EFFECTS OF FEEDING VARYING LEVELS OF DRIED BLOOD MEAL AND EJACULATION FREQUENCY ON SEMEN CHARACTERISTICS OF ISA BROWN COCKS

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CERTIFICATION

I certify that Ojukoko, Oghenetega Macellina carried out this research in the department of Animal Science, Delta State University, Asaba Campus.

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DECLARATION

I declare that this is an original research work carried out by Ojukoko, Oghenetega Macellina (PG/11/12/205452) in the Department of Animal Science, Delta State University, Asaba Campus.

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DEDICATION

This research work is dedicated to Almighty God for wisdom, strength and provision for the success of this work.

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ABSTRACT

In a study conducted to evaluate the effect of replacing fishmeal with blood meal (BM) and assessing the effect of different types of diluent and storage time on semen quality of cocks, 60 Isa brown breeder cocks aged 21 weeks were fed five experimental diets were formulated such that blood meal replaced 0%, 25%, 50%, 75% and 100% fishmeal in the diet. The diet with 0% BM served as the control. The experimental cocks were randomly allocated to the 5 experiment diets and measurement/computation of the categorization of semen and body performance. Semen was collected from cocks by lumber massage technique after feeding the cocks for one week with experimental diets. The semen evaluation on diluents and storage time was determined by 4 diluent types (Trisodium citrate, Trisodium buffer, Glucose solution and Glucose buffer) with refrigerated temperature of 5ºC stored for 5 days. Results revealed that the cocks fed diets containing 50% BM gave best performances in body weight gain and feed conversion ratio. The least cost expenditure (N241.07) was also recorded in 50% BM as compared to the control diet with N283.70). Frequency of semen collection has significant effect on semen volume, sperm motility, sperm concentration and percent live sperm. The scores decreased as ejaculation frequency increased. The highest sperm motility was observed when semen was ejaculated once weekly for 0% BM and 25% BM also, twice weekly in cocks in 50%, 75% and 100% BM. The fertilization ability of the spermatozoa was tested through its motility. Glucomate to egg solution and Trisodium to egg recorded highest motility of 60% at day 2. Semen could be stored for more than 24 hours without considerable loss of their motility at a steady temperature.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Poultry production is an important and diverse component of Nigeria's agriculture, with chicken production being the predominant part. It stands out as one of the most promising aspects of animal production in terms of the primary products (meat and eggs) which are in high demand (Madubuike and Ekenyen, 2005). Chickens play an important role in producing animal protein effectively within the shortest possible time, (Hossein Zadeh *et al*, 2012). This is because they are highly prolific, and grow fast. The quick turnover of chickens in terms of their ability to efficiently convert dietary protein and energy into meat and eggs is another advantage over other birds (Williamson, 2000).

According to Odunsi (2003), the rapid growth of human and livestock populations, which is creating increasing need for food and feed in the less developed countries, demands that alternative feed resources be identified and evaluated. The shortage of feed, particularly energy and protein feeds has been reported to be more severe in non-ruminant production which depends to a great extent on compounded feed (Akinde *et al*, 2007) than in ruminant production. An important part of raising poultry is feeding. It makes up the major cost of production, and good nutrition is reflected in the birds' performance and their products (NCAT, 1998). Since protein is generally one of the most expensive feed ingredients, basically, the crude protein ration in chickens' diet is calculated to meet its actual demand on chicken nutrition (NCAT, 1998).

There have been continuous replacement of the more expensive protein concentrates mainly fishmeal and soya beans meal, in poultry diets with cheaper and less competitively demanded protein feeding resources (Fanimo *et al*., 1998). Due to their high cost, there is need to substitute soya bean meal and fish meal with similar but cheaper high protein sources. Hence, making use of other protein sources, which have the capacity to yield the same output as conventional feed at cheaper cost (Piepenbrink *et al*., 1998; Maiga *et al*., 1996) has become necessary.

Blood meal contains mostly protein, and is used as a source of protein to supplement cereal-based diets, plant by-products and forages. It has also shown satisfactory performance on

animal production like dairy cattle, beef cattle, sheep, pigs, poultry, various fish species and silkworms (Fanimo *et al*., 1998).

Blood meal contains more essential amino acids than soya bean meal (Kleinesrud *et al*., 2000; Piepenbrink *et al*., 1998). Blood meal is a by-product of animal blood and is used as a protein source in the diets of non-ruminants and ruminants. It might offer a suitable cheap substitute of conventional feed ingredients. Blood meal is not very palatable, and it is thus not advisable to include high rates of blood meal in livestock diets (Seifdavati *et al*., 2008).

Reports on the effect of protein from blood meal on semen quality of chicken are scanty (Shafqat *et al*., 2002). Research has shown that deficiencies of various trace minerals, inadequate vitamin intakes, energy-protein imbalances and excessive protein intakes contribute to infertility and poor reproductive performances of animals including chickens. Miller *et al*. (2007) reported that feeding turkeys with high protein source had significant effects on growth and semen quality within 20-30 weeks of age, but no significant difference in sperm volume or sperm concentration after 34 weeks of age. Shafqat *et al*. (2002) investigated feeding un-degraded protein supplements (another source of protein) on the semen quality of bulls. The result shows that the absence of un-degraded protein affected semen volume and quality. In another research, Jubril *et al*. (2011) reported that 11–12% crude protein (CP) significantly increased semen volume while 14.69% CP significantly increased sperm concentration in rams. According to Meyer *et al*., (1980), Turkey male fed with a higher dietary protein content produced slightly greater volumes of semen than males fed with lower dietary protein diets. In addition, Sexton (1986) recorded significant different in fertility of the male fed with lowest protein diets. This is to show the relevance of appropriate dietary protein in the feed on semen quality. Blood meal, a typical dietary protein will also be examined in this study.

Semen evaluation is a simple way of determining a male's reproductive potentials and fertility. It was reported by Kosin (1999) that one of the constraints to rapid development of the poultry industry in most developing countries is poor fertility in chickens. He added that fertility, which is the ability of an organism to produce offspring, is an important quality for the poultry industry as a means of perpetuation of their species. One of the factors that can boost fertility in poultry is the use of high quality of semen and this is important to the poultry industry. A high concentration of viable sperm will yield good fertility. It was

reported that a concentration of $50x10^6$ /mL of semen is adequate for good fertility in chickens and turkeys (Bratte and Ibe, 1989).

1.2 Statement of problem

Increased production of animals with short generation intervals such as chickens seems to be a way out of meeting the current protein shortfalls in meat industry. However, low fertility in poultry appears to be one of the constraints to rapid progress in the sector, and is due, among other factors, to low fertility due to poor quality of semen in male poultry. Consequently, egg fertility and hatchability, and overall productivity are reduced (Heghe *et al*., 2010).

Protein level is a limiting factor in the diet of poultry. Its optimal content is a necessity not only for rapid growth, but also for the normal condition of breeders by influencing the quantitative and qualitative parameters of semen (Sotriv *et al*., 2012). The use of blood meal in poultry feed would not be without constraints. It is thus not possible to include high rates of blood meal in livestock diets because of its low digestibility and poor palatability (Rao *et al.*, 2009). Appropriate ration of blood meal which contains 77.35% of protein and energy of 2845k/cal (Aduku, 1993) is incorporated into the diet at various proportions for better performance. The studies of Meyer *et al.* (1980) about the volume of ejaculation and the concentration of spermatozoa showed that the feeding of male turkeys with diets containing 12% and 17% protein respectively resulted in non-significantly better results when the higher dietary protein level was used.

The management of feed resource is an important aspect of commercial poultry production (Bhatta and Sharma, 2001). One of the limitations to the expansion of poultry industry is the high cost of protein and energy ingredients. With the present trend of rising prices of conventional protein sources such as soya beans, fishmeal considerable attention has been placed on the search for alternative sources of dietary protein. In order to reduce this high cost, there is need to intensify research in the use of alternative and cheaper source of protein.

1.3 General Objective

The general objective of this study is to determine the effect of dietary blood meal, frequency of semen collection and use of different diluents on semen characteristics of Isa Brown cocks.

Specific objectives

The specific objectives of the study are to determine:

i. The proximate composition of dried blood meal and the experimental diet.
ii. The effect of replacing different levels of dried blood meal with fish meal on semen characteristics of Isa brown cocks subjected to different ejaculation frequencies (weekly, twice and thrice a week)
iii. The cost effectiveness of feeding at varying proportions in the diets of Isa brown cocks.
iv. The effect of blood meal and different semen diluents on semen progressive motility of sperm with storage time.

1.4 Project Justification

In Nigeria, a major limitation to animal production is poor reproductive performance (RIMS, 1992). Other factors are skyrocketing prices and scarcity of conventional animal feed rich in protein. NRC (1994) attributed many reproductive health disorders in animals to diet inadequacy. According to Arscott and Parker (1988), protein-deficient feeds reduce semen quality. There are findings that nutritional factors (especially protein) have reproductive performance in poultry (Memon *et al*. 2002). Thus, this study is designed to investigate the effect of different levels of blood meal in diets of cocks on semen characteristics.

Blood meal is an excellent but cheap source of protein. There is need to carry out this trial in order to determine the level of blood meal inclusion in the compounded feed that will produce optimal semen quality in chicken cocks at the lowest cost. Recent reviews on the subject by Seifdavati *et al*. (2008), Hassan *et al*. (2007); Donkoh *et al*. (2001) and others indicate that blood meal is an excellent source of protein and energy. Blood meal used as animal feed reduces the competition between man nd livestock, sice it is not generally consumed directly by man as food. It is much cheaper than the conventional protein feeds, and readily available as a by-product of the meat industry.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrition

In animal production, successful reproduction is an important factor in livestock production economy and it depends on nutrition, physical environment and management (Rasbech, 1984). Hence, the physiological fates of the nutrients is on the basis for formulating the nutritional requirement of the birds. To meet this requirement, the relationships among the different classes of nutrients in the diet should be considered. The National Research Council (NRC) recommendations covers the needs of crude protein in addition to its amino acids contents, energy, minerals and vitamins to the extent the information is available on them. However, there is no standard that is adequate for all environmental condition. However Nigeria has now developed its own standards, since feed consumption tends to be lower by about 20% in the tropics than temperate regions (Oluyemi and Robert, 2000)

To meet the nutrient daily requirement of poultry, the right ration-balancing diet is important. If diets are not properly balanced, then birds will suffer from nutritional diseases. The quality of protein in poultry diet is important since it is made up of amino acids. Animal protein supplement are the only source rich in all the essential amino acids (Batterham *et al.*, 1986). Chronic low protein intake in developing countries is a basic problem that needs urgent solution (Attah *et al*., 2006). The low animal protein intake may be attributed to low livestock production and therefore, available animal protein is very expensive for a population with a very low per capita. Blood meal is a rich source of protein. It is high a biological value where compared with vegetable protein supplements for poultry rations. Generally, vegetable protein supplements are deficient in two of the essential amino acids which are lysine and methionine whereas; blood meal is rich in both of these amino acids. Although, protein is the most costly nutrient of poultry ration, it is however essential and should supply all the essential amino acids.

Blood meal meets the requirements by firstly, it meeting the protein requirements of birds and secondly, it provides lysine.

2.2 Blood meal overview

Blood is a bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. Blood can be collected during the slaughtering of various livestock species (cattle, goats, chicken, sheep) under a wide range of conditions, (Divakaran *et al*.; 1988). Blood meal is available worldwide. It is highly perishable and must be processed as soon as possible after slaughter, (Tabinda *et al*.; 2000). The processing conditions of blood meal, especially those relating to use of steam, the temperature and pressure applied must be regulated. In addition, to the process duration of the blood meal products define its nutritional value to a large extent. It is advisable to consider the levels of blood meal in commercial diets, (Quatantelli *et al*, 1987). Blood is usually dried and made into blood meal so that it can be handled and incorporated into ration more easily. It is thus not possible to include high rates of blood meal in livestock diets, (Rao et al., 2009).

Blood meal contains about 800g/kg of protein and small amount of ash and oil. It also contain about 100g/kg of water (Donald *et al*., 1988). It is one of the richest sources of lysine, a rich source of arginine, methionine, cystine, leucine. Overall, it is best regarded as a food for boosting dietary lysine level (Donald *et al*., 1988). It is now being used as an animal protein in poultry ration in various proportion for better performance. (Rao *et a*l., 2009)

Blood meal can be prepared by a small-scale operation. It is somewhat hydroscopic and needs to be dried to less than 10-12% moisture and stored in a dry place in order for it not to deteriorate, (Dimitrova, 1975). There are different ways to prepare blood meal: solar drying, oven drying, drum drying, flash drying, and spray drying. The drying method used has an effect on the digestibility of the crude protein of the blood meal, because there is an inverse relationship between the amount of heat applied and protein digestibility. Particularly, lysine content and lysine availability decrease when the amount of heat increases (Fombad and Bryant, 2004). Overcooked blood meal are darker due to haemoglobin destruction are less palatable.

2.3 Blood meal Composition and Nutrients

2.3.1 Nutritional attributes

Blood meal is nutritive because of its high nutritive value since it contains mostly protein (about 90-95 % DM) and small amounts of fat (< 1% DM) and ash (< 5% DM), though non-

industrial blood meals may include other materials and thus be richer in ash. Unlike other animal protein sources, blood meal contain some essential amino acid , (King'ori *et al*., 1998) Its lysine content is relatively high (7-10% DM) which makes it an excellent supplemental protein source to use with plant-derived feed ingredients that are low in lysine. However, its isoleucine content is very low (about 1 % DM), so diets for monogastric animals must be formulated to contain enough isoleucine for the level of performance desired (Piepenbrink *et al.,* 1998; Maiga *et al*., 1996). Pepsin digestibility has been shown to be a good test for assessing the availability of the protein fraction of blood meal (Hegedüs *et al*., 1989). Blood meal is rich in iron (more than 1500 mg/kg DM).

Blood meal is somewhat unpalatable, particularly if overcooked, so care needs to be taken to not add more than 5 to 6 % blood meal to a ration, especially if high feed consumption and performance are desired,(Hoagland *et al*., 1992). Often an adaptation period is required to get animals used to eating blood meal. In broilers, blood meal is a good protein source. It can favourably replace 50 to 100 % fish meal (Rao *et al*., 2009; Nabizadeh *et al*., 2005), 50 % soybean meal (Onyimonyi *et al.,* 2007; Tyus *et al.,* 2008) also copra meal or groundnut meal (Donkoh *et al*., 2001; Donkoh *et al.,* 1999) resulting in better performance and economic results. These levels are equivalent to 3 to 9 % (DM basis) dietary level (Tabinda Khawaja *et al*., 2007; Matserushka, 1996; Quarantelli *et al*., 1987). In laying hens, blood meal is as palatable as other animal products. Sun-dried blood meal given at 4.5 % dietary level has a positive effect on layer performance (feed intake, live weight gain, egg weight and yolk colour) (Donkoh *et al.,* 2001). Blood meal improves Iron (Fe++) content in yolks (Revell *et al.,* 2009).

2.3.2 Environmental impact

Processing blood into feed removes potentially contaminating slaughter wastes from the environment. Modern drying techniques require high amounts of energy but solar drying is an interesting option in warm climates (Wahlstrom, 1977).

2.3.3 Pre-treatment

Blood can be coagulated to aid in the removal of water, by adding 1 % un-slaked or 3 % slaked lime. This method of water removal increases the amount of dry matter losses by 10–15 %, which includes many of the minerals.

In some situations, blood needs to be stored prior to being processed and dried. Raw blood can be stabilized and stored for one week by adding 0.7 % sulphuric acid or an equivalent amount of another acid. A method for preparing blood meal by adding 3 % sulphuric acid and storing 72 h before sun-drying has been described (Divakaran, 1987; Divakaran *et al*., 1988).

The tropical feedstuff analysis table shows the Blood meal analysis:

Table 2.1: Blood meal analysis

Feed stuff	Protein %	Fat %	Fibre %	Ca %	P %	Ash %	TDN %	ME kcal%	Lysine %	Methionine %	Cystein %	Arginine %	Tryptophan %
Blood meal	77.35	0.53	1.46	0.30	0.20	4.40	78.00	2845	5.30	1.00	1.40	2.50	1.00

Source: Aduku (1993)

Feed stuff	Ca%	P%	Na%	Cl%	K%	Cu ppm	Fe ppm	Zn ppm	Mn ppm	Mg ppm	I ppm	Se ppm	Ce ppm
Blood meal	0.28	0.22	0.32	0.30	0.09	10	3700	100	5	2200	0	0.22	0.08

Source: Aduku (1993)

2.3.4 Blood Meal Processing

Solar drying is well suited for small-scale operations or when advanced technical equipment is not affordable (Overton, 1976). Blood is collected in large pans and slowly boiled while stirring constantly. When moisture is sufficiently reduced (10-12 %), blood meal is spread on a clean cemented surface and then sun-dried, (Elamin and Elzubeir 1990). It can also be oven-dried. The blood may be spread on milling offals, rice bran or other plant products for better drying result in a complete feed mixture.

For large scale operations the following processes are used:

1. Drum drying

The raw blood is finely broken down to fragments to form free-flowing slurry and then deposited onto the descending side of the top of a heated drier drum and formed into a film by one or more spreader rolls. The film is rapidly dried and scraped in the form of a dried sheet which can either be flaked or pulverized to provide a high grade blood meal product. Vapours above the drying cylinder are scrubbed before being released to the atmosphere and represent the only effluents from the process (Overton, 1976).

2. Ring and flash drying

The blood is dispersed into the high velocity venturi section of the system. The blood first comes into contact with the hot drying airstream and the bulk of the evaporation occurs. The product is then dried as it is conveyed up through the drying column. The presence of a "manifold" or "internal classifier" in the ring drying system is what differentiates it from the flash dryer (GEA, 2009a; GEA, 2009b).

3. Spray drying

The blood is spray dried as whole blood, or after being separated into plasma and red albumin (GEA, 2010). Blood products have to be dried at low temperatures in order to prevent heat coagulation (GEA, 2009a; GEA, 2009b). Spray dried blood meals are also called spray dried blood powder or blood flour (Dipanjali Konwar *et al*., 2005).

Spray-dried porcine plasma is prepared as follows to the blood from slaughtered pigs is added an anticoagulant (generally sodium citrate) and then centrifuged to remove erythrocytes. The plasma obtained is subsequently spray-dried and used for production of animal feeds (Van Dijk *et al*., 2001). Products resulting from the 3 latter processes have an overall higher quality than sun-dried blood meals since the duration of the heating period is lower than with cooking. Proteins and amino acids are better preserved and lysine content is higher (Cromwell, 2009).

2.3.5 Potential constraints

For safety reasons, blood must be heated to be used in animal feeding: a minimal 100°C for 15 min is to be attained in order to destroy potentials pathogens (salmonella, mycotoxins, prions) (Göhl, 1982). It is recommended to avoid feeding a species with blood meal from the same species. In the European Union, blood meal has been criticised since 2000 for animal nutrition (Council Decision 2000/766/EC) though blood products from non-ruminants have been authorized for aquaculture in 2006 (Médale *et al*., 2009).

2.3.6 Blood meal pH

In blood meal manufacturing, liquid blood can be stored for several days post collection before it is dried. During this storage time, microbial populations in the liquid blood may increase and pH decrease. Anecdotal evidence suggests that blood meal with a low pH has an offensive odour, which may result in decreased nutritional value or a negative effect on

palatability. Past research, either by chemical laboratory analysis (Dimitrova and Brankova, 1975) or rat growth assays (DeRouchey, 2003) has indicated that blood meal can be irradiated to decrease bacteria without jeopardizing protein quality.

2.4 Semen Characteristics

Infertility has major public health, economic and psychological consequences. It affects approximately 15% of male and female animals of reproductive age. A male related factor is solely in 30% to 40%. Fertility evaluation plays a major role in identifying the underlying causes of poor fertility, directing treatment of reversible conditions to allow effective fertility, Nelson (2003).

It was observed by Belker (2002), Kolettis (2003) and WHO (1999) that knowledge of semen characteristics will enable the farmers or breeders pursue assisted reproductive options without delay. They added that semen analysis is the initial and most essential step of infertility evaluation which also includes a physical examination, hormonal evaluation, sperm-function testing and genetic analysis.

Semen quality is considered as a cornerstone of the laboratory evaluation of the infertile animals since it helps to define the severity of male factor infertility (MFI). Therefore, the cause of MFI in most instances is linked to an abnormality in one or more of the semen characteristics. Sharma (2003) reported that spermatozoa display marked heterogeneity and therefore, a variety of semen abnormalities may be found in the semen samples, even those from fertile animals. A normal spermiogram does not necessarily indicate satisfactory fertility potential. Owning to these inherent limitations in the methods of assessment, an accurate diagnosis of MFI can be made in only 40% of affected males seeking assistance.

2.5 Semen Extraction

Several techniques for extraction of semen of different animals have been devised from time to time. The older unsatisfactory methods have gradually been replaced by the new modern techniques. For chickens, Tay (2009) and Chia (2009) both revealed that semen can be extracted using special sperm collection devices made up of a 50mm square piece of rubber with a small pocket at its base. There are three common methods.

1. Use of artificial vagina
2. By Electro-stimulation method.
3. By massaging the ampulae of the *ductus defferentia* through the rectal wall.

The ideal method of semen collection is use of artificial vagina which is safe for sire and the collector also

2.5.1 Artificial Vagina Method

There are different methods in different species of animals i.e. speculum method, vaginal method and recto vaginal method. The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Improper AI techniques can negate all other efforts to obtain conception. Hand is passed through the vagina and the inseminating tube is guided by hand to the site of insemination and semen is deposited. Here there is a risk of contamination and injury of female genitalia.

The artificial vagina has the following parts:

- A heavy hard rubber 2" hose, open at both ends with nuzzle for air and water in and outlet.
- Inner sleeve of rubber or rubber liner.
- The semen receiving cone or rubber cone.
- Semen collection tube made of glass or plastic graduate in mL and its fraction correct to 0.1 mL

Insulating bag before using for semen collection all the parts are washed thoroughly and sterilized properly, and assembled as artificial vagina, the rubber pipe is inserted into the hose; inverting both ends back by folding back from either side opening, and fastening with rubber bands. Now the space between the hard rubber hose and inner rubber liner forms a water tight compartment. The nozzle at one end of the hose can be fixed turning through the threaded nut up or down. The water jacket of the Artificial vagina is filled with hot water at a temperature of 40-45°C Eduvie (2013) by opening the nuzzle. The graduated semen collection tube is fixed to the narrow end of the artificial vagina hose, and fastened by a rubber band. The inner side of the rubber liner on the anterior side of the artificial vagina is lubricated with sterile jelly to a length of 3 to 4 inches. Air is blown through nuzzle into the

water jacket, to create pressure in if, and the same is exerted the rubber linear, to simulate natural vagina. The temperature of the artificial vagina is to be checked, at each collection, and it should simulate natural vagina at mounting time if the artificial vagina is to mount later. If it is too cold, ejaculate may not be there after a thrust, or even if ejaculate is there; it may be contaminated with urine, and becomes unfit for use, Brilliard (1985)

Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates. Early methods of AI involved deposition of the semen in the vagina, as would occur in natural mating. Those methods are not satisfactory. Fertility is low and greater numbers of sperm are required. Another method which gained popularity was the "speculum" method. This method is easily learned, but proper cleaning and sterilizing of the equipment is necessary, making it more impractical to inseminate than with the recto-vaginal technique which is the most widely used AI method today Abdel-Aleem (2003).

2.5.2 Electro-Stimulation Technique

It can also be called Recto-virginal method. A sterile, disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix into the uterus. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is withdrawn. Expulsion of the semen should be accomplished slowly and deliberately to avoid excessive sperm losses in the catheter. The body of the uterus is short; therefore, care should be taken not to penetrate too deeply which might cause physical injury. In animals previously inseminated, the catheter should not be forced through the cervix since pregnancy is a possibility. Since research data show little variation in conception rates when semen is placed in the cervix, uterine body or uterine horns, some people recommend incomplete penetration of the cervical canal and deposition of semen in the cervix.

The recto-vaginal technique is more difficult to learn and practice is essential for acceptable proficiency but the advantages make this method of insemination more desirable than other known methods. With practice, the skilful technician soon learns to thread the cervix over the catheter with ease. If disposable catheters are used and proper sanitation measures are

followed, there is little chance of infection being carried from one cow to another, Coetzee (1998)

In cattle the safe and best method of insemination is “Recto vaginal method of insemination”. Cow which is in heat is well controlled placing it in a Travis. The inseminator will get ready by wearing a plastic apron, gumboots and gloves. The semen straw after thawing (keeping the semen straw in warm water for a minute to convert the freeze semen into liquid and the sperms become motile) is loaded in a sterilized A.I. gum and is covered with a plastic sheath. The inseminator will insert the gloved left hand into the rectum after applying the soft soap or other lubricant on the glove and back racked the animal, and the hand is further inserted and will catch hold the cervix through rectal wall. The A.I gum loaded with semen straw is passed.
Through the vulva to ‘vagina and cervix and observed with the hand in rectum that the A. I gum reaches the cervix, then the semen is deposited by injecting the gun, and after depositing the semen the gun is removed, the empty straw and sheath are disordered.

In this method spectrum is placed in the vagina of the cow, which provides passage outside to the site of insemination, then inseminating tube is passed through the speculum and semen is deposited at the cervix insemination method Foote (1999).

2.5.3 Massaging Method

For poultry, it was reported by Johnson (2003) that the commonest method used for semen extraction from the birds is the massage technique. This usually requires two operators; one operator holds the male and massage and massage the dorso-lateral lumbo-sacral region on the abdomen while the other operator to collect semen by milking the erect copultatory organ or appendages (phallus). However, Mohammed and Nestor (2003), Alhussaini (1998) and Abdel-Aleem (2003) revealed that new devices that would allow only one operator to collect semen were developed. Nestor (2004) suggested that the tip of the thumb and forefinger must be thrust deep into the soft of the base of the pygostyle (parson nose) in order to get behind the cloacal opening and the visible erected copulatory appendages. Erection and ejaculation in a fowl is an immediate response to massage and the bulk of spermatozoa are obtained in the first portion of the ejaculation; characterised by a thick consistency and pearly white colour. As soon as pressure is applied to the erectile copulatory appendages, semen will either squirt or flow readily from the ejaculatory papillae. Lawal (1991) described another method

which he called suction method that allows semen samples to be obtained clearer. Smyth (2000) and Brown (1993) described method of obtaining semen from birds without milking the erect copulatory appendages (phallus). Stewart (1978) described another technique that combines abdominal massage with vacuum pump.

Peter (1984) and Gallagher (1989) advised that males intended for semen extraction for AI should be separated from females for some days before being expected to yield semen artificially. Semen should be collected three times weekly on alternate days to obtain maximum volumes of good quality semen throughout the breeding season.

2.6 Semen Evaluation

A frequent question concerning AI is: What time during oestrus should cows is bred for greatest chance of conception? Since oestrus may last from 10 to 25 hours there is considerable latitude in possible time of insemination. Much research work has been conducted on this subject. Controlled investigations were conducted by Trim Berger and Davis at Nebraska in 1943. These and other studies show that conception rate is lower when cows are bred prior to mid oestrus or later than 6 hours after cessation of oestrus (standing heat in this case). Maximal conception is obtained when cows are inseminated between mid-oestrus and the end of standing oestrus, with good results up to 6 hours after oestrus (Hermen and Swanson, 2001).

Success in insemination timing is dependent upon a good heat detection program. In large herds, this means assigning individual responsibility for heat detection and a continued education program for labour. A successful heat detection program and subsequent proper timing of insemination will pay dividends in increasing reproductive efficiency.

Age at first semen production and semen characteristic of poultry are very important to the farmer. Lake (1988) reported that the semen of poultry immediately after collection should be immersed into water bath kept in a temperature of 38°C. The semen should be evaluated for the following:

i. Volume
ii. Motility
iii. Concentration

iv. Live and dead ratio using eosin-nigrosin stain

v. Percent abnormal sperm using buffer formal saline.

2.6.1 Ejaculated Volume

There are variations in ejaculation volume resulting to species differences, season, age and temperature Joshi (1980). Sexton (1997) added that frequency of semen collection, body weight, nutrition and number of male kept in each pen/cage. More or large amounts of semen are desirable since more semen will be required for extension and insemination purposes.

2.6.2 Motility

Semen motility can be estimated using subjective method of rating semen based on the swirling motion observed when fresh semen is examined under a light microscope. Herman and Swansom (2001) proposed 0 – 5 scale, (0 for no motility and 5 to highest motility). Some other workers have also used scales such as 1 to 6, Leighton (1999), 0 – 4 Smith (1990), Soller (2003) used 0 – 7, 1 – 5 was used by Bedaiwy (2003), 0 – 10 was used by Kurbarov *et al* (1980). The percentages for estimating motility of avian sperm were given by Schramm (1982).

Other methods such as determination of motility using a haemocytometer, the use of time exposed photographs, sperm penetration in thin egg albumen and use of electronic counters amongst others have also been used Kolettis (2003). Sperm motility is important because it proves the fertilizing capacity of spermatozoa (Brazil, 2001)

2.6.3 Semen concentration

Semen concentration may be determined by the direct count method using a haemocytometer Brilliard *et al.* (1985). Brown (2003) suggested the use of a flourometer and Hill (2009) said by determining the Packed Cell Volume (PCV) using micro-haematometer technique. A recommendation was made on the use of spectrophotometer especially where a large number of sample was involved, on account of its result to those of the haemocytometer and observed significant correlations of 76 between the haemocytometer and spectrophotometer techniques, 70 between fluorometer and haemocytometer method and 40 between the coulter counter and the haemocytometer method. In a comparative study of three methods of determining sperm concentration, Stewart *et al* (2009) obtained a highly significant

correlation .96 between photometry and naemecytometer and repeatability estimates of .99, .91, and .99 for haemocytometer, spermocytometer and photometry respectively.

Wilson (1999) reported that the relationship between sperm concentration and fertility is not very obvious. Lawal (1991) reported significant negative correlations (P<.05) between sperm concentration and fertility with artificial insemination and low non-significant positive correlations with natural mating in birds. However, Diamond (2003), reported sperm correlation of .45 between sperm concentration and fertility. Sperm concentration determines the number of spermatozoa introduced into the female reproductive tract during artificial insemination. There are variations in the number of spermatozoa that must be inseminated for maximum fertility. Delong (1998) started that about 100 million spermatozoa must be placed in the virginal to ensure high levels of fertility in chickens for a period of 10 days. Sexton (1997) recommended 100 million spermatozoa for chicken semen diluted 1:2 and 300 million for semen diluted 1:5 for maximum fertility.

2.6.4 Percent Dead Spermatozoa

Irvine (1995) reported that dead and live spermatozoa could be separated by differential staining as the cell membrane of the dead sperm easy permeable to certain stains. A mixture of eosin, opal blue and a phosphate buffer was used. Kamar (1999) used a combination of bromophenol blue, nigrosin and two phosphate buffers on chicken semen and obtained good result while Menkveld (2001) reported poor result with chicken semen when mixture of bromophenol blue and nigrosin was used. Other stains that have been used successfully for differential staining of live and dead spermatozoa are Bengal red and Victoria blue, Bonadonna (1986) and eosin-nigrosin mixture, Blom (1993). Ogasawre (1990) described a wet mount technique which involved the use of trypan blue for differential staining of sperm. Measurement was made on sperm cell membrane integrity by fluorometric assay technique as a means of estimating percent of dead spermatozoa. Measurement of the amount of Glutamic Oxaloacetic transamines (GOT) passed through the extracellular medium by dead spermatozoa, Howarth, (2009). Bilgili (2002) compared the fluorometric technique with the nigrosin-eosin differential staining procedure and with glutamic oxaloacetic transamines (GOT) concentration in seminal plasma and found very highly significant positive correlation ($r = .99$; $P<.001$) was also found between percent dead spermatozoa measured by fluorometric and GOT levels. It was reported by Wilson (1999) that wet amount technique in which tryptophan blue was used lower percent dead spermatozoa than nigrosin-eosin method.

Semen has to be alive to be capable of fertilization. Bilgili (2002) reported a highly significant negative correlation (P<.001) between percent dead spermatozoa and percent fertility (-.055), number of fertile eggs (-0.51) and duration of fertility. Significant negative correlations were also found between percent fertility and percent dead spermatozoa by Chia (2009).

2.6.5 Percent Abnormal Spermatozoa

Warren *et al.* (1990) reported negative correlation between percent abnormal spermatozoa and fertility. According to Thomas (2003), cocks producing semen containing more than 20% abnormal spermatozoa usually tend to be sterile. However, a negative correlation (-0.77) between the incidence of crooked-necked spermatozoa in semen and fertility was found by Saeki (1995) while Bunaciu *et al.* (1978) obtained a correlation of (-0.48) between the proportion of abnormal sperm and egg fertility in turkeys. The incidence of spermatozoa with bent neck was found to be correlated (-0.85) with sperm fertility in fowls by Nelson (2003). Percent abnormal spermatozoa may be determined by sperm counts using a microscope.

2.7 Semen Dilution and Storage

Semen is usually thick and viscous. It contain billions pf spermatozoa and are delicate. It requires a diluent using suitable extender. According to Lake (1989), as soon as semen are ejaculated a proportion of them loss their integrity. They advised that extenders containing buffers like sodium citrate can provide environmental *in vitro*, which is ideal for the survival of spermatozoa.

2.7.1 Semen Dilution Process

Semen dilution is essential in poultry production. This is to increase the volume of semen, so that a given quantity can fertilize more females. It also help to reduce the stress over short distance so as to fertilize female kept remote from the males and to store semen beyond an hour. It also provides a suitable medium that will sustain and protect spermatozoa thus preserving their fertilizing capacity until they are used for insemination (Salisbury *et al*., 1998). The negative effects of dilution according to Nelson *et al.* (2003), dilution reduces the number of spermatozoa per mL of Semen. The type of extender used for dilution of semen may appreciably influence observed levels of fertility (Howarth *et al*., 1999). Ledec *et al.*

(1980) carried out 13 diluents trials on poultry. They found motility of semen was greater than 65% in only 4 of the diluents. According to Shulman *et al.* (1990), Excessive dilution causes dilution of nutritive substances necessary for sperm survival. It can also change cell permeability. Introduction of substances toxic to spermatozoa is another possible effect.

2.7.2 Diluents Preparation

There are different preparations to preserve and extend poultry semen. More preparations contain ingredients that provide one or more of the following; energy, proper osmotic balance, sufficient buffering capability against pH changes, and chelating action against the effect of toxic ions (Mohamed *et al.,* 2003). According to Suidzinka and Luka (2008), the value of egg yolk in preserving semen was discovered by a researcher named Phillipe in 1939. They reported that he used extenders, which was a mixture of egg yolk and 2 phosphate buffers to preserve the fertility of refrigerated bull semen. In 1941, another researcher named Salisbury developed a yolk-citrate extender which contained equal volume of egg yolk and Sodium citrate dehydrates solution. The yolk-citrate diluent has been modified. Thomas (2003) reported that egg yolk protects spermatozoa against cold shock. It also play a part in dehydgenase reactions, provides substrates from sperm metabolism.

More researchers have created alternative proportion of egg yolk and sodium citrate, also, including new ingredients. In 2008, Suidzinska and Luka added prepared a diluent with these ingredients; Sodium glucomate, Potassium citrate, Magnesium citrate, D-Glucose and Distilled water. Another combination of Sodium Citrate, Potassium Citrate, Magnesium Acetate, D-Frutose, Sodium hydrogen phosphate, Sodium dihydrogen phosphate and distilled water. The inclusion of glycine in cock semen extenders was first suggested by Lorenz and Tyler (1990) as means of greatly extending motile life of spermatozoa.

2.7.3 Dilution Ratio

Due to the various effects of semen dilution, many researchers have generated interest in finding suitable dilution rates for semen in poultry production. Sexton (1997) reported that high fertility could be obtained by inseminating chickens weekly with semen diluted 1:4. His reported was backed up with the finding of Lake (1980), who observed no different in fertility in fowls within 1:1 to 1:3 for turkeys. Koll (1980) recommended dilution of 1:3 to 1:5 as optimum for ducks compared with 0, 1:1, 1:2, 1:3 in chickens. Lake (1980) found that

fertility of cock semen declined rapidly with increasing dilution and reached almost 0 at 1:64. Dilution of semen up to 1:9 did not give any significant lower fertility compared to those of lower dilutions, if the dose inseminated contains about 400 million spermatozoa. Steel et al (1998) observed that fertilizing capacity of fowl semen decreased greatly after dilution ratio of 1:10 had been reached. However, Lake (1980) observed 1:8 and 1:12 dilution ration provided the insemination dose that contained 100 million spermatozoa or more, high fertility level could be obtained in chickens with a dilution 1:10.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1. The Experimental Location

The study was carried out at the Poultry Unit of the Research and Teaching Farm of the Delta State University, Asaba Campus. The farm is located on longitude 6°45'E and latitude 6°12'N, and has annual rainfall that ranges from 1800mm to 3000mm and the maximum day temperatures that range from 25.8°C to 32.3°C and relative humidity of 65% (Asaba Meteorological Centre, 2014).

3.2 The Poultry House and its Preparation

One of the poultry houses at the Teaching and Research Farm was used for this study. The poultry house has dwarf walls and is netted fully with ½ inch wire mesh to permit good ventilation. The mesh was neatly fixed to prevent reptiles, rodents and other predators from entering. Both sides of the poultry house were partitioned into 15 unit pens of equal sizes, measuring 1.8m × 1.5m each with 4 birds per pen resulting to a total of 60 birds in the poultry house.

The poultry house and its environment were kept in good sanitary conditions with the interior properly swept, washed, disinfected and allowed to stand for two weeks prior to the arrival of the birds. The birds were raised on deep litter with fresh dried litters of wood shavings spread on the floor of the pens. Used engine oil was poured around the surroundings to prevent ants from entering the experimental house. Heat and ventilation were properly monitored to prevent adverse effects.

3.3 The Experiments

Two experiments were performed in this study. Experiment I investigated the effects of varying levels of dietary blood meal and different frequencies of ejaculation on semen characteristics of Isa Brown cocks as well as the performance and cost benefit of production while Experiment II was on the effects of blood meal and diluents on progressive motility of sperm with storage time.

3.3.1 Experimental Ia: Effect of varying levels of blood meal and frequencies ejaculation on semen characteristics of Isa brown cocks

i) The Experimental Birds and their Management

A total of 60 Isa brown cocks aged 21 weeks were used for this experiment. They were purchased from Adims Farms in Ughelli, Delta State, Nigeria. The cocks were randomly allotted to 5 treatments of 12 cocks each. Each group of 12 cocks was divided into 3 ejaculation frequency group of 4 birds each, which act as replicate. The cocks were weighed before the experiment started and given a pre-treatment feeding regime of commercial feed for a period of two weeks. Thereafter, they were fed on the experimental diets for 8 weeks. The cocks were provided with fresh cool clean water *ad libtum*.

ii) Experimental Diets

Samples of dried blood meal (BM) were analysed for their proximate composition. Based on the information obtained from the proximate analysis, blood meal was incorporated into five dietary treatments T1, T2, T3, T4 and T5 such that blood meal replaced 0%, 25%, 50%, 75% and 100% fish meal respectively. Each diet was formulated to contain approximately 15.96% crude protein and 2,649kcal kg-1 ME. The diets which are presented in Table 3.1 were self-prepared rations formulated with ingredients purchased from local markets.

Notably, the control diet, T1 contained no blood meal while in diets T2, T3, T4 and T5, blood meal were replaced 25%, 50%, 75% a2nd 100% of fish meal respectively (composition in Table 3.1). The diets were based on the following specific ingredients: maize, soya bean meal (SBM), wheat offal (W/O), bone meal, blood meal, fish meal (FM), oyster shell, methionine, lysine, breeder premix and common salt.

iii) Semen Collection and Evaluation

Prior to collecting semen from the cocks, the cocks were subjected to a training period of 1 week using double hand Lumber massage method (Burrow and Quinn, 1937). All the ejaculations were done between the hours of 7:45a.m to 10a.m during the collection period. Upon collection, the cocks were group per dietary treatment of 12 cocks. They were further divided into 3 ejaculation frequency (once weekly, twice weekly and thrice weekly) group of 4 birds each. See Table 3.2.

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A micro syringe was used to measure semen volume. Values were read to the nearest 0.01ml. Semen colour was also assessed directly while sperm progressive motility, sperm concentration, semen pH, ratio of live to dead sperms and sperm abnormality were estimated using methods described below:

- **Mass activity**

It was determined by placing a drop of semen on the pre-warmed microscope slide and examining the movement of seen sperm cells. Mass concentration of the sperm cells are categorised from high concentrations with dark waves, mixed activity with granules and no movement. It was scored as +++ (high), ++ (mixed activity), + (Spermatids seen), 0 (no movement).

- **Mass Density**

This was characterised by the colour of the semen and was assessed as;

Watery 0 – 200,000 sperm cell/cm^3

Opalescent 200,000 – 500,000 sperm cell/cm^3

Milky 500,000 – 1,000,000 spermcell/cm^3

Creamy Above 1,000,000 sperm cell/cm^3 (Sperm rich)

- **Sperm Motility**

Sperm motility was estimated by placing a drop of semen in a pre-warmed (37°C) microscope slide and examining it under a light microscope. Sperm movement based on the swirling motion observed was then subjectively rated, as proposed by Herman and Swanson, (1989) on 0 – 5 scale (0 for no motility, 5 for highest motility).

- **Sperm concentration**

Sperm concentration was determined in both diluted and undiluted semen by direct count using a haemocytometer (Brilliard, 1985).

- **Percent live and dead spermatozoa**

The method for examining the live and dead spermatozoa was counted under a light microscope after; it was expressed as number of sperm per mL of semen. Different stains with eosin, opal blue and a phosphate buffer were used to determine the percentage of live spermatozoa. A drop of semen was mixed with a drop of stains on a microscope slide. With the help of a second slide, a smear was made of the mixture, and quickly dried in the air. The dried smear was then examined under a light microscope at x400 magnification. Spermatozoa with head stained blue were regarded as dead because the dead cell membrane is easily permeable to the stains while those with unstained heads were regarded as live sperm.

iv) Statistical Model

The analysis was based on the following model:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

Where Y_{ijk} = the response variable.

μ = Overall mean

a_i = Effect of the ith diet (i ranging from 1,2,3,4,5)

b_j = Effect of the jth frequency of semen collection (j = 1,2,3)

ab_{ij} = Effect of the interaction of ith diet and the jth frequency of semen collection.

e_{ijk} = Experimental error associated with the experimental determinations.

v) Data Analysis

All data generated were subjected to a two-way analysis of variance (ANOVA) using the General Linear model of IBM's SPSS (version 20) of 2011. Means showing significant differences were separated using the Duncan Multiple Range Test of the same statistical package (Duncan, 1955).

3.3.2 Experiment Ib: Effect of blood meal on performance of Isa brown cocks

a) Experimental animals and management

A total of sixty (60) Isa brown cocks were used for the study. The management techniques were the same as reported in experiment Ia. The poultry house and its environment were kept in good sanitary condition all through the experiment.

b) Experimental Design

The experiment was arranged in a Complete Randomized Design (CRD). The cocks were allotted to 5 experimental treatments. Treatments were designated as T1, T2, T3, T4 and T5.

c) Data Collection

Feed intake was measured daily while average weight gain was recorded weekly on replicate basis for the period of the experiment (8 weeks). Feed conversion ratio was calculated weekly based on the result from feed intake and weight gain. The analyses of the data are as follows:

Average Feed Intake: The initial weight of the feed was taken before feeding the cocks; the left over weight was recorded. The difference between the initial weight of the feed and the left over was recorded as feed intake of the cocks per replicated. Average feed intake was calculated by dividing feed intake by the number of cocks in the replicate.

Average Weight Gain: Firstly, the cocks were weighed on arrival. The weight of the cocks were measured weekly throughout the experiment. At the end of the experiment, the initial weight was subtracted from the final weight of the birds to get the weight gain. The average weight gain per cock was measured by dividing the weight gain of the cocks per replicate by the number of the cocks in that replicate. The weekly weight gain was obtained by subtracting the weight of the cocks at the first week from the weight of the second week.

Feed Conversion Ratio (FCR): This is the ability for the cock to convert the feed taken into flesh**.** This is the ratio of feed intake to weight gain. It is determined by dividing the weight gain per cock per replicate by feed intake.

Economic benefit of Blood meal inclusion in the diets of Isa Brown cocks:

This was determined to ascertain the least-cost diet formulation that will not adversely depress semen characteristics of the cocks. This was based on the cost of the compounded diets, most specifically the protein sources. A lesser cost of protein source was determined and analysed in this study. All feed ingredients of the compounded diets were analysed in relation to the market price as at the time of the experiment. These prices were used to analyse the cost of feed that was consumed per kilogram (kg) weight gain per treatment. The total cost of feed consumed was determined by multiplying feed consumed by cost per kg of feed. the cost differential in relation to the control was also analysed as cost per kilogram weight gain less cost per kilogram weight gain of control diet (T1) while the Relative Cost Benefit was calculated as cost per kilogram (kg) weight gain of control (T1) divided by cost per kilogram (kg) weight gain of experimental diets.

d) Statistical Analysis

The model of the CRD given below:

$$Xij = \mu + Ti + Eij$$

Where;

Xij = the observed value of the response

μ = population mean

Ti = observed effect of the i^{th} dietary treatment

Eij = residual error

All data obtained were subjected to one-way analysis of variance in a completely randomized design and significant means were separated by Duncan's multiple Range Test using SPSS version 20.0.

3.4 Experiment II: The effects of blood meal and diluents on progressive motility of sperm with storage time

This phase involved the preparation of diluents, semen extraction and dilution, semen concentration and sperm motility progression test.

3.4.1 Experimental Location

The study was conducted both at the Poultry Unit of the Teaching and Research farm of Animal Science Department and the Animal Science Laboratory of the Delta State University, Asaba campus, Nigeria.

3.4.2 Experimental Animals and their Management

Fifteen (15) Isa brown cocks aged 28 weeks, and weighing 2.52 - 2.68kg were used for this experiment. The cocks were randomly allotted to 5 treatments of 3 cocks each. The cocks were weighed before the experiment started and given a pre-treatment feeding regime of commercial feed for a period of two weeks. Thereafter, they were fed on the experimental diets for 8 weeks. The cocks were provided with fresh cool clean water *ad libtum*.

3.4.3 Preparation of Diluents

The different diluents used for this study were:

Glucomate Buffer only (GB): This was prepared fresh prior to semen dilution. The mixture of 0.135g of Sodium glucomate, 0.125g of Potassium citrate, 0.108g of Magnesium citrate and 0.008g of D-Glucose. They were dissolved in 100ml of distilled water to obtain the buffer. The pH was taken.

Glucomate to egg yolk (GEY): This is the continuation of the above procedures of the buffer but in this case, after the buffer was obtained, it was mixed with raw egg yolk. The egg used was carefully sterilized by cleaning with70% alcohol thereafter, separating the yolk from the albumen. The diluent was prepared by filling 100ml graduated stopper cylinder with 80ml of the buffer prepared with the ingredients indicated in the formulation of the appropriate extender to 20ml of the egg yolk .0.5ml of antibiotic(penicillin) was added. This was mixed vigorously then centrifuged at 3000rpm. The decounted supernatant was used for the extension. The complete extender was attained on 1:2 bases i.e 0.25ml of semen to 0.50ml of extender. (Watababe *et al.*, 1998).

Trisodium Buffer only (CB): This was prepared using 2.9g of Trisodium citrate dissolved in 100ml of distilled water. The pH was taken.

Trisodium citrate to egg yolk (CEY): 80ml of Trisodium citrate buffer was mixed with 20ml of raw egg yolk. 0.5ml of penicillin was added. It was centrifuged at 3000rpm. The decounted supernactant was used for the extension. 1:2 bases were also used for the complete extender.

3.4.4 Semen Collection and Dilution

All the ejaculations were done between the hours of 7:45a.m to 10a.m during the collection period. The lumber massage method of collection was used. The semen were collected and pooled immediately. The dilution was attained at a ratio of 1:2 bases (semen to diluent). This was prepared within 5 minutes for quick storage while the undiluted semen were monitored under light microscope for as long as the last semen was seen alive right there at the farm. The diluent at 4 different pH were tested simultaneously on split sample of semen. The buffers were chosen and pH determined in order to maintain the concentration of the buffer for effectiveness.

3.4.5 Motility Progressive Test

Sperm motility was determined immediately after semen collection by examining a drop of undiluted semen under a light microscope at 100 x magnification. Each sample examined was rated subjectively on the basis of swirling motion that was observed on a scale of 0 to 100 scales (Leighton *et al*., 1998). A score of 0 was given for no motility and 100 for maximum motility. Diluted sperm motility test was carried out at daily intervals to determine the effect of the buffers and extenders on the semen storage and observe the extent of decrease in motility with increasing time. The semen was stored at 5°C refrigerated temperature.

3.4.6 Experimental Design and Data Analysis

The experiment was arranged in a two way analysis of variance (ANOVA) using the General Linear model of IBM's SPSS (version 20) of 2011. Means showing significant differences were separated using the Duncan Multiple Range Test of the same statistical package. (Duncan, 1955).

3.4.7 Statistical Model

The following model was used:

$X_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$

Where:

X_{ijk} = The observed value of each of the response variables

μ = The overall population mean

a_i = Observed effect of the dietary treatment

b_j = Effect of the jth days of motility progression

ab_{ij} = Effect of the interaction between dietary treatments and days

e_{ijk} = Random residual error due to the experiment

Table 3.1: Composition of the diet for the experimental Isa brown cocks.

Ingredients	T1 0%	T2 25%	T3 50%	T4 75%	T5 100%
Maize	57.00	57.00	57.00	57.00	57.00
Soyabean meal	12.00	12.00	12.00	12.00	12.00
Fish meal	2.00	1.50	1.00	0.50	-
Blood meal	-	0.50	1.00	1.50	2.00
Wheat offal	24.00	24.00	24.00	24.00	24.00
Bone meal	1.50	1.50	1.50	1.50	1.50
Oyster shell	2.50	2.50	2.50	2.50	2.50
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25
Premix*	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	15.05	15.72	15.86	15.92	15.94
M.E (Kcal kg⁻1)	2648.92	2649.15	2649.38	2648.56	2649.85

**vitamin-mineral premix (animal care ltd, Nigeria) provided the following vitamins and minerals per kg of diet: Vit. A,15,000 I.U; Vit. D3, 3,000 I.U; Vit. E, 30 I.U; Vit. K, 2.5mg; Vit. B12, 0.2mg; Niacin 40mg; Pantothenic acid 2.5mg; Folic acid 1.0mg; Biotin 0.08mg, Chlorine 500mg; Monodium 6mg; Iron 24mg; Copper 6mg; Iodine 1.4mg; Selenium 0.25mg; Cobalt 0.4mg; Antioxidant 125mg*.

Table 3.2: Semen Collection Plan

Blood meal at different levels	0%	25%	50%	75%	100%
Semen collection					
Once/week	4	4	4	4	4
Twice/week	4	4	4	4	4
Thrice/week	4	4	4	4	4

Table 3.3 Composition of the Diluents

Stock Solution	Diluent 1(GB)	Diluent 2(GEY)	Diluent 3(CB)	Diluent 4(CEY)
Sodium Gluconate	0.135g	0.135g	-	-
Potassium Citrate	0.125g	0.125g	-	-
Magnesium Citrate	0.108g	0.108g	-	-
Tri-sodium Citrate	-	-	2.9g	2.9g
D-Glucose	0.008g	0.008g		
Distilled water	100mL	80mL	100mL	80mL
Egg yolk	-	20mL	-	20mL
Penicillin(Antibiotic)	-	0.5mL	-	-

CHAPTER FOUR

4.0 RESULTS

4.1. Experiment I: Proximate composition of dried blood meal and experimental diets

The results of the proximate analysis of dried blood meal (BM) and experimental diets are presented in Tables 4.1 and 4.2 respectively. The BM results showed that, the crude protein (CP) content was 78.80%, crude fibre 1.59%. Dry matter (DM) and moisture content were 89.10% and 10.90% respectively, percentage of ash was 3.94, ether extract, 0.62% and nitrogen free extracts (NFE) was 5.72%.

The proximate analysis of the experimental diets which involved partial replacement of blood meal (BM) with fish meal (FM) revealed that percentage of dry matter for treatments 1 to 5 were 87.90%, 88.60%, 87.80%, 89.40% and 88.80% respectively. The percentage moisture in control treatment (0% BM and 100% FM) and treatment 3 (50% BM and 50% FM) were closely valued at 12.10% and 12.20% respectively. However, the ash content was highest in treatment 4 (75% BM and 25% FM) and treatment 5 (100% BM and 0% FM) with score of 4.90% and 4.70% respectively. The control treatment had the lowest score of 2.5%. Crude fibre content was highest (9.60%) in the control diet and lowest (4.70%) in the diet in treatment 5. Ether extract varied from 8.45% in the control diet to 12.23% in treatment 4. The percentage CP was from 15.07% in the control diet to 15.96% in treatment 5.According to Zhang *et al*., 1999 and NRC, 1994, this result falls within the recommended crude protein of cocks at age 20- 25 weeks old. Nitrogen Free Extracts (NFE) in control treatment was slightly higher than in the other dietary treatments.

4.2 Performance characteristics of Isa brown cocks fed varying levels of dietary blood meal

The results shown in Table 4.2 represent the performance characteristics of Isa brown cocks fed with different levels of blood meal. There were no significant ($p>0.05$) differences in the initial body weight among the treatments. However, there were significant ($p<0.05$) differences among treatments in the mean final body weight, mean body weight gain and feed conversion ratio.

Mean final body weight and mean weight gain at treatment 3 were significantly ($p<0.05$) different from T2 but were similar ($p>0.05$) to the control and T5. T2 had the lowest mean

body weight gain. The variations of feed intake showed that the control (0%) was significantly ($p<0.05$) higher than the T2 diet but similar to the T3, T4 and T5%. However, T2 was numerically lower than, but not significantly ($p<0.05$) different from the other treatments. In other words, T1, T3, T4 and T5 were similar ($p>0.05$) in feed intake but T1 was significantly ($p<0.05$) different from the T2. For feed conversion, ratio T3 was significantly ($p<0.05$) lower than T2 but similar ($p>0.05$) to T1, T4 and T5.

The results of the economic benefit of blood meal inclusion in the diets of Isa Brown cocks are presented in Table 4.4. The total feed consumed per bird recorded significant ($p<0.05$) differences among treatments. T1 was significantly ($p<0.05$) higher than the rest treatments. Although, T3, T4 and T5were similar ($p>0.05$) but significantly ($p<0.05$) lower than T2. Variations of the cost of feed consumed per bird in T1 recorded highly significant ($p<0.01$) to the rest treatment. T2 and T5 were similar ($p>0.05$) but significantly ($p<0.05$) lower than T3 and T4. The cost of per kilogram feed (₦/kg) revealed all treatments were significantly ($p<0.05$) different from each other. The control (0%) was the most expensive while T5 was the cheapest. The cost of feed showed significant ($p<0.05$) decrease as the blood meal inclusion was increasing.

The cost/kg of body weight gain (₦) recorded that T1, T2 and T4were similar ($p>0.05$) but, was significantly ($p<0.05$) lower that T3 and T5. T2 had the highest cost/kg body weight gain of ₦298.37 while 50% T3 had the least with ₦241.07. The cost differential body weight revealed that increased in blood meal and decrease in fish meal into the dietary treatment also increase the cost differential. The relative cost benefit scored the highest in T3 (117.68%) while the least was T2 with 95.08%.

4.3 Effect of Blood meal on body weight gain and semen characteristic of Isa brown cocks

Table 4.5 shows the effect of feeding varying levels of blood meal on the mean body weight gain and semen characteristics of Isa brown cocks. The results revealed that there were significant ($p<0.05$) differences in the body weight gain among the dietary treatments. T3 and T5 were significantly ($p<0.05$) higher than the T2, but similar ($p>0.05$) to the control (T1) and T4. The variation of semen volume between the different levels of blood meal inclusion showed, there were no significant differences ($p>0.05$) among the means. However, the control cocks produced more semen (0.30mL) when compared to the other treatment groups.

Variations in dietary blood meal had no significant ($p>0.05$) effect on mean mass activity, sperm motility, semen colour, sperm concentration and percent abnormal sperm.

Percent live sperm scores varied significantly ($p<0.05$) among the levels of blood meal inclusion in the diets. Although feeding blood meal led to a decrease in percent live sperm in the cocks, values obtained were not significantly ($p>0.05$) lower than that of the control group except at T2. However, there were significant ($p<0.05$) differences between T1, T2 and T4. Feeding of blood meal did not reduce percent live sperm below 97% in any of the treatments.

4.4 Effect of frequency of semen collection on semen characteristics of Isa brown cocks

The effect of frequency of semen collection on semen characteristics are presented in Table 4.6. The results revealed that frequency of semen collection had a significant ($p<0.05$) effect on semen volume, mass activity, sperm motility, semen colour, sperm concentration, and percent live sperm. Subjecting the cocks to more than two ejaculations per week significantly ($p<0.05$) reduced mean semen volume, mass activity, sperm motility, semen colour and sperm concentration. Variations in mean mass activity showed significant ($p<0.05$) effect in thrice/weekly, which is lower than once weekly and twice weekly. Mean motile sperm cell was significantly ($p<0.01$) higher in twice weekly and thrice weekly compare to once weekly. In other results, semen colour and sperm concentration increased significantly ($p<0.05$) at the weekly and twice weekly frequencies than thrice weekly. Frequency of semen collection showed that thrice weekly resulted to significant ($p<0.05$) effect higher than those of once weekly and twice weekly.

However, the live sperm percent had no significant ($p<0.05$) effect in among the different semen collection frequencies. The result showed decrease in live sperm with increase in the frequency of semen collection. More so, once weekly recorded the highest live sperm percent (98.20 ± 0.12). Percent abnormal sperm and semen pH of this study were unaffected ($p>0.05$) by variation in the frequency of semen collection.

Table 4.1 Proximate Composition of Dried Blood Meal

Proximate Fractions (%)	Composition
Dry matter	89.10
Moisture content	10.90
Ash	3.94
Crude Protein	78.80
Crude Fibre	1.59
Ether Extract	0.62
Nitrogen Free Extract	5.72

Table 4.2 Proximate Analysis of the experimental diet

Dietary Treatments	Dry Matter (%)	Moisture (%)	Ash (%)	Crude Fibre (%)	Ether Extract (%)	Crude Protein (%)	*NFE (%)
T1 (0%)	87.90	12.10	2.50	9.60	8.45	15.07	49.68
T2 (25%)	88.60	11.40	4.40	8.60	10.93	15.77	42.09
T3 (50%)	87.80	12.20	2.69	5.40	11.05	15.83	42.76
T4 (75%)	89.40	10.60	4.90	4.90	12.23	15.90	40.56
T5 (100%)	88.80	11.20	4.70	4.70	10.53	15.96	37.54

***NFE-** Nitrogen Free Extracts*

Table 4.3 Performance characteristics of Isa brown cocks fed with blood meal in the dietary treatment

*Characteristics	*T1 0%	T2 25%	T3 50%	T4 75%	T5 100%	SEM
Initial body weight	2096.67^{a}	2123.33^{a}	2136.67^{a}	2133.33^{a}	2116.67^{a}	14.64
Final body weight(g)	2550.00^{bc}	2533.33^{c}	2650.00^{a}	2603.33^{abc}	2620.09^{ab}	14.47
Body weight gain(g)	453.33^{ab}	410.00^{b}	513.33^{a}	470.00^{ab}	503.33^{a}	12.69
Feed intake(g)	1657.20^{a}	1620.60^{b}	1637.80^{ab}	1637.80^{ab}	1638.80^{ab}	4.74
Feed conversion ratio	3.71^{ab}	3.92^{a}	3.19^{b}	3.49^{ab}	3.25^{b}	0.09

***The results are calculated per cock. *T1 = Control diet*

Table 4.4 Economic benefit of blood meal inclusion in the diets of Isa brown cocks

*Characteristics	T1/0%	T2/25%	T3/50%	T4/75%	T5/100 %	SEM
Total feed consumed (kg/bird)	1.66^{a}	1.60^{c}	1.64^{b}	1.64^{b}	1.64^{b}	0.05
Cost of total feed consumed (₦)	127.04^{a}	121.65^{d}	123.62^{b}	123.50^{bc}	122.23^{cd}	0.36
Cost/kg feed (₦/k)	76.53^{a}	76.03^{b}	75.53^{c}	75.03^{d}	74.53^{e}	0.35
Cost/kg body weight gain (₦/kg)	283.70^{ab}	298.37^{a}	241.07^{b}	262.21^{ab}	242.50^{b}	7.92
Cost differential body weight gain (₦)	0.00^{b}	14.67^{a}	-42.63^{e}	-21.49^{c}	-18.13^{d}	11.29
Relative cost benefit body weight gain (%)	100.00^{d}	95.08^{e}	117.68^{a}	108.20^{b}	102.56^{c}	3.87

** Mean with different superscripts are significantly different from one another. Mean= Mean + SEM*

Table 4.5 The effect of Blood meal on body weight gain and Semen characteristics

*Semen characteristics	T1	T2	T3	T4	T5	SEM
Bodyweight gain (g)	453.33^{ab}	410.00^{b}	513.33^{a}	470.00^{ab}	503.33^{a}	12.69
Volume (mL)	0.30^{a}	0.23^{a}	0.20^{a}	0.13^{a}	0.13^{a}	0.05
Mass Activity	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	0.03
Motility	73.33^{a}	70.00^{a}	73.33^{a}	70.00^{a}	73.33^{a}	0.41
Semen Colour	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	5.00^{a}	0.05
Live sperm (%)	99.00^{a}	97.33^{c}	97.67^{bc}	98.33^{abc}	98.67^{ab}	0.19
Concentration (x10^{6})	33.33^{a}	34.67^{a}	33.33^{a}	35.33^{a}	35.67^{a}	0.57
pH	6.67^{b}	7.67^{a}	7.67^{a}	8.00^{a}	8.00^{a}	0.19
Abnormal sperm (%)	0.33^{a}	0.33^{a}	0.33^{a}	0.33^{a}	0.33^{a}	0.23

**Mean with different superscripts are significantly different from one another. Mean= Mean + SEM;*
^ On scale: 1 for watery, 5 for creamy; ^^On scale: 0 for no motion, 5 for vigorous wave motion.

Table 4.6 Effects of frequency of semen collection on semen characteristics of Isa brown cocks

*Semen characteristics	Once	Twice	Thrice
Volume(mL)	0.22±0.02^{a}	0.22±0.01^{a}	0.14±0.01^{b}
Mass Activity^	4.88±0.04^{a}	4.83±0.03^{a}	4.30±0.05^{b}
Motility (%)	70.52±0.91^{a}	69.60±0.50^{a}	62.03±0.62^{b}
Mass density^^	5.00±0.00^{a}	5.00±0.00^{a}	4.96±0.01^{b}
Concentration(×10^{6}/mL)	38.27±0.41^{a}	38.74±0.30^{a}	34.83±0.25^{b}
Live sperm (%)	98.20±0.12^{a}	97.99±0.09^{ab}	97.81±0.09^{b}
pH	7.92±0.08^{a}	8.19±0.05^{a}	8.49±0.43^{a}
Abnormality (%)	0.31±0.05^{a}	0.41±0.04^{a}	0.30±0.03^{a}

**Mean with different superscripts are significantly different from one another.Mean= Mean + SEM; ^ On scale: 1 for watery, 5 for creamy; ^^On scale: 0 for no motion, 5 for vigorous wave motion.*

4.5 Interactions between Blood meal and frequency of semen collection on semen characteristics

The interactions between blood meal and frequency of semen collection on semen characteristics of Isa brown cocks are presented in Figure 1. Three ejaculations per week generally led to significant ($p<0.05$) reductions in mean semen volume at each of the levels of dietary blood meal. However, T2 (25% BM) semen volume at thrice weekly ejaculation frequency was significantly ($p<0.05$) lower than once weekly and twice weekly. Interactions at T3(50% BM) revealed that the thrice weekly frequency was significantly ($p<0.05$) different from once weekly and twice weekly, while once weekly and twice weekly scored the same value of 0.19mL.

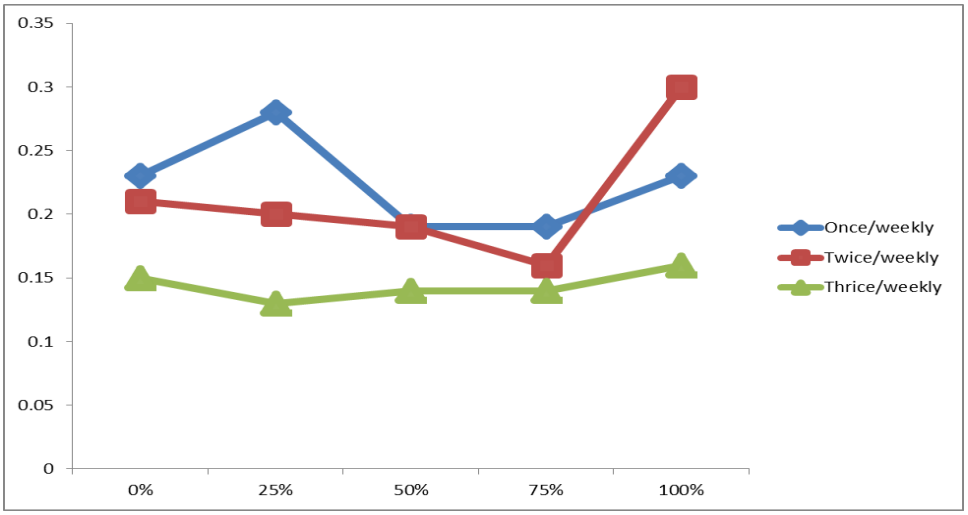

Figure 1: Interaction effects of blood meal on semen volume (mL) with ejaculation frequency

Mass activity of birds fed 0% BM (T1) showed similarity ($p>0.05$) between twice weekly and thrice weekly frequency, but significant ($p<0.05$) difference from once weekly ejaculation. At 25% (T2), 50%(T3), 75% (T4) and 100% (T5) dietary blood meal, mass activity of semen from affected birds showed significant ($p<0.05$) difference between once weekly and thrice weekly insemination frequencies, though, once weekly and twice weekly inseminations are similar ($p>0.05$) (Figure 2).

Figure 2: Interaction effects of blood meal on mass activity with ejaculation frequency

Mean sperm motility at 0% blood meal were similar ($p>0.05$) between twice weekly and thrice weekly but significantly ($p<0.05$) different from once weekly, while proportion of motile sperm was at its best at 74.74% at once a week ejaculation frequency. The results at 25% and 50% blood meal showed once weekly was significantly ($p<0.05$) higher than thrice weekly but similar to twice weekly frequency of ejaculation. At 75% blood meal, subjecting the cocks to ejaculation frequency more than once weekly resulted in significant ($p<0.05$) differences, though, once and thrice weekly frequency were similar ($p>0.05$). Variations at 100% blood meal revealed that scores at twice weekly (71.54%) are significantly ($p<0.05$) higher than once weekly (67.50%) and twice weekly (63.78%). See Figure 3.

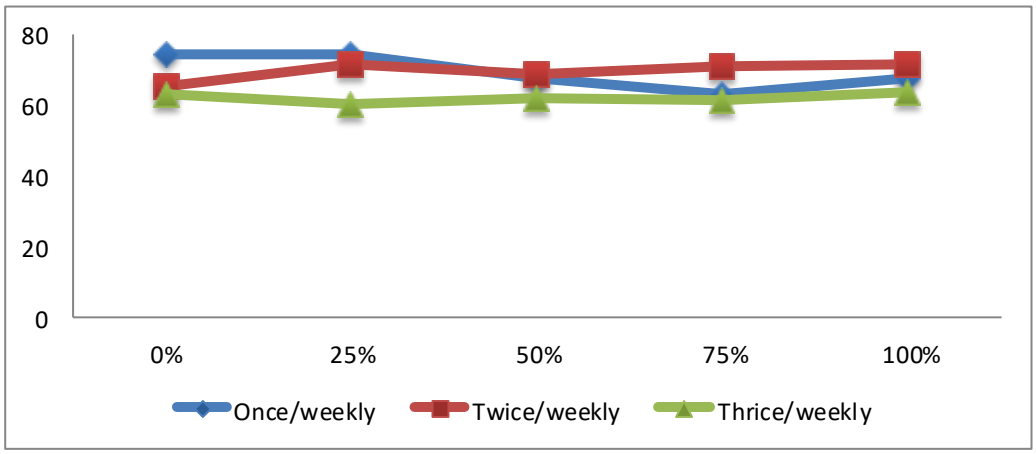

Figure 3: Interaction effects of blood meal on motility with ejaculation frequency

Semen colour was unaffected ($p>0.05$) by variations in blood meal at all levels and frequency of semen collection. The control and 25% blood meal groups had the same values at the different frequencies of semen collection. However, the 50%, 75% and 100% blood meal

groups were similar between once weekly, twice weekly and thrice weekly frequencies of insemination (Figure 4).

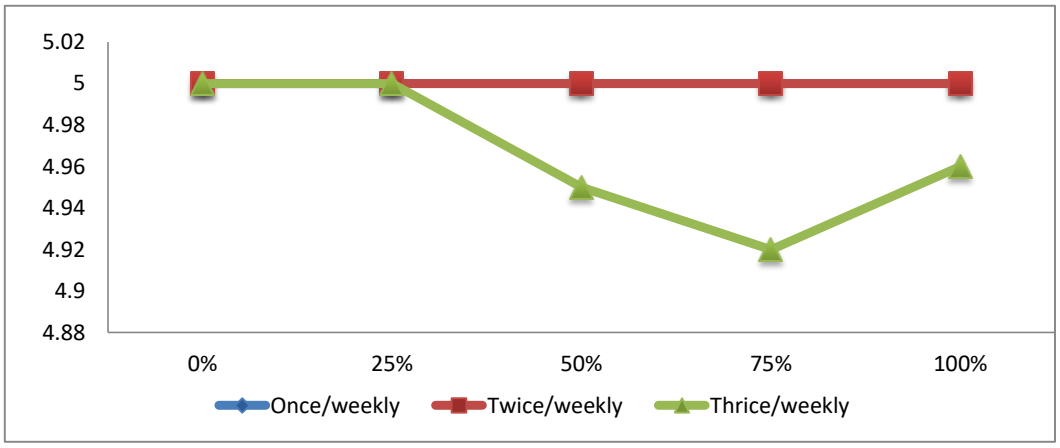

Figure 4: Interaction effects of blood meal on mass density (colour) with ejaculation frequency

Sperm concentration ($x10^6$/mL) at 0% and 25% varied significantly ($p<0.05$) between cocks ejaculated twice weekly and thrice weekly. However, there was similarity ($p>0.05$) between once weekly and twice weekly. At 50% blood meal, once weekly and thrice weekly were significantly ($p<0.05$) higher than twice weekly. The result at 75% BM revealed that thrice weekly was significantly ($p<0.05$) lower than once weekly but similar ($p>0.05$) to twice weekly. On the other hand, 100% BM twice weekly was significantly ($p<0.05$) higher than once weekly and three weekly while three weekly was significantly ($p<0.05$) lower than once weekly (Figure5).

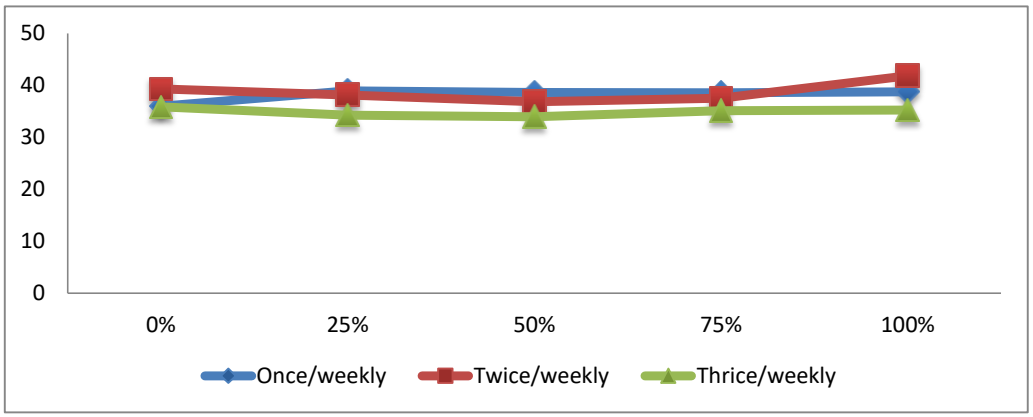

Figure 5: Interaction effects of blood meal on sperm concentration($x10^6$/mL) with ejaculation frequency

The variations of mean percent live sperm at 0% BM showed once weekly and thrice weekly were significantly ($p<0.05$) higher than twice weekly. Results are 25% BM revealed that twice weekly was significantly ($p<0.05$) higher than once weekly and twice weekly. The scores at 50% blood meal for twice weekly was numerically higher, though non-significantly ($p>0.05$), from once weekly and thrice weekly. However, 75% BM showed twice weekly was significantly ($p<0.05$) lower than once weekly though, twice weekly was similar ($p>0.05$) to thrice weekly. In addition, thrice weekly at 100% BM produced a significant ($p<0.05$) decrease in live sperm percent than the other semen collection frequencies

Table 4.7: Interaction between blood meal and ejaculation frequency on semen characteristics

*Blood meal (%)	Ejaculations/ Week	Volume (mL)	Mass Activity	Motility (%)	Semen colour	Conc. (x10^{6}mL)	Live Sperm (%)	pH	Abnormal sperm (%)
0 (T1)	1	0.23±0.03^{a}	5.00±0.00^{a}	74.74±1.18^{a}	5.00±0.00^{a}	36.00±1.01^{ab}	98.32±0.28^{a}	7.74±0.17^{ab}	0.21±0.10^{b}
	2	0.21±0.02^{ab}	4.61±0.12^{b}	65.56±1.51^{b}	5.00±0.00^{a}	39.28±0.74^{a}	97.33±0.26^{b}	8.67±0.12^{a}	0.42±0.08^{a}
	3	0.15±0.02^{b}	4.68±0.08^{b}	63.41±1.34^{b}	5.00±0.00^{a}	35.84±0.45^{b}	98.43±0.16^{a}	7.52±0.12^{b}	0.23±0.07^{b}
25 (T2)	1	0.28±0.05^{a}	5.00±0.00^{a}	74.44±0.61^{a}	5.00±0.00^{a}	38.94±0.86^{a}	97.67±0.37^{b}	8.17±0.17^{a}	0.22±0.10^{b}
	2	0.20±0.00^{b}	4.87±0.05^{ab}	71.62±0.61^{a}	5.00±0.00^{a}	38.14±0.31^{a}	98.35±0.18^{a}	8.11±0.10^{a}	0.46±0.11^{a}
	3	0.13±0.08^{c}	4.42±0.11^{b}	60.47±1.56^{b}	5.00±0.00^{a}	34.26±0.57^{b}	97.65±0.22^{b}	8.19±0.10^{a}	0.26±0.07^{b}
50 (T3)	1	0.19±0.02^{a}	4.80±0.09^{a}	67.50±1.76^{a}	5.00±0.00^{a}	38.60±0.78^{a}	98.00±0.23^{a}	7.60±0.21^{ab}	0.25±0.10^{b}
	2	0.19±0.14^{a}	4.65±0.09^{ab}	68.75±1.25^{a}	5.00±0.00^{a}	36.83±0.78^{b}	98.40±0.16^{a}	7.80±0.10^{ab}	0.38±0.18^{ab}
	3	0.14±0.01^{b}	4.37±0.11^{b}	62.09±1.35^{b}	4.95±0.03^{ab}	38.91±0.67^{a}	97.98±0.20^{a}	8.17±0.13^{a}	0.42±0.16^{a}
75 (T4)	1	0.19±0.02^{a}	5.00±0.00^{a}	63.33±2.18^{b}	5.00±0.00^{a}	38.56±1.16^{a}	98.67±0.20^{a}	8.28±0.18^{b}	0.22±0.10^{b}
	2	0.16±0.01^{ab}	5.00±0.00^{a}	71.35±0.88^{a}	5.00±0.00^{a}	37.51±0.48^{ab}	97.86±0.16^{b}	8.35±0.08^{b}	0.35±0.08^{ab}
	3	0.14±0.08^{b}	4.10±0.17^{b}	61.54±1.62^{b}	4.92±0.04^{a}	35.13±0.53^{b}	98.03±0.15^{ab}	10.49±2.36^{a}	0.42±0.16^{a}
100 (T5)	1	0.23±0.05^{ab}	4.65±0.13^{a}	67.50±2.20^{b}	5.00±0.00^{a}	38.75±0.68^{b}	98.55±0.23^{a}	7.85±0.15^{b}	0.60±0.15^{a}
	2	0.30±0.03^{a}	4.65±0.13^{a}	71.54±0.78^{a}	5.00±0.00^{a}	41.82±0.64^{a}	98.18±0.24^{a}	8.08±0.11^{ab}	0.46±0.11^{ab}
	3	0.16±0.02^{c}	4.51±0.08^{b}	63.78±1.07^{c}	4.96±0.03^{ab}	35.22±0.54^{c}	97.20±0.20^{b}	8.40±0.09^{a}	0.29±0.07^{b}

* Within each protein level, means with different superscripts in the same column are significantly different from one another. Mean= Mean + SEM; ^ On scale: 1 for watery, 5 for creamy; ^^On scale: 0 for no motion, 5 for vigorous wave motion

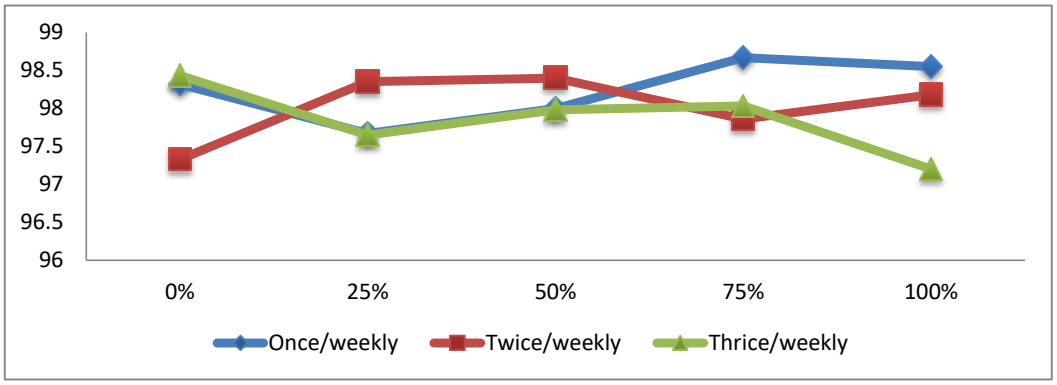

Figure 6: Interaction effects of blood meal on Live Sperm (%) with ejaculation frequency

Mean semen pH scores at 0% blood meal were significant ($p<0.05$) at twice weekly and thrice weekly ejaculation frequency. The results at 25% and 50% blood meal revealed that thrice weekly was numerically but non-significantly ($p>0.05$) higher than once weekly and twice weekly. At 75% and 100% blood meal, thrice weekly was significantly ($p<0.05$) higher than once weekly and twice weekly. Though, they were generally slightly alkaline from 7.44±0.17 to highly alkaline at 10.49±2.36 among blood meal treatments.

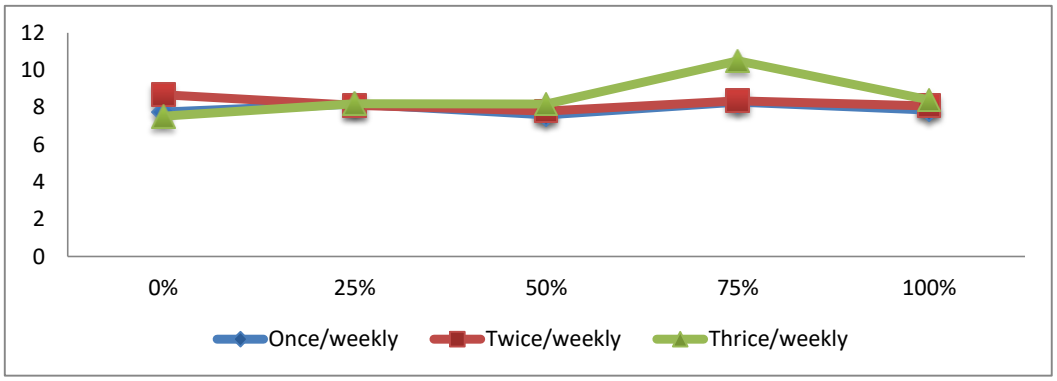

Figure 7: Interaction effects of blood meal on semen pH with ejaculation frequency

Furthermore, abnormal sperm result showed that blood meal at 0% and 25% for twice weekly were significantly ($p<0.05$) higher than once weekly and thrice weekly. The case was different at 25% and 50% blood meal , thrice weekly scores were higher and had significant ($p<0.05$) effect on once weekly. Variations at 100% blood meal showed once weekly was significantly ($p<0.05$) higher than twice weekly and thrice weekly.

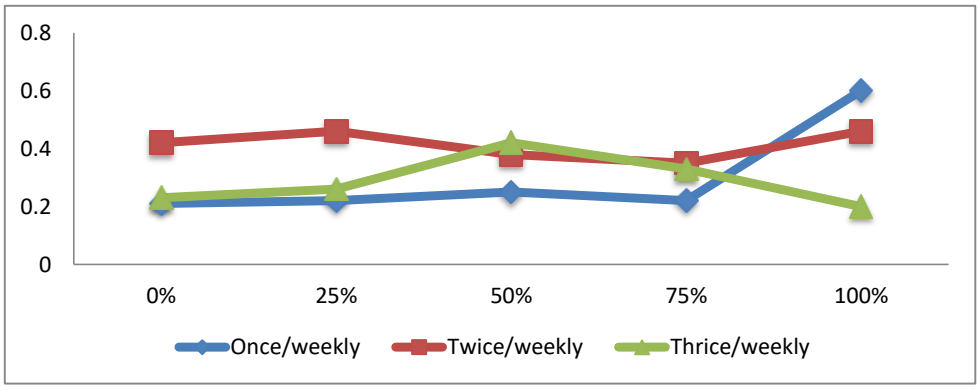

Figure 8: Interaction effects of blood meal on Abnormal sperm cell with ejaculation frequency

4.6 The effects of blood meal on progressive motility of sperm with storage time

The result of the effect of blood meal on progressive motility of sperm with storage time on Isa brown cocks is shown in Table 4.6. At day 1, the result were numerically higher, though non-significantly ($p>0.05$) different among dietary treatments. However, day 2 and 3, at 50%, 75% and 100% blood meal were significantly ($p<0.05$) higher than 0% and 25% blood meal. Results at day 4 show that they are numerically but non-significant ($p>0.05$) higher than the control (0%). Some motility was observed at day 5, but not significantly ($p>0.05$) different from all the others.

4.7 The effects of diluents on progressive motility of sperm with storage time

The result of the effects of diluents on progressive motility of sperm with storage time is presented in Table 4.7. There were no significant ($p>0.05$) differences among diluents between day 1 and day 5. However, mean progressive motility of sperm for Glucomate-buffer (GB) was significantly ($p<0.05$) different from the rest of the diluents at day 2. Values obtained were 50.67±1.82, 54.67±1.65 and 55.33±1.65% for CEY, CB, GEY respectively. GB scored the lowest motility at 48.00±2.00%. In days 3 and 4, CEY and GEY were significantly ($p<0.01$) higher than CB and GB while there was no sperm motility in CB and GB extenders. In addition, day 5 witnessed 0.67% sperm motility under diluent GEY while other diluents were without sperm motility.

4.8 The interaction between blood meal and diluents on progressive motility of sperm with storage time

The interaction between blood meal and diluents on progressive motility of sperm with storage time is presented in Table 4.8.

At 0% blood meal, the values had no significant ($p>0.05$) differences in sperm motility at days 1, 2 and 5. However, the differences were highly significant ($p<0.01$) at days 3 and 4. Sperm motility on day 3 was similar ($p>0.05$) between CEY and GEY but values obtained were highly significantly ($p<0.01$) higher than those recorded for CB and GB. In day 4, sperm motility for CB and GB buffers were similar ($p>0.05$).

For cocks fed 25% blood meal, differences in sperm motility were not significant ($p>0.05$) for the first two days, while day 3 recorded significant ($p<0.05$) differences between extenders, although CB was similar ($p>0.05$) to GB. On day 4, no motility was observed for semen in CB and GB buffers while motility for GEY was slightly but non-significantly ($p<0.05$) higher than that of CEY. Also, day 5 recorded sperm cell motility only in GEY extender while the rest had no sperm cell motility.

Variations in sperm motility of cocks fed 50%, 75% and 100% blood meal were not significant ($p>0.05$) on days 1, 2 and 5. At these levels of dietary blood meal, GEY consistently supported highest motility when compared to the other diluents on all days except on day 5 in which no diluent supported sperm motility. On days 3 and 4, the egg yolk-based diluents (CEY and GEY) supported higher levels of sperm motility than the buffers (CB and GB). Sperm motility on days 3 and 4 was similar for CEY and GEY while the latter was significantly higher than values obtained for CB and GB. On day 4, motility of semen diluted with GEY was significantly ($p<0.05$) higher than that of semen diluted in CEY only for birds fed 50% blood meal, while semen in CB and GB were no longer motile for all levels of blood meal. No sperm cell motility was recorded among diluents on day 5.

Table 4.8 Effects of blood meal on progressive motility of sperm with storage time

DAYS	0% (T1)	25% (T2)	50% (T3)	75% (T4)	100% (T5)
1	67.50±1.31^{a}	67.50±1.31^{a}	70.00±1.74^{a}	70.00±1.74^{a}	68.33±1.67^{a}
2	49.17±1.49^{b}	49.17±1.93^{b}	52.50±2.18^{ab}	55.83±2.29^{a}	54.17±2.29^{a}
3	21.67±3.86^{c}	25.00±3.80^{bc}	31.67±3.22^{ab}	30.83±5.43^{ab}	34.17±3.79^{a}
4	5.83±2.29^{a}	7.50±2.79^{a}	7.50±3.05^{a}	10.00±3.48^{a}	8.33±2.71^{a}
5	0.00±0.00^{a}	0.83±0.83^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Mean with different superscripts are significantly different from one another. Mean= Mean+SEM

Table 4.9: Effect of diluents on progressive motility (%) of sperm with storage time

*DAYS	CEY	CB	GEY	GB
1	70.00±1.38^{a}	68.00±1.45^{a}	69.33±1.18^{a}	67.33±1.18^{a}
2	54.67±1.65^{a}	50.67±1.82^{ab}	55.33±1.65^{a}	48.00±2.00^{b}
3	38.67±2.56^{a}	19.33±2.67^{b}	38.67±2.74^{a}	18.00±2.96^{b}
4	14.00±2.35^{a}	0.00±0.00^{b}	17.33±1.82^{a}	0.00±0.00^{b}
5	0.00±0.00^{a}	0.00±0.00^{a}	0.67±0.67^{a}	0.00±0.00^{a}

*Mean with different superscripts are significantly different from one another. Mean= Mean + SEM; CEY- Trisodium citrate to egg; CB- Trisodium Buffer; GEY- Glucomate to egg; GB- Glucomate Buffer.

Table 4.10 The interaction between blood meal and diluents on progressive motility of sperm with storage time

*Blood meal (%)	Diluent	Day 1	Day 2	Day 3	Day 4	Day 5
T1 (0)	CEY	70.00±0.00^{a}	50.00±0.00^{a}	30.00±5.77^{a}	10.00±5.77^{ab}	0.00±0.00^{b}
	CB	66.67±3.33^{a}	46.67±3.33^{a}	10.00±5.77^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	GEY	66.67±3.33^{a}	53.33±3.33^{a}	30.00±5.77^{a}	13.33±3.33^{a}	0.00±0.00^{b}
	GB	66.67±3.33^{a}	46.67±3.33^{a}	16.67±8.82^{b}	0.00±0.00^{b}	0.00±0.00^{b}
T2 (25)	CEY	66.67±3.33^{a}	53.33±3.33^{a}	33.33±6.67^{a}	13.33±6.67^{ab}	0.00±0.00^{b}
	CB	66.67±3.33^{a}	50.00±5.77^{a}	23.33±3.33^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	GEY	70.00±0.00^{a}	50.00±0.00^{a}	33.33±6.67^{a}	16.67±3.33^{a}	3.33±3.33^{a}
	GB	66.67±3.33^{a}	43.33±3.33^{a}	10.00±5.77^{b}	0.00±0.00^{b}	0.00±0.00^{b}
T3 (50)	CEY	66.67±3.33^{a}	50.00±5.77^{a}	36.67±3.33^{ab}	10.00±5.77^{c}	0.00±0.00^{b}
	CB	73.33±3.33^{a}	f53.33±3.33^{a}	23.33±3.33^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	GEY	70.00±0.00^{a}	60.00±0.00^{a}	43.33±6.67^{a}	20.00±5.77^{a}	0.00±0.00^{b}
	GB	70.00±0.00^{a}	46.67±3.33^{a}	23.33±3.33^{b}	0.00±0.00^{b}	0.00±0.00^{b}
T4 (75)	CEY	73.33±3.33^{a}	60.00±0.00^{a}	46.67±3.33^{a}	20.00±5.77^{a}	0.00±0.00^{b}
	CB	66.67±3.33^{a}	53.33±3.33^{a}	16.67±8.82^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	GEY	73.33±3.33^{a}	60.00±5.77^{a}	46.67±3.33^{a}	20.00±5.77^{a}	0.00±0.00^{b}
	GB	66.67±3.33^{a}	50.00±0.00^{a}	13.33±6.67^{b}	0.00±0.00^{b}	0.00±0.00^{b}
T5 (100)	CEY	73.33±3.33^{a}	60.00±3.33^{a}	46.67±3.33^{a}	16.67±3.33^{a}	0.00±0.00^{b}
	CB	66.67±3.33^{a}	50.00±5.77^{a}	23.33±6.67^{c}	0.00±0.00^{b}	0.00±0.00^{b}
	GEY	66.67±3.33^{a}	53.33±3.33^{a}	40.00±5.77^{ab}	16.67±3.33^{a}	0.00±0.00^{b}
	GB	66.67±3.33^{a}	53.33±6.67^{a}	26.00±6.67^{bc}	0.00±0.00^{b}	0.00±0.00^{b}

*Within each protein level, means with different superscripts in the same column are significantly different from one another. Mean= Mean + SEM; CEY-Trisodium citrate to egg; CB- Trisodium Buffer; GEY- Glucomate to egg; GB- Glucomate Buffer.

CHAPTER FIVE

5.0 DISCUSSION

5.1. Proximate composition of blood meal and experimental dietary treatments

The proximate composition of blood meal (BM) showed that dry matter, ash, crude protein, crude fibre, ether extract and nitrogen-free extract (NFE) occurred in relatively high amounts when compared with the report of Tabinda *et al*. (2007). With a crude protein value of 78.80%, BM is a high protein feed ingredient which can be of great value in reducing production costs in commercial poultry enterprises by substituting it with the more expensive commercial fishmeal.

There was a progressive increase in crude protein (CP) as dietary BM was used to replace 0% to 100% dietary fishmeal due to the fact that the protein content of blood meal (78.88%) is higher than that of fishmeal (65.00%). Consequently, dietary crude protein content increased slightly from 15.07% in the control treatment (0% BM and 100%FM) to 15.96% in treatment 5 (100% BM and 0% FM), and fall within the 15-18% dietary crude protein recommended for breeding chicken cocks (Zhang *et al.,* 1999).

5.2 Performance characteristics of Isa brown cocks fed with blood meal ration.

Data showing the effects of varying levels of dietary blood meal on growth performance of the cocks is shown in Table 4.2a. There were no significant ($p>0.05$) differences in the initial body weights among treatments. The final body weight, body weight gain, feed intake and feed conversion ratio varied between diets. Significant ($p<0.05$) differences were observed in final body weight and body weight gain in treatment 2 and treatment 3. In other words, cocks fed with diets containing 50% BM and 50% FM had the highest final weight and body weight gain. It appears that an equal ration of blood meal and fish meal produces a complementary effect which produced a better quality diet. This may be due to a possible improvement in the protein quality in the diet since blood meal is rich in lysine while fish meal is rich in methionine (AOAC, 2000, Khawaja *et al.,* 2007).

Furthermore, a significantly higher ($p<0.05$) score in average feed intake was observed in the control diet when compared to treatment 2. Their feed intake was the highest, but it did not show best performance on body weight gain. The differences among cocks of the latter three treatments (Treatments 3, 4 and 5) were non-significant although, numerically, higher amount

of feed intake were observed as inclusion of blood meal increases. This is against Castell *et al*., (2004) who stated that supplementation of broiler chicken diet with more than 3% blood meal had negative effect on feed intake and body weight gain of broilers.

Feed conversion ratio is a function of feed intake and body weight gain (Miles and Jacob, 1990). Best feed conversion ratio per bird was observed in treatment 3 (3.19) whereas poor feed conversion ratio was noted in treatment 2 (3.92). The data when subjected to analysis of variance revealed that treatment 2 was found to differ significantly ($p<0.05$) among cocks fed the different treatments. The result also indicated that the cocks on treatment 3 were more efficient than cocks in treatments 1, 2, 4 and 5. These results are in agreement with those of Naurantelli *et al*., (1987), who observed that significant differences were observed when birds were fed with different levels of blood meal. This attests to the quality of blood meal which possesses positive attributes of high quality protein as revealed by Khawaja *et al.*, (2007).

The economic benefit of blood meal inclusion in the diets of Isa brown cocks as shown in Table 4.2b recorded that the cost of total feed consumed per cock was significantly higher than the rest treatments. The scores at 50%, 75% and 100% BM were similar ($p>0.05$) with no effect. Cost/kg of feed decreases as the inclusion of blood meal increases and fish meal decreases. This is because blood meal is a cheaper source of protein; it produces a lower cost of feed when used to replace fish meal. This agrees with the findings of Sastry and Thomas (2012) who stated that the objective of producing poultry is to convert the low quality feeds like cereal grains, oil cakes and other farm by-product into high quality food like meat and eggs. Cost/kg feed per bird at treatment 1 was N76.53 while treatment 5 had a lower cost of N74.53. This is so because cheaper BM decreases the cost of feed. The return of feed production implies that N2.00 was realised for each bird fed with 100% BM. According to Adebambo *et al.*, (2010), to maximize profit, there must be a means of reducing cost of feed without compromising quality.

The cost differential scores had negative values in 50%, 75% and 100% BM level; it indicated that the amount of feed production cost is cheaper than the control treatment. It can be said that it is cheaper to include blood meal than fish meal in the diet of the birds.

5.3 Effect of Blood meal on body weight gain and semen characteristic of Isa brown cocks

The body weight gain of cocks differed, when blood meal (BM) instead of dietary fish meal (FM) was replaced in the experimental diet. The body weight gain of cocks fed the diet with 50% substitution of FM by BM (Treatment 3) was significantly ($P<0.05$) higher than cocks fed the diet with 25% replacement (Treatment 2). In other words, heavier body weight in cocks that consumed equal ration of 50% BM and 50% FM (513.33g) than cocks fed with 25% BM and 75% FM (410g). The body weight gain did not increase the mean semen volume in treatment 3, although, the value obtained met the recommended range of 0.25-0.20mL according to Cerolini *et al*., (2003). It is also in line with Roghanyeh *et al*, (2015) and Zhang *et al*, (1999). Among treatments, numerically, the overall mean body weight gain has not drastic effect on semen characteristics. In other words, the body weight gain had no direct influence on the semen characteristics. The result in Table 4.3 showed semen volume, mass activity, sperm motility, semen colour, sperm concentration and abnormal sperm in cocks didn`t influence by substitution ratio of blood meal and fish meal. The results suggest that blood meal inclusion into the trial diet were inferior to these semen characteristics. This is in line with the study of Ndubuisi *et al*., (2014) that reported dietary protein does not have effect on sperm motility but contrary to Lious *et al*., (1994), who recorded low protein intake results to poor semen characteristics standards. Also, Ladokun *et al*., (2013) who reported low motility resulted to low abnormal cell.

However, Live sperm cell in the control diet was significantly ($p<0.05$) higher than treatment 2. This may be influenced by the fish meal in the dietary treatment. There were similarity ($p>0.05$) between 50%, 75% and 100% BM with numerical value of 97.67, 98.33 and 98.67. Dietary treatment showed that increasing the dietary blood meal level affected the semen pH. It showed that the control diet was significantly ($p<0.05$) lower than the rest treatments. The scores range from 6.67 – 8.00. This falls within the standard range (6 – 9) of a good quality semen sample according to Osinowu, (2006).

5.4 Effect of frequency of semen collection on semen characteristics of Isa brown cocks

The results presented in Table 4.4 revealed that frequencies of semen collection on semen characteristics significantly ($p<0.05$) affected the output of volume, mass activity, motility, colour, mass concentration and live sperm. There were reductions in their semen output as the ejaculation frequency increases. This confirms the report of Umesiobi and Iloeje, (1999),

Iheukwumere *et al*., (2001), who had similar results on their output. Numerically, Semen concentration was higher on twice weekly (38.74) than once weekly (38.27) and thrice weekly (34.83). This affirms the report of Jones *et al*., (1977), who revealed that concentration of spermatozoa varies as a result of method of collection and ejaculation volume. However, the semen pH did not have significant ($p>0.05$) effect on the frequency of semen collection. This is in line with Allanah and Bratte, (2015) findings. Numerically, the pH of thrice weekly were more alkaline than once weekly and twice weekly. The pH scale increases as the ejaculation frequency increases resulting from 7.92 to 8.49. This finding is contrary to Osinowo (2006) and Suidinka (2008), they reported that semen do not need to be too alkaline in nature, a scale of 6.5-6.9 is the normal semen pH but chicken can tolerate pH range of 6.0-8.0.

There were no significant ($p>0.05$) differences on Abnormal sperm cell percent. Though, high abnormality was observed on twice weekly semen collection. This is against the report of Dauda (1984), Iheukuwere and Okere (1990) that frequency of semen collection can reduce percent of abnormality. Their reason was that frequency ejaculation could lead to disturbance of spermatogenesis.

5.5 Interactions between Blood meal and frequency of semen collection on semen characteristics

The result in Table 4.5 showed that variations in semen volume between frequencies of semen collection at the control of the dietary treatment were significantly ($p<0.05$) higher on cocks on once weekly than thrice weekly at each levels of dietary treatment except treatment 5 (100% BM), where twice weekly was significantly ($p<0.05$) higher than thrice weekly but similar ($p>0.05$) to once weekly. However, Treatment 2 (25% BM) semen volume at thrice weekly ejaculation frequency was significantly ($p<0.05$) lower than once weekly and twice weekly. Interactions at T3(50% BM) revealed that the thrice weekly frequency was significantly ($p<0.05$) different from once weekly and twice weekly, while once weekly and twice weekly scored the same value of 0.19mL.

Frequency of semen collection at each level of blood meal had significant ($p<0.05$) effect on mass activity. Once/weekly were significantly ($p<0.05$) higher among dietary treatments while thrice/weekly were significantly ($p<0.05$) lower except in the control diet, which recorded (4.68) higher than twice/weekly (4.61).

Semen motility differ significantly ($p<0.05$) among dietary treatment and ejaculation group. Twice weekly in treatments 3, 4 and 5 were significantly ($p<0.05$) higher than treatment 1 and 2. This is contrary to the report of Zahradden *et al.*, (2005) that no significant effect of ejaculation frequency on sperm cell motility. There was stability at the outcome of semen colour. In other words, neither the blood meal nor ejaculation frequency affects the cock semen colour at that particular time.

Variations in sperm concentration between ejaculation frequencies were significantly ($p<0.05$) lower in cocks on thrice weekly at difference level of dietary treatment expect a rare case in treatment 3 (50% BM and 50% FM). The reduction in sperm concentration on thrice weekly may be attributed to high ejaculation frequency, also low semen volume recorded earlier. This is in line with Brilliard *et al.*, (1985), that increases in spermatozoa is attributed by the semen volume and concentration of spermatozoa. This is against the report of Mkpughe and Bratte (2015) who revealed that no significant differences in sperm concentration between ejaculation frequencies but in agreement with Zahradden *et al.*, (2005).

Live sperm cell recorded significant ($p<0.05$) differences between ejaculation frequency and dietary treatments except treatment 3. Cocks had highest live sperm (98.67%) at once weekly of treatment 4 (75% BM and 25% FM) than the rest dietary treatment. This treatment group showed similarity ($p>0.05$) between them. It can be said; they had better adaptation to more ejaculation frequency than the rest group. The time of semen collection did not affect the semen pH on dietary treatments 1, 2 3 and 5. Thrice weekly of treatment 4 was significantly ($p<0.05$) higher than once and twice weekly. This group also recorded the highest value of semen pH of 10.49. High ejaculation frequency has drastic effect in the semen pH causing high alkaline scores which could form base.

Abnormal sperm cell percent scores varied significantly at all dietary level. It recorded the highest in treatment 5 100% BM and 0% FM) group of once weekly (0.60%). The lowest was revealed in 0% BM of once weekly (0.21%). Obviously, this result showed that blood meal has effect in sperm cell by producing more abnormal sperm cell when compared with the control.

5.6 The effects of blood meal on progressive motility of sperm with storage time

The effects of blood meal on progressive motility of sperm with storage time as shown in Table 4.6 revealed that cocks fed with 25% BM and 75% FM level (treatment 2) had the longest life span of sperm cells. However, there were no significant ($p>0.05$) differences among treatments means sperm motility at day one. This may be as a result of the effect of the ration of blood meal and fish meal trial on the dietary treatment in the two groups. At day two, there were general reductions in sperm motility. The result revealed significant ($p<0.05$) differences among treatments where the control diet and treatment 2 were significantly lower than the rest treatment groups. Treatment 4 and 5 were significantly ($p<0.05$) different from 0% and 25% though, 75% BM were similar ($p>0.05$) to 50%and 100%. The scores at day 3 showed 50%, 75% and 100% BM were similar though, 100% BM was significantly ($p>0.05$) different from 0% and 25% BM. This affirms the dietary treatment effect of a better ration composition of fish meal with blood meal. 0% blood meal group scored the least sperm motility of 21.67%. Day4 showed no significant ($p>0.05$) difference between treatments but numerically, 75% BM scored the highest sperm motility of 10% while the least was 5.83% as recorded in the control. These scores may also be influenced by the blood meal utilization in the dietary treatment. There were no sperm cell motility in Day 5 except in 25% BM which recorded 0.83% although, no significant ($p>0.05$) effect between the treatment groups. The poor results of the sperm cell durability may be attributed to poor handling as a result of in consistency in power supply to store the semen at 5℃ refrigerated temperature to attain its best result and longevity.

5.7 Effect of diluents on progressive motility (%) of sperm with storage time

There were no differences in the sperm motility parameters between samples diluted in CEY- Trisodium citrate to egg; CB- Trisodium Buffer; GEY- Glucomate to egg; GB- Glucomate Buffer at day 1 of storage at 5℃ refrigerated temperature. Though, CEY had the highest percentage of sperm cell of 70% while GB had the least with 67.33%. This value is comparable to the result of Peters *et al*., (2008). They reported that sperm cell of fresh semen from different chicken varied from 60% – 90%.mean sperm cell motility for day 2 showed GB diluents were significant. The proportion of spermatozoa in the samples diluted significantly decreases. Promising fertility of sperm motility at maximum of 55% was obtained after 24hours storage. This is similar to Pelge (1955) he used Glucose-citrate solution as a diluent and obtained 50% motility after 24 hours of storage.

Furthermore, at day 3 and 4, diluent GEY and CEY showed highly significant ($p<0.01$) differences when compared to GB and CB. Progressive decrease in sperm motility is observed in the various diluent used. This may be due to the specific composition of the commercial extenders. According to Vasicek *et al.,* (2015), they reported that, the diluent might decrease the sperm motility in order to preserve enough energy for the moment when spermatozoa will reach the female reproductive system. In addition, day 5 witnessed only 0.67% sperm cell motility with GEY diluent; other diluents were without sperm cell. This poor result can be as a result of temperature alteration due to inconsistent power supply. This can result to damage of spermatozoa at high temperature for storage. Watson and Morris (1987) reported that exposing sperm cell to temperature in the range of 0 - 20℃ may cause damage. Contrary to this report, Slanina *et al*., (2011) stated that the use of low temperature (4 - 8℃) for long term storage of turkey spermatozoa could maintain better motility than higher storage temperature.

5.8 The interaction between blood meal and diluents on progressive motility of sperm with storage time

The relationship between the dietary treatments and the different diluents did not show any significant ($p>0.05$) difference at day 1 and 2. This means the blood meal and diluent did not affect the spermatozoa. The effect was visible at day 3 and 4. The extenders (GEY and CEY) were significantly higher than the buffers (GB and CB) in all dietary treatment. As the days of storage increases, the motile sperm cell decreases. This might be attributed to the storage facility or diluent composition. The buffer had reduced spermatozoa activity than the extenders. Day 4 scored no spermatozoa in the buffer solutions. This shows that the extenders had better storage capability. Only 3.33% sperm cell motility was found at 25% blood meal x GEY. The rest had no sperm cell.

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATION AND CONTRIBUTION TO KNOWLEDGE

6.1. Conclusion

The cocks readily accepted all dietary treatment although; it appears that a combination of 50% blood meal and 50% fish meal in treatment 3 produced a complementary effect which raised the quality of the experimental diet. In other words, it had better feed utilisation efficiency than the rest treatments. The data when subjected to analysis of variance revealed that cocks fed with 25% blood meal (BM) and 75% fish meal (FM) group (treatment 2) was found to differ significantly ($p<0.05$) among cocks fed the different dietary treatments. Treatment 2 had the least body weight gain, least feed intake and highest feed conversion ratio. The body weight gain and semen characteristics were not affected significantly by the different dietary treatments although, numerically, they met the recommended requirements of a good semen quality. This is so because semen quality is not an entity on its own; it is influence by the semen characteristics.

This study revealed that ejaculation frequency of the cocks had effect on semen quality (expressed as sperm motility and percentage of live sperm) and semen quantity (expressed as sperm concentration and semen volume). The highest sperm motility and percentage live sperm cells were observed when semen was ejaculated once a week. Furthermore, once weekly and twice weekly ejaculation frequencies had better performances on semen volume and sperm concentration.

An appropriate dietary crude protein level and ejaculation frequency regimen is considered for improving semen production in broiler breeder cocks. In relation to the effects of blood meal and ejaculation frequencies on semen characteristics, once a week and twice weekly semen collection showed superiority in semen volume at 0%, 50%, 75% and 100% BM level (treatments 1,3,4 and 5) and they will significantly higher than thrice weekly. Variations in mass activity and sperm motility were significantly lower in thrice/weekly at all levels of dietary treatment. Highest motile cell was recorded in the control diet (0% BM, 100% FM) of once weekly. In addition, semen colour was not affected by the dietary treatment and ejaculation frequencies. It maintained its creamy colour of a rich semen quality. Treatment 5 (100% BM, 0% FM) of twice weekly recorded the highest sperm concentration while

percentage of live sperm was at its peak at once weekly of treatment 4 (75% BM and 25% FM).

The effect of blood meal and diluent types on sperm cell storage was investigation through the variations motile sperm cell. The blood meal did not show any effect on the spermatozoa at days 1, 4 and 5. The effect was observed at days 2 and 3, where sperm motility was significantly higher at 50%, 75% and 100% blood meal group. As the storage days increases, the motile sperm cell decrease in all dietary treatments and diluent types. The buffers (Trisodium-CB and Glucomate-GB) had reduced spermatozoa activities when compared with the extenders (Trisodium citrate to egg-CEY and Glucomate to egg-GEY) with significantly higher effect on days 3 and 4. From the result, it could be concluded that Glucomate to egg solution is a better diluent with potentials to store sperm cell because of its longest duration of sperm cell recorded in this study.

6.2 Recommendation

- Blood meal has significant effect on semen quality as regard to its result on semen characteristics. It is advisable to include blood meal at the maximum of 2kg per 100kg of feed for cocks between 20-25 weeks old, since these results showed better result on semen characteristics.
- Poultry farmers especially breeders should adopt the use of blood meal as a substitute of fish meal since it is richer in crude protein and contains good amount of amino acids. Blood meal also does not compete with human for food.
- Economically, blood meal is a cheaper and richer source of protein. It will reduce cost of production and maximize profit.
- The ejaculation frequency should be a matter of importance to breeders because it will affect the overall semen quality and quantity.
- This study affirms once/weekly and twice/weekly. It is recommendable for optimal result because thrice/weekly affected the outcome of volume, mass activity, sperm cell motility and sperm concentration.
- It is advisable to use Glucomate to egg solution in a 1:2 dilution. It has better storage efficiency.

6.4 Contribution to knowledge

- This study has revealed that blood meal a combination of 50% blood meal and 50% fish meal can be effectively utilised by breeder cocks undermining its characteristic smell and low digestibility.
- The results of this study will enlighten poultry farmers especially breeders that once/weekly and twice/weekly are effective for quality semen production.
- Poultry farmers will be exposed to cheaper and efficient source of protein.
- It has established the fact that dietary protein treatment has an advantage on quality semen production. In other words, the general performance of cocks is an attribute of the protein source.

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