose sperm

concentration did not differ significantly from those of their full-fed counterpart. One of the biggest problems of extensive poultry production is the unsteady nature of feed supply and availability, resulting in undernourished birds most of the time. It is of interest, therefore, to stipulate the feeding situation under extensive production system to assess how this may affect semen production and quality in the Nigerian local cocks. Ezekwe *et al.* (2003) found that the effects of underfeeding local cocks on the semen quality traits appear to be more severe on the physical rather than on the biochemical characteristics. This shows that the spermatogenic functions of the testes are more responsive to underfeeding than the secretory activity of the reproductive tract. Underfeeding diminished the ability of the gonads to respond to gonadotropin stimulation (Davies *et al.,* 1957). Moderate underfeeding or reduction of feed intake by 30% to 50% will not adversely affect semen production and quality (Ezekwe *et al.,* 2003). It is considered necessary to evolve a feeding programme for breeding birds that will reduce the high cost of feeding under the intensive production system without inflicting serious damage to their reproductive functions.

2.5 Physical and Chemical Properties of Semen

2.5.1 Morphology of Spermatozoa

Chicken and turkey spermatozoa are filiform in shape and are nearly indistinguishable by light and scanning electron microscopy. The head is slightly curved and consists of an acrosome and nucleus, followed by the midpiece and tail. The chicken and turkey sperm heads are morphologically simple when compared to the mammalian sperm heads (Lake, 1981; Bakst, 1998). The cone-shaped acrosome contains homogenous materials, and is enveloped by a continuous acrosomal membrane. The sub-acrosomal space separates the acrosome from the nucleus (Hafez,

1987). A centriolar complex forms the neck region, which joins the head with the tail segment. The anoxeme, the motor component of the tail originates from the distal centriole, while the mid-piece is formed by about 30 mitochondria (Hafez, 1987).

2.6 Mating Behaviour (Natural Mating)

With the exception of pigeons in which bonding of the pair is an essential part of the reproductive process; birds domesticated for meat and egg production are polygamous. One male is required for 7-14 females depending on the breed, body condition and age of the flock (Etches, 1996). The physical presence of the male is not required to stimulate ovulation in the female, and mating has no apparent effect on ovulation (Lake, 1981). The male to female ratio of broiler breeders is lower than that of breeding stocks used to produce laying hens. The addition of males to an established flock, however, is associated with aggressive encounters between the males as the social hierarchy (peck order) is re-established (Guhl, 1962). The establishment of a stable social ranking preceedes the onset of normal mating activity.

Maximum fertility is not obtained until the social order is established (Guhl, 1962; Lake, 1971). High-ranking males mate more often than low-ranking ones and interfere with the mating performance of low ranking-males, particularly if the mating occurs in close proximity (Lake, 1984). In large flocks, males establish a territory and attempts by a foreign male to mate within this territory are met with stiff resistance. Mating behaviour in chickens has been evaluated by direct observation (Siegel and Beare, 1965) and indirectly based on fertility (Bakst and Cecil, 1997).

Cocks may mate up to 30 times per day, and half of these mating may not be associated with semen transfer. Mating is usually preceeded by courtship of females by the males. Behaviours most strongly associated with mating in poultry have been well

described by Lake (1984) and Mellor (2001). These include: Waltzing; that is, the male drops one wing and approaches the hen with short shuffling side steps; and the rear approach in which the male holds the comb or neck of the hen or flaps his wings over her. Other courtship activities include circling around the hen with exaggerated high steps, "tidbitting" in which he pecks and scratches at the ground while giving food calls (Lake, 1984). The cock runs to a corner, stamps his feet and settles down in the litter with movement that makes a nest-like depression while vocalizing to the hens. This is called "cornering". Other behaviors like dust bathing, wing flapping, head shaking, feather ruffling, tail-wagging, bill-wiping, preening strutting and whining vocalization appear to transmit a courtship signal to the hens, even though these behaviours are not specifically associated with courtship (Mellor, 2001).

The males direct most of their courtship behaviours to the entire flock; waltzing and the rear approach are clearly directed to the individual females. Courtship by the male initiates a selective response from the female, who may accept or reject his advances. The receptive hen crouches before the male with her legs slightly spread. The cock mounts, grasps her neck feather with his beak and treads on the hen's back, the hen raises her tail and moves it to one side, while the male lowers his tail and slides over the rear of the hen. Semen is ejaculated through the engorged phallic folds (penis) onto the everted cloaca of the female as the two organs make brief contact. The everted cloaca of the hen is retracted immediately as the male dismounts the female. The female then stands up and shakes vigorously, while the male may circle or waltz (Lake, 1971). Cloaca contact lasts for less than one second as the semen is transferred from the male to the female.

2.7 Semen Production

The expression of various physiological traits, including semen production can be influenced by certain environmental features to which the birds are subjected to vizs: type of housing, staff changes, light, temperature, nutrition, diseases, drugs and pesticides, *etc* (Wishart *et al*., 2001). Adequate light intensity and duration are needed for good semen production. Fertilization requires good quality semen such that the spermatozoa will be able to reach and penetrate the egg yolk (Mellor, 2001).

Reproductive inefficiency is recognized as the costliest and limiting constraint to efficient animal production. Both qualitative and quantitative characteristics of semen have a marked effect on egg fertility (Kamar, 1960; Chalow, 1970). There has been an appreciable amount of work on semen production and quality in a variety of poultry breeds. Semen production is highly correlated with breed (Boone and Hughes, 1969; Bah *et al*., 2001), body weight and testis size (Burrow and Titus, 1939). Seasonal variations have been reported (Bah *et al*., 2001). Saeid and Alsoudi (1975) reported similarity in semen quality between local Iraqi cocks and some popular imported breeds.

Egbunike and Oluyemi (1979) in a comparative study of four breeds, vizs: White Rock, Rhode Island Red, White Leghorn and Nigerian local breeds found that the sperm volume and motility were highest in White Rock cocks and lowest in the Nigerian local breeds, the White Rock had the lowest sperm abnormality and the Rhode Island Red had the highest.

2.8 Collection of Semen

Apart from inherent physiological factors that influence the quality and production of spermatozoa, there are aspects of the collection and preparation of semen

for insemination, which have to be taken into consideration in order to achieve high fertility. There are several methods of semen collection in poultry.

2.8.1 Abdominal Massage

With minor modifications between species, massaging the back of the male commonly produces semen; and this stimulates the erection of the phallic structures and the papillae, which are the terminal portions of the *ducti deferentes* projecting into the cloacae. The semen is then squeezed into a collecting vessel (Donoghue *et al.*, 2003). Lymphatic folds within the cloacae of the fowl also become tumescent in response to the massage stimulus, and from these folds a variable amount of a transparent fluid is obtained when the copulatory organ is squeezed to obtain semen. The swelling is much greater generally in broiler type chickens than in the layer type chickens, resulting in more transparent fluid being released in the broiler type cock and semen samples with reduced concentration of spermatozoa.

In the drake (male), the fluid emanates from the cloacal wall and flows down the penis (Nishiyama *et al.*, 1976). The temperament of the male differs between breeds and strains of fowl and between different types of poultry. This governs the method with which the male can be captured and handled. This in turn determines to some extent, the amount of force needed to express semen (Lake, 1984). Very excitable males may yield little semen and there is a tendency for an inexperienced handler to express transparent fluid. Transparent fluid is believed to be ejected to some extent with semen from the *ductus deferens* at natural mating (Lake, 1984). It causes a rapid loss of fertilizing ability in fowl and duck spermatozoa after prolonged holding *in vitro* (Fujihara and Nishiyama, 1976), particularly at high ambient temperatures. In order to maintain high fertility using artificial insemination, semen contaminated with excessive

amount of transparent fluid should not be collected. Apart from the spermicidal effects, it decreases the density of spermatozoa in semen samples, leading to a decrease in fertility (Lake and Stewart, 1978).

Semen quality is also influenced by faecal and urine contamination in the cloaca during excitement caused by semen collection. These affect the quality of semen, particularly if the contaminated semen is held *in vitro* for too long. The higher the AT, the quicker the detrimental effect on spermatozoa. Semen should not be collected soon after birds have been fed and watered because the chances of excretion on massage are enhanced.

2.9 Semen Handling and Dilution

As soon as semen is ejected from the male, a proportion of the spermatozoa begin to lose their integrity and this continues during storage *in* v*itro*, leading to a decline in fertility after insemination (Lake, 1984; Rekwot *et al.*, 2005). After collection, the container with the semen is placed in a water-bath at about 40^{o}C. It is difficult to produce exactly *in vivo* environment, which is ideal for prolonging the survival of spermatozoa (Surai and Wishart, 1996). Research into diluents and storage techniques is aimed at providing the best possible conditions for their maintenance. If spermatozoa are to be stored beyond an hour they must be suspended in a synthetic diluent, careful handling being essential before and during the process of dilution to minimize the loss of viable spermatozoa. Moderate dilution (1 part of semen to 5 parts of extender) prolongs spermatozoa viability during *in vitro* storage. Excessive dilution reduces the concentration of the spermatozoa per cubic centimeter. (>1:10). This can lead to infertility (Lake, 1967). Excessive dilution, (the classic dilution effect) stimulates sperm motility and metabolic activity; which shortens the life-span of

spermatozoa *in vitro*. To minimize this, the semen dilution ratio should be 1:1 or 1:5 during storage of spermatozoa of both cock and turkey semen (Surai and Wishart, 1996).

2.10 Fertility in Female Avian Species

Fertilization involves binding and fusion of gametes. In birds, as in mammals, fertilization occurs internally. However, the avian egg at ovulation is much larger than the mammalian egg, and it is surrounded by a glycoprotein layer known as the inner perivitelline layer (Kido and Doi, 1988). This layer is similar to the *zona pellucida* and acts as an initial binding site for spermatozoa, and as an inducer of acrosomal exocytosis (the acrosome reaction) (Howarth, 1992). Significant progress has been made in understanding the physiological mechanisms that control sperm selection and storage in the oviduct of the turkey hen (Bakst *et al*., 1994).

Many factors influence the efficiency of sperm storage and subsequent fertility including the timing of insemination (Mclntyre and Christensen, 1985), the duration of egg production (Brillard, 1993) and age of the hens. AI before the onset of egg production maximizes the filling rate of the sperm storage tubules (SSTs) and results in higher fertility over the egg production period (Donoghue *et al*., 1995). In addition, inseminations performed just before or after oviposition result in a decreased fertility rate, possibly due to reduced numbers of spermatozoa in the SSTs (Christensen and Johnston, 1977). Fertility also declines over the egg production period after insemination with semen stored i*n vitro* for 24 hours (Thurston, 1995). However, the effect of storage for 24 hours *in vitro* on oviductal sperm storage and subsequent fertility is not finally understood (Donoghue *et al*., 1995).

Fertility in poultry is generally measured as the "percentage fertile eggs", laid by hens during a period of time following artificial insemination or natural mating (Wishart *et al.,* 2001). This parameter has presented some problems for the statistical analysis of fertility and its main limitations derived from the binary classification of an egg as fertile or infertile. An alternative measurement of breeding efficiency is the number of sperm transferred into hens. In several applications they have revealed differences in the efficiency of mating or artificial insemination that were not discernible by traditional estimations of fertility.

Individual males or females are fertile when they are "capable of breeding," and the result of their breeding is the "fertile" egg, "capable of hatching" (for the purposes of this review). A fertile egg is one that has been fertilized and has a formed embryo at oviposition. Fertility refers to the fertile status of groups of eggs laid over a period of time by single hens, by small groups of hens, or by commercial flocks. It is usually expressed as the percentage of the total eggs laid (Wishart *et al*., 2001).

2.10.1 Techniques for Investigating Embryonic Development

Kosin (1945) introduced a technique for estimating fertility by macroscopic visualization of the chicken embryonic blastoderm in unincubated eggs. This depends on the fact that, at oviposition, the "stage x" chicken blastoderm can be seen as an opague 2.5 mm discoid structure of about 50,000 cells. The degenerate germinal disc of an infertile chicken egg has the same size and position; but appears as a dense irregular "knot" of white material, surrounded by dark vacuoles. The fertile turkey embryo is less differentiated at lay, but its layer of embryonic cells is still obvious. The Kosin technique is valuable for distinguishing true infertility from the proportion of embryos that are lost during storage or incubation. Its disadvantages are that it requires

the sacrifice of a sample of eggs, and, more particularly, the classical fertile/infertile morphology is not always apparent.

"Break-out fertility" is investigated by opening eggs that do not appear to contain an embryo following candling (candling fertility) or complete incubation (hatchability). Although this does not involve the sacrifice of eggs that would otherwise have hatched after 2-3 weeks of incubation, it may not always be possible to distinguish between aborted early embryonic development and an infertile egg. Both of these may present disorganized sheets of cellular membranes that spread as opaque areas over the yolk (Thorne *et al.*, 1991).

2.10.2 Analysis of Fertility data

The simplest way of describing fertility data is as "percentage fertile eggs" laid over a period of time. Such data will be recorded routinely in commercial poultry operations and are usually expressed on a weekly basis. This is also the method of choice for experimental systems using naturally mated birds. However in experimental systems that involve artificial insemination, there are other options based on the fact that, following a single artificial insemination a hen will lay fertile eggs for only a limited period of time. Thus, the usual fertility trial reports the mean proportion of fertile eggs laid by groups of hens during days 2-8 or 2-15 after insemination.

A major problem in using such methods for comparing the fertilizing ability of different samples of semen is that the data are not distributed normally (Kirby and Froman, 1990). This problem can be circumvented by inseminating different doses of spermatozoa and describing the fertility in terms of the number of spermatozoa that enable fertilization of 50% of laid eggs (Wishart, 1985).

Alternatively, fertility can be described as the number of days until the last fertile or first infertile egg-or the median of these-is laid (Brillard *et al*., 1998). Another temporal mathematical interpretation of fertility data is to describe the fertility of groups of hens as the number of days corresponding to the 50% fertile eggs laid by groups of hens (Kirby and Froman, 1990).

Although these methods are statistically valid, most are dependent on the number of spermatozoa inseminated. If these are high, then because of the sigmoidal relationship between insemination dose and proportion of fertile eggs, (Wishart, 1985), large differences in sperm quality or quantity will include only very small differences in the fertility of inseminated hens. However, the fundamental limitation of all fertility data is that the binary fertile/infertile status of an egg reveals the fate of only one spermatozoa – the one that fertilized the egg.

While fertile eggs are the end-point of the breeding process, we are often concerned with analyzing mating or artificial insemination efficiency and, for this, fertility evaluation is insensitive. However, alternative systems are available that involve visualizing, and estimating the numbers of spermatozoa that associate with the egg around the time of fertilization.

A significant feature of avian reproductive physiology is the ability of hens to store spermatozoa for prolonged periods. This is due to the presence of sperm storage tubules (SSTs), which are epithelial invaginations, located mainly in the uterovaginal junction of the oviduct, but also present in small quantities in the infundibulum (Van Krey *et al.*, 1966). Spermatozoa transferred to the vagina by copulation or artificial insemination move through the vagina and ascend to the SSTs in the uterovaginal junction mucosa. Here spermatozoa enter the SST for variable periods, depending on

the species, reproductive status and age of the female. Stored spermatozoa are present at the site of fertilization (Bakst *et al.,* 1994). The mechanisms surrounding sperm selection before their entry into the SST, as well as their release from the SST are not well characterized, but it is known that only motile and morphologically normal spermatozoa enter the SST (Allen and Grigg, 1958). Maximum storage capacity of spermatozoa in the SST is reached within 2 days of AI in turkeys (Brillard and Bakst, 1990; Brillard, 1993). Quantification of the number of spermatozoa stored within the SST originally relied on histological procedures, but more recently has relied on moderate grinding (Brillard and Bakst, 1990), Collagenase digestion (Brillard, 1993) or homogenization of the SST (McLean and Froman, 1996).

2.11 Seasonal Variations of Fertility under Natural Mating or Artificial Insemination

The fertilizing potential of females along the reproductive season is at first dependent on their ability to store and maintain adequate populations of spermatozoa in their sperm storage tubules in order to repeatedly provide the site of fertilization with sufficient numbers of "fit" spermatozoa after each ovulation. (Wishart *et al.,* 2001*).* As a consequence in poultry species, eggs from females with prolonged sperm storage potential have also a natural tendency to maintain optimal fertility rate for prolonged periods. This situation may be highly desirable to sustain high fertility in the case of partial failure of the males during the season (Wishart *et al.,* 2001).

It has long been established that poultry species undertake more or less marked variation of their reproductive performances over the reproductive season, a consequence of their physiological age and photoperiod (Umeda *et al*., 1992). The physiological status of the hen at the onset of the reproductive season can play a major role on subsequent performances of fertility. Thus, in the turkey, Brillard and Bakst

(1990) demonstrated that hens inseminated just prior to the onset of the laying season store higher populations of sperm compared to hens inseminated just after onset of lay. The rationale for such higher sperm storage efficiency in pre-laying hens has not been established. Hypothetically, the absence of oviductal contractions in pre-laying hens may facilitate the migration of spermatozoa up to the storage sites. Notwithstanding the origin of such differences, it appears that high fertility rates obtained early in season generally sustain high fertility performances over the entire season. By contrast, low fertility performance at the onset of the season generally results in persistently poor fertility performances over the season. Whether or not such differences can be attributed to a more functional release of spermatozoa in hens having sperm storage tubules primarily filled with large populations of "fit" spermatozoa also remains to be established (Wishart *et al*. 2001).

A general feature of fertility in fowl and turkey hens is its progressive increase over the first 3-5 weeks of the reproductive period (up to peak of lay) followed by a plateau maintained up to two-thirds to three-quarter of this period. Following this plateau, fertility progressively declines with age, a consequence of a faster release of spermatozoa from the sperm storage tubules rather than impaired storage of spermatozoa in the oviduct (Brillard, 1993). In order to persistently obtain pre-cited profiles of fertility rather than profiles with successive, temporary drops revealing the existence of impaired sperm storage, an adequate management of the hens with regards to filling the sperm storage tubules is necessary (Young, 1999). Over the past years, research to assess the population of sperm present at the fertilization sites has provided a series of tools, which at many instances may reveal useful information to point out the existence of defective sperm deposition (Wishart and Staines, 1999) or low sperm

quality (Donoghue *et al*., 1995). These tools are either spermatozoa present in the perivitelline membrane (Wishart, 1987; Brillard and Antoine, 1990) or on the number of holes hydrolysed by spermatozoa in this membrane (Howarth and Donoghue, 1997; Robertson *et al*., 1997). Each of these techniques may be of practical interest in order to detect early the causes of impaired fertility over the reproductive season in breeder flocks or in parental lines.

2.11.1 Control of Fertility by extrinsic factors

Any factor impairing the initial sperm storage in the sperm storage tubules will also affect the population of "fit" spermatozoa at the infundibular level as the two populations are closely linked (Brillard and Antoine, 1990). From a practical stand-point, factors that may impair sperm motility include defective mating behaviour (in the fowl), or in artificially inseminated flocks, any factors capable of influencing the quantity and quality of sperm migration; for example, sperm dose, *in vitro* storage of semen, timing of insemination with oviposition (Brillard, 2003).

2.11.2 Semen Concentration and Quality

In contrast to sexual behaviour the rationale for successful use of artificial insemination procedures have been extensively studied in fowl and turkey breeders (Bakst and Brillard, 1995). Among factors known to influence the subsequent fertility performances, the initial concentration and quality of spermatozoa at the time of insemination exert a major role. This is because they directly influence the degree of selection exerted by the hen on subsequent sperm populations stored in the oviduct. A series of experiments conducted over the past years have revealed that following a single insemination, a maximum of approximately 1% of the initial population of spermatozoa deposited intravaginally reaches the storage sites both in the fowl and

turkey hen (Brillard and Bakst, 1990; Brillard, 1993); the quantity of spermatozoa present in the infundibulum at the time of fertilization, that is, during the 8-9 minutes following ovulation, can be estimated in freshly laid eggs (Wishart and Fair-weather, 1999). This is either directly obtained from their population trapped in the perivitelline membrane (Wishart, 1987) or indirectly from the number of holes created by the hydrolysis of the inner perivitelline layer (Howarth and Donoghue, 1997). The number of perivitelline sperm is itself highly correlated with the actual population of spermatozoa stored in the uterovaginal junction (Brillard, 1993).

From a practical standpoint, both techniques reveal appropriate methods to rapidly detect whether or not a fertility problem can originate from a lack of sperm storage efficiency. If such a problem is confirmed several quality tests are now available to pin-point whether or not the problem is qualitative or quantitative (Bakst and Cecil, 1997).

2.12 Timing of Insemination with Oviposition

The environment in the oviduct is most favoured for spermatozoa when the female is in good laying condition and usually the most intense layers are the most fertile females (Brillard, 2003). Linked to this is the well-known fact that individual birds at or around the peak of lay produce the most fertile eggs. From the point of view of artificial insemination, the oviduct is most receptive to semen just after an egg is laid (Brillard *et al.*, 1998). This is one of the reasons why it is recommended that for the best fertility, flock insemination should be performed at a time of day when most hens are without hard-shelled eggs in the shell gland (Brillard, 2003). A series of experiments conducted both in the chicken and in the turkey have demonstrated that most spermatozoa deposited within 1-3 hours prior to or just after oviposition are

eliminated by the vaginal contractions involved in the process of oviposition (Brillard *et al*., 1987; Brillard and Bakst, 1990). For example in the fowl hen, the relative efficiency of sperm storage around the time of oviposition can be reduced as much as 30-40 times that observed in hens inseminated well apart (>4 hours) from ovipostion.

From a practical standpoint, the period of insemination in a given breeder house should be carefully scheduled in order to minimize the risks of performing a majority of inseminations at or around the time of lay. Chicken hens at peak lay most of their eggs between the 5^{th} and 8^{th} hours following the onset of the photoperiod (model: 16L: 8D), while turkey breeder hens at peak would lay mainly from the 7th to the 9th hours of lay (Model: 14L: 10D). Both species would have their period delayed about 2 hours in ageing flocks.

2.12.1 Insemination Timing and Dosage of Semen

The dose of spermatozoa, depth of insemination and frequency of insemination vary for the different sperm of poultry and with treatment of the semen samples, that is, more cryopreserved spermatozoa are required for optimum fertility (Surai and Wishart, 1996). Chicken sperm are mostly inseminated 2-3 cm into the vagina (Artemenko and Tereshchenko, 1992), although occasionally 3-4 cm is recommended (Kurbatov *et al.*, 1984). Insemination of chicken sperm to depth of 5-6 cm penetrated the uterus and was found to reduce fertility (Artemenko and Tereshchenko, 1992). An interval of 7-10 days has been recommended for doses of 0.01 ml undiluted turkey semen (Besulin, 1982), and an average of 89.2% fertility was achieved during a 5-month breeding season by inseminating 60-65 million sperm weekly (Rubalsky, 1986).

A single insemination of 35, 46, 53, 64 and 80 million drake sperm produced fertilities of 84.0%, 92.5%, 96.1%, 93.4% and 94.5%, respectively (Davtian and

Starigin, 1982). For the guinea fowl, doses of 50-100 million sperm gave fertilities of 86.6 to 88.1%, although a lower dose of 35 million resulted in reduced fertility of 59.8% (Davtian and Roiter, 1975). In later experiments, fertility of over 90% in 3,940 eggs was achieved by inseminating 60 million sperm every 10 days (Roiter and Davtian, 1976), with little difference in fertility found after morning and afternoon inseminations (Roiter, 1988). It was then found that over 90% fertility could still be maintained in the guinea fowl inseminated with 23 or 35 million sperm (Davtian *et al.*, 1986).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Location

This study was carried out at the Poultry Research Programme of the National Animal Production Institute, Shika, Zaria. The Institute is located in the Northern Guinea Savannah zone of Nigeria between latitude 11^{o}N, 12^{o}N and longitude 7^{o}E, 8^{o}E at an elevation of 650 m above the sea level. An average annual maximum and minimum temperatures of 31.8$\pm$ 3.2^{o}C and 18.0 $\pm$ 3.7^{o}C, respectively characterize the climate of the area. The monthly average rainfall during the rainy season (May-October) is 148.1$\pm$ 68.4 mm (69.2-231.9 mm), while mean monthly relative humidity is 71.1$\pm$ 9.7%. This study was carried out during three seasons. Harmarttan (November-February), hot-dry (March-May) and rainy (June-November) seasons (Igono *et al*., 1982).

3.2 Experiment 1

3.2.1 Experimental Cocks

The experimental cocks comprised 20 each of Shikabrown White and Shikabrown Red breeders, maintained at the Poultry Breeding Unit of National Animal Production Research Iinstitute, Ahmadu Bello University, Zaria, Nigeria. The birds were hatched in the same Research Institute in mid-September, 2004.

3.2.2 Management System

The birds were fed *ad libitum* on breeders mash, containing 17% crude protein. Water was also provided *ad libitum*, while all the necessary vaccines and veterinary health care were given when due. Weekly liveweights of cocks were taken using weighing scale. Rectal temperatures were determined each time of semen collection

using a clinical thermometer (The Hartmans Company PLC, England). Each cock was tagged for easy identification.

3.2.3 Semen Collection

Forty breeders consisting of twenty 20 each of Shikabrown White and Shikabrown Red; aged 52 weeks with weights of 2.30 kg and 2.45 kg for Shikabrown White and Shikabrown Red, respectively were subjected to weekly semen collection for twelve months (October, 2005 to September, 2006). Semen was obtained by gentle massage (stroking) of the back feathers, two or three times with the palm of the hand; the abdomen being massaged towards the tail with the other hand. These stroking movements caused the erection of the copulatory appendages (phallic tumescence); that is, eversion of the phallus in the cloaca. The semen was immediately expressed from the swollen ejaculatory papillae into a collecting vial (Plate 3.1), and the volume of the semen recorded. Cloacal temperature of each cock was taken immediately after each collection.

3.2.4 Semen Evaluation

Four hundred and seventy six (476) ejaculates were collected and immediately evaluated for colour, volume, motility and concentration as described by Zemjanis (1970). This included the visual or gross evaluation of the ejaculate soon after collection in respect of volume, colour and absence or presence of foreign materials as well as microscopic examination of wave pattern (gross motility) and percentage sperm count. Three different colour categories of creamy, milky and watery were used for scoring semen as described by Zemjanis (1970). Gross motility and progressive status (wave pattern) were determined by examining a drop of raw undiluted semen on a pre-warmed slide under a light microscope at x 100 magnifications. The percentage dead sperm was calculated by using the eosin-nigrosin vital staining technique. This examination was based on the principle that nigrosin-eosin penetrates and stains dead sperm cells, while the live perm cells repel the stain. The slides were examined at x 430 magnification of the microscope. At least 100 stained and unstained cells were counted and percentage of each group estimated. Semen concentration was determined by the use of haemocytometer according to the method of Coles (1974). The concentration of spermatozoa was determined using the red blood cell counting chamber of a haemocytometer that was crossed with microscopic grids containing 25 large squares, with each containing smaller squares.

3.3 Experiment II

3.3.1 Artificial Insemination of Breeder Hens

A total of 40 breeder hens consisting of 20 hens each, of Shikabrown White and Shikabrown Red were used to examine the effect of time of insemination on fertility rates of hens. Breeder hens used for the study were about 48 weeks with average body

weights of 1.7 kg and 1.84 kg for the white and red strains, respectively. The two strains of breeder hens were equally subdivided into groups A and B. Group A consisted of ten Shikabrown White and Shikabrown Red breeder hens; group B also consisted of ten Shikabrown White and ten Shikabrown Red breeder hens.

Groups A and B breeder hens were inseminated weekly for four weeks each with 0.05ml of pooled semen from five Shikabrown White breeder cocks (Plate 3.3). Group A and B hens were inseminated at 10:00 h and 15:00 h respectively, using the graduated tuberculin syringe. Three persons were involved in the insemination process according to the method of Lake (1978). The first person helped in careful catching and restraining of the hen with his hands. The second person was responsible for abdominal massaging of the breeder hens; and as soon as there was partial eversion of the cloaca, he assisted in exerting a controlled pressure on the lower region of the hen's abdomen for proper eversion of the vagina. This was followed by inserting the tuberculin syringe with semen into the hen's vagina by the third person, who released the semen intravaginally as soon as the vagina started to relax.

A total of 623 fertile eggs (298 eggs for Shikabrown White and 325 eggs for Shikabrown Red) were collected and properly identified two days after the first insemination for 7 consecutive days, and stored at 14^0C for a maximum of seven days and then incubated. Percentage fertility and hatchability were determined following candling at day 18 of incubation and at hatching day 21, respectively.

3.4 Experiment III

3.4.1 Determination of Gonadal Sperm/Spermatid Reserves

Five cocks each of the Shikabrown White and Shikabrown Red were slaughtered. The two testes of one of the cocks are shown (Plate 3.3). The weight, length and volume of each testis were determined. Determination of sperm and spermatid reserves were done according to the method of Igboeli and Rakha (1971) and Rekwot *et al.* (1994). Each testis was homogenized in 20 ml of saline with antibiotics and centrifuged for about two minutes. Furthermore, the homogenate volume after rinsing the blender container with 50 ml of saline and adding the effluent, was measured. About 5 ml of the homogenate was transferred to a conical flask and further dilution was made with 30 ml of saline, and this homogenate was stored overnight at 5^{o}C to allow sperm cells and spermatids to ooze out of the tissues.

Finally, the gonadal sperm/spermatid concentrations were determined with a haemocytometer according to the method of Coles (1974). The concentration of spermatozoa was determined using the red blood cell counting chamber of a haemocytometer that was crossed with microscopic grids containing smaller squares. Sperm cells and spermatids were counted diagonally from left top to right bottom in five large squares according to the method of Rekwot *et al.* (1994).

CHAPTER FOUR

4.0 Results

4.1 Seminal Characteristics

A total of 476 ejaculates were collected and analysed. The results of the effects of season on ejaculate quality of the two strains of Shikabrown breeder cocks are presented in Tables 4.1- 4.8. The mean semen ejaculate volume was lowest during the hot-dry season compared to during the harmattan and rainy seasons for all the two strains. The mean ejaculate volume of 0.39 $\pm$ 0.01ml of the Shikabrown White cocks obtained during the hot-dry season was significantly lower than for the harmattan and rainy seasons ($P< 0.05$; Table 4.1). The volume of ejaculate recorded in the Shikabrown Red strain during the harmattan and rainy seasons were also not significantly different ($P>0.05$). In the Shikabrown Red strain the lowest ejaculate volume was recorded during the hot-dry season, and the ejaculate volume during the harmattan and rainy seasons were also not significantly different ($P>0.05$; Table 4.1). Similarly, the overall mean ejaculate volume for the two Shikabrown strains was lowest during the hot-dry season, while the overall mean values of the ejaculate volume recorded during the harmattan and rainy seasons did not differ from each other ($P< 0.05$;Table 4.1).

There was no significant difference between strains in ejaculate volume during the harmattan season. During the hot-dry season the value was higher ($P<0.01$) in the Shikabrown White the Shikabrown Red (0.39 ± 0.01ml and 0.30 ± 0.02ml, respectively; $P<0.01$). The volume of the ejaculate between strains was not significantly different ($P>0.05$) during the rainy season.

The sperm motility during the harmattan and rainy seasons did not differ significantly ($P>0.05$). However during the hot-dry season, the sperm motility was significantly ($P<0.01$) lower than during rainy season in the Shikabrown White cocks. In the Shikabrown Red, sperm motility was significantly ($P<0.01$) lower in the hot-dry season than during the rainy and harmattan seasons. In general for the Shikabrown breeder cocks the overall mean sperm motility significantly ($P<0.01$) differed from one season to the other and the highest sperm motility of 82.0 $\pm$0.2% was recorded during the rainy season. The highest overall mean sperm motility was obtained during the rainy season (Table 4.2).

As shown in Table 4.2, the ejaculate percent motility did not differ ($P>0.05$) between strains during the harmattan season. However, there was a significant difference ($P<0.01$) between strains in ejaculate percent motility during the hot-dry season. The two strains were not significantly different ($P>0.05$) in their ejaculate percent motility during the rainy season.

Table 4.1: Influence of Season on Semen Ejaculate Volume (ml., Mean ± SEM) of Shikabrown Breeder Cocks.

Shikabrown Strains	Season			Overall
	Harmattan (November – February)	Hot-dry (March – May)	Rainy (June – October)	
White (n=20)	0.49 ± 0.04^{a}	0.39 ± 0.01^{b}	0.50 ± 0.03^{a}	0.46 ± 0.03
Red (n=20)	0.44 ± 0.02^{a}	0.30 ± 0.02^{b}	0.45 ± 0.05^{a}	0.39 ± 0.04
Overall	0.47 ± 0.02	0.35 ± 0.03	0.48 ± 0.02	0.43 ± 0.03

[ab:] Means with different superscripts are significantly ($P < 0.01$) different.

Table 4.2: Seasonal Variations in Ejaculate Percent Motility of Shikabrown Breeder Cocks (Mean ± SEM)

Shikabrown Strains	Season			Overall
	Harmattan (November – February)	Hot-dry (March – May)	Rainy (June – October)	
White (n=20)	80.5 ± 1.1[a]	79.2 ± 0.2[b]	82.3 ± 0.5[a]	80.7 ± 0.7
Red (n=20)	81.3 ± 1.1[a]	78.0 ± 0.2[b]	81.8 ± 0.3[a]	80.4 ± 1.0
Overall	80.9 ± 0.3	78.6 ± 0.03	82.0 ± 0.2	80.5 ± 1.0

[ab:] Means with different superscripts are significantly ($P < 0.01$) different.

Sperm concentration ml^{-1} in the Shikabrown White strain was highest during the rainy season with a value of 3.60 $\pm$0.16x10^9 /ml. Similarly in the Shikabrown Red strain, the highest value of sperm concentration (3.53$\pm$ 0.09x10^9 ml^{-1}) was obtained during the rainy season. The sperm concentration value significantly fluctuated with seasons and the lowest value was obtained during the hot-dry season. The overall mean sperm concentration also significantly ($P<0.01$) fluctuated with seasons. It was highest during the rainy season and lowest during the hot-dry period. The spermatozoa concentration was not significantly different ($P>0.05$) between the two strains during the harmattan and rainy seasons but the concentration in Shikabrown White was higher ($P<0.01$) than that of Shikabrown Red breeder cocks during the hot-dry season (Table 4.3).

Table 4.3: Seasonal Variations in Ejaculate Concentration (10^9/ml) of Shikabrown Breeder Cocks (Mean ± SEM)

Shikabrown Strains	Season			Overall
	Harmattan (November – February)	Hot-dry (March – May)	Rainy (June – October)	
White (n=20)	3.53 ± 0.10^{a}	2.90 ± 0.04^{b}	3.60 ± 0.20^{a}	3.34 ± 0.20
Red (n=20)	3.44 ± 0.05^{a}	2.50 ± 0.08^{b}	3.53 ± 0.09^{a}	3.20 ± 0.30
Overall	3.50 ± 0.03	2.70 ± 0.14	3.60 ± 0.03	3.30 ± 0.05

[ab:] Means with different superscripts are significantly ($P < 0.01$) different.

Table 4.4: Seasonal Variations in Ejaculate Percent Dead of Shikabrown Breeder Cocks (Mean ± SEM)

Shikabrown Strains	Season			Overall
	Harmattan (November – February)	Hot-dry (March – May)	Rainy (June – October)	
White (n=20)	9.00 ± 1.0[a]	6.40 ± 0.5[b]	4.60 ± 0.8[a]	6.70 ± 1.0
Red (n=20)	8.40 ± 0.5[a]	6.20 ± 1.2[b]	5.50 ± 1.0[a]	6.70 ± 0.7
Overall	8.70 ± 0.2	6.30 ± 0.1	5.05 ± 0.3	6.68 ± 0.01

[ab:] Means with different superscripts are significantly ($P < 0.01$) different.

As shown in Table 4.4, the percent dead sperm cells were significantly ($P>0.01$) higher during the harmattan than during the hot-dry and rainy seasons. The percent dead sperm during the hot-dry and rainy seasons was not significantly ($P>0.05$) different. In the Shikabrown Red strain, percent dead sperm was also highest during the harmattan season and lowest during the rainy season. The percent dead spermatozoa for Shikabrown Red during the hot-dry season was 6.20 $\pm$ 1.2%, and this value did not differ significantly ($P>0.05$) from the value of 5.50$\pm$1.0% obtained for the strain during the rainy season. Similarly, the overall mean percent dead spermatozoa were highest during the harmattan season for the Shikabrown White breeder cocks, and lowest during the rainy season.

There was a significantly higher ($P<0.01$) percentage dead spermatozoa in semen collected from Shikabrown Red during the harmattan and hot-dry season. During the rainy season the percent dead sperm was lower in the Shikabrown White than Shikabrown Red with the values of 4.60 ± 0.8% and 5.5 ± 1.0%, respectively (Table 4.4).

Table 4.5: Seasonal Variations in Ejaculate Total Abnormality (%) of Shikabrown Breeder Cocks (Mean ± SEM)

Shikabrown Strains	Season			Overall
	Harmattan (November – February)	Hot-dry (March – May)	Rainy (June – October)	
White (n=20)	10.1 ± 1.8[a]	6.60 ± 1.0[b]	6.50 ± 0.1[a]	7.70 ± 1.0
Red (n=20)	10.1 ± 0.6[a]	8.0 ± 0.4[b]	7.80 ± 2.6[a]	8.6 ± 0.6
Overall	10.1 ± 0.0	7.30 ± 0.5	7.20 ± 0.8	8.20 ± 0.8

[ab:] Means with different superscripts are significantly ($P < 0.01$) different.

In the Shikabrown White strain the highest mean ejaculate total abnormality of spermatozoa was recorded during the harmattan season Simiarly, in the Shikabrown Red strain the percent total abnormality of 10.1±0.6% obtained during the harmattan was significantly (P<0.01) higher than the values of 8.0±0.4% and 7.8±2.6% obtained during the hot-dry and rainy seasons, respectively. The overall mean total abnormality for the two strains was highest during the harmattan season and lowest during the rainy season (Table 4.5).

During the harmattan and hot-dry seasons between strain percent sperm abnormalities were not significantly different (P>0.05). The percent sperm abnormalities of 6.50 ± 0.1% obtained in the Shikabrown White was lower (P<0.01) than the value of 7.80 ± 2.6% recorded for the Shikabrown Red during the rainy season, the difference was not significant (Table 4.5).

Although there was a slight variation in values of percent total abnormality of ejaculates obtained in the Shikabrown White during the three seasons, the differences in the values were not significant (P>0.05). In the Shikabrown Red breeder cocks, the percent abnormality of ejaculates during the harmattan was significantly (P<0.01) higher than during the hot-dry or rainy season (Table 4.5).

However, the percent ejaculate abnormalities during the hot-dry and rainy seasons were not significantly (P>0.05) different. The overall percent abnormalities of ejaculates obtained in Shikabrown White and Shikabrown Red breeder cocks were not significantly (P>0.05) different.

The overall percent abnormality of 7.20±0.5% obtained during the rainy season was significantly (P>0.01) lower than the value of 10.1±1.2%, recorded during the harmattan season for both strains of breeder cocks.

The highest percent dead in Shikabrown White breeder cocks spermamatozoa was recorded during the harmattan with the value of 9.0±1.0% while the lowest value of 4.60±0.80% was obtained during the rainy season. Although the value obtained during the harmattan season was higher than during the hot-dry and rainy seasons, percent live-dead sperm during the hot-dry and rainy seasons were not significantly ($P>0.05$) different (Table 4.5).

Similarly, in Shikabrown Red breeder cocks, highest percentage dead sperm of 8.40 ±0.5% was recorded during the harmattan seasons but this value was significantly ($P>0.01$) higher than that recorded during the rainy season. The overall mean value of percent dead in any one season was significantly ($P<0.01$) different from the other. Percent dead sperm in each season between strains did not differ significantly ($P>0.05$). On the whole, the overall percent dead sperm in the ejaculates recorded in Shikabrown White cocks throughout the experimental period and in Shikabrown Red breeder cocks did not differ significantly ($P>0.05$); that is, 6.7 ±1.0% and 6.7 ± 0.7%, respectively.

The maximum spermatozoa volume in the Shikabrown White was significantly ($P<0.01$) higher than that of the Shikabrown Red. Similarly, sperm motility in the Shikabrown White strain was significantly ($P<0.01$) higher than that of the Shikabrown Red. Other ejaculate characteristics including concentration, percent dead sperm and percent abnormality obtained in the two strains were not significantly ($P>0.05$) different. Similarly, the minimum and range values of the ejaculate characteristics in the two strains were not significantly ($P>0.05$) different. The overall mean of maximum, minimum and range of ejaculate characteristics obtained for the two strains were not significantly ($P>0.05$) different from each other (Appendix 1)

4.2 Correlation Co-efficients between Ejaculate Characteristics of Shikabrown Breeder Cocks

During the rainy season, the relationships between the ejaculate characteristics in Shikabrown White cocks were significant (Table 4.7), and predominantly higher than in Shikabrown Red ($P<0.01$). The relationship between volume and concentration was the strongest in Shikabrown White ($r = 0.638$, $P<0.01$) while in the Shikabrown Red this relationship was significant, but very weak ($r = 0.0157$, $P>0.05$). Although the relationship between volume and motility was highly significant ($P<0.01$) in both strains, the correlation co-efficient was higher in Shikabrown White ($r = 0.505$) than in Shikabrown Red ($r = 0.393$). Similarly, highly significant ($P<0.01$) correlation between motility and concentration was found in both strains ($r = 0.548$ and $r = 0.394$) of Shikabrown White and Shikabrown Red (Table 4.7) respectively.

The relationship between volume and concentration of the spermatozoa during the harmattan season was positive and significant for Shikabrown White and Shikabrown Red cocks ($r = 0.642$ and $r = 0.586$, respectively). The relationship between volume and motility was stronger in Shikabrown White spermatozoa than in Shikabrown Red spermatozoa ($r = 0.561$ and $r = 0.423$; Table 4.7) respectively. The higher the volume of spermatozoa, the higher the number of percent dead sperm in Shikabrown White and Shikabrown Red. Although this relationship was insignificant in both strains of cocks ($r = 0.119$) the correlation co-efficient obtained was higher than that recorded in Shikabrown White cocks ($r = 0.066$; $P>0.05$, Table 4.7).

The relationship between motility and concentration was positive and highly significant in Shikabrown White, but not in Shikabrown Red cocks ($r = 0.619$, $P<0.01$) and $r = 0.287$, $P>0.05$, respectively). It is noteworthy that during the harmattan, the

relationship between motility, percentage dead sperm and abnormality were insignificant (P>0.05) and low in both strains of cocks. However, these relationships were weaker in Shikabrown Red than Shikabrown White. This was also true of the relationship between percent dead and abnormality.

During the hot-dry season, the relationship between volume and concentration of spermatozoa in Shikabrown White cocks was higher than that of Shikabrown Red cocks (r = 0.470 and 0.464; P<0.01, respectively, Table 4.8). Similarly, the correlation co-efficient between volume and motility of spermatozoa was higher in Shikabrown White (r = 0.534, P<0.05) than Shikabrown Red cocks (r = 0.432, P<0.05). The relationship between motility and concentration was also higher in Shikabrown White than in Shikabrown Red cocks with significant (P<0.05) correlation co-efficient values of 0.557 and 0.479, respectively. The relationship between other parameters of the ejaculate, were very low and insignificant in both strains of cocks.

Table 4.6: Correlations Matrix for Semen Characteristics of Shikabrown Breeder Cocks during the Rainy Season

Correlated Parameters	Correlation Coefficients	
	White	Red
Volume and Concentration	0.638**	0.157
Volume and Motility	0.505**	0.393**
Volume and Percent Dead Sperm	0.173	0.156
Volume and Abnormality	0.073	0.118
Volume and Live weight	0.042	0.102
Volume and Rectal Temperature	0.162	0.047
Motility and Concentration	0.548**	0.394**
Motility and Percent Dead Sperm	-0.060	-0.141
Motility and Abnormality	-0.204	-0.146
Motility and Liveweight	0.073	-0.146
Motility and Rectal Temperature	-0148	-0.105
Concentration and Percent Dead Sperm	0.123	-0.002
Concentration and Abnormality	-0.106	0.058
Concentration and Liveweight	0.65	-0.002
Concentration and Rectal temperature	-0.130	0.016
Percent Dead and Abnormality	0.0103	0.454**
Percent Dead Sperm and Liveweight	0.075	0.342**
Live-dead and Rectal Temperature	-0.148	0.075
Abnormality and Liveweight	-0.111	-0.264
Abnormality and Rectal Temperature	.0124	0.090
Live-weight and Rectal temperature	.0010	-0.065

** Significantly correlated.parameters

Table 4.7: Correlations Matrix for semen characteristics of Shikabrown during the Hot-dry season

Correlated parameters	Correlation Coefficients	
	White	Red
Volume and Concentration	0.470**	0.464**
Volume and Mortality	0.534**	0.432**
Volume and Percent Dead Sperm	0.174	0.08
Volume and Abnormality	0.068	0.187
Volume and Liveweight	0.129	0.118
Volume and Rectal Temperature	0.082	0.042
Motility and Concentration	0.479**	0.557**
Motility and Percent Dead Sperm	0.096	0.060
Motility and Abnormality	0.002	0.213
Motility and Liveweight	0.233	0.005
Motility and Rectal Temperature	0.174	0.017
Concentration and Percent Dead Sperm	0.009	0.044
Concentration and Abnormality	0.092	0.066
Concentration and Liveweight	0.119	0.232
Concentration and Rectal Temperature	0.014	-0.096
Percent Dead and Abnormality	0.0512	-0.116
Percent dead and Rectal Temperature	0.017	0.004
Abnormality and Percent Dead Sperm	0.051	-0.116
Abnormality and Rectal Temperature	0.080	-0.013
Live-weight and Rectal Temperature	-0.187	.0.124

**Significantly correlated parameters

Table 4.8: Correlations Matrix for Semen Characteristics of Shikabrown Breeder Cocks during the Harmattan Season

Correlated parameters	Correlation Co-efficients	
	White	Red
Volume and Concentration	0.642**	0.586**
Volume and Motility	0.561**	0.423**
Volume and Percent Dead Sperm	0.066	0.119
Volume and Abnormality	0.101	-0.003
Volume and Live-Weight	0.097	0.046
Volume and Rectal Temperature	0.033	-0.142
Motility and Concentration	0.619**	0.287**
Motility and Percent Dead Sperm	0.139	0.063
Motility and Live-Weight	0.171	0.063
Motility and Abnormality	0.328**	0.074**
Motility and Rectal Temperature	-0.11.	-0.166
Concentration and Rectal Temperature	0.021	-0.158
Concentration and Percent Dead Sperm	0.226**	0.083
Concentration and Abnormality	0.280**	0.156
Concentration and Liveweight	.197	0.007
Percent dead and Abnormality	0.261	0.147
Percent Dead and Liveweight.	0.197	0.085
Live-weight and Rectal Temperature	-0.026	0.175
Abnormality and Live-weight	0.147	-0.142
Abnormality and Rectal Temperature	-0.126	-0.175
Live-weight and Rectal Temperature	-0.111	-0.128

** Significantly correlated parameters

4.3 Fertility and Hatchability

The effect of time of day on fertility and hatchability of eggs obtained from artificially inseminated Shikabrown White and Shikabrown Red hens are shown in Table 4.9. Eggs obtained from Shikabrown White hens, inseminated at about 10:00 h had a higher ($P<0.01$) percentage fertility than those obtained in Shikabrown White hens inseminated in the afternoon at about 15:00 h (82.1$\pm$1.0% and 76.0$\pm$5.2%, respectively). The percent fertility of the eggs obtained from Shikabrown Red was also higher ($P<0.01$) for hens inseminated in the morning hours than those inseminated in the afternoon hours (85.0$\pm$2.3% and 78.3$\pm$5.5%, respectively). On the whole the morning insemination yielded a higher percentage of fertile eggs than afternoon insemination of the hens (83.5$\pm$1.0% and 77.2$\pm$0.8, for the morning and afternoon inseminations respectively).

The percent hatchability of eggs obtained from hens inseminated during the morning hours was 75.0$\pm$2.5%, while that of the afternoon was 65.0$\pm$1.0% in Shikabrown White hens. In Shikabrown Red hens inseminated during the morning hours, the percent hatchability of eggs obtained was 67.0$\pm$3.3%, while that obtained from Shikabrown Red hens inseminated in the afternoon hours was 61.0$\pm$4.0%. The overall hatchability obtained in the two Shikabrown strains of hens inseminated were 72.5$\pm$2.8% and 63.0$\pm$1.1% at 10:00 h and 15:00 h respectively.

Table 4.9: Fertility and Hatchability of Inseminated Shikabrown Breeder Hens (Mean ± SEM, n =40)

	White		Red		Overall	
Parameter	10:00 h	15:00 h	10:00 h	15:00 h	10:00 h	15:00 h
Eggs set	35.0 ± 1.0	40.0 ±1.5	41.5 ±2.5	40.0 ±3.2	38.5±2.5	40.0±0.0
Eggs candled	6.30 ± 0.2[a]	9.3±1.8[b]	6.3±1.0 [a]	8.3±2.0[b]	6.3 ±0.0	8.8±0.4
% Fertility	82.1±1.0 [a]	76.0±5.2[b]	85.±2.3[a]	78.3±5.5[b]	83.5±1.0	77.2±0.8
% Hatchability	75.0±2.5 [a]	65.0±1.0[b]	67.0±3.3[a]	61.0±4.0[b]	72.5±2.8	63.0±1.1

[ab:] Means with different superscripts are significantly (P<0.01) different.

4.4 Gonadal Sperm/Spermatid Reserves

Gonadal sperm/spermatid reserves for the Shikabrown breeder cocks is presented (Table 4.11). The mean testicular lengths for the Shikabrown White were 4.22 $\pm$ 0.03 cm and 3.98 $\pm$ 0.04 cm for right and left testes, respectively. The mean testicular lengths for the Shikabrown Red were 4.40 $\pm$ 0.1cm and 4.44 $\pm$ 0.09 cm for right and left testes respectively. There was no significant ($P>0.05$) difference between the right testicular lengths of Shikabrown White and Shikabrown Red, but there was a highly significant ($P <0.01$) difference between the left testicular lengths of Shikabrown White and Shikabrown Red with an average of 3.98 $\pm$ 0.04 cm and 4.44 $\pm$ 0.09 cm, respectively. However, there was no statistical difference between the overall means of the testicular length in both strains.

The right testicular weights of Shikabrown White and Shikabrown Red cocks were statistically different. The mean right testicular weight of Shikabrown Red cocks was higher than that of Shikabrown White cocks (12.39 $\pm$ 0.076 g and 10.23 $\pm$ 0.25 g respectively). The mean weights of the left testes in Shikabrown White and Shikabrown Red cocks were not statistically ($P > 0\ 05$) different. There was a significant ($P < 0.01$) difference between their overall means.

The gonadal sperm/spermatid reserves for the right testes in Shikabrown White and Shikabrown Red cocks were 57.6$\pm$5.6 and 103.2$\pm$22.3 ($P<0.05$), respectively, while those of the left testes were 84.8$\pm$18.2 and 209.4$\pm$95.1, respectively. The gonadal sperm/spermatid reserves in the right testis of the Shikabrown White were significantly ($P<.0.01$) different from those of the left testes. Similarly, the gonadal sperm/spermatid reserves in the right testes of the Shikabrown White were significantly ($P<0.01$) different. There was a significant ($P<0.01$) difference between strains in those

parameters with higher values in the Shikabrown Red than the Shikabrown White (57.6 $\pm$5.6 x10^9 and 103.2 $\pm$22.3 x10^9/gram testis) in Shikabrown White and Shikabrown Red respectively.

Similarly the gonadal sperm/Spermatid reserves for the left testes in Shikabrown Red were significantly ($P<0.01$) higher than that of Shikabrown White (84.8$\pm$18.2 x10^9 and 209.4$\pm$95.1 x10^9per gram testis) for Shikabrown White and Shikabrown Red respectively.

Table 4.10: Gonadal Sperm/Spermatid Reserves of Shikabrown Breeder Cocks (Mean ± SEM)

Parameter	White	Red	Overall
Liveweight (kg)	3.70 ± 0.09	3.80 ± 0.09	3.75 ± 0.03
Testicular Length (cm):			
Right Testes	4.22 ± 0.03[a]	4.40 ± 0.1[a]	4.31 ± 0.5
Left Testes	3.98 ± 0.04[a]	4.44 ± 0.09[b]	4.21 ± 0.7
Testicular Weight (g):			
Right Testes	10.23 ± 05[a]	12.40 ± 0.76[b]	11.30 ± 0.4
Left Testes	11.25 ± 0.5[c]	12.46-± 0.8[a]	11.90 ± 0.55
Gonadal Sperm Reserves ($x10^9$/ml)			
Right Testes	57.6 ± 5.6[a]	103.2 ± 22.3[b]	80.4.0 ± 16.1
Left Testes	84.8 ± 18.2[a]	209.4 ± 95.1[b]	147.1 ± 44.0

[ab:] Means with different superscripts are significantly ($P<0.01$) different.

CHAPTER FIVE

5.0 DISCUSSION

The ejaculate characteristics collected from the Shikabrown breeder cocks in the present study demonstrated that season had significant influence on semen characteristics; and this corroborates earlier studies in bulls (Rekwot, 1986; Rekwot *et al.*, 1987) and Rhode Island Red breeder cocks (Rekwot *et al.*, 2005). This may be due to the deleterious effect of high temperature causing germ cell destruction as reported by Wetterman and Bazer (1985) in Duroc boars, Rekwot (1986) in bulls, Penfold *et al*. (2000) in Northern Pintail ducks and Machebe and Ezekwe (2005) in Nigerian local cocks. The result agreed with the findings of Nayak and Misra (1991) that the hot-dry season, characterized by elevated ambient temperature and relative humidity, causes a temporary decrease in sperm production and fertility.

The relatively low volume of ejaculates recorded during the hot-dry season agreed with the reports of Bah *et al*. (2001) who obtained similar results in breeder cocks in the Sahel region of Nigeria. The ejaculate volume in both strains showed that the White breeder cocks had higher semen volume regardless of the season of the year than the Red cocks This contradicts the finding of Nwagu *et al*. (1996), who reported a higher ejaculate volume for the Rhode Island Red breeder cocks. The difference between the findings of Nwagu *et al.* (1996) and the present study may be attributed to the short time (four weeks) Nwagu *et al.* carried out the study. Four weeks may be too short to draw significant conclusion between the two strains in ejaculate volume. The volume of semen collected in the hot-dry season in both breeder strains was the least. The hot-dry season has been shown to be thermally stressful to livestock especially to

the chicken, which lack cutaneous evaporative mechanisms for heat dissipation (Bianca, 1976).

Sperm concentration per ml in Shikabrown breeder cocks varied with season and the highest spermatozoa concentration were obtained during the rainy season, apparently due to the favourable meteorological conditions prevailing during the season, rather than management techniques including nutrition.

The single most important factor adversely affecting poultry production in the tropics is season; not only in those reared under extensive management system, but also in those reared intensively in poultry houses with non-artificially regulated microclimatic conditions (Mahmoud *et al*., 1996 and Ayo *et al*., 2005). The data obtained in this study are in agreement with those obtained by Bah *et al*. (2001) in the Sahel region of northern Nigeria who recorded the highest spermatozoa counts in local cocks during the rainy season in July- September. Similar findings were obtained by Rekwot *et al.* (2005), when higher concentration per ml of spermatozoa was obtained in Shikabrown Red breeder cocks during the rainy season than during the dry season. The lower value of seminal concentration observed in this study during the hot-dry season corroborated the results obtained by Machebe and Ezekwe (2005) in local breeder cocks in the Eastern part of the country. Similar seasonal variations in semen characteristics were observed by Cheon *et al.* (2002) in the boar and Christopher *et al.* (1996) in broiler breeders.

The observed genotypic effect that the seminal concentration was higher in Shikabrown White than Shikabrown Red in the present study is in agreement with the findings of Egbunike and Oluyemi (1979). However, the report of Nwagu *et al.* (1996)

on the higher value of semen concentration for the Red strain in comparison with the White is in contrast to the results obtained in the present study. Again, the observed differences between both results may be due to the short duration in the study conducted by Nwagu *et al.* (1996) and apparently the season.

The findings of Nwagu *et al.* (1996) on gross percent motility were also in agreement with the results obtained in the present study. However, the results disagreed with those obtained by Machebe and Ezekwe (2005), who reported percent gross sperm motility values of 70.11 ± 0.6%, 70.05 ± 0.89% and 67.92 ± 0.50% in the Nigerian local cocks during the rainy, harmattan and hot seasons respectively in the South-Eastern part of the country. This difference may be attributed to genetic differences; the difference may also be dependent on location. Although, there was no significant difference ($P>0.05$) in motility in the overall means for the breeder cock strains in the present study, there was an observed strain difference. The White cocks had higher gross motility value than the red during the hot-dry season. This is in agreement with the results obtained by Nwagu *et al.* (1996). The results on the impact of season on gross motility agreed with the findings of Penfold *et al.* (2000) for the Northern Pintail duck.

The findings that the ejaculates of cocks had the highest and lowest abnormality during the harmattan and rainy seasons, respectively agreed with the findings of Bah *et al.* (2001) in local Sahelian cocks in Nigeria that the rainy season is associated with high semen volume and total sperm count and, consequently, high fertility.

The results further corroborate the findings of Machebe and Ezekwe (2005), who recorded an average of 8.0% sperm abnormality in two genotypes of local cocks in

South-Eastern Nigeria. This value is close to the average value of 7.2% obtained in the present study in the two strains during the rainy season.

The seasonal variations in (total abnormality) obtained in the ejaculate characteristics agreed with the findings of Onuora (1982) in the guinea fowl, who reported higher percent sperm abnormalities in the ejaculates during the hot-dry than the rainy season. The findings that the hot-dry season adversely affects seminal traits have been demonstrated in bulls (Rekwot *et al.*, 1987); poultry (Egbunike and Oluyemi 1979); Rekwot *et al.* (2005); boars (Cheon *et al.*, 2002). Seasonal impact on seminal abnormality has also been reported for the Pintail duck (Penfold *et al.*, 2000).

Seasonal variations in percent dead ejaculates obtained both from the White and Red breeder cocks was demonstrated in this study. This finding is in agreement with the results obtained by Bah *et al.* (2001) and Rekwot *et al*. (2005), which showed that the hot-dry season adversely affected semen quality, leading to high percentage dead spermatozoa.

The finding that semen quality was better during the wet months of the year than during the hot-dry season agreed with the results of Rekwot *et al.* (2005) and showed that artificial insemination using ejaculates collected from Shikabrown breeder cocks may be more suitable during the rainy season than the hot-dry or harmattan seasons.

The result further indicated that the best season to conduct artificial insemination in the two strains of breeder cocks is the rainy season, which is known for its optimum meteorological conditions for reproductive activity. This result also confirms previous findings by Onuora, (1982), who demonstrated that meteorological conditions affect semen characteristics in guinea fowl. Further studies are required on

the effect of season on percent dead spermatozoa involving larger number of breeder cocks during the season.

The results of the fertility and hatchability of hens inseminated at different times of the day demonstrated that these parameters varied with time of insemination. The higher fertility and hatchability results were obtained from hens inseminated in the morning hours (10:00 h), indicating that for optimum insemination results, artificial insemination should be carried out in the morning hours in the Northern Guinea Savannah zone where Zaria is located. This phenomenon may be attributed to the higher volume of ejaculates and, hence higher concentration of sperm cells that are essential for invasion of sperm host glands (Wishart *et al*., 2001; King *et al*., 2002).

It is well established that the fertilization potential of breeder hens along the reproductive season is at first dependent on their ability to store and maintain adequate populations of high quality spermatozoa in their sperm storage tubules in order to repeatedly provide the site of fertilization with sufficient numbers of "fit" spermatozoa after each ovulation (Brillard, 2003). The higher fertility and hatchability values observed in the present study may be subject to higher percentage of such superior spermatozoa essential for optimum fertility in the morning semen than in the afternoon especially in an environment subject to diurnal fluctuations as reported by Piccione and Caola (2002).

The higher fertility result obtained in this study did not agree with Lake (1978), who demonstrated that greater numbers of spermatozoa were obtained in collections made between 18:00 h and 19:00 h than in the morning or mid-day. This difference may be due to prevailing environmental influences and different timing of semen collection. However, the lower fertility of insemination of hens performed at 15:00 h is

in agreement with Lake (1971), who demonstrated that lower concentration of spermatozoa was obtained from semen collections made in the afternoon. The apparent differences observed in semen concentration at different times of the day, which invariably impacted on fertility and hatchability in the current research may be attributed to diurnal rhythm; that is, possible changes in spermatogenesis in response to changes in body temperature and metabolic rate at different hours of the day (McDaniel *et al*., 1995; Piccione and Caola, 2002).

The present research has confirmed the negative influence of high ambient temperature on fertility in breeder hens. It has been established that high environmental temperature is one of the most serious factors affecting adversely the production performance of laying hens in both tropical and temperate countries (Mahmoud *et al*., 1996). In the current research the higher ambient temperature at 15:00 h may have reduced the efficiency of the sperm host glands in the utero-vaginal junction, indicating that fewer sperm cells are trapped in the sperm host glands in the utero-vaginal junction, for controlled release for fertilization at the infundibulum (McDaniel *et al.*, 1995; Brillard , 2003).

The effect of strain differences in fertility and hatchabilty is obvious in this study. This study has shown a significantly higher hatchability ($P<0.01$) for Shikabrown White breeder hens than their Shikabrown Red counterparts although there was no significant ($P>0.05$) strain differences in candling fertility. Further research is required in this respect. From the present study it is noteworthy that the Shikabrown White breeder hens may be better breeders for artificial insemination in the Northern Guinea Savannah zone.

The results of the gonadal sperm/spermatid reserve for the two strains of Shikabrown breeder cocks showed that the left testes had higher significant weights, than the right testes. This finding agrees with the results of Thurnston and Korn, (2000), who made a similar report for the turkey tom. Similar findings were reported by Etches (1996) in Plymouth Rock breeder cocks. It is noteworthy that mean weights of paired testes observed in this study is within testicular weights reported by Lake (1978) for the exotic breeder cocks. In both strains it is noteworthy to observe that gonadal sperm/spermatids reserves was higher ($P<0.01$) in the left testes than the right. This may not be unconnected with the higher left testicular weights recorded in both strains which further corroborates earlier reports by Etches (1996).

The higher sperm/spermatids reserve counts for the left testes in both strains may be due to a higher spermatogenic activity than the right. There is an observed significant ($P<0.01$) difference between strains in gonadal sperm/spermatids reserves with the Shikabrown Red having higher values in the right and left testes than the Shikabrown White. This observation may be due to genetic differences. In the present study, the Shikabrown Red had a significantly ($P<0.01$) higher testicular weight than Shikabrown White, which may also be attributed to strain differences. The observed higher values in the gonadal sperm/spermatid reserves for the left testes than the right may be attributed to the higher weight recorded for the left testis in both strains (Etches, 1996).

It is noteworthy in the present study that within and between strains gonadal sperm/spermatid reserves are within the limit reported by Lake (1978) for optimum fertility. In a general conclusion, it is imperative that further research be conducted to determine and improve the reproductive capability of the Shikabrown breeder birds in our environment.

5.1 Conclusion

Seasonal impacts on seminal characteristics of Shikabrown White and Shikabrown Red breeder cocks were observed for the harmattan, hot- dry and rainy seasons in Zaria .The overall mean volume, concentration, % motility, % dead and % total abnormality for Shikabrown White were 0.46 $\pm$ 0.03 ml, 3.34 $\pm$ 0.2x10^9/ml, 80.7 $\pm$ 0.7%, 6.7 $\pm$ 1.0% and 7.70 $\pm$ 1.0%, respectively, while that of Shikabrown Red were 0.39 $\pm$ 0.04 ml, 3.20 $\pm$ 0.3x10^9/ml, 80.4 $\pm$ 1.0%, 6.7 $\pm$ 0.7% and 8.6 $\pm$ 0.6%, respectively.

Of all the three seasons prevailing in the Northern Guinea Savannah zone of Nigeria, the hot-dry season is particularly thermally stressful to avian species and has the highest adverse effects on ejaculate characteristics.

The optimum time for artificial insemination in order to obtain maximum fertility and hatchability was established as 10:00h.

Overall fertility and hatchability were significantly ($P<0.01$) higher in Shikabrown White than in Shikabrown Red breeder hens.

The gonadal sperm/spermatid reserves of Shikabrown White and Shikabrown Red breeder cocks did not differ significantly, but the Shikabrown Red cocks had higher testicular weight.

5.2 Recommendations

(1) Adequate scientific and management measures should be adopted during the thermally stressful hot-dry season, in order to ameliorate the adverse effects of the season on animal health and productivity.

(2) The adoption of artificial insemination in poultry species in Nigeria is highly recommended for parent stock farmers to enhance rapid multiplication of excellent genetic merits or traits in the poultry industry for maximum economic return.

(3) Further research on various interactive factors affecting fertility and hatchability in breeder stock should be intensified.

(4) From a practical standpoint, the period of artificial insemination in Shikabrown breeder hens should be carefully scheduled in order to minimize the risks of performing a majority of inseminations at the time of lowest fertility.

(5) Human factors such as adequate training of artificial insemination crews should be given a high priority. Large flocks of hens should be inseminated with sufficient numbers of technicians in order to shorten the daily duration of artificial insemination period.

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Appendix 1: Maximum, Minimum and Range Values of Ejaculate Characteristics of Shikabrown Breeder Cocks (Mean± SEM).

	Season		
Rhode Island strain	Maximum	Minimum	Range
White (n=20)			
Volume (ml)	0.9±0.05	0.13±0.02	0.77±.05
Mortality (%)	90.0±0.0	70.0±1.4	20.0±1.4
Concentration (109)	4.34±0.10	1.67±0.3	2.70±0.2
Per cent Dead	14.50±1.2	1.0±0.4	13.50±0.9
Total Abnormality (%)	17.10±1.2	2.20±1.0	5.0 ±1.0
Red (n=20)			
Volume (ml)	0.75±0.6	0.12±0.2	0.63 ± 0.5
Motility (%)	86.50±0.6	71.20±2.0	15.30 ± 2.0
Concentration (10^9 /ml)	4.20±0.1	2.0±0.16	2.23± 0.1
Per cent Dead	15.10±1.0	0.5±0.3	14.60± 1.0
Total Abnormality (%)	18.0±1.1	2.10±0.6	16.0± 1.0
Overall Mean ± SEM			
Volume	0.90±0.04	0.1± 0.0	0.8± 0.04
Motility (%)	89.0±0.9	68.0± 1.4	21.0± 1.5
Concentration (10^9 /ml)	4.32±1.0	1.53±0.3	2.80±0.2
Per cent Dead	16.40±1.2	0.2±0.2	16.0±1.2
Total Abnormality (%)	18.4±1.1	0.50±0.3	17.8±1.0