LIVEWEIGHT CHANGES, SPERMIOGRAM AND GONADAL SPERM RESERVES OF YANKASA RAMS FED GRADED LEVELS OF DIETARY PROTEIN USING COTTON SEED AND PALM KERNEL CAKES

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF SCIENCE IN THERIOGENOLOGY,

DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

NOVEMBER, 2017

DECLARATION

I declare that the work reported in this dissertation, titled **"**Liveweight Changes, Spermiogram and Gonadal Sperm Reserves of Yankasa Rams Fed Graded levels of Dietary Protein using Cotton Seed and Palm Kernel Cakes**"** has been carried out by me in the Department of Theriogenology and Production, Ahmadu Bello University, Zaria, Nigeria, under the supervision of Prof. P.I. Rekwot and Prof. J.O. Ayo. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

TAIWO KAMARDEEN BELLO	____________________	____________________
Name of Student	Signature	Date

CERTIFICATION

This dissertation, entitled **"Liveweight changes, Spermiogram and Gonadal Sperm Reserves of Yankasa Rams Fed Graded Levels of Dietary Protein Using Cotton seed and Palm Kernel Cakes"** by TaiwoKamar-deen Bello meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. P.I. Rekwot Chairman, Supervisory Committee Department of Theriogenology and Production, Ahmadu Bello University, Zaria.	____________________ Signature	______________ Date
Prof. J.O. Ayo Member, Supervisory Committee Department of Veterinary Physiology Ahmadu Bello University, Zaria.	____________________ Signature	______________ Date
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Prof. S.Z. Abubakar Dean School of Post-graduate Studies Ahmadu Bello University, Zaria.	____________________ Signature	______________ Date

ACKNOWLEDGEMENTS

All praise and adoration to Almighty Allah (S. W. T.) and May peace and blessings of Allah be upon the Noble Prophet, Muhammed (S. A. W.). I thank Allah for providing me with the strength, good health and guidance for this study. My gratitude also goes to members of my supervisory committee, Prof. P.I. Rekwot and Prof. J.O. Ayo, for their immense inputs, contributions and guidance throughout this study. I appreciate their efforts at the repeated perusal, meticulous corrections and guidance, all of which brought out the best in this dissertation. I can't forget my Programme Leader, Prof. (Mrs) N.P. Chiezey because of her constant advice in making sure this study came out good. My appreciation also goes to my colleagues namely; Dr. F.U. Samuel, Dr. R. Hassan, Mrs. F. Maikaji, Mr. Jerry Luka, Mallam S. Ambo and Late Dr. M.H. Joseph for their inputs. Many thanks to Mr. S.I.B. Bolaji, Mr. Y. Lekwot, Mrs. B. Aderibigbe and Madam Justina Joseph for their assistance during sample analyses. I also want to recognize the contribution of staff of Artificial Insemination Unit during the study period. I am also grateful to the farm attendants, at the Biotechnology Research Programme for their services during this study. Their assistance and contributions were vital at bringing this study to completion. Words cannot express my gratitude to my parents, Prof. and Mrs. I.A. Bello, for nurturing me since childhood, for their constant love, support and sponsorship, may Allah reward them abundantly, Amin. I will also appreciate my parent in-laws, Arc. A.A. Oyelowo and Hajia, W. T. Oyelowo, for their prayers and support. To my loving siblings, Dr. K.K. Bello, Miss I.B. Bello, Engr. Kabeerah Bello, Dr. F. Abdulraheem, Dr. N. Oyelowo, Mrs. J. Oyelowo, Mr. H. Oyelowo, Dr. N. Oyelowo. I appreciate you all. Thanks for being there always. To my loving Wife, Dr. Balkisu Oyelowo-Bello, you have been a constant source of support, encouragement and inspiration. Thanks for being my pillar of strength always.

DEDICATION

I dedicate this work to Almighty Allah, my Parents, my lovely Wife and Daughter.

ABSTRACT

Aim of this study was to assess the effects of graded levels of dietary protein on scrotal circumference,liveweight changes, semen characteristics, gonadal and extragonadal sperm reserves and serum testosterone profile in Yankasa rams. Fifteen rams were used in this study aged 19.06 ± 2.4 months and weighing 19.4 ±1.6 kg with body condition scores of 3.5. They were divided into three treatment groups (A, B and C) based on dietary protein level. Groups A, B and C received 10%, 15% and 20% crude protein respectively. The rams were managed under intensive system, kept in separate pens, fed individually and allowed two weeks adjustment period. All rams were fed a basal diet of hay (*Digitaria*spp) *ad-libitum* and given a supplement ration of concentrate mixture at 2% body weight/day. Body weights and scrotal circumference were recorded weekly. Semen samples were collected weekly for analyses using battery controlled electro ejaculator. Three rams from each group were sacrificed for determination of gonadal and extragonadal sperm reserves. The overall mean scrotal circumferences obtained through the study period for the groups revealed significant ($P < 0.05$) difference in the increase between group A and B with values of 22.9 ± 0.6 cm and 26.2 ± 0.7 cm. There was also a significant ($P < 0.05$) increase between group A and C (22.9 ± 0.60 cm and 26.8 ± 0.8 cm) respectively. Rams fed 10% crude protein had significantly ($P < 0.05$) lower mean live weights (18.8 ± 0.4kg) than those fed 15% (22.1 ± 0.6kg) and 20% (24.1 ± 0.1kg) crude protein. Mean Packed cell volumes obtained in this study revealed rams fed 15% crude protein had significantly ($P < 0.05$) higher values (31.3 ± 0.9%) than those fed 10% (25.9± 0.7%) and 20% (25.9 ± 0.7%) crude protein. There were no significant ($P > 0.05$) difference in mean white blood cells counts between all groups. Rams fed 10% had the lowest value of 8.4 ± 0.3 $x10^6$/µl as compared to other groups (8.4 ± 0.2 $x10^6$/µl and 8.4 ± 0.2 $x10^6$/µl). Rams fed 15% crude protein had significantly ($P< 0.05$) higher RBC values (5.2 ± 0.11 X10^6 µl) than other groups (4.2 ± 0.1 X10^6 µl and 5.1 ± 0.01 X10^6 µl). Semen concentration was significantly ($P < 0.05$) higher in rams fed 15% crude protein (163.1 ± 20.6 x10^6/ml) than group A (96.4 ± 16.3 x10^6/ml) and group C (98.8 ± 8.5 x10^6/ml). Motility patterns recorded revealed rams fed 15% crude protein had significantly ($P < 0.05$) higher values (75.1 ± 3.0%) than group A (54.7 ± 3.8%), and group C (53.9 ± 3.9%). There was no significant difference in reaction time, but rams fed 15% crude protein had the lowest reaction time. Regardless of crude protein levels, left testes weighed more than the right testes. Testicular weights and gonadal reserves were highest in rams fed 20%, but the difference with other groups were not statistically ($P > 0.05$) different. Regardless of crude protein intake, the *corpus* epididymis had the lowest reserves, while the *cauda* had the highest reserves. Rams fed 20% crude protein had the highest abnormalities when compared with those fed 10% and 15% crude protein. This difference was however not statistically ($P > 0.05$) different. Feeding rams 15 % C.P gave significantly higher values in terms of body weight, scrotal circumference, semen concentration, sperm motility and percentage live sperm, than in rams fed 10 % C.P. From this study,Yankasa rams should be fed crude protein levels of 15% for better reproductive performance. However, studies should also be conducted to investigate if antioxidants could ameliorate some of the effects of low and high levels of protein diets that were observed in this study.

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ANOVA Analysis of Variance

BMI Body Mass Index

CORT Corticosterone

CP Crude Protein

DHA Docosa-Hexaenoic Acid

DNA Deoxyribonucleic Acid

ER- Epididymal Reserves

FSH Follicle Stimulating Hormone

GnRH Gonadotropic Releasing Hormone

GR Gonadal Reserves

HPG Hypothalamic-Pituitary Gonadal axis

IU International Unit

LAC L-Acetyl-Carnitine

LC L-Carnitine

LH luteinizing Hormone

LW Live-weight

MDA Malondialdehyde

MMP2 Metalloproteinase2

PCV Packed Cell Volume

PUFA Poly-Unsaturated Fatty Acids

RBC Red Blood Cell

RIM Resource Inventory and Management

RNA	Ribonucleic Acid
SC	Scrotal Circumference
SHBG	Sex Hormone Binding Globulin
SOD	Super-Oxide Dismutase
TST	Testosterone
WAD	West African Dwarf
WBC	White Blood Cell

CHAPTER ONE

INTRODUCTION

1.1Background of the study

Chronic low animal protein intake in developing countries is a basic problem that needs an urgent solution (Attah*et al.*, 2006). The low animal protein intake may be attributed to low livestock productivity and therefore available animal protein are very expensive for the growing population with a very low per capita income. Successful reproduction as an important factor in livestock production economy depends on genetic merit, physical environment, nutrition and management (Rasbech, 1984). 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. Enjalbert (2006) attributed many reproductive health disorders in animals to diet inadequacy. It is well documented that protein deficient feeds reduce semen quality and sexual activity in bulls (Brown, 1994). The relationship between nutrition and reproduction is an increasingly important topic and the interactions have important implications for the reproductive performance of farm animals (Bindari*et al*., 2013). The major reason for low levels of animal production in numerous African countries is the inadequate supply and low level of feeding due to serious shortage of feedstuffs (Amata, 2014). Nutrition plays a major role in enhancing reproductive efficiency in all animals (Bindari*et al.*, 2013). The effects of nutrition particularly underfeeding and flush-feeding on female fertility have been documented (Lozano *et al*., 2003). The reproductive ability in the male comprises the production of semen containing normal spermatozoa in adequate concentration, together with the desire and ability to mate (Oyeyemi and Ubiogoro, 2005). Several studies have documented the interrelationship between energy intake and reproductive performance in adult rams (Murray *et al*., 1990) and there is no doubt that protein deficient feeding can reduce semen quality and sexual activity (Okolski*et al*., 1971; Brown, 1994). Carbohydrate, protein and nucleic acid metabolism and their deficiency may impair spermatogenesis and libido in males, embryonic development and survival, post-partum recovery activities, milk production, offspring development and their survival in females (Smith and Akinbamijo, 2000; Alejandro *et al*., 2002; and Mitchell *et al*., 2003). Nutrition is a major factor in the effectiveness of reproductive function. It may affect the efficiency of related hormone production and the growth of reproductive organs. Studies have demonstrated that luteinizing hormone (LH) secretion during early gonadotropin rise is elevated and can be sustained for a longer period when calves are fed with improved nutrition during calfhood (Cheah and Yang, 2011). The efficiency of sperm production, libido and quality of spermatozoa tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs and chemicals (Togun and Egbunike, 2006). Under-nutrition poses adverse effect on the reproductive capacity of males, therefore successful reproduction requires complete provisions of macro and micro-nutrients (Zambrano, 2005; Abdu, 2008). Nutrition may affect the efficiency of related hormone production and the growth of reproductive organs (Almeida *et al*., 2007). The fertility of males and females in a particular herd/flock, as well as level of nutrition to a large extent, determines the rate of production in any livestock industry (Abdulrashid and Darren, 2016). Restricted diets significantly decrease the number of cells of the spermatogenic series at all stages and the number of sertoli cells. Thus, it is apparent that the cellularity of the tubular epithelium is significantly lower in low nutrition animals compared to those of high nutrition (Almeida *et al*., 2007). Nutritional deficiency is usually caused by general underfeeding with rations of poor quality (Rekwot*et al*., 1988). These effects on reproduction are usually directed at the level of anterior pituitary gland, which fails to secrete enough gonadotropins to stimulate the testis to produce testosterone and semen (Asdell, 1955).

1.2 Statement of research problem

In Nigeria, consumption of animal protein is still low, as such improving the level of protein intake in most tropical countries is important, given the fact that daily protein intake has been shown to be below standard requirements in most developing countries. Meat supply plays a significant role in addressing problems of macro and micro nutrients deficiency in diets of many parts of the world, particularly among the urban areas of developing countries (Boland *et al*., 2013). Reproductive well-being and performance of farm animals is largely dependent on their nutritional status (Fernandez *et al*., 2004). Protein deficient rats have a reduced hypothalamic content of GnRH and reduced serum concentrations of LH, FSH, and testosterone (Glass *et al*., 1979). Low protein intakes result in decreased sperm production or output in bulls (Rekwot*et al*., 1988) and rats (Vawda and Mandlwana, 1990). There is need for increased livestock production to meet the growing demand for animal protein.

1.3 Justification for the study

Previous study conducted by Jibril*et al*. (2011) investigated effects of protein diets on reproductive performance using Dried Layer Litter as source of protein in Yankasa rams, which is an unconventional source of protein. Studies are scarce on effects of dietary protein on reproductive performance using conventional feed source, hence this study investigated the effects of graded levels of dietary protein of Yankasa rams on liveweight changes, spermiogram and gonadal sperm reserves.

1.4

Aim of the study

The aim of this study was to assess the effects of graded levels of dietary protein using cotton seedand palm kernel cakes on haematological parameters, live-weight changes, semen characteristics, gonadal and extragonadal sperm, spermatid reserves and serum testosterone profiles in Yankasa rams.

1.5 Objectives of the study

The specific objectives of the study are to determine the:

I. Effect of graded levels of protein diets (cotton seed and palm kernel cakes) on Live-weight changes in Yankasa rams.

II. Effect of graded levels of protein diets (cotton seed and palm kernel cakes) on scrotal circumference in Yankasa rams.

III. Effect of graded levels of protein diets (cotton seed and palm kernel cakes) on Haematological parameters in Yankasa rams.

IV. Effect of graded levels of protein diets (cotton seed and palm kernel cakes) on Semen characteristics (spermiogram) in Yankasa rams.

V. Effect of graded levels of protein diets (cotton seed and palm kernel cakes) on Gonadal and extragonadal sperm and spermatid reserves in Yankasa rams.

1.6 Research Questions

I. What are the effects of graded levels of dietary protein (cotton seed and palm kernel cakes) onliveweight changes in Yankasa rams?

II. What are the effects of graded levels of dietary protein (cotton seed and palm kernel cakes) on scrotal circumference in Yankasa rams?

III. What are the effects of graded levels of dietary protein (cotton seed and palm kernel cakes) onhaematological parameters in Yankasa rams?

IV. What are the effects of graded levels of dietary protein (cotton seed and palm kernel cakes) on semen characteristics (spermiogram) in Yankasa rams?

V. What are the effects of graded levels of dietary protein (cotton seed and palm kernel cakes) gonadal and extragonadal sperm and spermatid reserves in Yankasa rams?

CHAPTER TWO

LITERATURE REVIEW

2.1 Sheep Breeds and Distribution in Nigeria

Sheep are kept everywhere in Nigeria, with a broad distinction between their importance and ubiquity in the North, and the more dispersed populations of the humid zone. Sheep and goats are seen as having secondary importance in relation to crops. There are generally considered to be four breeds of sheep native to Nigeria namely, the Balami, Uda, Yankasa and West African Dwarf (WAD) (Bello *et al*., 2017).

2.1.1 Balami

The balami is the largest bodied native sheep in Nigeria and is confined to the semiarid North, but it is favoured as a stall-fed breed by Muslims throughout the Nigerian Middle Belt (Agangaet *al*., 1988). It is white and hairy with pendulous ears and a long thin tail; rams have throat ruffs and are horned but ewes are normally polled (without horn). Another feature that makes the Balami distinctly recognizable is its Roman nose; a large bulbous nose that distinguishes it from the Yankasa (Bello *et al*., 2017). As a pastoral animal, it is confined to the semi arid north but it is favoured as a stall fed breed by muslims throughout the Nigerianmiddle belt. It is white and hairy with pendulous ears long leg and a long thin tail. It has good potential as a meat producer. The weight of mature males ranges from 40 to 80kg while that of female lies between 30 and 40kg. The milk yield per lactation lies between 28 and 33kg in 70days(Bello *et al*., 2017).

2.1.2 Uda

The Uda is slightly smaller-bodied than the Balami, although its size ranges overlap. It is easily recognized by a distinctive coat colour pattern, entirely brown or black forequarters and white behind. Uda sheep derives its name from a Fulani clan, the Uda'an, who herd large flocks of this breed between Niger and the northern parts of the Nigerian Middle Belt (Benoit *et al*., 2017).The Uda is found in northern Nigeria, southern Niger, central Chad, northern Cameroon and western Sudan, the Uda is one of the hair sheep breeds of the Sahel type. It is a meat breed. It is a long legged breed of sheep with distinctive coat colour. They are large with straight and long face. The rams of the Uda are horned and the ewes are usually polled. The Uda is slightly smaller bodied than the Balami, although their size ranges overlap. The weight of mature females could be 30 to 40kg while mature rams weigh 30 to 60kg. Milk yield per lactation lies between 32 and 36kg for an average lactation length of 91 days (Benoit *et al*., 2017).

2.1.3 Yankasa

The Yankasa has been the most extensively studied in Nigeria. The body colour is white with black patches around the eyes and sometimes on the feet. The muzzle and ears are usually black too. Rams have curved horns and hairy white mane while ewes are polled. The yankasa is a meat breed found in North central Nigeria. The tail is long and thin, ears moderately long (Sanchi*et al*., 2015). Yankasa sheep have been recorded in all parts of Nigeria, though the populations attenuate towards the northern border and the sea-coast (Sanchi*et al*., 2015). The Yankasa is a meat breed found in north and north central Nigeria. The Yankasa is a Medium-sized breed of sheep.The tail is long and thin, the ears moderately long and somewhat droopy. They have white coat colour with black patches around the eyes, ears and muzzle. Yankasa rams stand 70 to 80 cm at the withers and weigh 55 to 60kg at maturity. Mature females could weigh 25 to 40kg while male weighs between 35 and 50kg. The milk yield (kg) per lactation is between 30 and 56kg and has a lactation length of 91 days. The peak milk yield per day is 960 grammes(Sanchi*et al*., 2015).

2.1.4 West African Dwarf (WAD)

The West African Dwarf breed of sheep is a small-bodied, compact breed which may be all white, black, Brown, or spotted black or brown on a white coat. It is a compact breed with small mature size and horizontal lop ears (Benoit *et al*., 2017).

2.2 Anatomy of Male Genitalia

The male ganitalia consist of the testis, epidiymis, vas deferens, ampulla, spermatic cord, vesicular gland, prostate gland, Copwer's gland and penis.

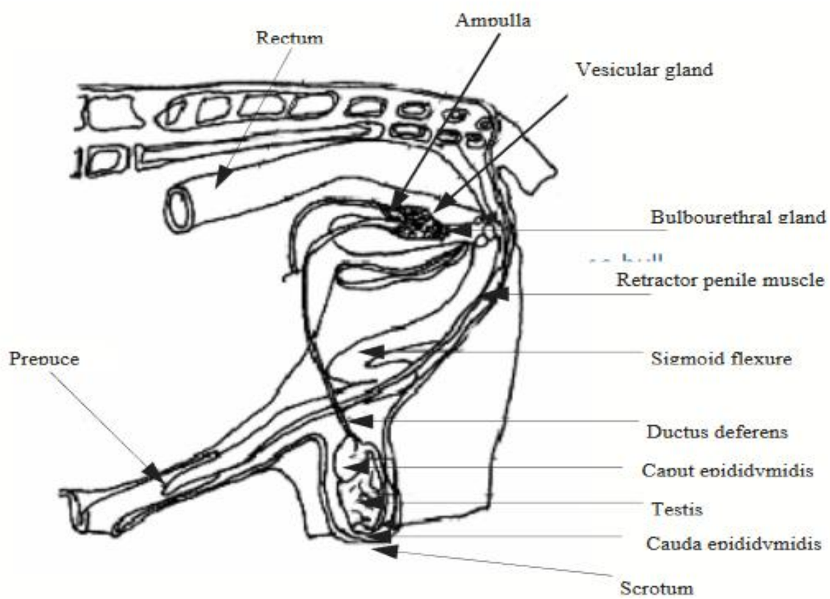

Figure 2.1: Structures of the male reproductive system (Amann and Schanbacher, 1983).

2.2.1 Testes

The testes are paired organs which descend from the abdominal cavity during foetal development to lie in the scrotum. They are the primary organs of reproduction in males just as ovaries are in females. Testes are considered primary because they produce the male gametes (Spermatozoa) and secrete the male sex hormone, testosterone. The ram, like other male farm animals, has 2 testes which are suspended outside the body but in a sac called scrotum (Etim, 2015).

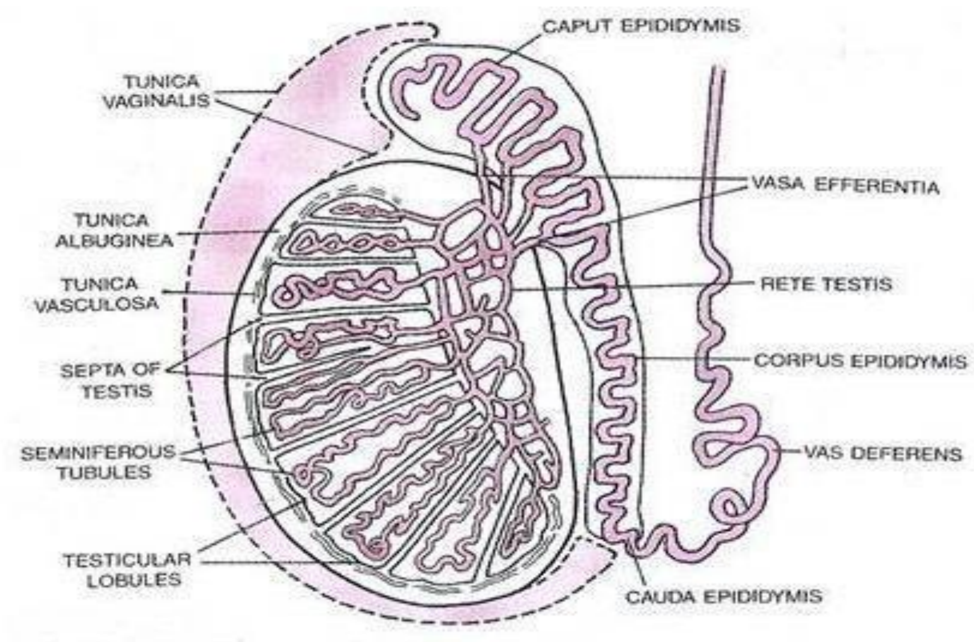

Figure 2.2: Sagittal section of testis illustrating segments of parenchymal tissue which contain the seminiferous tubules, rete testis, vasa efferentis and epididymis.
(https://www.google.com.my/search?q=diagram+of+animal+testis)

2.2.1.1 Functional morphology of the testesandblood-testis barrier

The testis in bulls is of similar size in boars, but is smaller in rams, bucks (goats) and stallions. In all species, testes are covered with the tunica vaginalis. This out pocketing of the body wall that surrounds the testes is a serous tissue, which is an extension of the peritoneum. This serous coat is obtained as the testes descend into the scrotum and is attached along the line of the epididymis. The outer layer of the testes, the tunica albuginea testis, is a thin white membrane of elastic connective tissue in which divides the testicular parenchyma into lobes and lobules containing the seminiferous tubules and inbetween them are the interstitial cells (Albert, 1961). Numerous blood vessels are visible just under its surface. Beneath the tunica albuginea testis is the parenchyma, the functional layer of the testes. The parenchyma has a yellowish color and is divided into segments by incomplete septa of connective tissues. Located within these segments of parenchyma tissue are the seminiferous tubules. Seminiferous tubules are formed from primary sex cords. They contain germ cells (Spermatogonia) and nurse cells (Sertoli cells). Sertoli cells are larger and less numerous than spermatogonia. With stimulation by follicle stimulating hormones (FSH), sertoli cells produce both androgen-binding protein and inhibin. Seminiferous tubules are the sites of spermatozoa production. They are small, convoluted tubules approximately 200um in diameter. It has been estimated that the seminiferous tubules from a pair of bull testes, stretched out and laid end to end, approach 5 km in length. They make up 80% of the weight of the testes (Albert, 1961). Seminiferous tubules join a network of tubules, the rete testis, which connects to 12 to 15 small ducts, the vasa efferentia, which converge into the head of the epididymis. Leydig (interstitial) cells are found in the parenchyma of the testes between the seminiferous tubules. Luteinising Hormone (LH) stimulates Leydig cells to produce testosterone and small quantities of other androgens. Testicular Leydig cells are the primary source of testosterone in males. Adult Leydig cells have been shown to arise from stem cells present in the neonatal testis. Once established, adult Leydig cells turn over only slowly during adult life, but when these cells are eliminated experimentally from the adult testis, new Leydig cells rapidly reappear (Li *et al*., 2016). The blood-testis barrier, which is essential for spermatogenesis, is located near the base of the seminiferous tubule, where it divides the epithelium into 2 distinct compartments, basal and adluminal (Fijak and Meinhardt, 2006). Spermatogonia and preleptotenespermatocytes reside in the basal compartment, whereas other primary and secondary spermatocytes, round spermatids, and elongating or elongated spermatids reside in the adluminal compartment. Thus, the function of the blood testis barrier is to sequester germ cells residing in the adluminal compartment from the circulatory and lymphatic systems, and together with local immune suppression, to provide an immune privileged microenvironment for the completion of meiosis (Li *et al*., 2012).

Spermatogenesis is the cellular process by which spermatogonia develop into mature spermatids within seminiferous tubules, the functional unit of the mammalian testis, under the structural and nutritional support of Sertoli cells and the precise regulation of endocrine factors (Dolores and Cheng, 2015). As germ cells develop, they traverse the seminiferous epithelium, a process that involves restructuring of Sertoli-germ cell junctions, as well as Sertoli-Sertoli cell junctions at the blood-testis barrier. The blood-testis barrier, one of the tightest tissue barriers in the mammalian body, divides the seminiferous epithelium into two compartments, basal and adluminal (Van Itallie and Anderson, 2014). The blood-testis barrier is different from most other tissue barriers in that it is not only comprised of tight junctions. Instead, tight junctions coexist and cofunction with ectoplasmic specializations, desmosomes, and gap junctions to create a unique microenvironment for the completion of meiosis and the subsequent development of spermatids into spermatozoa via spermiogenesis. The key structural, scaffolding, and signaling proteins of the blood-testis barrier has been identified and more recent studies have defined the regulatory mechanisms that underlie blood-testis barrier function (Dolores and Cheng, 2015).

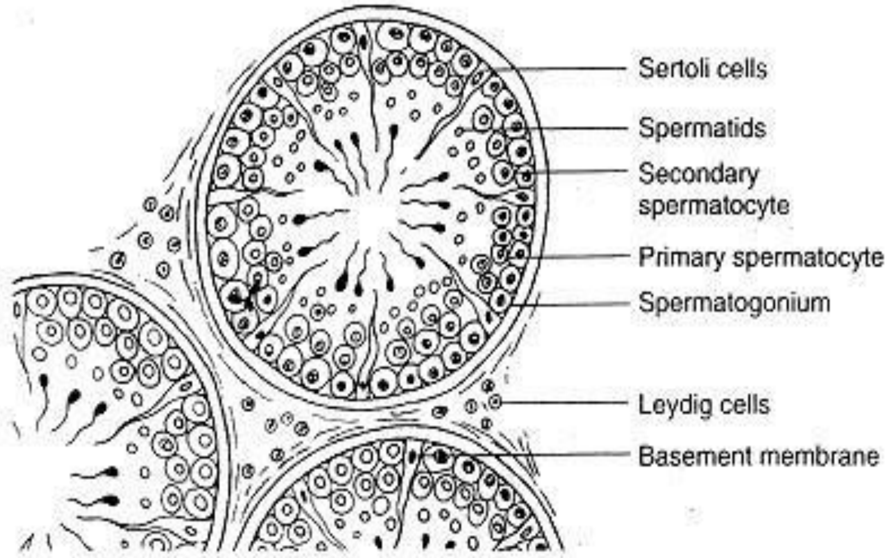

Figure 2.3: Cross section of parenchymal tissue showing relationship between the seminiferous tubules and interstitial tissue containing Leydig cells (Amann and Schanbacher, 1983).

2.2.2 Spermatic Cord

The spermatic cord connects the testis to its life support mechanisms, the convoluted testicular arteries and surrounding pampiniform plexus, and nerve trunks. In addition, the spermatic cord is composed of smooth muscle fibres, connective tissue, and a portion of the vas deferens both the spermatic cords and scrotum contribute to the support of the testes. Also, they have a joint function in regulating the temperature of the testes (Bedford, 1978).

If a ram's scrotum is insulated, or the testes are tied against the abdomen, sterility results. The higher temperature causes degeneration of the cells lining the wall of the seminiferous tubules. Fertility will be restored if the testes and scrotum are returned to their natural state before total degeneration occurs. However, a few weeks will be required before fertile semen is again produced. The bilateral cryptorchid is sterile, again illustrating that production of spermatozoa stops when the temperature inside the testes is as high as normal body temperature (Bedford, 1978). Low fertility semen produced by several species of farm animals during the summer has been attributed to the inability of the body’s cooling mechanism to keep the testes cool enough (Bedford, 1978).

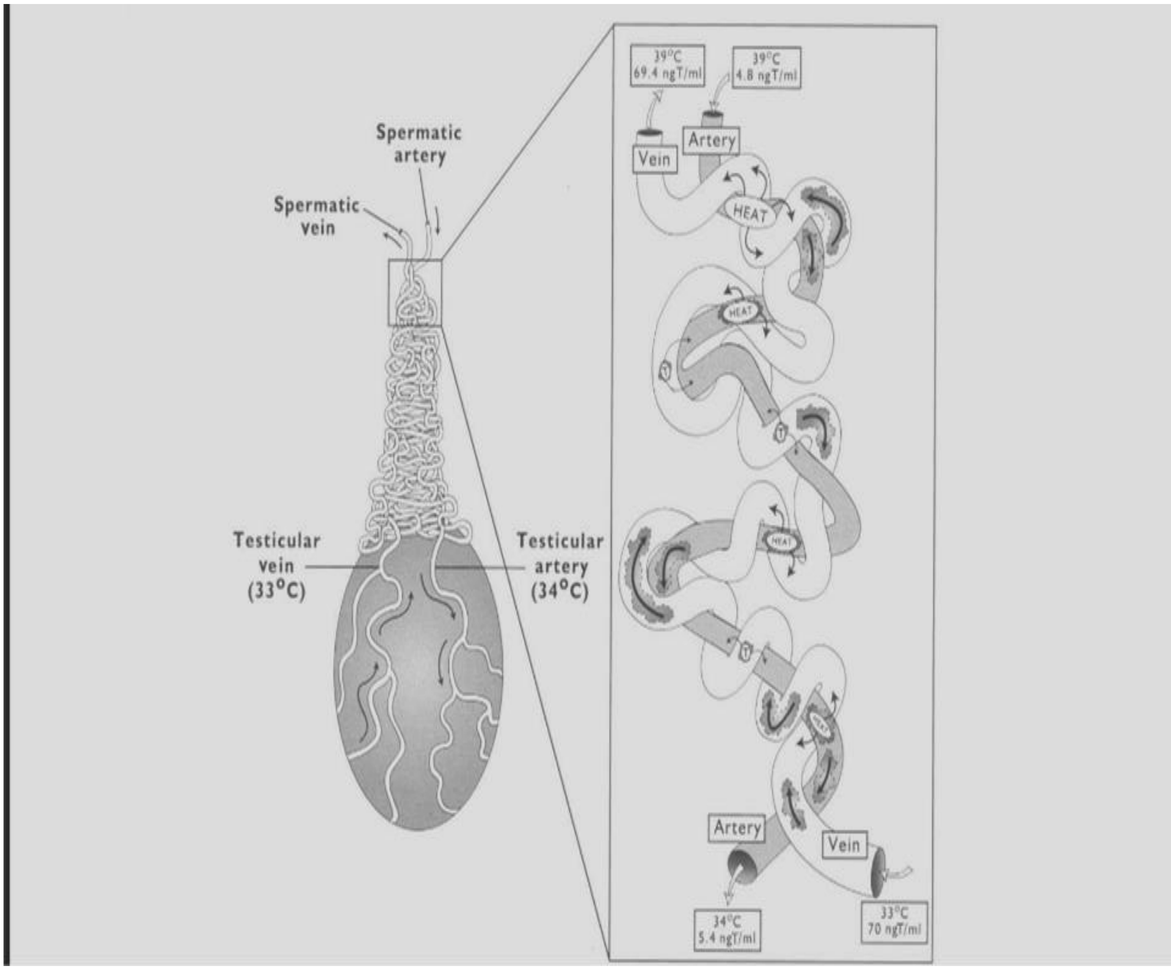

Figure 2.4: The pampiniform plexus (Kummar, 2014)

The pampiniform plexus is a network of many small veinsfound in male animal’s spermatic cord.It is formed by the union of multiple spermatic veinsfrom the back of the testisand tributaries from the epididymis(Kummar, 2014). The veins of the plexus ascend along the cord in front of the ductus deferens.Below the superficial inguinal ringthey unite to form three or four veins, which pass along the inguinal canal,and, entering the abdomen through the deep inguinal ring,coalesce to form two veins. These again unite to form a single vein, the testicular vein,which opens on the right side into the inferior vena cava,at an acute angle, and on the left side into the left renal vein,at a right angle. The pampiniform plexus forms the chief mass of the cord (Kumar and Kumar, 2008). In addition to its function in venous return from the testes, the pampiniform plexus also plays a role in the temperature regulation of the testes. It acts as a heat exchanger,cooling blood in adjacent arteries (Kumar and Hegde, 2012). An abnormal enlargement of the pampiniform plexus is a medical condition called varicocele(Kumar and Hegde, 2012).

2.2.3 Epididymis

The epididymis is a long and convoluted tube in which sperm cells produced by the testicles are stored and mature to a stage capable of fertilization. This maturation change occurs as sperm cells move from the head to the body of the epididymis with mature sperm being stored in the tail of the epididymis. The first external duct leading from the testis is fused longitudinally to the surface of the testis and is encased in the tunica vaginalis with the testis. Each epididymis leads to longer tubes called *vas deferens* (Etim, 2015). The single convoluted duct is covered with an extension of the tunica albuginea testis. The captut (head) of the epididymis is a flattened area at the apex of the testis, where 12 to 15 small ducts, the vasa efferentia, merge into a single duct. The corpus (body) extending along the longitudinal axis of the testis is a single duct which becomes continuous with the cauda (tail). The total length convulated duct is about 34 meters in the bull and longer in the ram, boar and stallion. The lumen of the cauda is wider than the lumen of the corpus. The structures of the epididymis and other external ducts (vas deferens and urethra) are similar to that of the tubular portion of the female tract. The tunica serosa (outer layer) is followed by a smooth muscle layer (middle) and an epithelial layer (innermost) (Bedford, 1978).

2.2.4Vas deferens and urethra

The vas deferens is a pair of ducts, each leading from the distal end of the cauda of each epididymis. Initially supported by folds of the peritoneum, it passes along the spermatic cord, through inguinal canal to the pelvic region, where it merges with the urethra at its origin near the opening to the bladder (Etim, 2015). The enlarged end of the vas deferens near the urethra is the ampulla. The vas deferens has a thick layer of smooth muscles in its walls and appears to have single function of transport of spermatozoa. Vas deference may be considered the extra-testicular continuation from cauda of the epididymis, and it is the portion of the reproductive tract fundamentally associated with transportation of the sperm-containing fluid from each epididymis to the urethra for their finishing discharge (Hamayun*et al*., 2015).

2.2.5 Accessory glands

The accessory glands are located along the pelvic portion of the urethra, with ducts which empty their secretions into the urethra. They include the vesicular glands, the prostate gland and the bulbourethral glands. Accessory glands secrete additional fluids, which when combined with the sperm and other secretions from the epididymis, form the semen. Accessory sex glands including vesicular gland and ampullae of vas deferens are characteristically essential for reproductive process (Hamayun*et al*., 2015). The prostate gland consists of two portions — the compact or external portion (corpus prostate) and the disseminate or internal portion (*pars disseminata*) (Pathak *et al*., 2012).

2.2.6 The Penis

The penis functions as the organ of copulation in males. It forms dorsally around the urethra from the point where the urethra leaves the pelvis with the external urethral orifice at the free end of the penis (Bedford, 1978). Bulls, boars and rams have sigmoid flexure, an S-shaped bend in the penis which permits it to be retracted completely into the body. These three species and the stallion have retractor penis muscles, a pair of smooth muscles which relax to permit extension of the penis and contract to draw the penis back into the body. These retractor penis muscles arise from the vertebrae in the coccygeal region and are fused to the ventral penis just anterior to the sigmoid flexure. The glans penis, which is the free end of the penis, is well supplied with sensory nerves and is homologous to the clitoris in the female. In most species the penis is fibro elastic, containing small amounts of erectile tissues. The penis of stallion contains more erectile tissues than are found in bulls, boars, bucks and rams (Bedford, 1978).

2.2.7 Scrotum

The scrotum is a two-lobed muscular sac enclosing the testes and it supports and protects the testes and also plays a major role in temperature regulation. It maintains the temperature 3 to 5 ^{0}C below body temperature for optimal function. It is located in the inguinal region between the rear legs of most species. The scrotum has the same embryonic origin as the labia majora in the female. It is composed of an outer layer of thick skin with numerous large sweat and sebaceous glands. This outer layer is lined with a layer of smooth muscle fibres, the tunica dartos, which is interspersed with connective tissue. The tunica dartos divides the scrotum into two pouches and is attached to the tunica vaginalis at the bottom of each pouch (Etim, 2015).

2.3 Hormonal control of sperm production

The production of sperm is usually regulated by hormones such as testosterone (TST), luteinizing hormone (LH), follicle stimulating hormone (FSH), corticosterone (CORT) and throxine amongst others, and these hormones were shown to be associated in regulation and maintenance of spermatogenesis as well as protein metabolism (Tajangookee*et al*., 2007). In addition modulation of the hypothalamus pituitary gonadal and or adrenal axes has been shown to influence the production and release of the productive and reproductive hormone (Aguirre *et al.*, 2007; Kishk, 2008). However, environmental factors such as ambient temperature, photoperiod, and nutrition have also been shown to influence the production of reproductive hormones on activity of reproductive hormones with commensurate effect on activity of reproductive organs (Okab, 2007). Some scientific evidence indicate higher thyroid activity and testosterone (Tajangookeh*et al.*, 2007) levels in ram during the summer period compared to the winter. Other related reports implicated prolactin and cortisol in reducing sperm production (Osinowo, 2006; Aguirre *et al.*, 2007), via reduction in LH and subsequently testosterone levels. However, it is an established fact that successful maintenance of testicular function is dependent on the hypothalamic secretion of GnRH which In turn stimulates pituitary hormone (FSH and LH) to act on the testis, there by initiating sperm production process as well as testosterone production (El-Masry*et al.*, 1994). The two functions of the testis, spermatogenesis and steroidogenesis, depend on normal stimulation by the pituitary gonadotropins, FSH and LH, which in turn are stimulated by GnRH (Matsumoto *et al.*, 1986). The male sex hormone, testosterone, is secreted by the Leydig cell under LH stimulation and plays a key role in the virilization of peripheral tissues and in promoting spermatogenesis.Sertoli cells have the capacity to bind FSH and contain mRNA for the FSH receptor (Kliesch*et al.*, 1992). Although there has been a report of FSH receptors on spermatogonia (Orth and Christensen 1978), and it is generally argued that the action of FSH on spermatogenesis is mediated through the Sertoli cells. Many studies have shown that FSH stimulates mRNA and protein synthesis in immature Sertoli cells (Means *et al.*, 1976) and increases the secretion of specific proteins such as androgen- binding protein, transferin, and inhibin. LH receptors are found only on Leydig cells, the action of LH is through the stimulation of T (and possibly other growth factors) secretion by Leydig cells. As with FSH, the presence of androgen receptors on germ cells has not been confirmed, although some immunocytochemical evidence for their presence on spermatids was recently reported (Vornberger*et al*., 1994). Androgen receptors are certainly abundant on Sertoli, peritubular myoid, and Leydig cells (Mulder *et al*., 1975).

2.4 Testosterone

Potent androgen testosterone, which is produced in the testes, the most important sex hormone in the ram, is responsible for the development of primary and secondary sexual characteristics, sperm production, and the regulation of sexual behavior (Maksimović*et al*., 2016). Spermatogenesis and steroid secretion are dependent on the individual activities of two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). However, both processes are intimately linked toadequate levels of testosterone necessary for normal sperm production and maturation (Maksimović*et al*., 2016). In vertebrates, around 95% of the body's TST is produced by Leydig cells that are located within the interstitial of the testes, intimately associated with the seminiferous tubules. Given the importance of Testosterone on traits associated with reproductive ability, individual variation in TST production appears likely to be a key factor underpinning differential reproductive success in male vertebrates (Preston *et al*., 2012).

Testosterone TST appears to be a good marker of semen production as well as libido (Kishk, 2008). LH and FSH level had been shown to exert an influence on TST biosynthesis, since both hormones are essential for the successful development of normal spermatogenesis (Talebi*et al*., 2009). It was pointed out that FSH and TST, act through the sertoli cells since the receptors of these hormones are located on these cells and are not located on germ cells (El-Masry*et al*., 1994). The major action of androgens appears to be on sertoli cells rather than directly on germ cells (Johnson *et al*., 2008). As such through the stimulation of the leydig cells (receptors) by LH, TST is produced locally within intertubular regions of the testis in high concentration and exerts its influence on spermatogenesis (Gesquiere*et al*., 2011).

The production and secretion of TST as well as its specific functions could be influenced by several factors such as breed, nutritional level/type, season and age, as such, its production has been shown to be altered when there was decreased feed intake as result of high ambient temperature, and consequently suppressed the physiological activity of the sertoli cells and leydig cells via reduced sensitivity to GnRH, with probably subsequent reduction in FSH and LH respectively. Thus ambient temperature, nutrition and photoperiod had been shown to directly or indirectly influence the activity of hormone and accessory glands (Hafez and Hafez, 2000).

2.5 Factors affecting reproductive performance of male animals

2.5.1Season

Testicular development in animals, during post weaning period, is associated with the age and breed of the animals, environmental conditions and nutritional regime. Maina*et al.*, (2006) reported a significant increase in ejaculate mass activity, motility, sperm concentration and percent live spermatozoa during the dry season than in the rainy season. They also observed the total morphological defective spermatozoa to be higher in rainy season than dry season. They concluded that ejaculate quality was better during the dry season. Consequently, semen collected and frozen during the dry season may produce higher fertility rates in an artificial insemination programme. In the work of Bitto and Egbunike (2005) on the effect of season and acute heat stress on the ejaculate characteristics of West African Dwarf buck, they found semen colour, volume and the proportion of live/dead sperm to be stable throughout the year, while total spermatozoa per ejaculate, mass activity, sperm concentration and normal sperm morphology were affected by season. In rams, the long-term, seasonal changes in testicular size and sperm production that are driven by photoperiod are implemented by long-term changes in GnRH pulse frequency and thus LH and FSH secretion (Lincoln and Short, 1980). High altitude had negative effect on the expression of some studied male fertility related genes, but sperm parameters were not significantly affected (Nabil *et al*., 2015). It has also been shown that heat stress alongside poor forage quality have resulted in serious challenges to efficient reproductive and productive performance in sheep (Marai*et al*., 2008), and in most livestock production (Oseni, 2012) in the tropics.

2.5.2 Disease and parasites

Disease and heavy parasitic infections reduce sexual performance. Viral, bacterial and other disease conditions incriminated with fever and loss of body condition are known to affect spermatogenesis (Rekwot*et al*., 1998). Rekwot*et al*., (1998) reported a decrease in reproductive performance of bulls with chronic dermatophilosis. Sekoni (1993) incriminated scrotal insulation caused by severe chronic dermatophilosis as an important causative agent of sterility in bulls in the tropics. Disease and parasites could be controlled through proper preventive measures against internal and external parasites such as prompt treatment of animals showing sign of infection, isolation of new animals and animals returning from shows for a period of about 30 days. Protozoan disease like trypanosomosis could also cause substantial percentage of sperm morphological abnormalities (Okunbanjo*et al*., 2015). In the male reproductive tract, pathological disorders attributed to trypanosomosis include testicular degeneration, scrotal inflammation, penile protrusion, prepucial inflammation, testicular odema, epididymitis and abnormal spermatogenesis (Victor *et al*., 2012). It is well established that obesity induces dysfunction of the hypothalamic-pituitary gonadal axis (HPG) and contributes to infertility in males. In obese humans, blood levels of testosterone and SHBG were decreased and total sperm count and sperm concentration were inversely related to BMI (Paasch*et al*., 2010), while rats fed high fat-diet show a decrease of sperm motility by altering the physical andmolecular structure of germ cells in the testes and mature sperm cells (Fernandez *et al*., 2011; Du *et al*., 2010) and gonadotropins secretion (Olivares *et al*., 2010). Fasciola*gigantica*infection has been reported to affect rams meant for breeding purposes due to its effect on semen quality. Semen quality is affected due to the anorexia, pyrexia and also deleterious effects fasciolosis have on reproductive organs (Iliyasu*et al*., 2014).

2.5.3 Age

Age plays an important role, in sperm production in ruminants. It was observed that there was significant and positive correlation between age and semen concentration, individual sperm motility and morphology KumiDiaka*et al*., (1981). There was an increase in sperm abnormalities, mainly percentage of proximal droplets in the ejaculates of younger animals which decreased as the animal matured. Percentage spermatogenesis was lower in older bulls than younger bulls (KumiDiaka*et al*., 1981).

2.5.4 Breed

A study conducted by Wahid and Yunus (1994) to determine the correlation between testicular measurements, libido and semen quality in five breeds of rams (Malin, Siamese long tail, Merino X Border, Leicester, Dorset and Suffolk), recorded breed difference in volume and semen quality where the tropical breed had better semen compared to temperate breeds. There was positive and significant correlation between testicular length and semen volume in all the breeds. Testicular length was found to be positively and significantly correlated with motility and sperm concentration in the tropical breeds (Wahid and Yunus 1994).

2.5.5 Frequency of semen collection and stimulation

Proper stimulation of animals prior to semen collection improves the quality and quantity of semen (Amann, 1970). In young Holstein bulls given adequate nutrition, the volume and concentration of semen decreased with increase in number of ejaculation from bi-weekly to six times per week

(Almquist 1976). Oyeyemi*et al*., (2000) reported that body weight, withers height and scrotal circumference were not affected by successive ejaculations. Ejaculate volume decreased as the frequency of ejaculation increased (Oyeyemi*et al*., 2001). Semen colour was affected as frequency of ejaculation increased from milky/creamy to white watery. The percentage live spermatozoa were also affected by frequency of ejaculation, but sperm concentration decreased as the number of ejaculations increased.

2.5.6 Nutrition

2.5.6.1 Energy

Energy deficits, caused by low intake or by excessive expenditure, decrease gonadotrophin secretion in both sexes of many species, including humans, and restoration of normal feeding patterns reverses the gonadotrophin deficit (Cameron, 1996). This is because the metabolic cue transmitting information to the reproductive axis is dependent on calorie intake (Cameron, 1996). Energy supplementation increased the sensitivity of testes to low testosterone levels in bulls fed high energy diets, as testosterone-leuteinizing hormone feedback mechanism responded to low quantities of testosterone (Alabi, 2005). In mature male sheep, similar effects have been observed, including the reduction of LH pulse frequency after reduction of feed intake (Martin and Walkden-Brown, 1995).

2.5.6.2 Protein

Dietary protein which contains important amino acids is essential for vital reproductive processes. Basically, protein is a critical nutrient that plays a vital role in livestock production and reproduction, which include body building (growth) and repair of body tissues, synthesis of hormones, nucleoproteins, enzymes and antibodies. The protein used in diets of livestock originates from variety of sources, such as cereal, legumes, forages, animal by-products and various by-products (Boland *et al*., 2013). Groundnut cake and soybean meal are the conventional sources of protein in livestock production (Boland *et al*., 2013). However, the utilisation of these protein sources would largely depend on their type and or animal species. Research findings on restricted protein intake in developing male rats have shown far reaching effects on spermatogenesis and epididymal sperm maturation. Rats fed with 3% protein revealed lower testosterone levels, inhibited spermatogenesis, immature testes, and delayed puberty compared to rats fed with 10% and 20% protein. The capability of protein deficient rats producing mature sperm was not completely impaired, but the initiation of spermatogenesis was retarded by 2.5 to 3 weeks. It is suggested that protein deficiency has an impact not only on normal gonadal organ function and spermatogenesis, but also on tubular atrophy. In a study about effects of maternal protein restriction on the male reproductive system, protein deficiency resulted in delayed testicular development and low sperm counts (Zambrano *et al*., 2005), indicating that maternal protein restriction during development changes testicular function and gonadal hormone secretion at 110 days. Protein deprivation in ratsdepresses gonadotropin production, either through elevated testosterone levels or LH levels, and negatively affects testicular development (Zambrano *et al*., 2005).

Some protein sources like cotton seed cake and whole cotton seed which are known to contain gossypol, can significantly have negative effects on semen quality of Yankasa rams if fed above 46% (Babashani*et al*., 2015). Amao*et al.,* (2012) reported marked severe reductions in testicular sperm reserve, daily sperm output and daily sperm output per gram of testis in rabbit bucks fed cotton seed cake. Overall, increased protein intake has been reported to favour spermatogenesis (Jibril*et al*., 2011). Arginine can be obtained from seafoods, watermelon juice, nuts, meat, soy proteins and others. It is suggested that the improvement of reproduction capacity is due to the increased synthesis of polyamines and Arginine-rich basic proteins in sperm cells. The regulatory role of arginine for sperm motility and capacitation can improve reproduction capacity (Wu *et al*., 2009).

L-carnitine (L-3-hydroxy-4-N, N-trimethylaminobu- tyrate) and its acyl derivatives L-acetylcarnitine (LAC) has long been well known as a conditionally essential amino acid for its physiologic role in health and disease. Carnitine is assumed as a nonessential protein in human diet as it can be biosynthesized in vitro through hepatic methylation from dietary amino acids (Ng *et al*., 2004). The exogenous source of L-Carnitine in the diet is meat, dairy products and fish. In contrast to blood, high concentrations of L-Carnitine and L-acetyl-Carnitine can be found in epididymal fluids and in spermatozoa. During transit from the caput to the cauda region of the epididymis, spermatozoa which are originally immotile gradually attain the capacity of flagellar motion and accumulate high concentrations of free LC. Several reports have demonstrated that the proportion of progressive sperm motility is parallel with the concentration of free LC and LAC. Therefore, supplementing diets with carnitine could provide a potential therapy in improving the male reproductive ability (Zhou *et al*., 2007).

2.5.6.3 Lipids

Lipids facilitate the take-up of crucial elements for our diet and serve as sources of energy and carriers of fat-soluble vitamins (Rooke*et al*., 2001). Fats also supply essential fatty acids that are vital constituents of cell membranes, and precursors of hormone-like compounds such as prostaglandins. It is well known that testes contain a high content of C20 and C22 carbon atoms of polyunsaturated fatty acids (PUFAs), especially in mammalian sperm (Conquer *et al*., 2000). As spermatozoa are rich in lipids with phospholipids occupying approximately 70% of the total lipids, lipids have an important role in maintaining sperm cell viability, maturity, fertility and function (Zalata*et al*., 1998). Phospholipids and cholesterol are major components which form the lipid bilayer of cell membranes (Rooke*et al*., 2001). The normal structure of the sperm membrane is

crucial for successful fertilization as both the acrosome reaction and spermatocyte fusion are membrane-associated events. In most mammals, docosahexaenoic acid (DHA, 22: 6n-3) is the major PUFA (more than 60%) and it constitutes the dominant part of sperm cell membranes. Research showed that the amount of DHA in spermatozoa is proportional to sperm motility and DHA is concentrated in the sperm tail (Lin *et al*., 2004). Since sperm cells are susceptible to free radical damage, defence mechanisms in sperm cells are important to neutralize the toxic intermediates. Malondialdehyde (MDA) which is the by-products of lipid peroxidation, can be used to monitor the destruction level of peroxidation in spermatozoa. Previous studies indicated that high MDA levels are concurrent with high SOD activity in human and boar spermatozoa. SOD is the major enzymatic defense against lipid peroxidation and contributes to the prevention of peroxidative damages by dismutating the superoxide anion radical into hydrogen peroxide
(Conquer *et al*., 2000).

2.5.6.4 Vitamins

Vitamins are well known for their importance in the process of metabolism. The influence of various vitamins on the reproductive capacity has been studied by several researchers. Early histological studies have shown that vitamin deprivation in dietary intake has an impact on the male reproductive system. Vitamin B12 in its diverse forms are crucial in cell replication, particularly RNA and DNA synthesis (Abdu, 2008). Retinoic acid, the alternative metabolite form of vitamin A (retinol) controls spermatogonia differentiation and spermatid adhesion characteristics (Abdu, 2008). Several investigations tried to elucidate the importance of natural antioxidants, such as vitamins A and E in improving semen quality and sperm production through the protection of spermatozoa membranes, but a study of boar sperm and supplementation with vitamins (B2, B6, B9, B12, and E) showed reverse results (Akins *et al*., 2013). An experiment of selenium and vitamin E diet supplementary in boar showed improvement in sperm quality (Tareq*et al*., 2010). Moreover, supplemental Vitamin E at a concentration of 200 IU per sheep a day increased semen quality and quantity and reduced the malondialdehyde (MDA) level significantly (Wang *et al*., 2007). A combination of SeMet and SeMet + Vit-E is reported to protect sperm from oxidative damage induced by ammonia and improve sperm motility, subsequently increasing the rate of acrosome reaction (Tareq*et al.*, 2010).

2.5.6.5 Minerals

Minerals are important for reproductive performance in livestock (Kumar *et al*., 2011) because their supplementation improves reproduction (Grace and Knowles, 2012). Studies show that the ovarian activity of ruminants is influenced by mineral deficiency. They are also involved in synthesis of hormones that are important for reproduction. Their deficiency affects both steroid and thyroid hormone (Abdollahi*et al*., 2013). Copper and zinc play an important role in regulating progesterone production by luteal cells via the involvement of superoxide dismutase. Copper is also involved in steroidogenic enzymes cytochrome P450, 17á-hydroxylase and cytochrome P450 side-chain cleavage and lysyl oxidase. Zinc is involved in the reorganization of ovarian follicles which are the source of progesterone. This occurs through the involvement of metalloproteinase2 (MMP-2) enzyme, which is a member of zinc endopeptidase family (Sales *et al*., 2011). Zinc is also involved in the secretion and function of male hormone testosterone through the enzymes that control the arachidonic acid cascade. Zinc is essential for thyroid hormone secretion and function. Thus, zinc plays an essential role in sexual development and spermatogenesis. Involvement of manganese in the synthesis and production of oestrogen and progesterone may be due to the fact that it acts as a cofactor in the synthesis of cholesterol, a precursor for steroids,including estrogen and progesterone. Iron also plays an important role in ovarian activity. Positive correlation was reported between serum progesterone level and copper-zinc in cows. Trace elements are important for reproduction (Machadoa*et al*., 2013) via contributing to the normal health of reproductive organs and reproductive cycles. Selenium is important in normal cattle production systems as its apparent direct link to postpartum uterine involution.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location

The study was carried out at the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University Zaria, situated in the Northern Guinea Savannah, and lying between latitudes 11º 8′ 19.5′′ N and between longitude 7º 45′ 51.22′′ E at an elevation of 646 m above sea level.

3.2 Experimental Rams

A total of 15 rams aged 19.06 ± 2.4 months and weighing 19.4 ±1.6 kg with good body condition scores of 3.5 were divided into three treatment groups (A, B and C) according to the dietary protein level.

Group A (n=5) received 10% crude protein level feed, group B (n=5) received 15% crude protein level feed, while group C (n=5) received 20% crude protein level feed. The rams were screened for blood and helminth parasites, and appropriate treatment carried out before the commencement of the research.

3.3 Experimental diets

The three levels of protein were formulated to contain 10% crude protein, 15% crude protein and 20% crude protein.

Table 3.1: Ingredients and nutrient composition of experimental diets

Groups/Ingredients (%)	A (10% C.P)	B (15% C.P)	C (20% C.P)
Maize Bran	23.5	10	2
Bagasses	20	8.5	2.5
Palm Kernel Cake	10	30	45
Cotton Seed Cake	15	30	48
Rice Bran	30	20	1
Bone meal	1	1	1
Common Salt	0.5	0.5	0.5
Total	100.00	100.00	100.00

Table 3.2: Proximate analysis of feeds (10%, 15% and 20% CP levels respectively)

Group and %CP	% DM	% Ash	% CF	% N	% CP	Energy (MJ/Kg DM ME)
A(10%)	96.21	11.94	35.69	1.67	10.44	2.060
B (15%)	95.57	9.26	31.15	2.43	15.19	2.120
C (20%)	96.05	6.70	30.50	3.30	20.63	2.210

DM= Dry matter, CF= Crude fibre, N= Nitrogen, CP= Crude protein

The rams were managed under intensive system, kept in separate pens, fed individually and allowed two weeks adjustment period. All rams under study were fed a basal diet of hay (*Digitaria*spp) *ad-*

libitum and given a supplement ration of concentrate mixture at 2% body weight/head/day. All test diets were subjected to proximate analysis using the method of Association of Official Analytical Chemist (1990).

The animals were fed for a period of three months.

3.4 Body Weights and Scrotal Circumference

Body weights of individual ram were taken using a weighing scale. The scrotal circumference was measured with a flexible tape at the broadest part of the scrotum. This was done by applying gentle pressure with hand above the head of the epididymis thereby forcing the testes into the scrotum. The rams were restrained in a standing position during the measurements. These parameters were taken in the morning (8 am) on a weekly basis throughout the study period.

3.5 Haematological Evaluation

3.5.1 Determination of packed cell volume

The *Packed Cell Volume* (PCV) was determined as described by Benjamin (1978) using the microhaematocrit method.

3.5.2 Red Blood Cell and White blood cell count

Red Blood Cell (RBC) and white blood cell (WBC) counts were done using haemocytometer method as described by Schalm*et al*. (1975).

3.5.3 Determination of total protein (TP)

Total protein was determined according to the method described by Benjamin (1978), known as Biuret reaction which is based on the principles that protein forms coloured complex with copper ions (Cu^{2+}) in alkaline solution as a result of nitrogen reaction complex.

3.6 Semen collection

Semen was collected in the morning (8 am) from rams adequately restrained in standing position. Semen samples were collected with the aid of a battery-operated electro-ejaculator. The probe was lubricated with petroleum jelly for easy insertion into the rectum and pushed forward slowly and a series of short electrical stimulations were done intermittently for approximately 2-5 seconds, until erection and ejaculation was achieved. Ejaculates were placed in a water bath at 37^0C before microscopic evaluations. Semen samples were collected once weekly from each ram for 3 months using electroejaculator.

3.7 Reaction time

The time from onset of application electrical pulses to ejaculation was determined using a stop watch (Casio HS3).

3.8 Semen evaluation

Semen samples were evaluated according to the method described by Oyeyemi*et al*., (2000). The gross semen characteristics examined were volume, colour, and presence or absence of foreign bodies as described by Maina*et al*., (2006).

3.8.1 Volume

The semen was collected and read from the graduated collecting tubes immediately.

3.8.2 Sperm concentration

This was determined by using the haemocytometer method. Semen volume were sucked into the red blood cell diluting pipette up to 0.1 mark and the volume made up to 101 mark with 10% formal saline and mixed thoroughly. A drop of the mixture was allowed to spread under the cover-slip placed tightly on the haemocytometer after discarding few drops. The cells were allowed to settle before counting under x 40 magnification. Sperm cells were counted diagonally from top left to right bottom in 5 small squares of the improved Neubauerhaemocytometer. Sperm output of individual ram was calculated as described by Verstegen*et al*., (2002) using the following equation:

Concentration (sperm cells/ ml) = Number of sperm cells counted in the 25 small squares x dilution factor x 10^4.

3.8.3 Percentage Live spermatozoa

This was determined as described by (Esteso*et al*., 2006). A thin smear of the semen sample was made on clean, grease-free glass slides and stained with Eosin-Nigrosin stain. The dead sperm cells were identified as those that absorbed the stain, while the live cells don't absorb the stain. At least 400 sperm cells were counted in a regular sequence using light microscope at x40 magnification.

3.8.4 Sperm Abnormalities

These were determined according to the method of Esteso*et al*.(2006). A thin smear of the semen sample was made on a clean grease-free glass slide and fixed with buffered formal saline. The preparation was examined and abnormal sperm cells counted in a regular sequence using light microscope at x 100 magnification with oil immersion. A total of 400 well-spaced spermatozoa were carefully examined in each preparation and the percentage of head, midpiece and tail sperm abnormalities will be determined as described by Melrose and Laing (1970).

3.9 Gonadal sperm reserves

Gonadal and epididymal spermatozoa reserves were determined as described by Ogunlade*et al*., (2006). Three rams from each treatment group were sacrificed, the length and weight of each testis were determined using a measuring tape and the digital weighing balance respectively. The *tunica albuginea* was removed from each testis. The epididymis were separated from each testis and divided into caput, corpus and cauda which was measured and weighed using a measuring tape and a digital weighing balance. Thereafter, testicular and epididymal spermatozoa numbers were determined by homogenization (Egbunike*et al*., 1976). Each fraction was homogenised in 25ml physiological saline solution using mortar and pestle. Antibiotics (Streptomycin sulphate 1mg/ml and Sodium G 100 IU/ml) were added to saline. The homogenate volume was measured after rinsing the mortar with 10ml of physiological saline solution and adding the effluent. Exactly 2.5 ml of the homogenate was transferred into a conical flask and further diluted with 40ml of saline. The diluted testicular homogenate sample were stored overnight at 5^0C and filtered through gauze, and then the filtrate volume will be measured. Spermatozoa/spermatids concentrations were determined using a haemocytometer according to the method of Kwari and Waziri (2001). The determination of spermatozoa and spermatid reserves was done according to the method of Oyeyemi*et al* (2000).

3.10 Epididymal (Extragonadal) Sperm Reserves

The epididymis was carefully separated from the testis with a scalpel and the lengths and weights of the *caput*, *corpus* and *cauda* portions were determined using a measuring tape and a digital weighing balance. The *caput*, *corpus* and *cauda* epididymis were separated using sharp scissors, minced separately in 20 ml of saline and stored overnight at 5^0C and filtered through gauze, then the filtrate volume measured. Spermatozoa/spermatid concentration was determined using a haemocytometer according to the method of Kwari and Waziri (2001).

3.11Statistical Analysis

Data collected were expressed as means and standard error of the mean (±SEM). Significance of differences between treatments means were estimated at $P \leq 0.05$ with Tukey-Kramer multiple comparison test of repeated measure analysis of variance (ANOVA). Statistical analysis was conducted using the GraphpadInstat computer programme (GRAPHPAD for Windows, Inc., version 3.05 of 2000).

CHAPTER FOUR

RESULTS

4.1 Live Weights

From this study, it was observed that there was an increase in the live-weights of rams from the beginning to the end of the study across the groups. The mean highest weight recorded in group A was 20.8 ± 1.3 kg, group B was 24.8 ± 3.0 kg and group C with 27.8 ± 1.4 kg (Figure 4.1), while the lowest mean weights recorded were 17.1 ± 1.5 kg and 19.2 ± 2.4 kg and 19.2 ± 1.59 kg for groups A, B and C respectively. Comparison of the mean live-weights changes through the study period showed a decrease in rams fed 10% crude protein being significantly ($P \leq 0.05$) lower (18.8 ± 0.8 kg) than those fed 15% and 20% crude protein which had mean live-weight changes of 22.1 ± 0.6 kg and 24.1 ± 1.0 kg, respectively. Group B had a lower mean live-weight throughout the study period with a value of 22.1 ± 0.6 kg compared to group C with mean value of 24.1 ± 1.0 kg, although there was no statistically significant ($P > 0.05$) difference between the weights obtained in groups B and C.

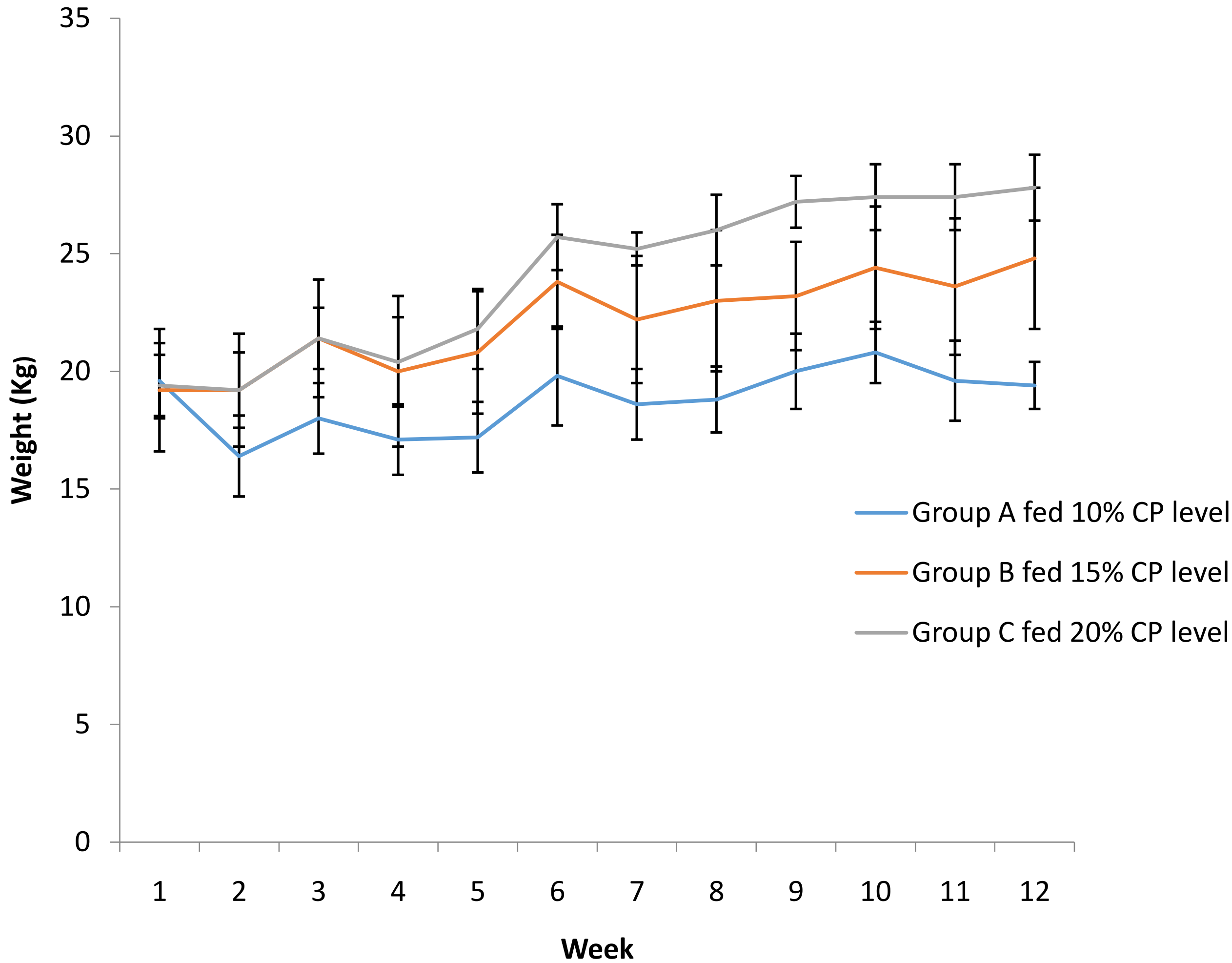

Figure 4.1: Mean weekly Live-weights (kg) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

4.2 Red Blood Cell

The mean RBC values obtained for the rams fed 10%, 15% and 20% crude protein ranged from 3.8 ± 0.1 X10^6 µl to 4.9 ± 0.5 X10^6 µl, 4.5 ± 0.5 X10^6 µl to 5.8 ± 0.2 X10^6 µl and 4.7 ± 0.2 to 5.7 ± 0.2 X10^6 µl respectively (Figure 4.2). There was significant ($P \leq 0.05$) difference between values for groups A and B and groups B and C. The overall mean RBC values obtained were 4.2 ± 0.1 X10^6 µl, 5.2 ± 0.1 X10^6 µl and 5.1 ± 0.1 X10^6 µl, for groups A, B and C respectively (Figure 4.2). The lowest value obtained was for rams fed 10% (3.8 ± 0.1X10^6 µl) was significantly lower ($P < 0.05$) than that for rams fed 15% (4.5 ± 0.5 X10^6 µl) and 20% (4.7 ± 0.2 X10^6 µl) respectively. The difference between values obtained for group B and C were not significantly different ($P > 0.05$), although group C had lower values than group B.

4.3 Packed Cell Volume

There was significant difference ($P < 0.05$) between PCV values obtained for groups A and B. and for groups B and C, but not ($P > 0.05$) between group A and C. The packed cell volume level of rams fed 20% CP ranged from 28.4 ± 1.2 % to 34.4 ± 1.4 % (Figure 4.3). That of those fed 15% and 10% CP ranged from 26.8 ± 2.8% to 35.0 ± 1.6% and 22.6 ± 0.8% to 29.6 ± 2.2% respectively. At the end of the study, the overall mean PCV of rams fed 10% CP was 25.9 ± 0.7% (Figure 4.3) which was significantly lower ($P < 0.05$) than values obtained for rams fed 15% and 20% CP. Rams fed 15% CP had an overall mean value of 31.3 ± 0.9 which was higher than what was obtained for group C(30.3 ± 0.6%), though the difference was not statistically significant.

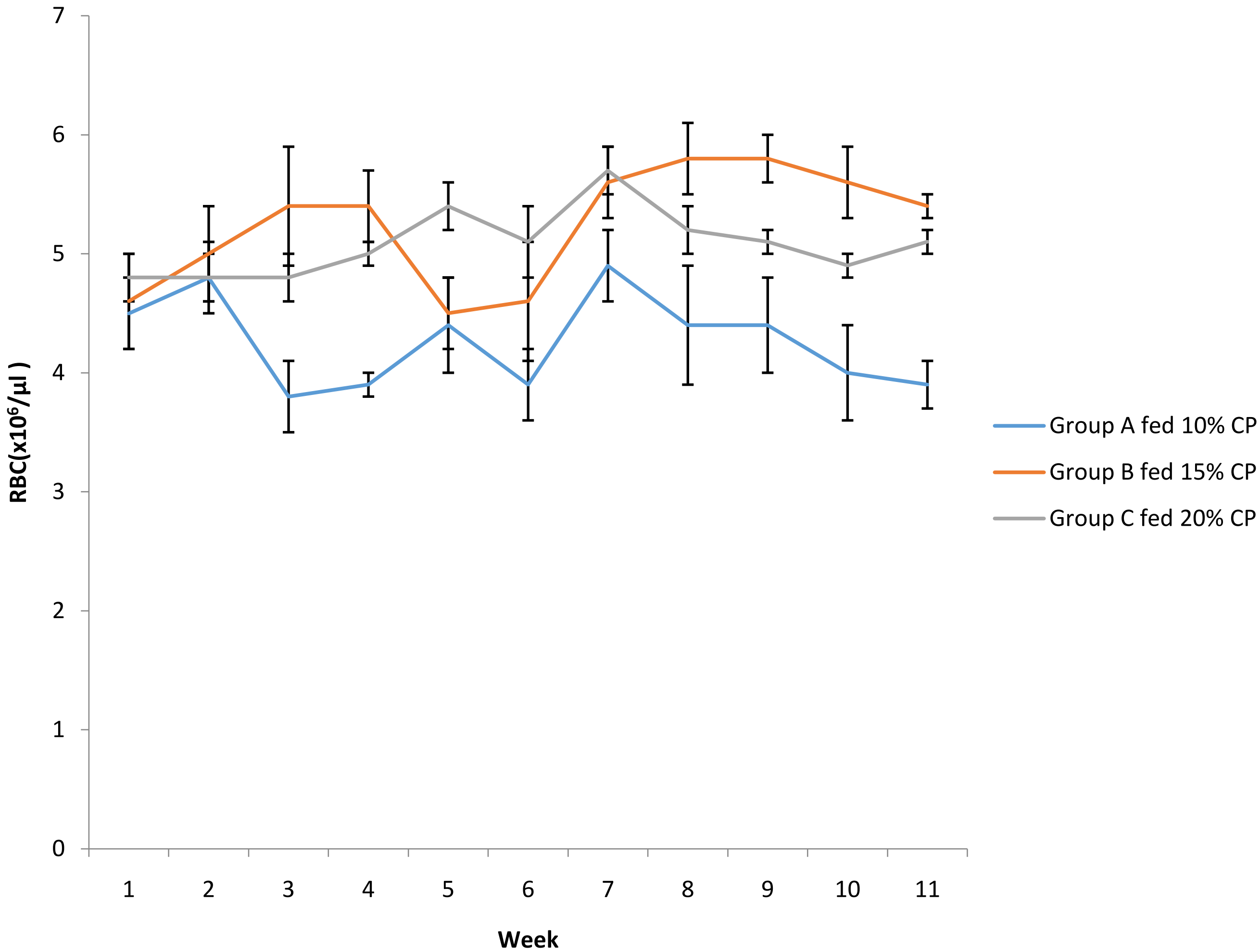

Figure 4.2: Mean weekly Red Blood Cell Counts (x10^6/µl) of Yankasa rams fed graded levels of dietary proteinfrom cotton seed and palm kernel cakes

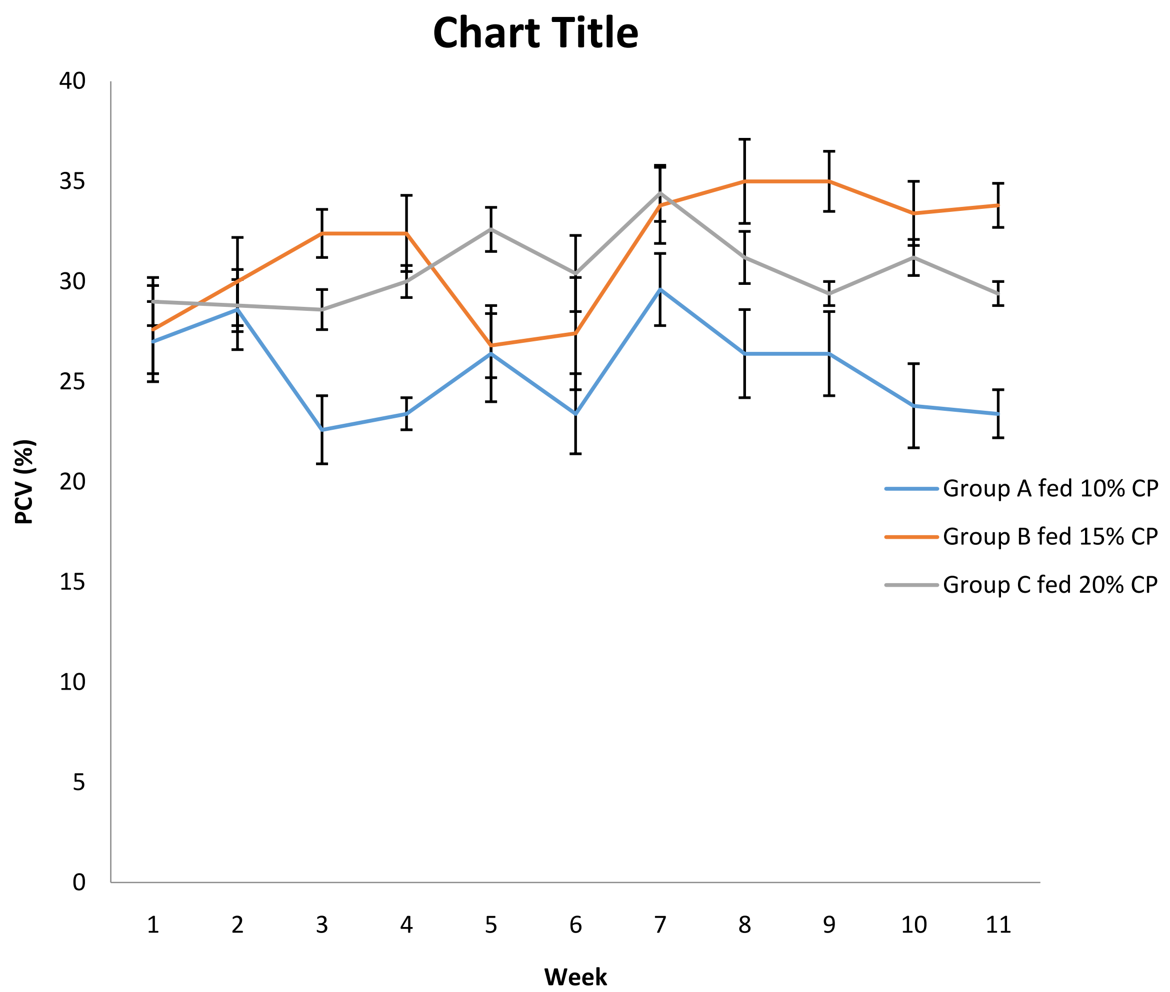

Figure 4.3: Mean weekly Packed cell volumes (%) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

4.4 White Blood Cells

Rams fed 10% CP had mean white blood cell counts ranging from 7.5 ± 0.7 $x10^6$/µl to 10.0 ± 2.1 $x10^6$/µl (Figure 4.4), while those rams fed 15% and 20% CP had values that ranged from 7.4 ± 0.8 $x10^6$/µl to 9.5 ± 1.0 $x10^6$/µl and 6.6 ± 0.7 $x10^6$/µl to 9.2 ± 1.5, respectively. The overall mean WBC obtained for rams fed 10%, 15% and 20% were 8.4 ± 0.7 $x10^6$/µl, 8.4 ± 0.2 $x10^6$/µl and 8.4 ± 0.2 $x10^6$/µl, respectively, thus values obtained for rams fed 10% CP were highest, while those fed 20% and 15% CP gave lesser values (Figure 4.4) but these differences were not statistically ($P \leq 0.05$) significant.

4.5 Total Protein

Those rams fed 10% had total protein values, ranging from 5.1 ± 0.3 g/l to 7.00 ± 0.71 g/l, rams fed 15% CP had values ranging from 6.4 ± 0.1 g/l to 7.3 ± 0.3 g/l, while those fed 20% CP had values ranging from 5.8 ± 0.2 g/l to 7.8 ± 0.5 g/l, (Figure 4.5).The overall mean total protein values obtained for the groups were 5.9 ± 0.2 g/l, 6.8 ± 0.0 g/l and 7.0 ± 0.2 g/l for group A, B and C respectively. There were statistically ($P \leq 0.05$) significant differences between groups A and B, and groups A and C, but not between groups B and C.

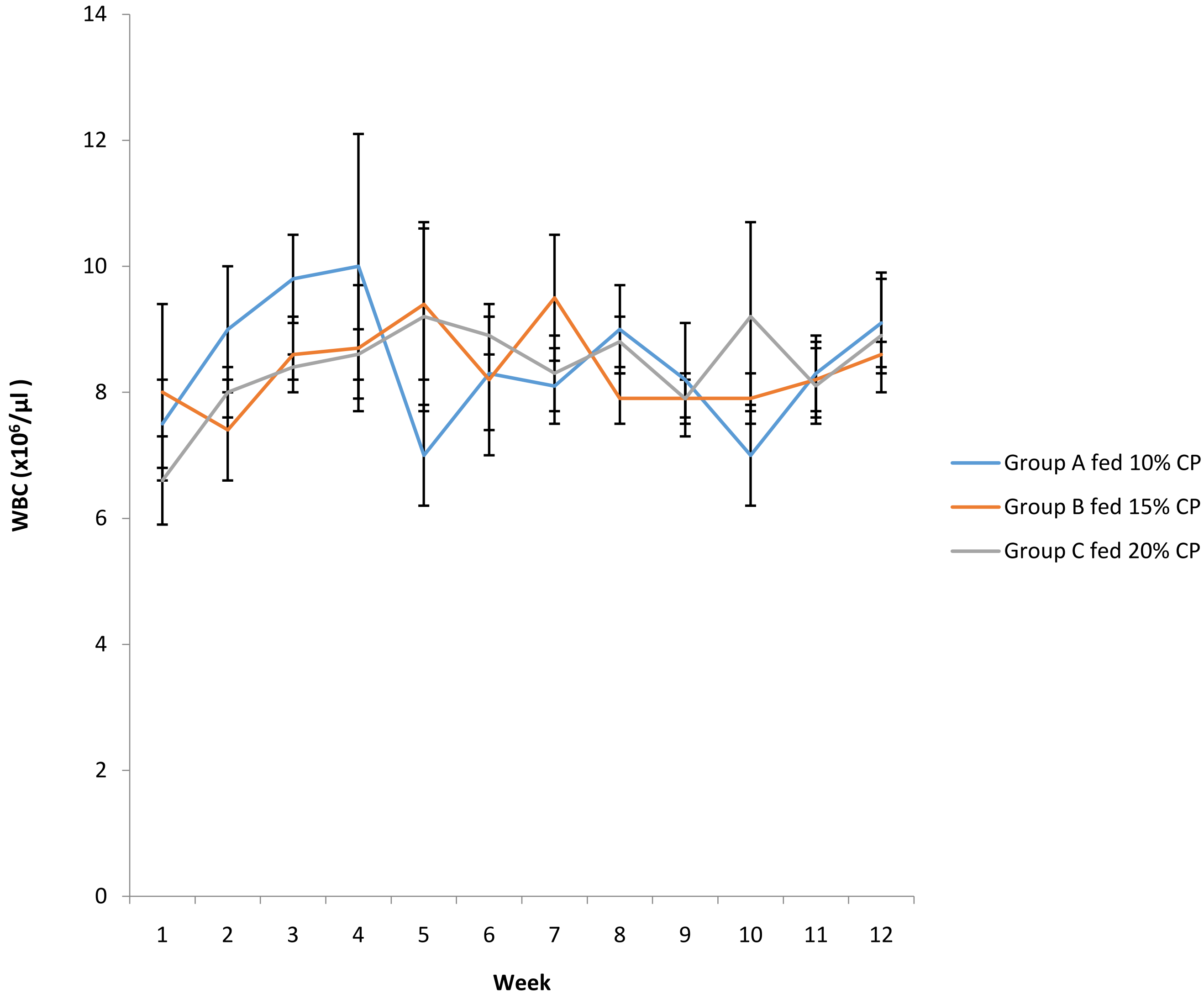

Figure 4.4: Mean weekly White blood cell counts (x10^6/µl) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

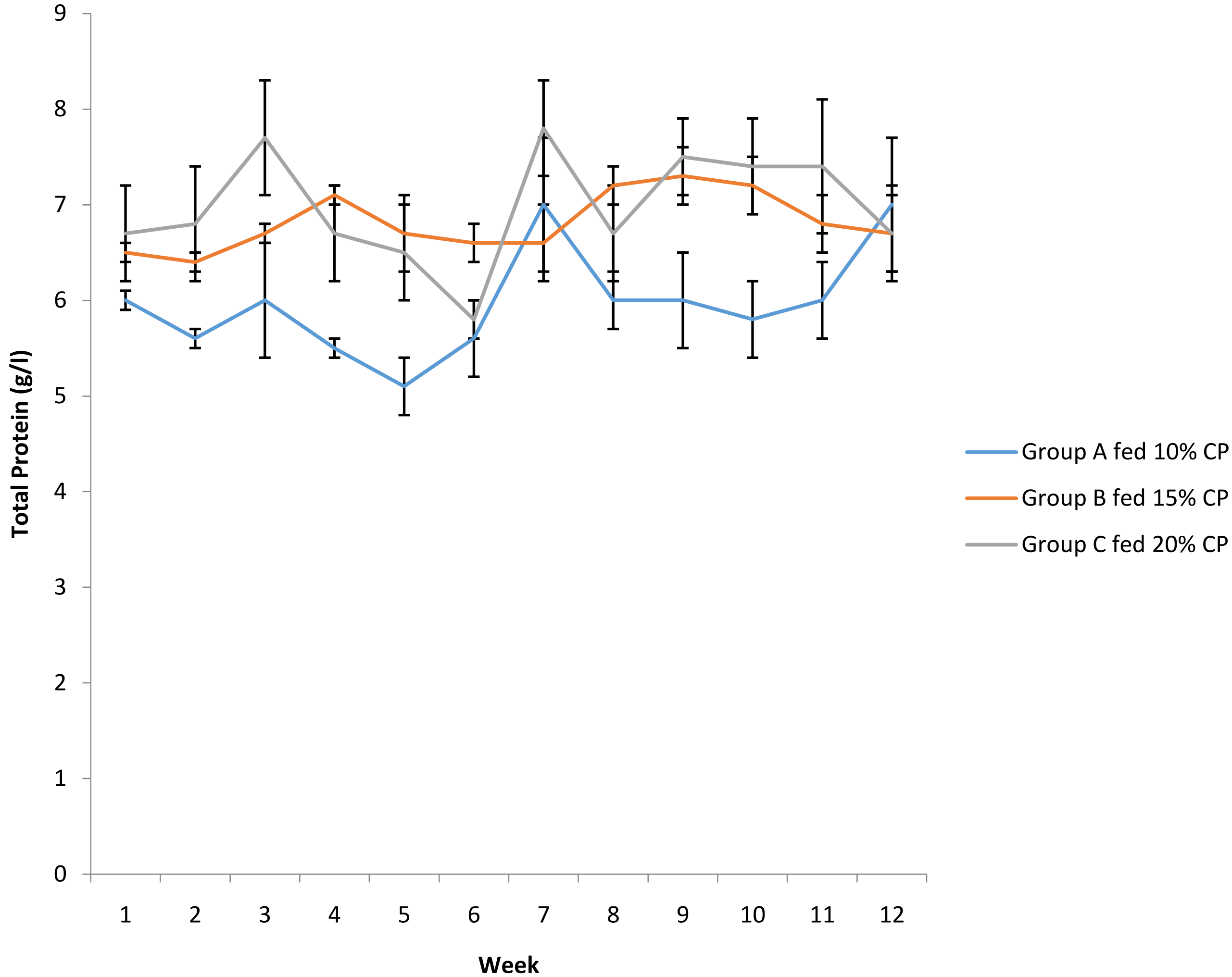

Figure 4.5: Mean weekly Total protein Concentration (g/l) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

4.6 Scrotal Circumference

Maximum values for scrotal circumference in groups A, B, and C were 25.8 ± 1.7 cm, 28.9 ± 1.7 cm and 30.2 ±1.1 cm, respectively (Figure 4.6) and minimum values of 20.8 ± 1.0 cm, 21.3 ± 3.0 cm and 21.8 ± 1.7 cm, respectively (Figure 4.6). Comparing the overall mean scrotal circumferences for the groups, there was a significant ($P \leq 0.05$) difference between groups A and B, and between groups A and C. There was a difference in the overall mean scrotal circumference between groups B and C with values of 26.2 ± 0.7 cm and 26.9 ± 0.8 cm, respectively, though these differences were not statistically ($P > 0.05$) different.

4.7 Semen Volume

The minimum mean semen volume recorded were 0.1 ± 0.1 ml, 0.1 ± 0.0 ml and 0.1 ± 0.1 ml, for groups A, B and C rams respectively (Figure 4.7). All the minimum values were recorded at the first week of the study. The maximum mean volumes of the groups were 0.5 ± 0.3 ml, 0.5 ± 0.0 ml and 0.5 ± 0.4 ml, respectively. The overall mean volumes for groups A, B and C at the end of the study were 0.2 ± 0.01 ml, 0.4 ± 0.01 ml and 0.4 ± 0.0 ml, respectively, with group A value being significantly ($P \leq 0.05$) lower than that of group B and C. The group B value was not significantly ($P > 0.05$) different from that of group C. The Overall mean value for group B was also higher than that of group A.

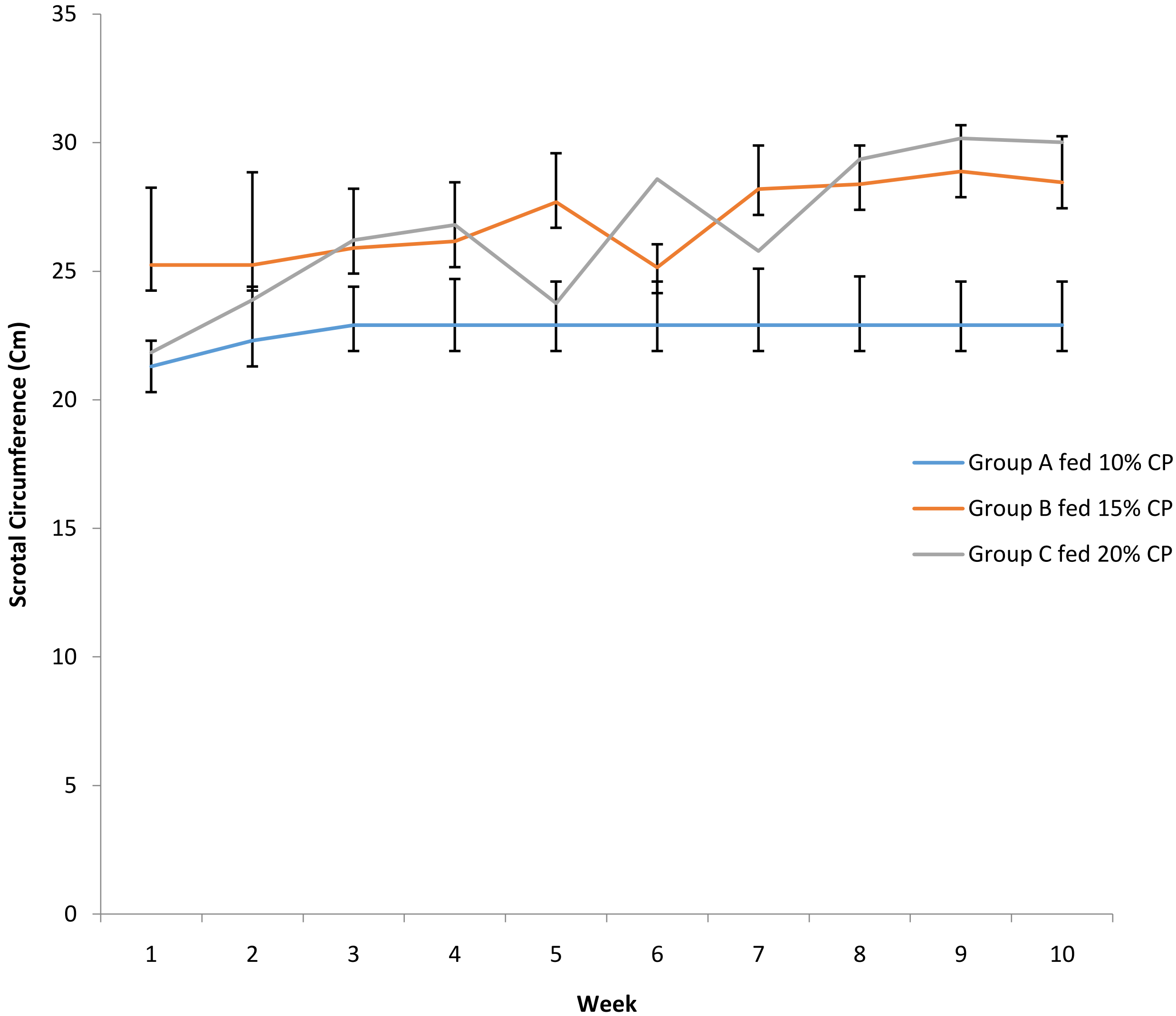

Figure 4.6: Mean weekly Scrotal Circumferences (cm) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

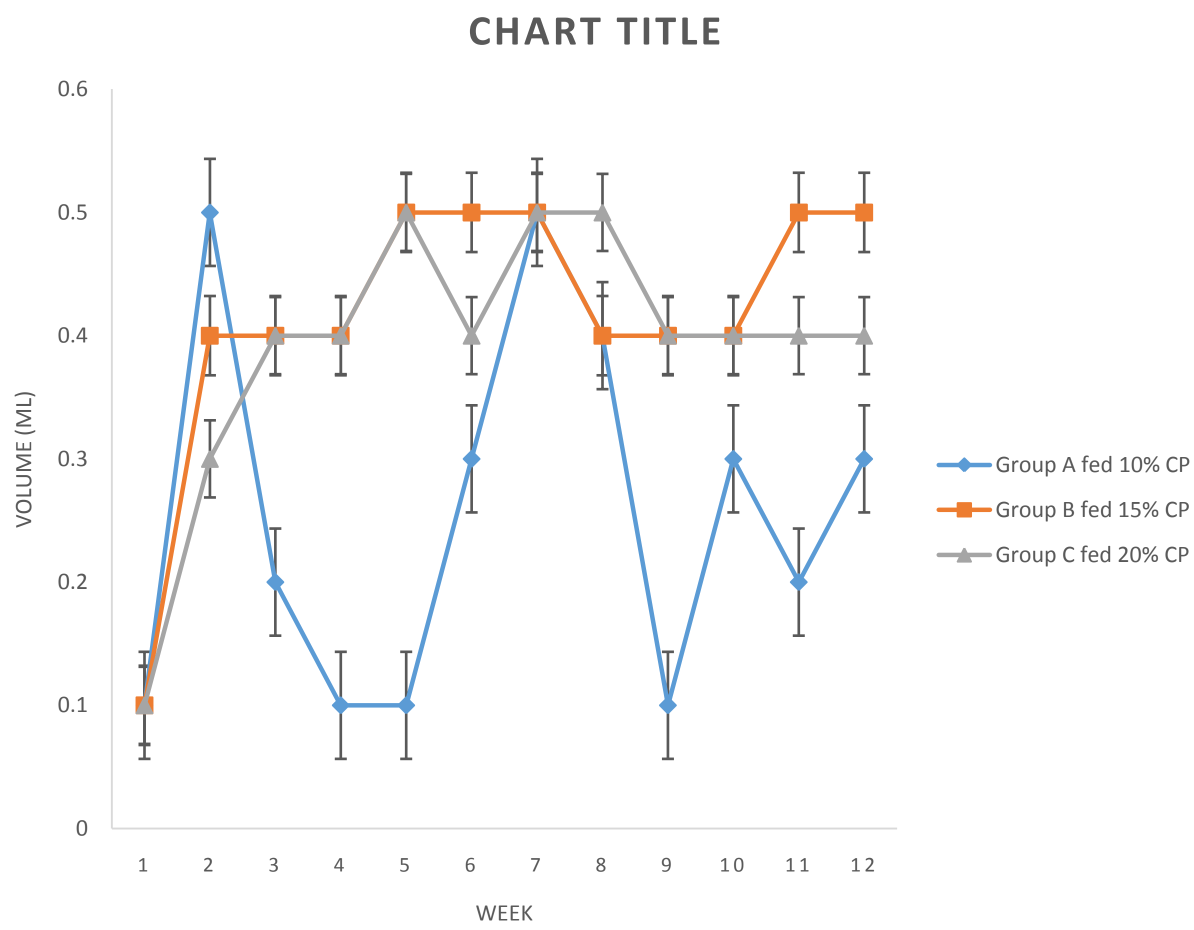

Figure 4.7: Mean weekly Semen Volume (ml) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cake

4.8 Sperm Concentration

The overall mean semen concentration for groups A, B and C were 96.3 ± 16.3x10^6/ ml, 163.1 ± 20.6 x10^6/ ml and 98.8 ± 8.5 x10^6/ ml, respectively, (Figure 4.8). The mean semen concentration throughout the study for group A (96.4 ± 16.4 x10^6/ ml) was significantly ($P \leq 0.05$) lower than that of group B (163.1 ± 20.60 x10^6/ ml) and also lower than the overall mean concentration for group C (98.8 ± 50 x10^6/ ml), though the difference was not significant ($P > 0.05$). There was significant ($P \leq 0.05$) difference between the mean concentration of group B and C with values of 163.1 ± 20.6 (x10^6/ ml) and 98.8 ± 8.5 (x10^6/ ml), respectively. The mean highest semen concentrations observed for group A, B and C were 156.8 ± 84.2 (x10^6/ ml), 264.8 ± 16.6 (x10^6/ ml) and 130 ± 54.4 (x10^6/ ml) respectively, while the minimum were 21.8 ± 14.7 (x10^6/ ml), 85.7 ± 27.6 (x10^6/ ml) and 36.2 ± 23.3 (x10^6/ ml) for groups A, B and C respectively.

4.9 Sperm Motility

The minimum mean motility for groups A, B and C were 42.5 ± 18.7 %, 42.0 ± 5.4 % and 40.0 ± 15.5 %, respectively (Figure 4.9), while the maximum mean motility were 78.0 ± 12.3 %, 90.0 ±3.5 % and 67.0 ± 10.7 % respectively in that order. The overall mean motility for group A was 54.5 ± 3.8 %, which was significantly ($P \leq 0.05$) lower than that obtained for group B with value of 75.1 ± 3.0 %, and higher than the value obtained for group C with the value of 53.9 ± 3.9 %. There was significant ($P \leq 0.05$) difference between the overall means for motility between group B and C.

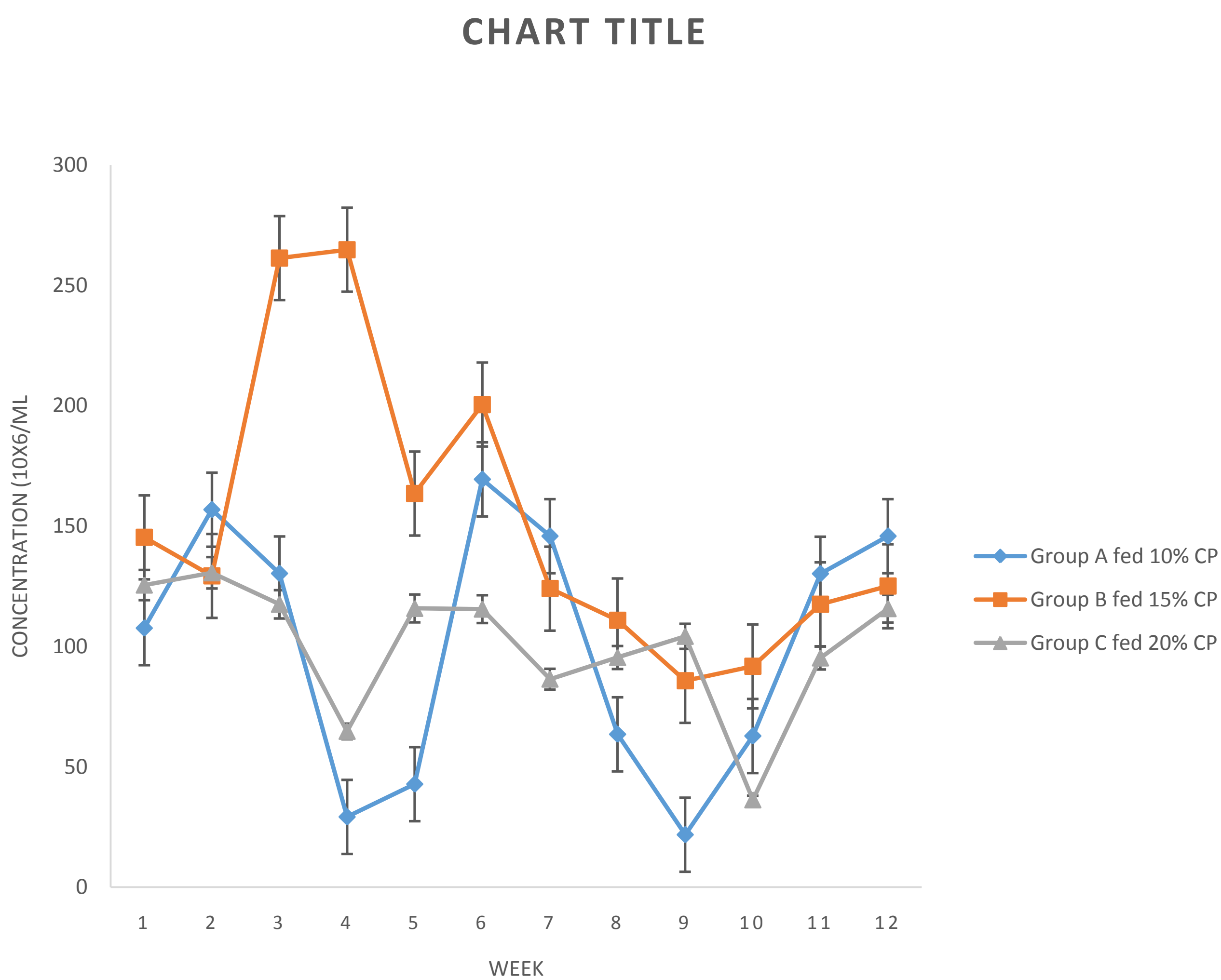

Figure 4.8: Mean weekly Semen Concentration (x10^6/ ml) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cake

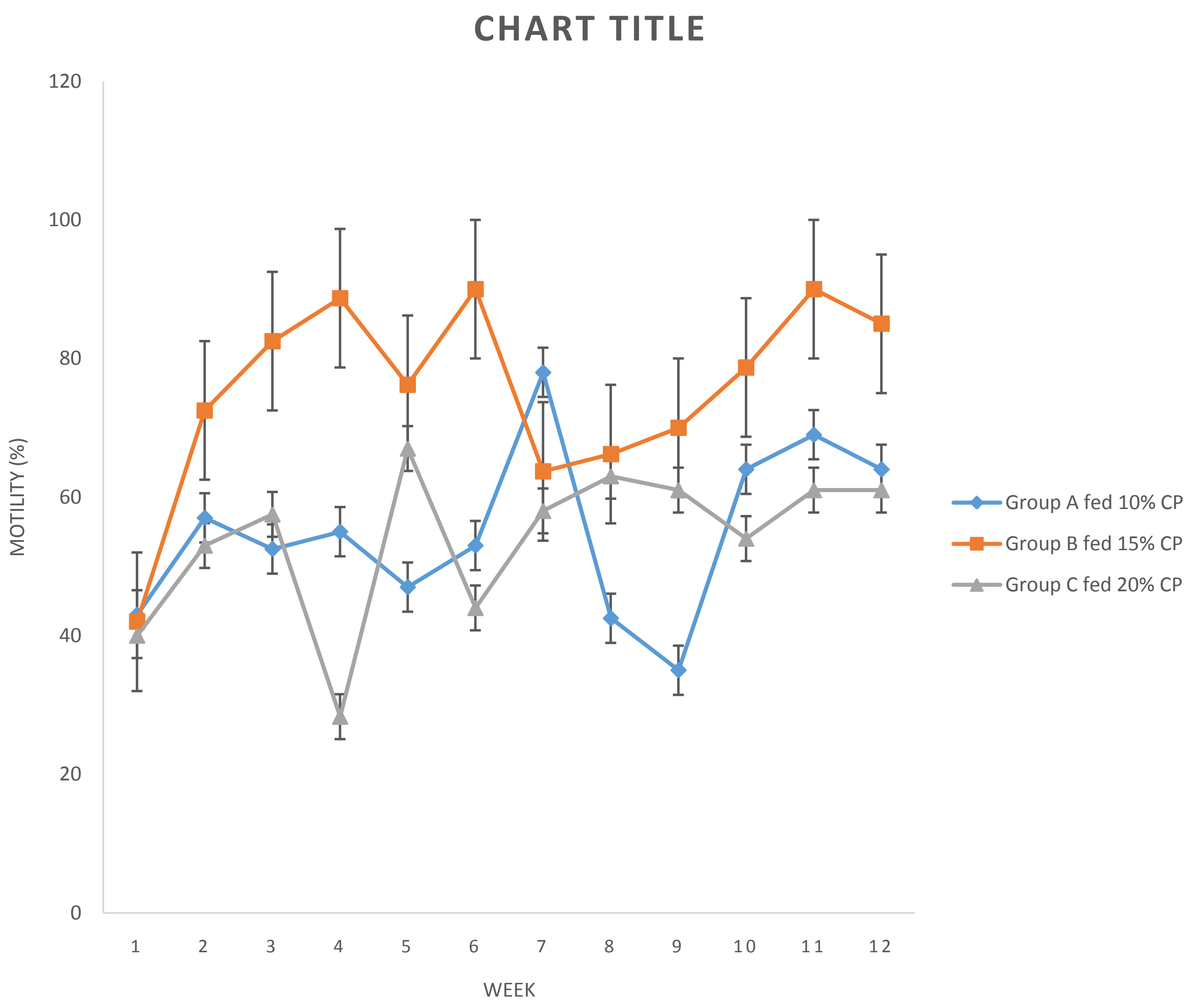

Figure 4.9: Mean weekly Sperm Motility (%) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cake

4.10 Percentage Live spermatozoa

Minimum mean values obtained for live sperm ratio for groups A, B and C were 56.0 ± 8.1 %, 71.2 ± 8.3 % and 66.0 ± 1.9 %, respectively (Figure 4.10), while the maximum mean values were 81.0 ± 5.6 %, 85.0 ± 2.9 % and 6.0 ± 6.0 % for groups A, B and C respectively. The overall mean value of live sperm ratio of sperm for group A, B and C were 71.0 ± 1.9 %, 80.0 ± 1.5 % and 72.2 ± 1.4 %. That obtained for group A was significantly lower (P < 0.05) than the value for group B, although lower than that of group C, not significant (P > 0.05). The value for group B is significantly higher (P < 0.05) than values obtained for groups A and C.

4.11 Reaction Time

Minimum mean reaction times for groups A, B and C were 0.4 ± 0.1 min, 0.5 ± 0.1 min and 0.4 ± 0.04 min, respectively (Figure 4.11) while the maximum mean reaction times were; 1.5 ± 0.5 min, 1.43 ± 0.49 min and 1.6 ± 0.3 min respectively. The overall mean reaction times at the end of the study were 1.1 ± 0.1 min, 0.9 ± 0.1 min and 1.0 ± 0.1 min for groups A, B and C respectively. Group B had the lowest mean reaction time, but there was no significant (P > 0.05) difference between the groups, while Group A had the highest overall mean reaction time (1.1 ± 0.1 min).

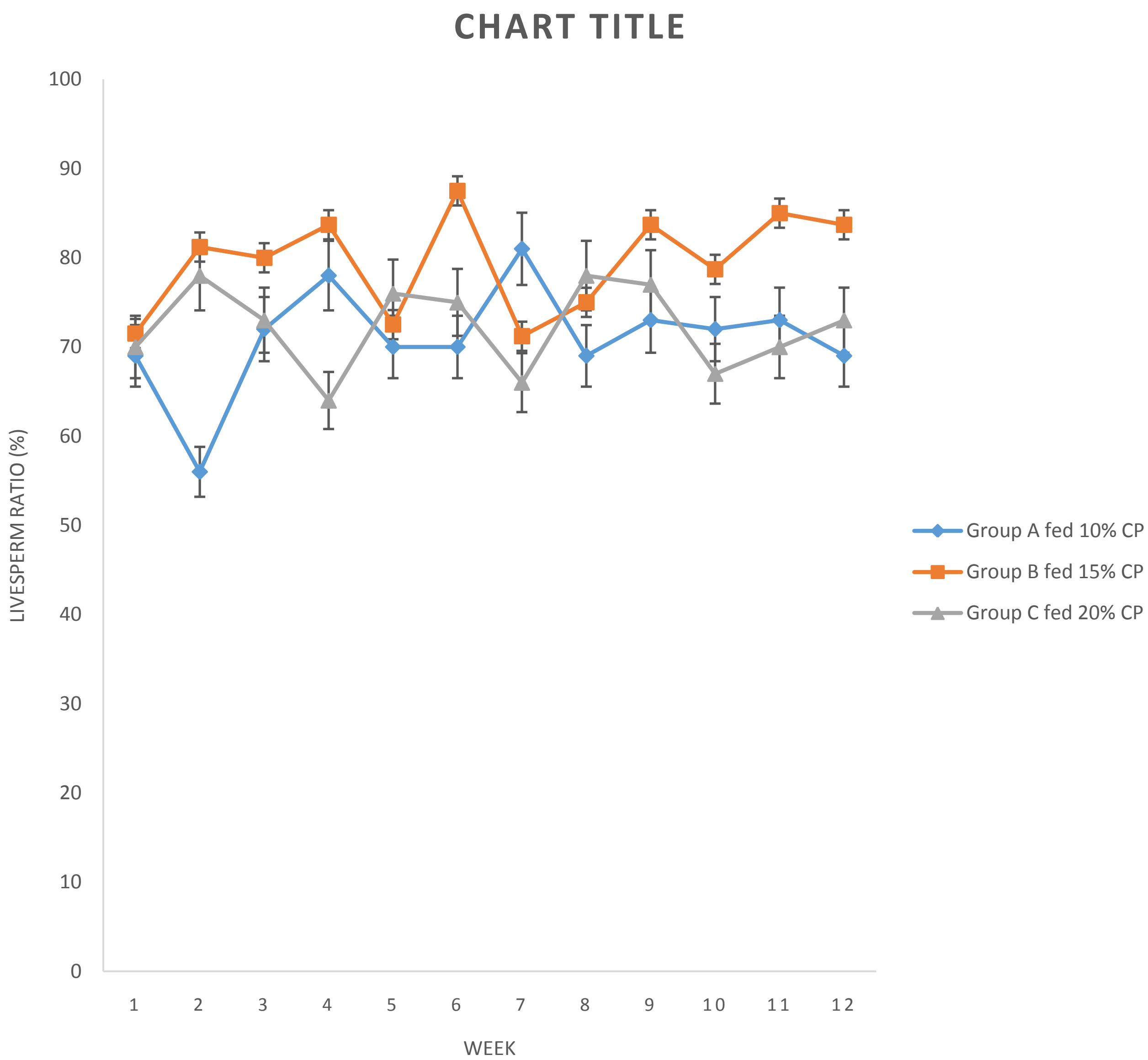

Figure 4.10: Mean weekly Live Sperm (%) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cake

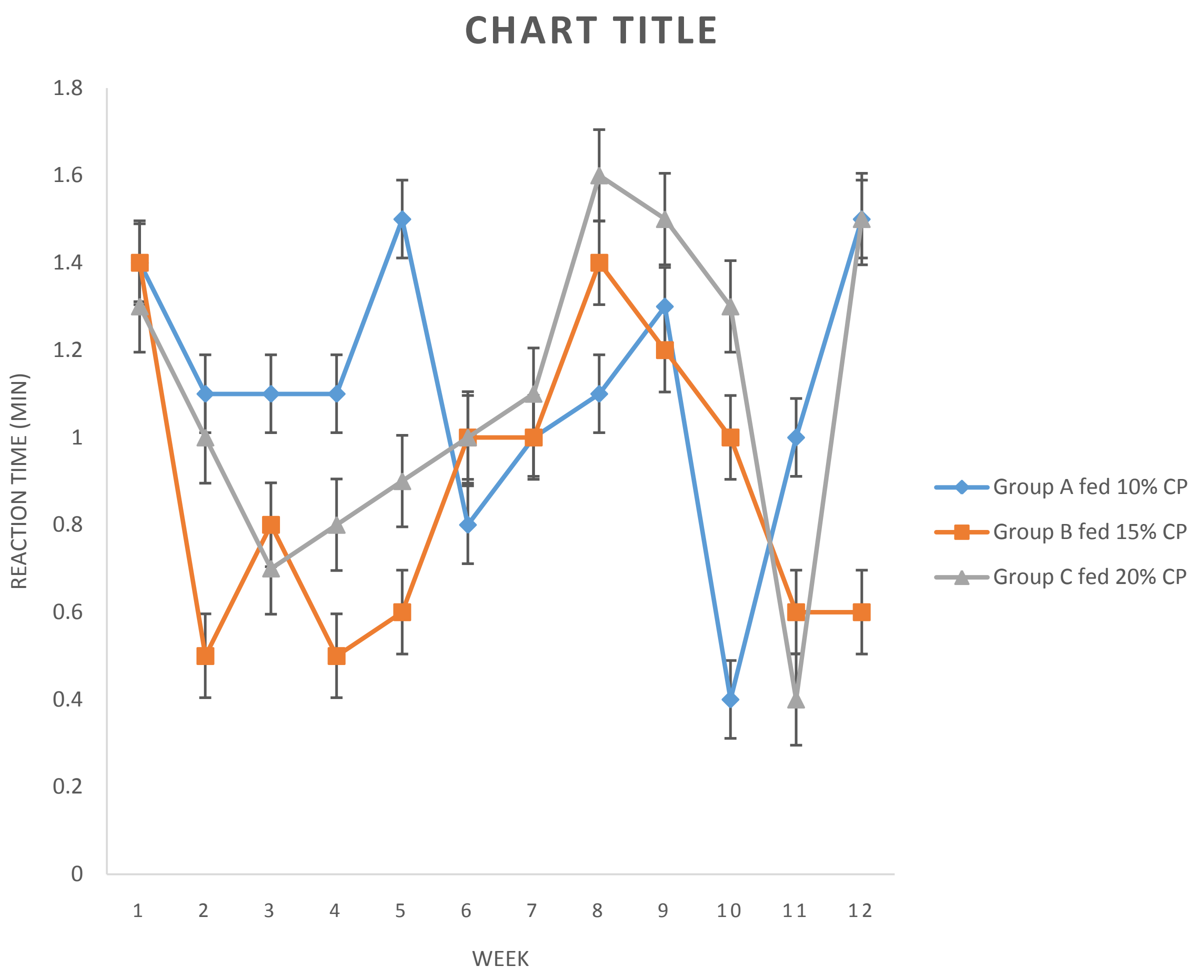

Figure 4.11: Mean weeklyReaction Time (Min) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

4.12 Sperm Abnormalities

Rams fed 15% CP (group B) had the highest values for normal sperm cells with 88.0 ± 4.6 % (Table 4.1). This was followed by rams in group A and then group C, with values of 84.43 ± 5.6% and 78.7 ± 6.2%, respectively. These differences were not significant ($P > 0.05$). Abnormalities recorded were; detached head, coiled tail, folded tail and bent tail. Rams in group C had the highest values for bent tails, detached head and curved tail (Table 4.1).

4.13 Gonadal and Epididymal Sperm Concentration

Rams fed 10% crude protein had the lowest testicular weights for both left and right testes with values of 98 ± 23.0g and 98.8 ± 26.3g, respectively. Rams fed 20% crude protein had the highest testicular weights with values of 149.4 ± 19.9 and 158.6 ± 15.7 (Table 4.2). Rams fed 15% had values of 131.6 ± 8.2g and 137.3 ± 9.7g (Table 4.2). Left testes of groups fed 10% and 15% C.P and 20% had higher weights than right testes. All the differences were not statistically ($P > 0.05$) different. Gonadal reserves of rams fed 20% was highest with values of 43.3 ± 3.9 ($x10^6$/g) and 47.0 ± 2.5 ($x10^6$/g). This was followed by rams fed 15% C.P. and 10% in decreasing order (28.0 ± 3.9 $x10^6$/g, 37.7 ± 2.5 $x10^6$/g and 20.7 ± 10.1 $x10^6$/g and 36.0 ± 14.6 $x10^6$/g) . These differences were not statistically ($P > 0.05$) different. Rams fed 15% had the highest *caput*epididymal reserves with values of 15.3 ± 0.7 ($x10^6$/g) for right epididymis and 16.7 ± 11.6 ($x10^6$/g) for left epididymis. This is followed by rams fed 20% C.P and 15% C.P. in decreasing order. *Corpus*epididymal reserves was highest in rams fed 20% C.P. followed by rams fed 15% and 10% in decreasing order. *Cauda*epididymal reserves was highest in rams fed 15% C.P., this was followed by rams fed 20% and 10% in decreasing order. These differences however were not statistically different

Table 4.1: Mean weekly Sperm abnormalities (%) of Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cakes

Sperm features (%)	% of sperm characteristics based on % dietary CP level		
	Group A (10%C.P.)	Group B (15% C.P)	Group C (20% C.P)
Normal cells	84.3 ± 5.6	88.0 ± 4.6	78.7 ± 6.2
Detached heads	4.9 ± 2.6	4.7 ± 1.1	6.5 ± 1.9
Folded tails	4.1 ± 1.2	3.3 ± 1.5	4.0 ± 1.3
Coiled tails	2.8 ± 1.2	2.3 ± 0.6	3.0 ± 1.3
Bent tails	3.4 ± 1.4	3.0 ± 1.2	3.7 ± 1.4

[ab] =Means in the same row with different letters are significantly ($P \leq 0.05$) different n = 5

C.P. = Crude Protein

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Table 4.2: Mean Gonadal and Epididymal Sperm/Spermatid Reservesof Yankasa rams fed graded levels of dietary protein from cotton seed and palm kernel cake

Parameters		Group A (10%)		Group B (15%)		Group C (20%)	
LW(kg)		19.4±1.0^{a}		24.8±3.0^{b}		27.8.±1.4^{b}	
SC (cm)		24.7 ±1.7^{a}		28.4±1.6^{b}		30.0±1.0^{b}	
Testes		Right	Left	Right	Left	Right	Left
	Length (cm)	8.2 ± 0.6	8.7±0.2	8.3 ± 0.3	8.5 ± 0.3	9.3 ± 0.3	9.3 ± 0.3
	Weights (g)	98.7±23	99.8±26.3	131.6 ± 8.2	137.3 ± 9.7	149.4±19.9	158.6±15.7
	GR($x10^{6}$/g)	20.7±10.1	36±14.6	28.0 ± 3.9	37.7 ± 2.9	43.3 ± 3.9	47.0 ± 2.5
Epididymis		Right	Left	Right	Left	Right	Left
Caput	Length (cm)	4.9 ± 0.6	4.8 ± 0.3	5.4 ± 0.4	5.3 ± 0.3	5.9 ± 0.7	5.4 ± 0.2
	Weight (g)	6.1 ± 1.8	6.3 ± 1.7	8.2 ± 0.4	8.0 ± 0.1	7.7 ± 0.5	7.83 ± 0.3
	Epididymal reserves ($x10^{6}$/g)	11.0± 9.2	12.3 ± 3.7	15.3 ± 0.9	16.7 ± 11.6	14.7 ± 2.9	10.3 ± 5.6
Corpus	Length (cm)	4.6 ± 0.7	4.0 ± 0.0	3.8 ± 0.6	4.2 ± 0.2	5.5 ± 1.2	4.8 ± 0.7
	Weight (g)	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.7 ± 0.0
	Epididymal reserves ($x10^{6}$/g)	7.3 ± 6.4	2.0 ± 1.1	2.7 ± 1.7	13.3 ± 9.6	6.7 ± 2.7	13.0 ± 7.5
Cauda	Length (cm)	4.4 ± 0.7	4.6 ± 0.9	5.9 ±0.1	5.1 ± 0.3	5.4 ± 0.0	5.6 ± 0.7
	Weight (g)	7.9 ± 2.0	8.1 ± 2.2	11.3 ± 0.5	11.5 ± 0.5	10.1 ± 0.6	10.6 ± 0.8
	Epididymal reserves ($x10^{6}$/g)	103.1±59	61.6±38.1	141.2 ± 26.6	168.8±24.1	142.3± 16.4	133.0 ± 14.0

^{ab}Means in the same row with different letters are significantly ($P \leq 0.05$) different

n = 3, LW- Liveweight, SC- Scrotal Circumference, GR- Epididymal Reserves

CHAPTER FIVE

DISCUSSION

Levels of protein in diets appeared to influence scrotal circumference (SC). Rams fed 15% CP had significantly higher SC than those on 10% crude protein and this finding agrees with that of Rekwot (1987) who found that bulls on high protein diets of 14.45% had higher SC than those on 8.15% CP. This result could be attributed to optimum utilization of dietary protein at 15% inclusion levels.The lack of significant increase in SC between rams fed 15% and 20% CP in this present study could be because of production of high level of ammonia associated with high rumen digestible protein and nitrogen retention (Jibril*et al*., 2013). The high content of protein in the feed, consisting of 20% CP probably resulted in high nitrogen content in the diet, leading to high nitrogen retentionwhich makes it unavailable for uptake by the body as reported by Al Haboby*et al*., (1999). This finding disagrees with that of Jibril*et al*, (2011), who reported a higher SC for rams fed 12.11% CP level. This difference may be due to difference in protein source used, which was dried layer litter, and these animals utilize non protein nitrogen at lower levels than at higher levels (Jibril*et al*., 2013). This finding also agrees with Allaoui*et al*., (2014), who reported that there is a positive correlation between scrotal measurements and body weight. From this study, rams fed 10% CP had the lowest live weights (kg) than those on 15% and 20% CP. This could be explained by the reduced feed intake by rams fed 10% CP, resulting in lower available of essential nutrients to build their body mass and subsequent decrease in live weights. This result differed from that obtained by Jibril*et al*., (2013) where rams fed 12.11% CP had the highest live weight as compared to 14% and 18% CP. This difference could be explained by the type of ingredients used as protein source. The authors used dried layer litter, while in this study, cotton seed and palm kernel cakes were used.

Liveweightincrease in rams fed 15% CP in this study are similar to those reported by Rekwot*et al*., (1988) and Elmaz*et al*., (2007), where bulls and rams fed higher protein diets (14% CP) had significant increase in live-weight when compared to those on low protein diet (8% CP). The lack of significant increase in live weights between rams fed 15% and 20% CP in this present study could be because of production of high level of ammonia associated with high rumen digestible protein and nitrogen retention, which makes nitrogen unavailable for uptake (Jibril*et al*., 2013). The high content of protein in the feed, consisting of 20% CP probably resulted in high nitrogen retention in the diet, thus making nitrogen unavailable for uptake by the body as reported by Al Habobby*et al*., (1999).

The Red blood cell values that were recorded in this study ranged from 3.76 to 5.83 $x10^6/\mu l$ and these values are lower than what was reported for sheep (8.9 - 9.3 $x10^6/\mu l$) by Etim *et al*. (2014). The difference might be due to nutrition, age or sex difference as stated by Etim*et al*. (2014). Rams fed 15% C.P had the highest values for RBC which indicated that feed improved the erythropoetic process in the animals (Togun*et al*., 2007). Rams fed 10% C.P had the lowest values of RBC which could be as a result of decreased protein to drive erythropoesis. This study revealed that animals on 15% CP and 20% CP had similar RBC levels which in turn would lead to an increased transport of oxygen and absorbed nutrients (Isaac *et al*., 2013).

The mean Packed Cell Volume (PCV) recorded for rams fed 10% CP in this study (25.9%) were lower than values reported by Njidda*et al*. (2014), who reported a normal value of 28.90% for Yankasa rams. Rams fed 15% CP had higher PCV values (31.3%) than what was reported (28.9%) by Njidda*et al*. (2014). Rams fed 10% C.P had the lowest PCV which was significantly lower than values obtained for rams fed 15% and 20% C.P, and this finding is similar to report by Babeker and Abdalbagi (2015), who reported a significant difference in goats fed (14% CP) compared to those fed (20%). This agrees with reports by Babeker andAbdalbagi (2015), who reported that reduction in PCV and red blood cells values are indicative of low protein intake. The high PCV values obtained in rams fed 15% CP level is similar to reports by Addass*et al*., (2010) who reported high PCV as signs of healthy and high productive animals.

The range of white blood cell count observed in this study is similar to what was reported for rams by Etim (2014), who gave a normal range of 4 – 12 $x10^6$/µl. This could be a pointer to proper health management system, as the animals were given prophylactic treatment against haemoparasites and helminth infections before commencement of this study, although this differed from reports by Babeker and Abdalbagi, (2015) who reported higher WBC 4 – $6x10^6$/µl in goats. WBC within normal values is an indication that there were no microbial infections or presence of foreign bodies or parasites (Bello and Tsado, 2013). The rams (group B) fed 15% CP and (group C) fed 20% had the lowest WBC; meaning they were in a healthy state with resultant production of lower WBC (Etim*et al*., 2014). This result differs from reports by Bello and Tsado (2013), who fed 15% C.P to rams and had a higher WBC (10.7 $x10^6$/µl) than those fed 7% CP, 13% CP and 14% CP ($10.6x10^6$/µl, $9.5x10^6$/µl and $7.0x10^6$/µl).

Rams fed 10% CP had the lowest value for total blood protein level. This is in contrast to reports by Babeker and Abdalbagi (2015), who reported highest values of total blood protein in goats, fed 15% CP level nutrition, while goats fed high levels of nutrition (18% CP) had lower values. Total blood protein is an indication of the quantity or quality of protein in the diet (Mohammed, 2012). The synthesis of plasma blood protein is markedly reduced when the supply of amino acid for digestive process is not adequate. This finding is similar to what was recorded in animals fed low level of Lucene hay due to low levels of total protein (Ahmed and Abdelatif, 1992; Kheir and Ahmed, 2008). Plasma protein helps to transport calcium and phosphorous and other substances in the blood (Njidda*et al*., 2014). Result from this study is similar to reports by (Mohammed, 2012) who stated that rams maintained on low protein levels (10%) showed significantly lower total protein. Total protein was high in rams fed 14% C.P (Bello and Tsado, 2013) and this corroborates the findings in this present study.

The ejaculate volume was highest in rams fed 15% CP and this is Similar to the report by Kheradmand*et al*., (2006) that the ejaculate volume tends to be higher with improvement of nutrition in Bakhtary rams. The ejaculate volume in the present study corroborates the report of Suhair and Abdalla (2010), where ejaculate volume in desert rams was significantly lower with low level of nutrition. The low ejaculate volume obtained in group A rams(10% CP) could be attributed to the decreased function of the pituitary gland and testis due to decrease in their sizes because of the decreased production of GnRH (Alkass*et al*., 1982). Consequently, the secretion of androgen-dependent organs (epididymis, testis and accessory glands) are expected to decrease, resulting in low ejaculate volume (Suhair and Abdalla 2010). Rams fed 15% and 20% CP had higher semen volume than rams fed 10% C.P corroborating the finding of Ososanya *et al*., (2014), but disagrees with that of Abi-Saab *et al*., (2008) who reported non-significant differences in semen volume of young bucks fed two different protein diets (11% and 18% CP). There was no significant difference in semen volume between rams fed 15% and 20% CP, and this agrees with the findings of Kheradmand*et al*. (2006).

In this study, rams on 15% CP had significantly higher semen concentration when compared to those fed 10% and 20% CP. This highest increase in semen concentration might be an indication that though an increased protein intake above 10% enhanced spermatogenesis, higher levels of CP in diets could result in excess urea and more available ammonia, which could alter the physiology of reproduction (Butler, 1993). This is similar to the reports of Rekwot*et al*., (1987), and Fernandez *et* *al*, (2004) where they reported that increased protein supply to 14% CPfavours spermatogenesis, but lower protein levels below 14% didn't favour spermatogenesis

The rams fed 15% CP had a higher sperm motility than those fed 10% and 20%, supporting the report by Ososanya*et al*., (2014), who reported that West African Dwarf goats fed 14% CP had greater semen motility than those given 12% CP. Kumar *et al*. (2015) also reported that feed restriction reduced the total motile spermatozoa, and slow spermatozoa were significantly higher in the nutritionally stressed rams. Sperm motility was significantly lower with poor feeding. This is clearly related to decline in the nutritional status of rams, which could have induced low fructose level in seminal plasma and consequently decreased semen motility as these parameters are positively correlated (Amir and Volcani, 1965). Low sperm motility might be related to low plane of nutrition and protein intake, resulting in relatively low concentration of seminal plasma metabolites (Chandrasekhar *et al*., 1986). The result from this study differs from what was reported by Jibril*et al*. (2011), in which there was no significant difference between groups fed different levels of CP.

The study revealed significantly lower live sperm values (71.0%) in rams maintained on low level of protein (10% CP). The group fed 20% CP also had low live sperm values (72.2 %) and this could be related to the high nutrition, resulting in increased metabolic activity of testicular cells thus causing testicular hypoxia (Suhair and Abdalla, 2010). This is similar to the study conducted by (Suhair and Abdalla 2010) in desert rams and Ososanya*et al*. (2014) in West African Dwarf rams. Rams that were fed 15% CP had highest value of 80.1 %.

Rams fed 15% CP had the lowest ejaculation time, which was an indication of high libido (Ososanya*et al*., 2014), althoughthere were no significant differences between all groups

From this study, it was observed that cauda epididymal sperm reserves was highest in all groups as compared to caput and corpus epididymal reserves. This is due to the fact that the major site of sperm storage within the male reproductive tract is the caudal epididymis as it has a relatively wider lumen in which high concentration of spermatozoa are stored. Similar findings were made by Iliyasu*et al*. (2014) that cauda epididymis had higher concentration of sperm cells in Yankasa rams.

Testicular weights observed in this study had a similar trend with gonadal reserves. This is similar to findings ofOgbuegbu*et al*. (1985) and Iliyasu*et al*. (2014). Also,Okwun*et al*. (1996) stated that rams with larger testes tend to produce more spermatozoa, a findingwhich was similar to what was observed in this study. Oyeyemi*et al*. (2002) also reported a positive correlation between testicular weights and gonadal reserves in West African Dwarf bucks. It was observed from this study that the left testes were heavier than the right testes in all groups, regardless of level of crude protein fed. This finding is similar to the observations ofRaji and Njidda, (2014), in Red Sokoto bucks fed with*Moringaoliefera*-supplemented diets. Also similar finding regarding the left testes having higher sperm reserves was observed in camel by Ibrahim *et al*. (2012). Babashani*et al*. (2015) reported similar findings in Yankasarams that were fed non gossypol containing diets. This increase in weight of the left testes might be due to higher proportion of Sertoli cells and seminiferous tubules in the left testes, which have been reported to be responsible for sustenance of spermatogenesis (Obidi*et al*., 2008).

The corpus of the epididymis had the lowest sperm reserves across all groups, compared with the *caput* and *cauda epididymis*. This result is similar to reports by Nasir *et al*. (2014), regarding gonadal reserves in Red Sokoto bucks fed cotton seed cake. This could be explained by the fact that the major storage point of spermatozoa is the *cauda epididymis,* and therefore the *corpus* might be serving as a transit point only. Similar findings were made by Yunusa*et al*., (2016), that the *corpus epididymis* had the lowest sperm reserves in goats.

From this study, rams fed 15% C.P. had higher extragonadal sperm reserves than rams fed 10% and 20% CP. This could be because at 15% CP, there is optimum utilization of protein to facilitate spermatogenesis. Rams fed 20% had higher gonadal sperm reserves compared to other groups. This is positively correlated with testicular weights recorded, which could be as a result of presence of more sertoli cells and seminiferous tubules, leadings to increased production of sperm cells.

It was observed that rams fed 20% C.P. had the highest percentage of abnormal spermatozoa. Similar reports were made by Alabi (2005) who observed higher sperm defects in bulls fed on both high and low energy diets than those on medium energy diet. Jibril*et al*. (2013), reported similar findings in rams fed high protein diet. The rams fed 20% C.P. had higher level of cotton seed cake in their diet. Cotton seed cake has a higher amount of bound gossypol, which could affect sperm morphology (Babashani*et al*., 2015). This occurs through gossypol induced inhibition of the synthesis of sperm cells histones and other nuclear proteins that stabilise the structure of DNA. (Ye *et al*., 1989).

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CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

I. The study has shown that feeding rams with diets containing 15 %CP (cotton seed cake and palm kernel cake) gave better results in terms of body weight (22.1 ± 0.6 kg), scrotal circumference (26.2 ± 0.7 cm), than 10% CP which had 18.8 ± 0.4 kg and 22.9 ± 0.6 cm respectively. Rams fed 20% CP also gave better results in terms of body weights (24.1 ± 1.0 kg) and scrotal circumference (26.9 ± 0.8cm) than rams fed 10% CP. No difference in values obtained between rams fed 15% CP and 20% CP.

II. Feeding rams with diets containing 15% CP (cotton seed cake and palm kernel cake) gave higher values as regards Packed Cell Volume (31.3 ± 0.9%), Red Blood Cell Counts (5.2 ± 0.1 $x10^6$µl), than feeding rams with diets containing 10% CP which had 25.9± 0.7% and 4.2 ± 0.1$x10^6$µl respectively. Rams fed 20% CP gave higher values as regards Packed Cell Volume (30.3 ± 0.6%), Red Blood Cell Counts (5.1 ± 0.1$x10^6$µl) than feeding rams 10% CP. No difference in values obtained between rams fed 15% CP and 20% CP

III. Feeding rams with diets containing 15% CP (cotton seed cake and palm kernel cake) gave higher values in terms of semen concentration (163.1 ± 20.6$x10^6$/ml), semen volume (0.4 ± 0.01ml), Percentage live sperm (80.1 ± 1.5%) and sperm motility (75.1 ± 3.0%) compared to diets containing 10% CP which had 96.4 ± 16.3$x10^6$/ml, 0.2 ± 0.01ml 71.0 ± 1.9% and 54.7 ± 3.8% respectively. Rams fed 20% CP had lower values in terms of semen concentration (98.8 ± 8.5$x10^6$/ml), percentage live sperm (72.2 ± 1.4%) and sperm motility (53.91 ± 3.9%) compared to rams fed 15% CP.

IV. Graded levels of dietary protein (10% to 20%) had no significant effect on total protein, white blood cell counts, reaction time, gonadal and extragonadal sperm reserves.

6.2 Recommendations

I. Yankasa rams should be fed crude protein levels of 15 % for better reproductive performance.

II. Studies should be carried out to investigate if antioxidants (Ascorbic Acid) could ameliorate some of the negative effects of low and high levels of protein that were observed in this study.

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