SEMEN CHARACTERISTICS, TESTICULAR HISTOLOGY AND HAEMATOLOGY OF YANKASA RAMS FED RATIONS OF WHOLE COTTONSEED AND COTTONSEED CAKE

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DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION
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DECLARATION

I declare that the work in this Thesis entitled "**Semen Characteristics, Testicular Histology and Haematology of Yankasa Rams Fed Rations of Whole Cottonseed and Cottonseed Cake**"has been carried out by me in the Department of Theriogenology and Production, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled, "**SEMEN CHARACTERISTICS, TESTICULAR HISTOLOGY AND HAEMATOLOGY OF YANKASA RAMS FED RATIONS OF WHOLE COTTONSEED AND COTTONSEED CAKE**" by Mohammed BABASHANI, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Cottonseed and its by-products are used extensively for supplementing ruminant rations to increase productivity. However, they contain gossypol which is a reproductive toxicant in males of both ruminant and non-ruminants. This study evaluated the effect of dietary gossypol from whole cottonseed (WCS) and cottonseed cake (CSC) on the semen characteristics and testicular morphology of Yankasa rams. Fifteen (15) Yankasa rams weighing an average of 20 $\pm$ 3 Kg were randomly distributed into three groups (A, B and C) of five rams each and were fed diets containing 48 % WCS, 48 % CSC and control diet (gossypol-free), respectively for 14 weeks. The semen was collected weekly using an electro-ejaculator and was evaluated for volume, gross motility, concentration, live sperm cells and morphology. Similarly, the body weight, scrotal circumference and some haematological parameters were evaluated on a weekly basis. An ultrasonographic examination of the testis of each ram was done every month for 3 months. At the end of the feeding period, the rams were slaughtered and histopathogical examination of the testicular tissues (n = 2) as well as gonadal and epididymal sperm reserves (n = 3) were conducted. The data obtained were statistically analysed using GraphPad Prism version 5.0. Significance of differences between treatments means were determined at $P \leq 0.05$ with Tukey-Kramer multiple comparison test of repeated measure analysis of variance (ANOVA). The mean semen volume and concentration, body weights, white blood cell counts and total proteins of the rams in the experimental groups A and B did not differ significantly ($p > 0.05$) from that of the rams in the control group. However, the mean values of semen gross motility ($p < 0.0001$), live spermatozoa ($p < 0.05$) and scrotal circumference ($p < 0.05$) were significantly lower in the treatment groups than in the control groups while the mean

totalsperm abnormalities ($p < 0.0001$)of the rams in the experimental groups A and B were significantly higher than those of the control group. Similarly, mean packed cell volume (PCV) and haemoglobin (Hb) concentration differed significantly ($P < 0.0001$) between experimental and control groups at weeks 8 to 14. Gonadal and epididymal lengths, weights and sperm reserves did not differ significantly ($p > 0.05$) between the experimental and the control groups. Histopathological changes observed in the testes of the rams in the treatment groups were depletion of spermatogenic cell layers and arrest of spermatogenesis at the spermatocyte and spermatids levels and an exfoliation of the spermatogonia. There was vacuolation of the germ layers in the seminiferous tubules. These changes were indicative of testicular degeneration. Ultrasonograms of the testis of rams from experimental groups showed loss of homogeneity and an increased hyperechoic appearance which were also indicative of testicular degeneration. It is concluded that, gossypol from whole cottonseed and cottonseed cake has detrimental effects on both semen characteristics and testicular histology. We recommend that these cotton products should be minimised in feeds forrams intended for breeding purposes.

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ABBREVIATIONS, GLOSSARY AND SYMBOLS

Symbols	Meaning
ABP	Androgen binding protein
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic acid
FAOSTAT	Food and Agriculture Organization Statistics
Kg	Kilogrammes
g	Grammes
cm	Centimetres
ILRI	International Livestock Research Institute
FAO	Food and Agriculture Organization
NDD	NationalDairy Database
mm	millimetres
m	metres
µm	micrometres
ST	Seminiferous tubules
LH	Luteinizing Hormone
FSH	Follicular stimulating hormone
DHT	Dihydrotestosterone
^{0}C	Degree celcius
BCS	Body condition score
ml	millilitre
OIE	Office International des Epizooties (World Organisation for Animal Health)
BVD	Bovine viral diarrhoea
IBR	Infectious Bovine Rhinotracheitis
MJ	Mega joules
CP	Crude protein
GnRH	Gonadotropin Releasing Hormone
HPG	Hypothalamo-Pituitary-Gonadal
CSF	Celebrospinal fluid
IGF 1	Insulin-like growth factor 1
GT1-7	Immortalized GnRH neurons
Ob-R	Leptin Receptor
DM	Dry Matter
g/d	Gramme per day
UV	Ultra Violet
PG	Plasma Gossypol
LD_{50}	Leathal Dose 50
BW	Body Weight
CSM	Cottonseed meal
GST	Glutathione S transferase
HSD	Hydroxy-Steroid dehydrogenase
GJIC	Gap junction intercellular communication
^{0}N	Degrees North
^{0}E	Degrees East
m^2	metre square
WCS	Whole Cottonseed

CF	Crude fibre
EE	Ester extract
NFE	Nitrogen free extract
MHz	Mega Hertz
EDTA	Ethylenediaminetetraacetic acid
PCV	Packed cell volume
NaCl	Sodium Chloride
µL	Micro litre
DF	Dilution factor
H & E	Haematoxylin and Eosin
FT	Free tail
CT	Coiled tail
BT	Bent tail
DH	Detached head
MPD	Mid-piece defect
SC	Scrotal circumference
e.t.c.	et cetera

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The relationship between nutrition and reproduction is an increasingly important topic. This interaction has been known to have important implications for the reproductive performance of farm animals (Bindari*et al*., 2013). Nutrition plays a major role in enhancing reproductive efficiency in all animals (Bindari*et al.,* 2013). However, it could also hinder this reproductive efficiency (Santos, 2010).

Ruminants require supplementation of nutrients in order to achieve optimal reproductive performance. This becomes more important during the usually prolonged and stressful cold-dry season of the year during which crop residues and grasses are dry and fibrous with low digestibility and devoid of most essential nutrients (Osuji *et al.*, 1993). These feed supplements include cereal grains such as maize, sorghum; cereal bran such as wheat offal, maize bran, rice; and oil seed residues such as groundnut cake, soya bean meal, cottonseed cake (Asaolu, 2012).

Cottonseed and its by-products are used extensively for supplementing ruminant rations to increase productivity (Zahid *et al*., 2002). Whole cottonseed provides a unique blend of energy, protein and fibre in the diets of ruminants (Santos, 2008). Cottonseed cake has some natural limiting factors that must be considered for safe use. Chief among these factors are protein level and quality, fiber level and gossypol content (Martin, 2005). Ruminants can tolerate higher levels of gossypol as free gossypol seems to be bound to proteins or complexed in the rumen (Coppock *et al*., 1997; Randel *et al.*, 1992). There may also be other incompletely defined mechanisms for rumen

detoxification of gossypol involved. The type of cottonseed product (meal versus whole seed), and gossypol enantiomer (+ or -) may determine the extent of the toxicological effect that will occur. It has been suggested that detoxification of gossypol in the rumen is more efficient with whole seed diets than with cottonseed meal diets (Reiser and Fu, 1962). Generally the free gossypol in cottonseed is extensively bound in the rumen and is much less available to cattle and sheep than free gossypol in cottonseed meal, regardless, of the process used for oil extraction (Calhoun and Wan, 1995).

It was known that young ruminants, prior to the development of a fully functional rumen, were susceptible to gossypol poisoning (Calhoun and Holmberg, 1991), and that mature ruminants were very tolerant; because, during digestion of cottonseed and cottonseed meal, gossypol was bound in the rumen so that it was mostly unavailable. However, Lindsey *et al.* (1980) demonstrated that the ability of the rumen to detoxify gossypol can be exceeded when very high levels of free gossypol are consumed by mature cows.

The recent interest in gossypol's effect on male reproduction is a result of reports in the 1970s of the contraceptive effect of gossypol in men in China. These observations were the basis of research to determine the value of gossypol as a male anti-fertility agent in laboratory animals and humans (Guo-zhen *et al.*, 1985).

The antifertility effect of gossypol has been studied most in males of non-ruminant species. The effects of gossypol on males are both dose- and time-dependent (Randel *et al.*, 1992). At effective doses, gossypol causes infertility by reducing spermatozoa motility and depressed sperm concentrations. Specific mitochondrial damage in the tails of spermatozoa seems to render them immotile, and extensive damage to germinal

epithelium may be responsible for depressions in spermatogenesis (Chase *et al.* 1994). In ruminant males fed diets containing gossypol, the integrity of the membrane of sperm cells may be damaged. Extensive damage to the germinal epithelium has been shown in both rams and bulls fed diets containing gossypol and is of major concern (Randel *et al.*, 1992).

1.2 Statement of Research Problem

Cottonseed and cottonseed meal are excellent sources of protein. Cottonseed is high in protein, energy and fiber, and is a good source of phosphorus and vitamin E (Arieli, 1998). Cottonseed meal is high in protein, and is a good source of fiber and phosphorus (Calhoun, 2004). However, both contain gossypol that can produce toxic effects in animals. The unique physical characteristics of cottonseed, along with its nutritional value, have resulted in it being extensively used in diets for ruminants which are tolerant to gossypol toxicity (Martin, 2005). However, evidence from various studies suggests that gossypol may be a cause of infertility in male ruminants (Stahnke, 1986; Arshami and Ruttle, 1989; Chase *et al.*, 1989; Jimenez *et al*., 1989; Randel *et al.*, 1992). The commonest protein supplements for livestock feed in Nigeria in periods of low yield and availability of poor quality forage are groundnut cake and cottonseed cake (Aruwayo and Maigandi, 2013). The indiscriminate use of cottonseeds or their by-products may adversely affect the reproductive performance of male ruminants, including rams (Arshami and Ruttle, 1989). Despite the extensive use of cottonseed and its products (Aruwayo and Maigandi, 2013), there effect on reproductive performance of Yankasa rams has not been well studied in Nigeria.

1.3 Justification

The importance of small ruminants for meat production in Nigeria is tremendous. Bayer (1986) found that 11% of the meat supplied from slaughter-houses in Nigeria came from sheep, and they stated that the importance of sheep was greater if rural unregistered slaughters were taken into account. In rural areas, sheep and goat supply three times more consumable meat than beef (Bayer, 1986).

However, the level of domestic livestock production falls short of the demand. According to GEMS (2012), in addition to the supply shortage and population growth, red meat consumption growth is driven by consumers (in Nigeriaand globally) being increasingly demanding on qualityissues. Demand for meat is largely driven by the Federation of Nigeria, as Nigerians make up 50% of meat consumers in ECOWAS. Nigeria is experiencing a historic demographic expansion and a spectacular change in food habits (Bonnet *et al.,* 2011). With a population growth nearing 2.8% per year, the country's own domestic production is far from being able to meet demand. Thus, in relation to the total population, meat consumption in Nigeria is still below the regional average (2 kg per person and per year in Nigeria, compared to 8 kg per person per year in ECOWAS) (Bonnet *et al.,* 2011).

Hence, there is a need to increase production of small ruminants to meet this demand (Aruwayo and Maigandi, 2013). Most successful sheep-rearing enterprises depend on the number of lambs raised and sold per ewe per year which in turn depends on the fertility of the ram (Gimenez, 2007).

In Nigeria, a major limitation to animal production is poor reproductive performance mostly due to diseases, environmental stressors, toxic substances and malnutrition

(RIM, 1992, Belay*et al.*, 1994). Therefore, anything that affects the fertility of the ram poses a serious threat to the production of sheep.

There is therefore a need to investigate the effects of gossypol containing feeds on fertility of male ruminants in Nigeria. The findings of this study will provide information about the effect of dietary gossypol on fertility of Yankasa rams in Nigeria.

1.4 Aim of the Study

The aim of this study is to evaluate the effect of feeding whole cottonseed and cottonseed cake on some reproductive characteristics and haematology of Yankasa rams.

1.5 Objectives

The objectives of the study are to:

i. evaluate the effects of feeding whole cottonseed and cottonseed cake on semen characteristics of Yankasa rams.
ii. determine the effects of feeding whole cottonseed and cottonseed cake on some haematological parameters of Yankasa rams.
iii. evaluate the effects of feeding whole cottonseed and cottonseed cake on histological morphology of the testis of Yankasa rams.
iv. determine the effect of whole cottonseed and cottonseed cake on gonadal and epididymal spermatozoa reserves of Yankasa rams

1.6 Research Questions

1. What is the effect of dietary whole cottonseed and cottonseed cake on semen characteristics, testicular histology and haematological parameters of Yankasa rams?

2. Is the effect of gossypol from whole cottonseed on semen characteristics and testicular morphology and haematological parameters of Yankasa same as that from cottonseed cake?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sheep Production and Management 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 (Blench, 1999). The population of sheep in Nigeria is currently estimated at 33.9 million making up 3.1 % of the world's total (FAOSTAT, 2011).

The Yankasa breed of sheep has been the most populous and extensively studied in Nigeria (Blench, 1999). It is a meat breed found in north and north central parts of the country. The Yankasa is a medium-sized breed of sheep. The tail is long and thin, the ears moderately long and somewhat droopy. Rams have curved horns and a hairy white mane and ewes are polled (Mason, 1996). They have white coat colour with black patches around the eyes, ears and muzzle (Blench, 1999). Yankasa rams stand 70 to 80 cm at the withers. 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 0.96 kg (Blench, 1999).

Yankasa sheep have been recorded in all parts of Nigeria, though the populations attenuate towards the northern border and the sea-coast. Some tentative studies have been made of its ecological adaptations. Yankasa sheep do not need daily watering in the wet season and watering once a day suffices in the dry season (Aganga *et al.*, 1988).

Sheep is an important livestock species in the socio-economic lives of people around the world including Nigerians (Yakubu and Ibrahim, 2011). FAO (2011) recorded the first increase in the global sheep population since 2007. The figures rose by 18 million to a total of 1,096 million heads of sheep, driven by increases in some of the largest flocks in the world, with the top three of China (138 million), India (74.5 million) and Australia (73 million) all reporting considerable increases on 2010 levels (FAOSTAT, 2011).These animals can be reared for various reasons such as income generation, religious purpose, household consumption and hobby and as security against crop failure. According to Adu *et al*. (1996) small ruminants in Nigeria are integral component of the household, where they contribute to the cultural, food and socio-economic life of the people, while Upton (1984) stated that the potential returns from sheep and goat keeping under the traditional management system are high. In South West Nigeria, goats are used for customary rites in addition to meat production and religious purpose (Odeyinka and Ajayi, 2004). It has been documented that sheep and goats are the principal domesticated small ruminants in terms of total numbers and production of food and fibre products (Winrock, 1983). This attribute may partly be due to their lower feed requirements compared to cattle, because of their body size (Okunlola, *et al*., 2010). This, however, allows for easy integration of small ruminants into different farming systems (Hirpa and Abebe, 2008).

Traditionally, sheep and goats have served as means of ready cash and a reserve against economic and agricultural production hardship (Hamito, 2008). In temperate zones, sheep are kept for wool and meat production (NDD, 2004). Okunlola *et al.* (2010) reported a similar trend in tropical Africa where a majority of small ruminants

areowned by individuals or families in rural areas and the number per group is small. Small ruminant animals contribute significantly to the economy of third world countries; for example, about 50% of the sheep and over 80% of the goats of the world are found in the third world countries (Ademosun, 1973).

In Nigeria, the systems of management of small ruminants are dictated by climate, cropping and population density. The small ruminant management system in the traditional sector can be broadly grouped into three; namely extensive, semi-intensive and intensive systems (Bonnet *et al.*, 2011).

Small ruminant rearing is an age long traditional production system in the Northern Guinea Savanna where animals are managed under the extensive system but a system that started to evolve in the last decade in which keepers combine small ruminant production with cultivation. As a result of this metamorphosis, new opportunities for growth are opening up and also new challenges are being faced (Bonnet *et al.*, 2011).

Horst (1976) blames the poor performance of the Nigerian sheep and goats on the predominance of the existing system of management and argues further that this poor performance of the Nigerian small ruminants can be limited or entirely circumvented via an improvement in the management.

There is a rapid increase in livestock meat (including sheep) demand in the West African sub-region. The strong rise in demand for animal products is due not only to the high rate of urbanisation (60% of Nigerians are city dwellers), but above all to consumers' greater purchasing power and the emergence of a new middle class(Bonnet *et al.*, 2011). Furthermore, this trade giant accounts for nearly 60% of international trad e in the region (Bonnet *et al.*, 2011).

Although the meat product market is growing, it still accounts for less than 10% of the overall meat product market. Despite these emerging dynamics, many people still do not have access to adequate meat, which is deemed to be too expensive by less-well-off segments of the population. Thus, in relation to the total population, meat consumption in Nigeria is still below the regional average (2 kg per person and per year) (Bonnet *et al.*, 2011).

2.2 Management Systems of Sheep Production

The management systems of sheep can be classified into two broad categories, that is, traditional and modern systems (Kisulaka and Kambarage, 1996). The traditional management system can be further sub-divided into three groups; the extensive (pastoral) and the semi-intensive (agro-pastoral) and the modern system which is the intensive systems (Kisulaka and Kambarage, 1996).

2.2.1 Traditional management systems

2.2.1.1 The extensive system

This system is common in the arid and semi-arid areas receiving rainfall below 700 mm per annum and because of the unreliability of rainfall, crop agriculture is restricted to irrigated areas where only drought resistant crops such as millet, sorghum and cassava can be grown (Adeniji, 1975). Forage and water sources for the animals in these areas are scarce and subsistence is mainly derived from livestock use, sale or exchange (Ademosun, 1988).

Two sub-systems, nomadism and transhumance are found under the extensive system of management (Kisulaka and Kambarage, 1996). In the nomadic system, people and animals are constantly moving in search of pasture and water or to avoid disease epidemics and tribal conflicts and subsistence is based wholly on animals. Examples of nomadic tribes are Maasai (Kenya and Tanzania) and Iraqwis (Mang'ati) of Tanzania (Das and Sendalo, 1989). In the transhumance system, livestock production is associated with rain-fed agriculture. Examples of transhumant tribes are Fulani in West Africa and the Wagogo and Wasukuma of Tanzania (Ademosun, 1988).

Malnutrition is the major limiting factor for profitable production of small ruminants because of shortage of water and forage particularly during the dry season. Heavy mortalities of animals have been recorded during severe drought periods in Somalia, Ethiopia and Sudan (Devendra, 1986). Intermingling of animals in communal grazing land facilitates spread of infectious diseases such as peste des petits ruminants, contagious ecthyma sheep pox e.t.c. (Kisulaka and Kambarage, 1996). Unrestricted movement of herds in search of feed grazing, to avoid diseases or tribal conflicts have resulted in spread of diseases beyond country borders (Kisulaka and Kambarage, 1996). Since the extensive system of management is characterised by low inputs, poor husbandry systems, poor nutrition, poor veterinary care and poor marketing systems, productivity under this system is extremely low and losses resulting from mortality and poor growth rates are high (Kisulaka and Kambarage, 1996).

2.2.1.2 The semi-intensive system

This system is common in areas where the annual rainfall is more than 1000 mm and therefore suitable for crop production. Thus the subsistence is gained mainly from crops

and, small ruminants are kept as an insurance against crop failure and as a source of income (Adeniji, 1975). Under this system, between 10 and 50 % of household revenue is derived from livestock or livestock products and in years of drought they may be the only means of subsistence (Ademosun, 1988). Livestock are sedentary, although movement of animals for a short-distance is not uncommon. Most commonly, animals are grazed for a restricted period of time depending on the availability of labour and feed (Kisulaka and Kambarage, 1996).

Under the semi-intensive system, since most of the land is utilised for crop production, land available for animals is limited. As a result, herd sizes are smaller than in the extensive system (Devendra, 1986). The restriction of feeding and husbandry practices associated with this system results in lowered productivity of the animals just as in the extensive system. Supplementary feeding of animals under system has a great potential for improving productivity and because animals are sedentary, it is possible to institute sheep improvement programmes (Kisulaka and Kambarage, 1996).

2.2.2 The intensive system

This system is common in areas with high population density and intense agricultural activity. Land scarcity and labour shortages are the major limiting factors to small ruminant production. Four feeding systems are found within this system; tethering, stall-feeding, cultivated forages/pastures and integration with tree crops (Adeniji, 1975).

Tethering is a widespread system of sheep management by small-holder farmers in sub-saharan Africa and has been adopted in order to prevent animals from destroying crops and to enable farmers to carry out other farm activities (Kisulaka and Kambarage,

1996). Due to scarcity of land and shortage of labour, fewer sheep are per household. In this system, the sheep are secured with a rope and tied to a peg, tree or post close to the owner's house, by the roadside or on nearby uncultivated lands. Supplements such as banana leaves, bean leaves and pods, brewers' or maize brans/grains may be provided by some farmers (Adeniji, 1975).

Stall-feeding (zero-grazing) is commonly practised in densely populated and intensively cultivated areas. Stall-feeding is mainly identified with small-holder fattening programmes and research institutes (Ademosun, 1988). The system is also increasingly being adopted in urban and peri-urban areas. Flock sizes are determined by owner's capital base and land availability. Animals are confined in houses and feed is provided to them. Household wastes are used as supplements. Fodder crops are sometimes grown for the sheep and concentrates are also provided (Kisulaka and Kambarage, 1996). Poor hygiene in stall-feeding units may be associated with high incidences of endoparasites, ectoparasites and pneumonia (Kisulaka and Kambarage, 1996).

Intensive use of cultivated forages/pastures is a high capital investment system, with carrying capacity of up to 60 sheep per hectare depending on the nutritive value of the grass, level of fertilizer and presence or absence of legumes. The system has a high potential for increased productivity, but it is less commonly practised in Africa (Kisulaka and Kambarage, 1996).

Integration with tree crops is a common system in many West African countries and the. This is an efficient mixed farming system where animals graze below crops and are protected from heat stress by the tree canopy shade. In return, they fertilize the land

with their excreta, graze waste herbage and control of weeds (Kisulaka and Kambarage, 1996).

2.3 Cotton Production in Nigeria

Cotton is a natural fibre and makes up just under half of all the fibre sold in the world. Cotton belongs to the family *Malvaceae* and genus *Gossypium* having about 40 species of annual, perennial and small trees (Frywell, 1969; Faulkner, 1974). By nature it is a perennial shrub which reaches a height of 3.5m. Commercially it is grown as an annual and only reaches a height of 1.2m (Faulkner, 1974).

Cotton as a major cash crop is of considerable social and economic importance to Nigeria (Alam *et al.*, 2013). Cotton/textile activities are widespread in the country. Cotton production in Nigeria dates back to 1903 with the British Cotton Growers Association taking the lead until 1974, when it was disbanded and replaced by the Cotton Marketing Board to develop, gin and market the produce (Gbadegesin and Uyovnisere, 1994). In Nigeria, prior to the oil boom, cotton was one of the main source of foreign exchange and second largest employer of labour after the public sector (Gbadegesin and Uyovnisere 1994).

According to Choyce, (1968) the production of improved varieties of cotton in Nigeria started with the introduction of an American upland type called “Allen long staple” from Uganda in 1912. Through breeding and selection of this variety, the Nigerian Allen and later the Samaru Allen were developed. Several commercial varieties (Samaru 26J, Samaru 68, Samaru 70 and Samaru 71) were developed from Samaru Allen which is the basic stock in Nigerian Breeding Programme (Choyce, 1968).

Cotton is predominantly produced in the North Western and North Eastern zones of Nigeria. The North Western zone comprising Kaduna, Katsina, Zamfara, Sokoto, Kebbi, Jigawa and Kano States accounts for about 65% of the total cotton in Nigeria while the North Eastern zone comprising Borno, Adamawa, Bauchi and Gombe states account for 30% of the total cotton production in Nigeria. The remaining 5% comes from the North Central zone of Nigeria comprising Kwara, Niger, Kogi, Nasarawa, Taraba, Benue and Plateau states (Choyce, 1968; Faulkner, 1974).

Cotton seeds are available where cotton fibre is produced. Cottonseed production(both for oil and feed use) was 42 million tons in 2010; cotton seeds (9.2 million tons) were used for feed in 2009 (FAO, 2012). Cotton seeds are mainly used locally and only few amounts are exchanged (0.74 million exported and 0.73 million ton imported in 2009). The main producers are China, India, USA, Pakistan, Uzbekistan and Brazil, which represented 96% of cottonseed world production in 2010 (FAO, 2012)

Cottonseed, meal or cake is primarily a protein and phosphorus source while cottonseed hulls, gin trash and textile mill waste are primarily roughage or fiber sources (Rogers *etal*., 2002). Whole cottonseed is composed of about 9 % cotton lint which is a short cellulose fibre used in paper manufacturing and other cellulose applications, 25 % is the hull which is used as ruminant feed and other specialized industrial applications, 16 % is crude cotton oil which is used for salad and other lipid applications for human consumption. The bulk of the seed (45 %) is the cottonseed meal, a high protein supplement used in animal feeds. The remaining 5 % is attributed to waste. Whole cottonseed can be fed to adult ruminant animals either alone or in combination with other feedstuffs and does not require any processing (Faulkner, 1974).

2.4 Cottonseed Products

2.4.1 Nutrient composition of whole cottonseed

Whole cottonseed, a by-product of cotton industry has been used as a supplemental feedstuff for livestock for over 100 years (Solaiman, 2007). Whole cottonseed can be used as a practical source of supplemental protein and energy to reduce cost of production. One of the major advantages of feeding cottonseed is its reduced cost of handling and processing (Solaiman, 2007).

Bernard (1999) reported that whole cottonseed contains high concentration of energy 14 MJ/kg, digestible fiber, protein (22-24 %) and phosphorus. On the average, whole cottonseed is composed of lint 12.21 %, hull 18.24 % and kernel 70.32% (NRC, 1990; Mujahid *et al*., 2000). These authors reported that whole cottonseed has about 17.74 % fibre and 4.27 % ash content while the average contents of the following: calcium 0.12 %, phosphorus 0.07 %, magnesium 0.09 %, potassium 0.15 %, sodium 0.025 %, chlorine 0.03 %, iron 141.35 mg, zinc 24,55 mg, copper 18.65 mg and manganese 27.12, mg. (Rogers *et al*., 2002). Vieira *et al.* (2010) reported an inclusion rate of whole cottonseed in diets of sheep without adverse effects. There was better meat quality and no effect on dry matter intake (Vieira *et al*., 2010). Whole cottonseed typically contains 1.5 to 2.0 % gossypol, all in the form of free gossypol, but levels can vary to as low as 0.4 % in some commercial species (Nomeir and Abou-Donia, 1985; Calhoun *et al*., 1995).

2.4.2 Nutrient composition of cottonseed cake

Cottonseed meal is valued as a protein feed, but the protein content is highly variable as it depends on the amount of dehulling and on the efficiency of oil extraction. The range of protein content goes from 30 % DM for non-dehulled cottonseed meal to up to 50 % DM for fully dehulled meals. Lower and higher values than these extrema have also been recorded. The fibre content varies accordingly, from 25 % (non-dehulled) to 5 % (fully dehulled) crude fibre (Feedipedia, 2012). The various methods used for oil extraction also explain the large range of residual oil present in cottonseed meal. Some solvent-extracted meals contain less than 2 % oil, like the other major oilseed meals, but many cottonseed meals contain higher oil values, often in the 5-10 % range but sometimes over 20 %. The cottonseed meal protein is less rich in lysine than soybean meal (4 % vs 6 % of the protein) and since the protein content is generally lower, the total content in lysine and essential amino acids is lower for cottonseed meal (Feedipedia, 2012).

According to Tanksley (1990) the most common is 41% crude protein products even though some Official Feed Definitions require a minimum of 36% crude protein for both the cake and meal cottonseed products. Some workers have reported values of between 24 and 26% crude protein content for undecordicated cottonseed cakes and meals (Oyenuga, 1968; Balogun *et al*., 1990; Aduku, 1992; Lamidi, 2006). Cottonseed cake is high in lysine and it is also adequate in other essential amino acids. The following ranges of percentage values had been recorded for arginine 4.40 – 6.63, lysine, 1.57 – 1.79, methionine, 0.62 – 0.73, cystine, 0.64 - 0.78, tryptophan, 0.51 – 0.57, threonine, 1.44 – 1.52, isoleucine, 1.27 – 1.56, leucine, 2.50 – 2.74, histidine, 1.15 – 1.45 and valine, 1.80 -2.05% (NRC, 1998; NCPA, 2002).

The energy content of cottonseed cake depends on the method of oil extraction of the whole cottonseed. The meals produced by the mechanical oil extraction have higher energy values compared with those produced by solvent oil extraction. The range values of 4200 – 4600 kcal/kg have been reported for gross energy of cottonseed meal samples (NRC 1998; NCPA, 2002). Analyses have shown that the fat and crude fiber contents of cottonseed meal samples ranged between 4.0 – 9.6% and 10.2 – 18.4% respectively, while the ash content is between 6.2 and 6.8% (NRC, 1998; NCPA, 1999). Cottonseed meal is said to be rich in phosphorus with value ranging between 1.14 and 1.20%. The values of various minerals recorded for samples of cottonseed meal are calcium, 0.16 – 0.22 %, magnesium, 0.56 – 0.66%, iron, 0.01 – 0.03%, potassium, 1.40 – 1.68%, and sodium, 0.04 – 0.14% (NRC, 1998; NCPA, 1999).

Cottonseed meal can replace soyabean or groundnut meal as protein source in diets for rams with a similar daily weight gain of 76.3 g/d and a better feed conversion ratio of 0.85 (Nagalakshmi *et al*., 2003). Cottonseed meal used in diets for growing lambs gave the same performance with other oilseed meals (groundnut, sesame, canola, soybean). Heat treatment (cooking) improved performance, probably due to a reduction of gossypol combined with increased undegradable protein (Nagalakshmi *et al*., 2003). Khan *et al*. (2000) reported the use of 40 % inclusion rate of cottonseed meal in lambs with positive effect of higher average daily weight gain of 185 g/day and dry matter intake (DMI) of 1.6 DM. Nagalakshmi *et al.* (2003) similarly reported an inclusion of 40 % of cottonseed meal with higher average daily weight gain of 88 g/day. Intoxication with gossypol resulting in decreasing hemoglobin and increasing

erythrocyte fragility has been found with dairy cows fed a high level of cottonseed meal (45 % in the diet) (Coppock *et al*., 1997).

2.5 Reproduction in the Ram

2.5.1 Anatomy of the reproductive system of the ram

The male gonads, the testes, lie outside the abdomen within the scrotum, which is a purse-like structure derived from the skin and fascia of the abdominal wall (Hafez, 2000). The testes in most domestic mammals are located in the inguinal region within the scrotum. The scrotum is two-lobed sac which enclosed the testes. It is located in the inguinal region between the hind limbs (as shown in Figure 1). The scrotum has the same embryonic origin as the labia majora in the female (Akers, 2013). The scrotum consists of an outer layer, the skin or epidermis, is heavily populated with sweat glands to assist in maintaining proper testicular temperature. The tunica dartos consists of fibro-elastic tissue and smooth muscle, which is connected closely with the tunica vaginalis at the bottom of the scrotum. The scrotum consists of two pouches, which contain a testicle. The tunica dartos acts as a thermoregulator by contracting to draw the testes close to the abdomen when cold and relaxing when the testes are warm (Akers, 2013).

The stratum subdarticum is a loosely woven layer of connective tissue between the tunica dartos and tunica vaginalis-parietal layer. This tissue allows the testes extraordinarily free mobility in an upward and downward direction. The tunica vaginalis-parietal layer (lines the scrotum) is continuous with the peritoneum. The

tunica vaginalis-visceral layer (covers the testis) is continuous with the peritoneum (Akers, 2013).

In the ram, the scrotum is long and pendulous and has a well-marked neck when not contracted. It is covered with short hairs with four (sometimes two) rudimentary teats in front of it. The spermatic cord is the link between the testis and the abdominal cavity. Each spermatic cord is circumscribed by a cremaster muscle and contains blood vessels, nerves and the vas deferens (Hafez, 2000). The outer layer of the peritoneum, processus vaginalis (tunica vaginalis reflexa) is reflected onto the testes to form the serous outer layer of the organ, tunica vaginalis propria. Accompanying this outer layer through the inguinal canal is a canal diverticulum of the internal oblique abdominal muscle, which inserts the cremaster fascia and vaginal tunics. The cremaster muscle lowers or rises the testis in response to temperature or noxious stimuli (Parkinson, 2009).

The testis is surrounded by a thick capsule, the tunica albuginea, from which a conical mass of connective tissue, the mediastinum testis, projects into the testis. The tunica albuginea is covered externally by a serosa (Lutz, 2009). This layer is composed of fibrous connective tissue but has smooth muscles. Overlying the capsule is the tunica vaginalis propria.

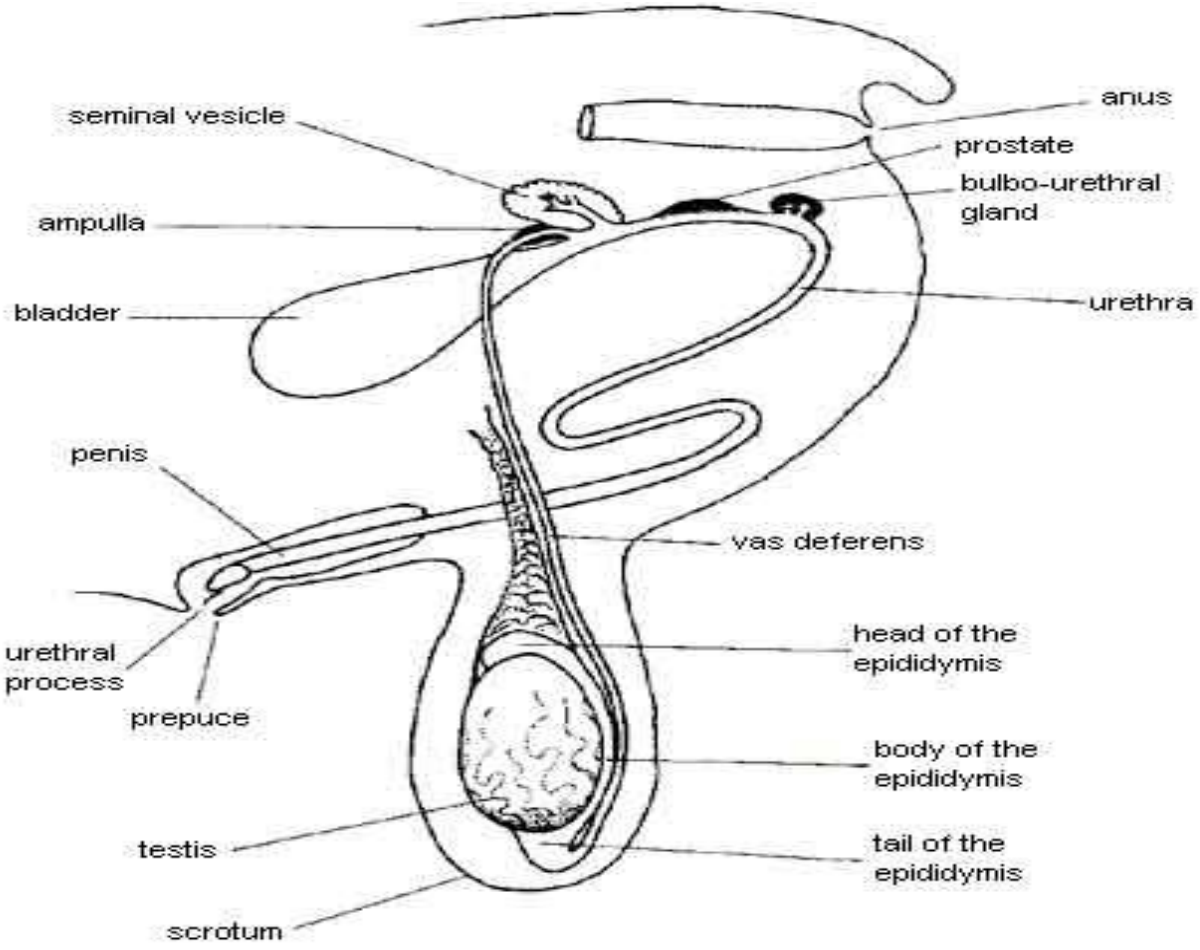

Figure 1: Genital tract of the ram (Source: Geisert, 2006)

The main blood vessel of the testes are distributed over the surface of the tunica albuginea before penetrating the capsule to supply the testicular parenchyma; while the innervations of the testes is mainly confined to the periphery and little nervous tissue is found in its structure (Parkinson, 2009).

From the mediastinum, delicate fibrous septa radiate towards the tunica albuginea and divide the parenchyma of the testes into about 300 lobuli testis, which communicates peripherally. Each testicular lobule is occupied by 1-4 U-shaped, double-ended seminiferous. A typical tubule is about 150-300 μm in diameter and 30-70 cm long. The tubules are tightly packed in the testes and each tubule can be cut several times in a

histological section.tubules. A typical tubule is about 150-300 μm in diameter and 30-70 cm long. The tubules are tightly packed in the testes and each tubule can be cut several times in a histological section (Fawcett *et al.*, 1973).

The substance of the testis is composed of two main tissues; the seminiferous tubules and the interstitial tissue. Each seminiferous tubule is a highly convoluted, unbranched tube which opens into the rete testis. The seminiferous tubules are limited by basement membrane which partially surrounded by contractile myoid cells. Within the tubule, the seminiferous epithelium is composed of two main cell lines; the somatic sertoli cells and the sperm producing germinal cells (spermatogonia) as shown in figure 2 below (Tim, 2009).

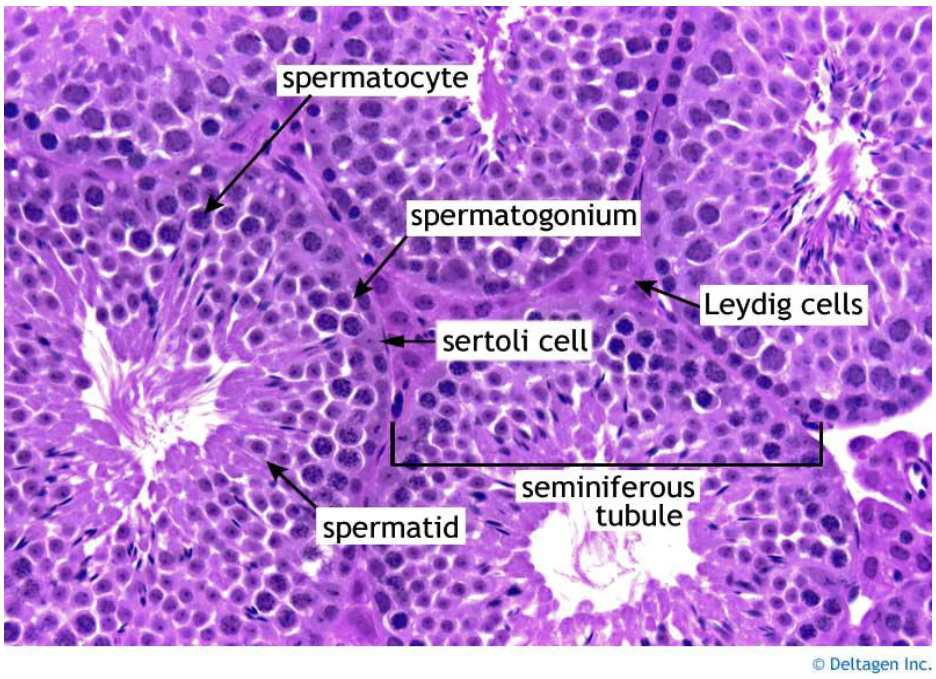

Figure 2: Histological features of the testis (Deltagen.com, 2014)

The interstitial tissue consists of steroid-producing Leydig cells, blood vessels and lymphatics. In the ram, the Leydig cells occur in small clusters around blood vessels, interspersed in relatively large lymphatics (Fawcett *et al.*, 1973). At the ends of each

seminiferous tubule (ST), there is a transitional zone between spermatogonic tissue and the rete testis. The number of ST openings varies between species as do the channels comprising the rete testis (Setchell *etal*., 1994).

The rete testis consists of a network of interconnecting channels with a simple epithelial lining that moves sperm into the epididymis. The rete opens into the epididymis via 13-20 efferent ducts (Hemeida *et al*., 1978). The tubules are initially relatively straight but have a long convoluted section before opening into the epididymal duct. Fluid resorption occurs in both rete testis and efferent ducts (Parkinson, 2009).

The epididymis is a single, highly convoluted tube into which the vasa efferentia drain the STs. spermatozoa which becomes turgid with store spermatozoa in sexually active animals (Parkinson, 2009). The Epididymis is fused longitudinally to the surface of the testis and is encased in the tunica vaginalis with the testis. The single convoluted duct is covered with an extension of the tunica albuginea testis. The caput (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 of this convoluted duct is about 36 meters in the ram (Setchell *et al.*, 1994).The lumen of the cauda is wider than the lumen of the corpus. The structure of the epididymis and other external ducts (vas deferens and urethra is 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) (Setchell *et al.*, 1994).

The ductus deferens is simply a continuation of the epididymis extending from the tail of the epididymis to the pelvic urethra. Its primary function is to transport spermatozoa

from the epididymis to the urethra. The wall of the ductus is thick and heavily muscled while the lumen is very small. The ductus deferens passes upward parallel to the testis and body of the epididymis into the spermatic cord, through the inguinal canal, and to the pelvic urethra, where the wall becomes thickened and folded to form a structure known as the ampulla. The thickening of the duct is due to abundant embedding of glands in the wall of the ductus deferens. The ampulla may serve as a limited storage place for spermatozoa. The ampullae narrow and pass under the body of the prostate to empty into the colliculis seminalis or cranial portion of the pelvic urethra. In some species, ejaculatory ducts may be formed by a union of the ductus deferens and the proximal portion of the duct of the seminal vesicle (Setchell *et al.,* 1994; Akers, 2013).

In the ram, the walls of the ductus deferens are not as thick as in other species, but still possess a firm cord-like character. On reaching the bladder, the two ducts are close together for a distance of about 10 cm where they form very distinct ampullae about 16 cm long and 1 to 1.5 cm in diameter. The ducts pass under the body of the prostate and end just in front of the ducts of the seminal vesicles as slit-like openings on top of the colliculis seminalis (Akers, 2013).

The accessory glands include the ampullae, prostate, vesicular glands and bulbourethral (or Cowper's) glands. Ampullae are present as dilations of the terminal portion of the vasa deferentia, just before they enter the pelvic urethra, where their main function is to act as reservoirs of sperm. In the ram, the ampullary glands that are present, contribute slightly to the seminal plasma (Mann *et al.,* 1956).

Vesicular glandsare prominent in ruminants. They are firm, lobulated structures in the ram. The glands are adjacent to the neck of the bladder and lateral to the ampullae. They open into the urethra just distally to the vasa deferentia. Their secretion, which is generally watery, contributes substantially to the volume of the semen. In all species its secretion contains large quantities of citrate, while in the ruminants it also contains fructose (Marley *et al*., 1977).

The prostateis intimately related to the pelvic urethra and, in most species, is in two parts; its body surrounds the neck of the bladder and its disseminated part spreads around the pelvic urethra into which it has several openings. The prostatic secretion is watery (Parkinson, 2009).

In the ram, the bulbourethral(or Cowper's) glandsare small, rounded structureslying between the anus and urethra. Theirwatery secretion is discharged prior to coitus andis considered to cleanse the urethra of urine (Boursnell *et al*., 1970).

2.5.2 Puberty

Spermatogenesis does not commence until the time of puberty. Thus, the seminiferous tubules of the prepubertal animal consist of immature Sertoli cells and relatively undifferentiated spermatogonial stem cells. As puberty approaches, the number and complexity of Sertoli cells increase, to reach maximal numbers at the onset of spermatogenesis (Parkinson, 2009). Mitoses in the spermatogenic cells occur at an increasing rate as puberty approaches, with puberty itself being characterized by the onset of meiosis and sperm production (Parkinson, 2009). The maximum

spermatogenic rate is not reached until sometime after the occurrence of puberty, for both the rate of cell divisions and the size of the testis continue to increase into adulthood (Hochereau-de-Reviers *et al*., 1987).

2.5.3 Spermatogenesis

Spermatogenesis is a multi-step process of germ cell expansion and development which occurs within the seminiferous tubules of the testis that determines male fertility (Hafez, 2000). Spermatogenesis comprises three phases: stem cell renewal and germ cell proliferation, meiosis and differentiation, and spermiogenesis (Hunter, 1980; Hafez, 2000). Stem cells located along the basement membrane of the seminiferous tubules divide, resulting in another stem cell and a committed cell called a spermatogonium (Sharpe, 1994). The spermatogonia undergo a species-specific number of mitotic divisions, with the final division resulting in differentiated type B spermatogonia (Hafez, 2000). The type B spermatogonia then divide to form preleptotene spermatocytes that detach from the basement membrane as they undergo meiosis to form round spermatids. After undergoing extensive differentiation (spermiogenesis) the differentiated elongated spermatids (now spermatozoa) are released into the tubule lumen (spermiation) (Sharpe 1994; Parkinson, 2009).

In the mammalian male, the germinal epithelium is located within the seminiferous tubules. The precursor cells of either male or female gametes, called *gonocytes*, originate from extra-embryonic endodermal tissue. These migrate to the gonadal zone where they differentiate either to oogonia or spermatogonia (Hafez, 2000).

Spermatogenesis begins with the mitotic division of spermatogonia in close proximity to the basement membrane and proceeds toward the lumen. Spermatogonia are divided into A, intermediate and B classes, with each class further subdivided according to morphology and degree of differentiation. Spermatogonia are activated to form active, type A spermatogonia (Parkinson, 2009). In the ram, A0, A1, A2, A3, intermediate,B1 and B2 spermatogonia occur (Hochereau-de-Reviers *et al.*, 1990).

A-series spermatogonia are the least differentiated and form the reservoir of stem cells within the seminiferous tubule. It is likely that stem cells are regenerated by asymmetrical divisions of early A-series spermatogonia; with one daughter cell remaining as an uncommitted stem cell, the other being committed to undergo further mitotic and meiotic divisions. All spermatogonia remain in contact with the basement membrane, but, as the final meiotic division of spermatogonia gives rise to the primary spermatocytes, the cytoplasm of the sertoli cells starts to intervene between the basement membrane and the primary spermatocytes. DNA synthesis occurs during mitotic divisions and then, to its greatest extent, during the formation of tetraploid nuclei during meiosis (Hochereau-de-Reviers *et al*., 1990). RNA synthesis occurs during preleptotene and late pachytene (Parkinson, 2009). The first meiotic division then proceeds through the highly sensitive zygotene and pachytene stages. The pachytene stage is particularly sensitive to noxious damage, such as by high testicular temperature and inadequate maintenance of spermatogenesis by inappropriate gonadotrophin levels. During the first meiotic division, the cells move deeper into the seminiferous epithelium and the tight cell junctions of the Sertoli cells form beneath the

spermatocytes and degenerate above them, so that the cells effectively pass through the blood–testis barrier (Parkinson, 2009).

Thus, the progeny of the first meiotic division, the secondary spermatocytes, move from the basal to the apical compartment of the seminiferous epithelium and are thereafter separated from the general tissue fluid compartment. The second meiotic division produces spermatids, which do not divide further. The spermatids thereafter differentiate into spermatozoa (Parkinson, 2009).At the end of meiosis, spermatids are round cells with round nuclei, which have to then undergo the very marked changes in cell function and morphology that occur during spermiogenesis.Immediately after completion of meiosis, the spermatids undergo a period of RNA synthesis, which is then followed by the beginnings of nuclear chromatin condensation (Garner and Hafez, 2000).

Simultaneously, acrosomal contents are synthesized in the Golgi, whose vesicles progressively fuse to form the acrosome. As the nucleus condenses and elongates the acrosome forms over the basal pole of the nucleus (Courtens and Fournier-Delpech, 1979), while at the opposite pole the flagellum starts to form from one of the centrioles. A transient microtubular structure, the manchette, appears during the formation of the flagellum in the postnuclear cytoplasm of the elongating spermatid. The function of the manchette is unknown and it disappears after the flagellum is formed (Zirkin, 1971; Fawcett*et al.*, 1973). The last stage of flagellum formation is the development of the mid-piece, when a helix of mitochondria condenses around the proximal part of the flagellum. During formation of the acrosome and flagellum, the cytoplasm of the spermatid is deeply invaded by a process of the Sertoli cell that extends between the

forming flagellum and the residual cytoplasm. It is suggested that this process is responsible for the reduction in cytoplasmic volume of the spermatid that occurs during spermiogenesis. Finally, most remaining cytoplasm is engulfed by the Sertoli cell as the formed spermatozoon, with its remnant cytoplasmic droplet, is expelled from the crypt of the Sertoli cell into the lumen of the seminiferous tubule (Fouquet, 1972).

Various researchers have classified the cellular associations in the cycle of the seminiferous epithelium into more or less distinct stages (Clermont, 1972). The duration of the cycle of the seminiferous epithelium and the duration of the spermatogenic cycle in the ram are 10.4 and 49 days respectively (Clermont, 1972, Garner and Hafez, 2000; Pineda, 2003). The duration of the spermatogenic cycle can be calculated by multiplying that of the seminiferous epithelium by 4 because spermatogenesis extends over four consecutive cycles of the seminiferous epithelium (Pineda, 2003). Epididymal transit takes a further 8–14 days. Thus, the interval between the most sensitive stage of spermatogenesis, meiotic prophase, and ejaculation, is approximately 30 days (Amann and Walker, 1983). Hence, the interval between damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 60 days, depending upon the site of damage (Amann and Walker, 1983).

The seminiferous epithelium appears as concentric layers of spermatogonia, spermatocytes and spermatids, with characteristic associations between generations of cells throughout the depth of the seminiferous epithelium. Each generation of seminiferous cells is linked by cytoplasmic bridges, so that developmental stages are

synchronous within each generation, and substantial areas of seminiferous epithelium exhibit cells at a similar stage of development (Parkinson, 2009).

Cellular associations are generally classified into type I, where two generations of primary spermatocytes and one of spermatids are present, and type II, where there is only one generation of primary spermatocytes but two of spermatids (Hochereau-de-Reviers *et al*., 1990). Transition between type I and type II occurs after the maturation divisions, while type II changes into type I with the release of spermatozoa and the arrival of a new generation of spermatocytes from the last spermatogonial division (Parkinson, 2009).

Although the Sertoli cells are the only non-germinal cells in the seminiferous epithelium, they are fundamental to normal spermatogenesis. Cytoplasmic processes from the Sertoli cells surround clusters of germinal epithelial cells. This allows the Sertoli cells to receive and convey signals and metabolic products from the extratubular environment through the basement membrane to the meiotically dividing germinal cells (Tiba *et al.*, 1994).

Considerable changes occur to spermatozoa as they pass through the epididymis (Hammerstedt and Parks, 1987). The epididymis is highly androgen-dependent; thus, if androgen levels are suppressed, epididymal function is immediately impaired. The protoplasmic remnant, which is initially sited close behind the sperm head, migrates distally to the end of the midpiece, before being finally shed in the tail of the epididymis. Sperm are immotile in the head of the epididymis, but they acquire the capacity for motility as they pass through its body (Hammerstedt and Parks, 1987).

Similarly, in the head of epididymis, sperm do not have the ability to fertilize, but this is acquired during passage of the epididymal body. Less obvious, but of equal or greater importance to the morphological changes exhibited by sperm during their passage of the epididymis, are the changes in their plasma membrane, to which surface glycoproteins are added or modified by epididymal secretions and luminal cells. It is likely that these act to stabilize the acrosome while the sperm is within the female genital tract, to reduce the surface immunogenicity of the sperm and to enhance the ability of the sperm membrane to bind to the zona pellucida (Hammerstedt and Parks, 1987).

Spermatozoa take between 8 and 13 days to traverse the epididymis (Pineda, 2003). In the ram, sperm take 5 days to pass through the head and body of the epididymis and a further 4-7 days to traverse the epididymal tail. The transit times of the head and body are fixed, but the tail of the epididymis has dual functions of both a site of maturation and storage, so that, in periods of high ejaculation frequency, the passage time of the tail may be reduced and relatively immature sperm ejaculated (Parkinson, 2009). Although sperm held in the tail of the epididymis have the capacity for motility, motility is not itself acquired until the time of ejaculation. Thus, sperm within the epididymis exhibit little motility, but are rapidly activated upon mixing with seminal plasma during ejaculation (Parkinson, 2009).

2.5.4 Structure and function of spermatozoa

Spermatozoa are divided into three main segments: the head, midpiece and tail. The head consists of little other than the condensed nucleus and the overlaying acrosome.

Of the enzymes contained within the acrosome, the main two are acrosin and hyaluronidase (Garner and Hafez, 2000). During the acrosome reaction, the outer acrosomal membrane fuses with the plasmalemma, under the control of intra- and extracellular calcium, whereupon exocytosis of the contents of the acrosome occurs (Harrison and Roldan, 1990). The main functions ascribed to the acrosomal enzymes are dispersal of the cumulus oophorus and local lysis of the zona pellucida; although it has been questioned recently whether the latter function is indeed a function of the released acrosomal enzymes per se (Pineda, 2003). The inner membrane of the acrosome is relatively stable and remains intact after the acrosome reaction has occurred, and some of the acrosomal enzymes are probably bound to the inner acrosomal membrane. Penetration of the zona pellucida and fusion with the oolemma are both receptor-mediated events, with specific areas of the sperm head binding to target components of the oocyte (Wassarman, 1990).

The midpiece and tail of the sperm may be considered to form a single functional entity. The tail itself consists of a central axoneme, which, in the region of the midpiece, is sheathed in a helix of mitochondria (Bedford and Hoskins, 1990). Sperm metabolizes simple molecules, principally sugars and their derivatives (fructose, glucose, mannose and pyruvate), by both aerobic and anaerobic pathways, to provide energy for motility and the maintenance of ionic gradients across membranes (Harrison and Roldan, 1990). Forward motility of sperm results from coordinated waves of flagellar bending progressing from neck along the length of the tail. Bending of the tail occurs as the result of forces generated between adjacent peripheral doublets of the axoneme (Satir *et al*., 1981).

2.5.5 Endocrinology of reproduction in the ram

All aspects of male reproductive physiology are under the endocrine control of the two major gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The secretion of LH is pulsatile, with irregular episodes of secretion occurring every 2–4 hours. The actions of LH are primarily upon the Leydig cell, where, acting through adenylate cyclase, it promotes steroidogenesis by regulating the rate-limiting step of steroidogenesis; namely, conversion of cholesterol into the testosterone precursor, pregnenolone (Parkinson, 2009). Peak testosterone concentrations follow those of LH by about 40 minutes and decline back to prestimulation values over a further 40–80 minutes (D'Occhio *et al*., 1982).

Testosterone is required for the production of sperm in the testis and their subsequent maturation in the epididymis, for the function of the accessory sex glands and for the development of masculine secondary sexual characteristics. After aromatization into oestrogen within the brain, testosterone is also responsible for negative feedback regulation of LH secretion and for male behaviour. Curiously, in long-term castrated animals, neither negative feedback nor libido can be restored by testosterone administration, for brain aromatase activity is eventually lostand oestrogen itself has to be given for the restoration of these effects (D'Occhio *et al*., 1982). Within the lumen of the seminiferous tubule, testosterone is converted by 5-reduction into 5-dihydrotestosterone (DHT), which is not susceptible to aromatization and is a more potent androgen than testosterone itself. Both testosterone and DHT are bound within the tubule lumen by the secretory product of the Sertoli cells, androgen binding protein (ABP). The role of ABP therefore appears to be to maintain high androgen

concentrations in the lumina of the seminiferous tubule and epididymis (Parkinson, 2009).

The main target of FSH is the Sertoli cell, where it also acts through adenylate cyclase linked enzyme systems. Under the influence of FSH, Sertoli cells secrete ABP and aromatize testosterone into oestrogens (Setchell *et al.,* 1983). Adequate FSH stimulation is also required to permit Sertoli cells to support spermatogenesis. Some evidence suggests that the production of pyruvate and lactate, which act as energy substrates for germ cells, may be a key role of the FSH-stimulated activity of the Sertoli cell in maintaining spermatogenesis. Debate remains over the pattern of secretion of FSH; some consider it to be pulsatile, a manner analogous to LH, while others consider its secretion only to exhibit longer-term fluctuations. FSH secretion is regulated by both gonadal steroids and inhibin, the regulatory protein secreted by Sertoli cells (Baird *et al*., 1991).

The actions of the gonadotrophins are, however, not limited to the somatic cells of the testis, as both LH and FSH regulate aspects of germ cell activity. Courot and Ortavant (1981) reported that experiments upon hypophysectomized rams indicated that the rate of division of stem spermatogonia is controlled by LH, while the rate of subsequent divisions and the ability of cells to undergo meiosis are regulated by FSH. Whilst these actions upon germ cells are, in part, mediated through the stimulation of activity in the somatic component of the testis by the gonadotrophins, circumstantial evidence indicates direct actions upon germ cells themselves (Amann and Walker, 1983).

2.5.6 Semen and semen characteristics of the ram

Semen is the liquid cellular suspension containing spermatozoa, the male gametes, and secretions from the accessory organs. The fluid portion of this suspension which is formed at ejaculation is known as seminal plasma (Hafez, 2000). The functional significance of seminal plasma is questionable in that pregnancy can be induced in some species by insemination with epididymal spermatozoa (Garner and Hafez, 2000). It, however, appears to be an essential component in natural mating because it serves as a carrier and protector of the spermatozoa (Mann and Lutwak-Mann, 1981). The importance of this role varies because spermatozoa are ejaculated directly into the uterus in some species such as mare and sow (Mann and Lutwak-Mann, 1981). Seminal plasma appears to be more important in natural mating of the ewe and cow where the ejaculate is deposited in the vagina (Garner and Hafez, 2000).

2.5.6.1 Semen characteristics of the ram

Good quality semen will have a milky or creamy appearance and, when examined under a microscope, will give an impression of boiling or rolling due to the intense motion of the spermatozoa (Ptaszynska, 2009). Table 2.1 gives the normal parameters of sperm expected in a mature ram.

Table 2.1: Normal Semen Parameters in Mature Ram

Parameter	Normal value
Volume	1 (0.5 to 1.5) ml
Colour	Milky to creamy
Sperm concentration (10^6/ml)	250 (100 to 800)
Percentage of motile spermatozoa	75 (60 to 80) %
Percentage of morphologically normal spermatozoa	90 (80 to 95) %
pH	5.9-7.3

Sources: Ptaszynska, 2009; Arthurs *et al.*1989; Ihukwumere and Okere, 1990

The normal colour of the semen of a ram is milky to creamy. Watery semen samples are usually oligospermic, and samples that are not homogeneous often contain pus. Some normal rams have semen that is bright yellow in colour, due to excretion of grass pigments into the seminal plasma (Parkinson, 2009).

Sperm motility is markedly influenced by temperature, so temperature control during semen examination for motility is critical. Ideally, this is achieved by using prewarmed slides on a heated microscope stage, but in the field improvised methods are needed. Temperatures must also be kept below 50°C, above which sperm rapidly die (Parkinson, 2009). A good semen sample should have greater than 40% progressively motile sperm (Kimberling and Parsons, 2009). Motility can be affected by a number of extraneous factors, such as urine contamination, cold shock, and the number of stimulations required for obtaining an ejaculate. Therefore, motility alone is not always

a good indicator of potential fertility (Kimberling and Parsons, 2009). Semen concentration is variable and depends on a number of factors such as breed, age, nutrition, environment and frequency and method of collection as well as handling of the semen (Garner and Hafez, 2000).

Sperm abnormalities have long been associated with male infertility and sterility in most species studied (Chenoweth, 2005). These abnormalities vary from morphological defects that are evident upon clinical examination, to those, which are more subtly defective. In general, sperm structure can play a substantial role in both fertilization and pregnancy outcome (Saacke, 2008). The causes of defective sperm structure may be environmental, genetic, or a combination of both. Although environmental causes are considered to be most common, there is a growing list of sperm structural defects, which are considered to be of genetic origin (Chenoweth, 2005).

Three main classifications of sperm morphology have been proposed (Parkinson, 2009). Firstly, defects can be classified according to their site on the sperm. By this classification, sperm are classified into head, midpiece and tail defects and sperm bearing protoplasmic droplets. A rather more useful classification is that based upon the site within the genital tract where the sperm defect has arisen (Blom, 1950). By this classification, defects are divided into primary abnormalities, which arise during spermatogenesis, secondary defects, which arise within the epididymis, and tertiary defects, which arise after ejaculation (for example from inadequate temperature, pH or osmotic control during handling of the semen). Thus, defects of the head and midpiece are mostly primary, protoplasmic droplets secondary and looped tails tertiary.

Blom, (1983) categorises defects, according to empirical observations upon their effects on fertility in the bull, into major and minor abnormalities. Major abnormalities include most defects of the head, proximal protoplasmic droplets and congenital acrosomal defects, while most other defects, including, somewhat surprisingly, detached heads, are classified as minor abnormalities.

2.6 Factors affecting Reproductive Performance of the Ram

Reproductive performance must be considered in light of the management system and genetics of the breed (Menzies, 2011). Semen quality, like other phenotypic expressions, no doubt consists of a genetic component, an environmental component and a variety of interactions between the two. The genetic component is generally thought to be small because the heritability of fertility usually is low (Rollinson, 1955; Foote, 1970). A number of factors may affect semen production and quality. They include age, genetics (breeds), scrotal circumference, diseases, environment, season, management, nutrition e.t.c

2.6.1 Genetics (breeds)

Genetics/heredity affects the fertility of farm animals in a variety of ways. Some animals may be genetically infertile. Reproductive traits have low heritabilities, a discrete phenotypic expression, and are expressed only in sexually mature ewes leading to low selection intensities and long generation intervals (Petrovic *et al.,* 2007). Breed selection can greatly influence reproductive performance, particularly prolificacy and

age at first lambing. Sheep breeds around the world are very diverse in performance, and it is advisable to be familiar with the traits of some of the more popular ones (Menzies, 2011). Use of physiological quantitative parameters in sheep improvement in increase of fertility is one of the procedures which can lead to success in sheep selection on fertility (Petrovic *et al.,* 2007). Favourable environmental conditions like good feeding and management have the same influence, as selection, and the results attained in this manner are faster and more spectacular (Petrovic *et al.,* 2007).

In the bovine species, *Bos indicus* was reported in Africa to have had higher sperm production sperm concentration and sperm viability than *Bos taurus*(Brito *et al.*, 2002). Fields *et al.* (1979) and Chenoweth (2002) observed a greater sperm production and quality in *Bos taurus*than *Bos indicus* bulls. The higher sperm quality reported for *Bos indicus* by Brito *et al.* (2002) could have been due to better adaptation to tropical environment. Wildeus and Hammond (1993) also observed greater sperm production in an adapted *B. taurus* breed than in non-adapted breed in the tropics. Semen quality decreased faster and to a greater extent in *B. taurus* bulls than in *B.* indicus bulls and cross-bred bulls exposed to high ambient temperatures (Johnston *et al.,* 1963; Skinner and Louw, 1966).

2.6.2 Age

Age has been reported to be a major cause of variation of semen quality in male animals due to physiological changes that occur as they grow to sexual maturity (Coe, 1999). In general, sexual development of ram lamb appears to be more closely associated with body growth than with chronological age (Dyrmundsson and Lees,

1972). David *et al*. (2007) found that in Lacaune and Manech tete rousse sheep, motility, concentration and number of spermatozoa were higher up to the age between 2-3 years then declined chronologically. Okukpe *et al.* (2001) reported that semen characteristics were significantly affected by age of Yankasa rams. They observed that higher sperm concentration, motility and live spermatozoa were observed in adult rams. Semen production efficiency was also highest in the adult rams. Similar findings were reported in rams by Colas *et al.* (1990) and Salhab *et al.*(2001).

Serrano (1984) indicated that bulls from 2-5 years of age produce larger volumes of ejaculate compared to bulls less than 2 years of age and bulls older than 5 years of age. Several other researchers reported that the volume of ejaculate, sperm concentration and semen motility improved with an increase in age of the bull (Almquist and Amann, 1976; Morris *et al*., 1978; Everett and Bean, 1982; Lunstra and Coulter, 1997; Shannon and Vishwanath, 1995; Chenoweth *et al.,* 1996; Garner *et al.,* 1996, Mathevon *et al.,* 1998; Coe 1999; Sekoni and Rekwot, 2003 and Hassan *et al.*, 2009).

An increase in the frequency of abnormal spermatozoa has been reported with advancing age in bulls (Rao, 1971, Wolfe *et al.,* 2000). A similar finding was obtained in humans by Kidd *et al.* (2001) who reported an increase in ejaculate volume of 3 to 22 %, sperm motility of 3 to 37 % and normal sperm cells of 4 to 18 % to be associated with an increase in age. Gustafsson and Sekoni (1980) reported that old bulls (5 to 8 years of age) had higher incidence of sperm abnormalities than young bulls (1.5 to 2 years of age) and younger bulls less than 13 months had higher incidence of head abnormalities, proximal and cytoplasmic droplets than in bulls of 1.5 to 2 years of age.

2.6.3 Scrotal circumference

Scrotal circumference (SC) is a very important trait in male animal reproduction, which can be used as a measure of fertility (Vilakazi, 2003). According to Lasley (1978), SC has a heritability estimate of 50%, which means that is it influenced mainly by genetic rather than environmental factors. SC is positive correlated to age of the animals (Brito *et al.* 2002). Several researchers have reported a high positive correlation between scrotal circumference and sperm production as well as semen quality (Palasz *et al.*, 1994; ValeFilho *et al.,* 1997, Elmaz *et al.* 2007, Akpa *et al.* 2012). Hoogenboezem and Swanepoel (2000) reported that SC is positively correlated with the overall potential breeding efficiency and seminal characteristics. They also noted that semen quality and scrotal circumference are affected by factors related to underdevelopment of the testes and testicular degeneration.

Small scrotal circumference size may also result from lack of testicular development (King, 1993) due to thermal and nutritional factors which interfere with the endocrine regulation of spermatogenesis (Bearden and Fuquay, 1997).

2.6.4 Lameness and physical fitness

Many males fail to achieve normal fertility due to lameness/injury and while some of this may be unavoidable for example, musculoskeletal injury. Many foot problems could be avoided by better selection of males and routine foot care (Penny, 2014). Sudden change of diet from high concentrate/low forage diet to silage or grass based

diet may induce laminitis leading to a lifetime of foot problems and premature culling (Penny, 2014). Regular hoof trimming may be required for some bulls, bucks or rams in order to prevent reproductive failure due lameness induced by hoof problems (Menzies, 2011). In addition to reducing libido and ability to mate, lameness also have dramatic effects on semen quality which may last for several months. Other causes of lameness include congenital limb defects (Gyang *et al.*, 1986), claw deformities, footrot, hoof cracks (Gyang, 2005), laminitis (Berg, 1981), foot abscess, tick bite lameness, interdigital pouch inflammation (Gyang *et al*., 1986) as well as nutritional (such as selenium and vitamin E deficiency) and infectious lameness such as FMD and polyarthritis (Gyang, 2005).

2.6.5 Environment

The major contribution to semen variation is environment (Curtis, 1983; Chandler *et al.*, 1985; Cupps, 1991; Bearden and Fuquay, 1997). The environment could be defined in terms of all factors surrounding the animal, which includes environmental temperature, nutrition, season and management of the animal (Bonsma, 1980).

2.6.5.1 Effects of temperature on semen characteristics

Ambient temperature is one of the most important factors affecting reproduction in domestic animals (King, 1993). The effect of temperature on semen quality should be treated with caution because it often modifies feed intake in animals and thus, changes ascribed to temperature may be due to altered nutrition. Furthermore, temperature and

photoperiods are positively correlated particularly in temperate regions of the world and under natural conditions; it might be difficult to differentiate which portion of the effect is due to temperature and which is due to photoperiod (McDowell, 1972).

In the tropics, sperm production and semen quality decrease during the hot season (Fields *et al.,* 1979; Kumi-Diaka *et al*., 1981; Rekwot *et al.,* 1987), however, seasonal variations cannot be attributed only to greater ambient temperature. Reproduction can be affected by heat stress; under high ambient temperature and/or humidity, body thermoregulatory mechanisms are unable to increase body heat loss and internal temperature increases above physiological limits (Chemineau, 1994). Direct exposure of the testis at high temperatures, causes changes in certain critical stages of spermatogenic cycle, which is also directly related to the quality of the ejaculate (Alejandro *et al.*, 2014).

Heat stress can decrease semen quality in bulls (Johnston *et al*., 1963; Skinner and Louw, 1966) and rams (El-Darawany, 1999; Mohammed and Abdelatif, 2010; Mohammed *et al*., 2012). Waites (1970) and Setchell (1978) reported thermo-regulation of the testis to be essential for semen production and quality. The detrimental impact of high ambient temperature on sperm quality in breeder cocks was reported by Rekwot *et al.* (2005) in the Northern Guinea Savannah zone of Nigeria.

However, Semen characteristics are not immediately affected by changes in testicular temperature because damaged spermatogenic cells do not enter ejaculates for some time after heat stress (Hansen, 2009). Increase in the frequency of abnormal cells was found to be associated with extreme temperatures such as heat stress (Rathore, 1970; Stephan *et al.,* 1971). During a period of heat stress, degenerative changes in the seminiferous

tubules (Kumi-Diaka *et al*., 1981), testicular degeneration (Mamabolo, 1999) and abnormal scrotal thermogram (Lunstra and Coulter, 1997) were reported in bulls. Skinner (1965) reported the effect of ambient temperature on semen quality to be dependent on the period of exposure to heat stress. He observed a reduction in sperm motility, percentage live spermatozoa and an increase in the number of morphologically abnormal spermatozoa in the ejaculates of bulls exposed to a temperature of 40^{o}C for 6 to 8 weeks.

2.6.5.2 Effects of season on semen characteristics

The influence of season on seminal characteristics of domestic animals reared in the temperate regions of the world has been documented (Mahmoud *et al.,* 1996; Penfold *et al*., 2000). It has been established that the tropical environment of high ambient temperature and relative humidity have adverse effects on livestock and poultry species (McDaniel *et al.,* 1995; Ayo *et al.,* 1996; McDaniel *et al.,* 1996; Bah *et al.,* 2001; Ayo and Sinkalu, 2007). Onuora (1982) reported that the hot humid environment of South-Eastern Nigeria has negative effects on semen characteristics in the guinea fowl. The detrimental impact of high ambient temperature on sperm quality in breeder cocks was reported by Rekwot *et al.* (2005) in the Northern Guinea Savannah zone of Nigeria.

Ahmad and Noakes (1996); Ibrahim (1997) and Mathevon *et al.* (1998) reported that the month and season of year have a significant effect on semen characteristics. Areas with marked seasonal variation in environmental temperature, bull semen quality tend to be lower during summer months as these results in heat stress which causes testicular

degeneration and abnormal scrotal thermogram and hence lower semen quality (Curtis, 1983). When considering the effect of season on semen quality, ambient temperature and humidity has to be interpreted carefully.

Taylor *et al.* (1985) reported that sperm production in Holstein bulls (ejaculate volume, sperm concentration and total sperm number) was greater during the summer in temperate environments. Higher semen quality in dairy *Bostaurus* bulls during the summer was also reported (Chandler *et al.*, 1985; Soderquist *et al.*, 1996, 1997). However, Mathevon *et al.* (1998) reported that Holstein bulls produced more sperm (higher sperm concentration and total sperm number) with greater motility during the winter and the spring. Diarra *et al*. (1997) observed an inverse seasonal variation in ejaculate volume and sperm concentration that resulted in non-significant variation in sperm production during the year. Godfrey *et al*. (1990) reported that in a temperate climate, *Bos indicus* (but not *Bos taurus*) bulls suffered from cold stress that was reflected in decreased sperm production and semen quality during the winter.

In the tropics and semi-tropics, sperm production and semen quality decreased during the hot season only in *Bos taurus* and crossbred bulls, but *Bos indicus* bulls were not affected (Fields *et al.*, 1979; Kumi-Diaka *et al.*, 1981). However, some authors showed that in Africa, sperm production (ejaculate volume, sperm concentration and total sperm number) and percentage of normal sperm cells decreased during the hot season in *Bos indicus* bulls (Igboeli and Rakha, 1971; Rekwot *et al*., 1987).

There is obvious influence of photoperiod on semen characteristics in rams. Moghaddam *et al.* (2012) reported on the seasonal variations in seminal characteristics of the two crossbred rams Ghezel×Baluchi and Arkharmerino×Ghezel in Iran.

The effect of season and/or photoperiod on semen quality and quantity has been previously studied in different breeds of rams (Amir *et al*., 1986; Karagiannidis *et al.,* 2000; Kafi *et al.,* 2004; Zamiri and Khodaei, 2005) and other seasonal breeding animals such as buck (Barkawi *et al*., 2006; Karagiannidis *et al*., 1999) and stallion (Janett *et al*., 2003). Among the quality characteristics of semen, a significant effect of season was recorded on spermatozoa progressive motility, percentage of live spermatozoa and abnormal spermatozoa, semen pH and semen index. Moreover, the effect of photoperiod was also observed in semen quantity characteristics. These seasonal variations in both semen quality and quantity are mainly due to the changes in daylight length throughout the year (Chemineau *et al*., 1992).

Malejane *et al.* (2014) reported that there was significantly higher semen quality in Dorper rams in South Africa during summer, autumn and spring than during winter. Similarly, Moghaddam *et al.* (2012) reported that the best semen is produced during late summer up to the second month of autumn. Similar findings were reported in Poyaya bucks in Spain by Zarazaga *et al*. (2009).

In Nigeria, Nayak and Misra (1991) reported that the hot-dry season, characterized by elevated ambient temperature and high relative humidity causes a temporary decrease in sperm production and fertility than in the cold-dry season. Obidi *et al.* (2008)reported that sperm concentration per ml in the Shikabrown breeder cocks varied with season, and that the highest spermatozoa concentration was obtained during the rainy season.

2.6.6 Diseases

The effect of diseases on fertility in male animals may be temporary or permanent depending on the type of infectious agent (virus, fungi, bacteria, and protozoan) and the lesions produced on reproductive tract organs (Pefa, 2011). A large number of microorganisms have been isolated from semen and the prepuce (Pefa, 2011).The direct effect of pathogens has been mainly focused on the testicles and glands forming part of the reproductive tract. Infection could be limited to a single organ (seminal vesicles) or spread extensively to other organs such as the epididymis, seminiferous ampoules, prostate, bulbourethral glands and urethra; in other cases they could reach the urinary bladder, urethra and kidneys (McCauley, 1980). The inflammatory processes producing these infections are complex and difficult to differentiate amongst the affected organs; they have thus been brought together under the term seminal vesiculitis syndrome (McCauley, 1980).

The World Animal Health Organization (OIE, 2011) has listed several diseases as having proven importance in transmission through semen. Such diseases have been grouped into two groups according to whether their transmission through semen has been demonstrated. The first group are diseases whose presence and transmission through semen has already been demonstrated and these include; foot and mouth disease, vesicular stomatitis, infectious bovine rhinotracheitis (IBR), bovine virus diarrhoea (BVD), papillomatosis, leptospirosis, tuberculosis, paratuberculosis, mycopla smosis, anaplasmosis, brucellosis, campylobacteriosis and trichomoniasis (OIE, 2011). The second group are diseases whose presence through semen has been demonstrated

but not their transmission and they include; babesiosis, leucosis (when there is contamination with blood) and trypanosomiasis (OIE, 2011). The most studied agents have been brucellosis and tuberculosis, possibly because they have been involved in eradication programmes (Thibier and Guerin, 2000).

Brucellosis is produced by a facultative intracellular, gram-negative coccobacillary bacteria; it does not form a capsule or spores and is not mobile (Seleem *et al.,* 2010). Ten species from the genus *Brucella* have been identified to date (Tiller *et al.,* 2010). *B. abortus, B.ovis and B. melitensis* are species affecting cattle and small ruminants (Seleem *et al*., 2010). *Brucella ovis* is the primary cause of lowered fertility in rams in most multi-sired breeding systems (Kimberling and Parson, 2007).

The lesions produced by *B. abortus* directly affect the testicular parenchyma where it could become cultured; genital tract cells produce erythritol promoting this pathogen's growth and are thus its preferred localization (Givens and Marley, 2008). It is an important cause of vesiculitis in regions having a high disease incidence (McCauley, 1980). Pathological lesions are caused by ampullitis (unilateral orchitis and epididymitis) and are accompanied by fibrosis of the vaginal tunic and the presence of abscesses and the presence of abscesses (Nicoleti, 1980). *B. ovis* affects sheep but not goats. This organism can cause epididymitis, orchitis and impaired fertility in rams. Initially, only poor quality semen may be seen; later, lesions may be palpable in the epididymis and scrotum. Epididymitis may be unilateral or, occasionally, bilateral. The testes may atrophy. Some rams shed *B. ovis* for long periods without clinically apparent lesions (Givens and Marley, 2008).

Trypanosomosis causes reproductive disorders such as testicular degeneration, scrotal

inflammation, penile protrusion, posthitis, epididymitis, abnormal spermatogenesis and deterioration of semen characteristics (Sekoni *et al.,* 1990, Adamu *et al.,* 2007, Mbaya *et al.,* 2011, Victor *et al.,* 2012, Okubanjo *et al.,* 2014). Okubanjo *et al.* (2014) reported epididymal damage and depletion of epididymal sperm reserve in Yankasa rams experimentally infected with *Trypanosoma congolense*. Similar findings were rep orted in *Trypanosoma evansi* infected bucks (Boly *et al.,* 1993), *T. brucei* infected gazelles (Mbaya *et al.,* 2011), *T. congolense* infected bulls (Sekoni *et al.,* 1990) and *T. vivax* infected bulls (Adamu *et al.,* 2007). Decrease or lack of epididymal sperm reserve is a consequence to testicular degeneration (Omekea and Igboeli, 2000). Trypanosomes are known to localize in nutrient-rich gonads where they drain nutrients and damage testicular parenchyma (Shehu *et al.,* 2006). However, other researchers have stated anorexia due to anaemia, immunological factors, physical swelling, production of active and toxic substances by trypanosomes and increased vascular permeability as other mechanism involved in the degenerative changes (Omekea and Onuora, 1992, Okubanjo *et al.,* 2014).

2.6.7 Nutrition

The interaction between nutrition and reproduction has long been known to have important implications for the reproductive performance (Martin *et al.,* 2004). Nutrition is known to affect many aspects of the reproductive process and much research effort has been devoted to puberty, gamete production and hormonal regulation (Martin *et al.,* 2004). The inter-relationships between nutrition, growth rate and age at puberty in the male are very similar to those in the female. Thus, males reared on high as opposed to

low planes of nutrition reach puberty at earlier ages and heavier body-weights (Brown, 1994). In common with the female, nutritional influences are much more pronounced if they occur in early life rather than post weaning (Pruitt *et al.* 1986). Feed restriction of Merino ram lambs prevented them attaining puberty in their first potential breeding season (Martin and Walkden-Brown, 1995). More liberal subsequent feeding allowed puberty to occur the following year with those growing at 130 g/day during the re-alimentation period reaching puberty 4 weeks earlier and sustaining higher sperm concentrations than those growing at 80 g/day. Similar reductions in sperm concentrations leading to overall reductions in sperm production have been noted by Alkass *et al.* (1982) following feed restriction of rams to 75% of their estimated maintenance needs for periods in excess of the 5-6 weeks required to develop sperm from the spherical spermatids in the germinal epithelium to fully mature spermatozoa in the distal cauda epididymidis (Amann, 1987).

Semen quality and quantity can adversely be affected by nutrition (Brown, 1994). Reduction in feed intake in animals exposed to high temperatures may lead to a deficient protein and energy intake (Vilakazi, 2003). There is evidence that the effect of nutrition on semen characteristics is mediated by the effect of dietary constituents on the hypothalamo-pituitary axis, although there are also some indications that dietary changes affect the testis growth indirectly (Brown, 1994). Diet restriction is associated with a decrease in the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn affects semen production and quality through their effect on testicular size and function (King, 1993). Similar observations were made by Martin and Thomas (1987) where the level of nutrition was associated with a reduction in

testicular size. During periods of nutritional stress, the animal body secretes stress hormone- glucocorticoids which lower the circulation or secretion of FSH and LH and hence causes inefficient spermatogenesis and poor semen quality (Bearden and Fuquay, 1997).

Growth and subsequent body weight appears to be a better guide to the onset of puberty in rams than age (Dunn, 1955; Watson *et al.*, 1956; Moule, 1970; Dyrmundsson, 1973; Rattray, 1977). Ragab *et al*. (1966) and Pretorius and Marincowitz, (1968), reported that rams reared on higher planes of nutrition attained puberty (age at which sperm first appeared in the ejaculate) at younger ages and at heavier body weights than rams on lower levels of feeding. Similarly, Krishnan (2014) observed that the level of nutrition markedly influences puberty in livestock. The faster an animal grows the earlier it reaches sexual maturity .In cattle, puberty occur at a particular live weight or body size than at a fixed age.

Krishnan (2014) reported that energy deficiency in young animals affects age at puberty, and sexual development due to reduced endocrinal activity. When mature animals are fed low energy diets for long libido and testosterone production are negatively affected which subsequently affect semen quality. Obesity or overfeeding reduces libido and sexual maturity in male animals. It was observed that excess dietary energy may adversely affect sperm production and semen quality due to fat deposition in the scrotum, thereby reducing heat radiated from the scrotal skin and increasing scrotal and testicular temperatures (Coulter and Kastelic, 1999). In another study, bulls fed high-nutrition diets had greater scrotal circumference than those fed medium-nutrition diets, but paired testes weights were the same (Coulter and Kastelic, 1999).

Since scrotal weight was greater in bulls fed high nutrition, perhaps fat deposition in the scrotum increased scrotal circumference in these bulls.

Furthermore, the low-plane rations considerably delayed descent of the testicles, penile development and first appearance of spermatozoa in the ejaculate (Petrovic *et al.*, 2007). As these processes are androgen-dependent, it was concluded that restricted feeding had inhibited gonadotrophic hormone release which resulted in a decrease in androgen activity as well as semen quality (Pretorius and Marincowitz, 1968).

2.6.7.1 Energy

Energy balance is probably the single most important nutritional factor related to poor reproductive function in animals (Scramuzzal and Matin, 2006). High-plane feeding of boars leading to leg weakness and lack of libido prompted the recommendation of lower planes of nutrition for boars in Dutch artificial insemination centres (Bindari *et al.*, 2013). Kemp *et al.* (1989) showed that maintenance feeding regimens, without a feed increment for the energy cost on the day of mating (1.3 MJ metabolizable energy (ME)), reduced spermatozoa numbers, but not their quality, by 32 and 41 % compared with those receiving twice and thrice maintenance rations.

Generally, increased dietary energy may adversely affect sperm production and semen quality due to fat deposition in the scrotum, thereby reducing heat radiated from the scrotal skin and increasing scrotal and testicular temperatures (Coulter and Kastelic, 1999). In a study, bulls fed high-nutrition diets had greater scrotal circumference than those fed medium-nutrition diets, but paired testes weights were the same (Coulter and Kastelic, 1999). Since scrotal weight was greater in bulls fed high nutrition, perhaps fat

deposition in the scrotum increased scrotal circumference in these bulls. In addition to the deleterious effects of high-energy diets on reproductive function, these diets may also result in abnormal foot growth due to laminitis, as well as adverse effects on bone and cartilage growth, and may hinder reproductive performance by altering the ability of the male animal to mount the female (Coulter and Kastelic, 1999).

Conversely, low nutrition in calves during young age suppressed LH secretion before 25 weeks, delayed puberty, and reduced testicular development at maturity (Kastelic, 2013). The initial suppression of testicular development and delayed puberty was not corrected in bulls fed reduced nutrition before approximately 26 weeks of age and subsequently placed on the high-nutrition regimen. Therefore, it is not possible to compensate for the effects of low nutrition early in life by subsequently giving supplemental feed (Kastelic, 2013).

2.6.7.2 Protein

The effect of dietary protein on reproduction is complex (Funston, 2007). Prolonged inadequate protein intake has been reported to reduce reproductive performance. More recently it has been found that reproductive performance may be impaired if protein is fed in amounts that greatly exceed the bull's requirements (Bindari *et al.*, 2013).

Feeding a low protein diet to adult bulls for about 1 year, resulted in smaller testes and epididymides in bulls and their gonadal and extragonadal reserves of spermatozoa were reduced to 70-75% of that for control bulls (Laszczka *et al.*, 1969). Though the volume of ejaculates and the percentage of live spermatozoa were also in decline after 8 months on this diet, there were no apparent morphological changes in the spermatozoa

(Laszczka *et al.*, 1969). Similarly, Rekwot *et al*. (1987) and Rekwot *et al*. (1988) found that bulls fed low protein diets had poorer semen quality than those fed high protein diets. Jibril *et al.* (2011) reported an increase in semen concentration in rams fed high protein diets. They observed that even though an increased protein intake above the minimum requirement (12% CP) enhanced spermatogenesis, higher levels of proteins in diets resulted in excess urea and more available ammonia which might have influenced the physiology and reproduction often associated with decline in fertility as reported by other workers (Jordan and Swanson, 1979; Kaim *et al.*, 1983; Canfield *et al*., 1990; Elrod and Butler, 1993).

2.6.7.3 Fats

The impact of fats on reproduction is a focus of considerable research (Clark and Henry, 1999). Because fatty acids and cholesterol are substrates for hormone synthesis, increasing fat in the diet may increase levels of reproductive hormones (testosterone, progesterone, prostaglandins) or fats may act directly on the reproductive axis (Martin *et al.*, 2004). Therefore, the effects of fat may be independent of or additive to those of increased energy availability (Engstrom *et al*. 1994). Ruminant diets usually contain less than 2 to 5 % fat (Brandt and Anderson, 1990). Supplementing fat to improve reproduction was initially attempted to increase the energy density in the diet. High fat diets for cattle contain 6% to 8 % fat. Exceeding these dietary fat levels impairs rumen function (Marx, 2000).

Obesity is a major health problem contributing to increased subfertility in males, as well as increased morbidity for diseases related to a decline in testosterone production

with aging (Landry *et al.*, 2013). Excess dietary fats can cause fat deposition in the scrotum, thereby reducing heat radiated from the scrotal skin and increasing scrotal and testicular temperatures (Coulter and Kastelic, 1999).

2.6.7.4 Minerals and Vitamins

For the minerals and vitamins the high zinc content of semen and the importance of this element in spermatogenesis coupled with its synergic role in the uptake by the spermatozoa of vitamin A is noteworthy, particularly as vitamin A itself plays an essential role in the attainment of puberty and in the maintenance of both libido and the integrity of the testicular germinal epithelium (Hurley and Doane, 1989).

Cupps (1991) reported that deficient nutrient intake such as iodine, zinc, cobalt and vitamins A and E are associated with a reduction in semen quality in terms of morphology, concentration and motility.In general all the vitamins are essential for reproduction due to their specific roles in cellular metabolism maintenance and growth. Vitamin A and E are of great importance because of their role in maintaining cellular integrity and antioxidant property in the semen respectively. Vitamin A is necessary for normal epithelial development in all species. Failure of epithelial development negatively impacts spermatogenesis (Krishnan, 2014).

A deficiency of selenium affects the morphology and motility of the spermatozoa and may be linked with subfertility in many domestic livestock (Scott *et al*., 1998). The spermatozoa membranes are attacked by the increasing formation of reactive oxygen species which lower the viability of the spermatozoa (Irvine, 1996). Selenium increases formation of the anti-oxidant glutathione peroxide, which in turn decreases the reactive

oxygen species and hence increases spermatozoa viability and fertility (Bray *et al.*, 1997).

2.6.8 Live weight and body condition score

Effect of body condition score (BCS), live weight (static effects) and changes in BCS and live weight (dynamic effects) before mating, during mating and after mating period, on reproductive efficiency of different breeds of sheep in the different rearing systems have been reported (Cam *et al.,* 2010, Aliyari *et al.,* 2012*).* Most of researchers have reported that, the absolute effects of BCS and live weight than their variations have greater impact on sheep reproduction efficiency, which suggest the importance of breed and interactions with nutritional and physiological conditions and its impact on reproduction efficiency (Gunn, 1983; Koycegiz *et al.*,2009).A correlation exists between BCS, live weight and amount of reserving body fat (Oregui *et al.*, 1997). BCS is also suitable for prediction of adult weight of sheep with different genotypes (Zygoyiannis *et al.*, 1997). Live weight is a combination of skeleton size and BCS and it's not a good representative to evaluate the reproductive efficiency. Adult weight is affected by factors such as skeleton scheme (body size), fullness or hunger trap (filled or empty digestive tract) (Adedeji and Gbadamosi, 1999; Koycegiz *et al.*,2009).Many researchers have reported that, fertility affected by BCS (Koyuncu, 2005; Madani *et al.*, 2009). Garcia *et al.* (2002) showed the significant effect of body to initiation of lambs' puberty.

Akpa *et al.* (2012) reported that sperm production and quantity can be affected by both animal size and physiological status. There is a positive and significant correlation between testicular dimensions and body measurements. These suggest that males with larger scrotal size might possess larger body size and good reproductive ability. Testicular length and circumference are measures of testicular size which had been found to be significantly correlated with body weight (Adedeji and Gbadamosi, 1999; Bratte *et al,* 1999). The strong positive correlation between BCS and scrotal circumference indicates that an increase in body condition would increase testicular measurement; particularly scrotal circumference which may invariably improve the fertility of the animal. Similar findings were reported for works conducted on stallions (Naden *et al.,* 1990; Blanchard *et al.*, 2001).

2.7 Nutritional Mechanisms of Control of Reproduction

Nutrition is the most important factor affecting reproduction and the responses to it can be divided into short-term effects that act mainly on the neuroendocrine system controlling testicular activity (Martin *et al.*, 1994a) and long-term effects that act on testicular growth and sperm production (Oldham *et al.*, 1978).

The beneficial effects of nutrition on reproduction in sheep are well known (Blache *et al.*2007). In particular, nutrition is one of the main factors affecting ovulation rate and spermatogenesis. Generally sheep will be at grass for half of the year, however during winter or at lambing they may be housed or given extra feed at grass. It is important to get the nutrition right to prevent loss of body condition (Martin *et al.*, 2004).

2.7.1 Effect of nutrition on the hypothalamo-pituitary axis

The most fundamental driver of reproductive function is a group of neuroendocrine cells in the preoptic–hypothalamic continuum that synthesizes GnRH (Martin *et al.,* 2004). These cells secrete discrete pulses of GnRH into the portal blood system leading to the anterior pituitary gland where they elicit pulses of LH and a relatively continuous stream of FSH. Both LH and FSH are involved in the regulation of the production of both spermatozoa and hormones by the testis (Martin *et al.,* 2004). The frequency of GnRH pulses, and consequently LH pulses, is the code used by the nervous system to control testicular function. Direct measurement of the activity of GnRH cells is difficult, but usually unnecessary because LH pulses are easily detected in peripheral blood and their frequency can be used as a bioassay of GnRH cellular activity (Thiéry and Martin, 1991). The network of neurones that controls GnRH secretion is not yet defined, but it is thought to be the final common pathway via which many factors influence gonadal activity, including metabolic status (Cameron *et al.,* 1993; Martin *et al.,* 1994a).

Glucose is the principal source of metabolic energy for the ram testis (Setchell and Hinks 1967) and for the ruminant brain (Pell and Bergman 1983). Insulin was implicated because, in rats, it can cross the blood-brain barrier into the cerebrospinal fluid (CSF), from where it is taken up by neural tissues (Baskin *et al*., 1987; Schwartz *et al*., 1990), presumably those that contain insulin receptors, such as the median eminence and mediobasal hypothalamus (Van Houten *et al*., 1980; Baskin *et al*., 1987). These areas are important in the control of GnRH release in sheep (Thiéry and Martin 1991). In rams, the lupin supplement increases insulin concentrations in peripheral

plasma as well as celebrospinal fluid (CSF), where the values are about 10% of those in plasma (Miller *et al*., 1993; Martin *et al*., 1994b). In both plasma and CSF, there is a tendency for glucose concentrations to increase too, but they generally differ little from control values, presumably due the regulatory influence of the extra insulin.

Martin and Walkden-Brown (1995) tested the role of the glucose-insulin system by intravenous and intra-abomasal infusions of glucose. These treatments greatly increased the circulating concentrations of both glucose and insulin, but had no effect on gonadotrophin secretion or testicular growth (Boukhliq *et al.* 1991; Boukhliq *et al* 1992). Intracerebral route for administration of insulin by injecting it into the third ventricle increased LH pulse frequency in mature Merino rams on a maintenance diet (Miller *et al.*, 1994).

Bergman (1990) reported that 70% of the energy requirement of ruminants is met by the volatile fatty acids, primarily acetate, propionate and butyrate that are produced by fermentation in the rumen. They are rapidly absorbed into the rumenal epithelium, where most of the butyrate is metabolised. Propionate is the only glucogenic volatile fatty acid and virtually all of it is absorbed immediately from the portal bloodstream by the liver where it is used for the production of glucose. Most of the acetate enters the general circulation and is used directly as an energy substrate by most peripheral tissues (Bergman 1990). The possibility that volatile fatty acids could act as a nutritional signal was suggested by Murray *et al*. (1990) who observed a tendency for testicular growth to increase in rams fed a mixture of volatile fatty acids as a dietary supplement. This effect was subsequently confirmed by Boukhliq *et al.* (1992) who also found that this treatment increased LH pulse frequency and FSH concentrations. Intra-abomasal

infusion of casein did not affect testicular growth in Merino rams, whether in the presence or absence of exogenous glucose (Boukhliq *et al*., 1992), but it did increase FSH secretion (Martin *et al.*, 1992) and tended to increase LH pulse frequency.

When low nutrition was accomplished by restricted feed intake, hypothalamic and pituitary function were suppressed, with LH secretion most severely affected (Brito, 2007). It appeared that insulin-like growth factor I (IGF-I) was a possible signal to the central "metabolic sensor" involved in translating body nutritional status to the gonadotropin releasing hormone (GnRH) pulse generator (Brito, 2007). Nutrition also affected blood testosterone concentrations, indicating effects on the number and/or function of Leydig cells. Age-related increases in physiological and GnRH-stimulated serum testosterone concentrations occurred earlier in bulls receiving high nutrition but were delayed in bulls receiving low nutrition; these effects were probably mediated by both LH and IGF-I (Brito, 2007). Circulating leptin and insulin apparently have only permissive roles on GnRH secretion but may enhance testicular development. Growth hormone concentrations decreased concomitantly with increasing IGF-I concentrations during sexual development in bulls, suggesting the testes could contribute considerable amounts of circulating IGF-I (Brito, 2007).

Leptin is a hormone produced by adipose tissue whose production increases with the amount of body fat (Landry *et al.*, 2013). Several studies have supported a relationship between increased leptin production and regulation of reproductive function (Kiess *et al.*, 1998; Clarke and Henry, 1999; Tena-Sempere*et al.*, 1999, Tena-Sempere and Barreiro, 2002). Indeed, leptin acts at all levels of the hypothalamus-pituitary-gonadal (HPG) axis in males. However, most of the obese individuals become insensitive to

increased endogenous leptin production and develop a functional leptin resistance (Tena-Sempere and Barreiro, 2002). This deregulation of leptin signaling might result in abnormal endocrine and reproductive functions. Altered leptin dynamics may contribute to male infertility in different ways, leading to hypogonadism. These include leptin resistance or leptin insufficiency at the hypothalamus and leptin modulation of testicular physiology (Landry *et al.,* 2013).

The mechanisms whereby leptin regulates reproductive function are multifaceted and likely involve actions at different levels of the hypothalamic-pituitary-gonadal axis. It is well established that the hypothalamus, a key element in the control of food intake and neuroendocrine regulation of reproductive function, is the primary target of leptin (Schwartz *et al.*, 1996; Casanueva and Dieguez, 1999; Ahima *et al.*, 2000). In the context of reproductive control, the hypothalamic effects of leptin likely involve stimulation of GnRH release, as predicted by experimental studies using hypothalamic tissue and immortalized GnRH-producing GT1-7 neurons in vitro (Yu *et al*., 1997; Magni *et al*., 1999). This action likely accounts for the stimulatory effects of systemic administration of leptin upon serum gonadotropins (Nagatani *et al*., 1998).

2.7.2 Effect of nutrition on the testis

Changing nutrition alters not only the total amount of testicular tissue, but also the efficiency with which the gametes are produced by that tissue. This is evident from the fact that changes in sperm production are consistently greater than those in testicular mass. Oldham *et al*. (1978) found that a 25% increase in testicular size led to an 81% increase in production of spermatozoa, and Cameron *et al*. (1988) found that an 86%

increase in testicular size led to a 250% increase in production of spermatozoa. The number of ejaculated spermatozoa is not affected until 7 weeks after a change in diet, suggesting that the effects on spermatogenic efficiency are exerted after the last spermatogonial division. This is similar to the effects of stimulatory photoperiod in more seasonal breeds of sheep, where efficiency is increased by reducing the rate of degeneration of germ cells following the mitotic and meiotic divisions of the spermatogenic cycle (Hochereau-de Reviers *et al*., 1985).

There is a need for detailed cytological analysis to determine whether nutrition operates by this mechanism, and also to determine the relative roles of FSH, testosterone and LH in mediating these responses at testicular level. Histological studies show that nutrition markedly affects the diameter of the seminiferous tubules (Setchell *et al*., 1965), the relative proportion of the testis occupied by the seminiferous tubules and the proportion of the seminiferous tubule occupied by the seminiferous epithelium (Oldham *et al*., 1978).

If changes in production of spermatozoa result primarily from alterations in Sertoli cell function, it is peculiar that peripheral inhibin concentrations do not seem to be affected by diet in mature rams, despite clear effects on FSH concentrations and testicular mass (Martin *et al*., 1994a). The effects of nutrition on the activity of the interstitial tissue should be reflected in rates of production of testosterone (Martin and Walkden-Brown, 1994). However, in mature rams, significant changes in testicular mass induced by nutritional treatments were not associated with changes in the size of the testosterone response to LH (Ritar *et al.,* 1984; Martin *et al.,* 1994a).

Tena-Sempere and Barreiro (2002) reported the direct effects of leptin at the gonadal level. Initial evidence for an inhibitory role of leptin on ovarian function was presented (Spicer and Francisco, 1997; Zachow and Magoffin, 1997). These studies paved the way for the characterization of the biological effects and mechanisms of action of leptin upon the testis (Tena-Sempere and Barreiro, 2002).On the basis of evidence showing the expression of the Ob-R gene in rat testis (Zamorano *et al*., 1997), characterization of direct testicular actions of leptin was undertaken. Tena-Sempere and Barreiro (2002) evaluated the effect of leptin upon testicular testosterone secretion *in vitro.* Leptin was found to inhibit testosterone secretion by the Leydig cells.

2.8 Gossypol

Gossypol is a yellow pigment occurring in free or bound form in all parts of cotton plants, with the highest levels found in seeds (Adams *et al.*, 1960; Markman and Rzhekhin, 1969; Jaroszewski, 1998; Dodou, 2005). Non-processed whole cottonseeds, as well as processed cottonseed meal may therefore contain large amounts of free gossypol, which may cause adverse and toxic effects if used as a feeding stuff. Cotton also contains terpenoid phytoalexins other than gossypol such as hemigossypol, desoxyhemigossypol, 2,7dihy-droxycadalene, hemigossypolone and heliocides H1 and H2, but in low quantities (Bell *et al.*, 1975; Stipanovic *et al.*, 1978). In the seed, gossypol is found in small pigment glands. When the seed kernels are flaked and heated as part of the oil extraction process, these small glands are ruptured and the gossypol is released. Some of the gossypol will bind with components in the seed (Adams *et al.*, 1960; Jones, 1985). This is the reason for the terms “bound” and

"free" gossypol. All of the gossypol in whole seed is in the freeform (Pons *et al*., 1953), but as a result of processing, both free and bound gossypol will be present in meal (Jones, 1985). During processing, free gossypol is bound to cottonseed protein resulting in bound gossypol and unavailable amino acids. This binding reduces the protein quality, especially with regard to lysine availability. Lysine is believed to be the primary amino acid that is bound to free gossypol (Kuiken and Lyman, 1948; Baliga and Lyman, 1957). Gossypol is found in both vegetative and reproductive tissues of the cotton plant. The pigment is located almost exclusively in pigment gland structures appearing as dark dots in the plant tissue. The presence of gossypol in cotton protein products is counteracted in part by appropriate processing technologies, and in part by use of glandless cotton varieties, which contain only traces of gossypol. However, glandless cotton varieties are more susceptible to pests (Alexander*et al.*, 2008).

2.8.1 Chemistry of gossypol

Gossypol, 1,1',6,6',7,7'-Hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-2,2'-binaphthalene-8,8'-dicarbaldehyde, $C_30H_{30}O_8$ (Molecular weight= 518.55) is crystalline, intensely yellow, insoluble in water and soluble in organic solvents and fats. The chemical structure of gossypol is shown in figure 3. It has an ultraviolet (UV) absorption maximum at about 385 nm (solvent dependent) (Alexander*et al.*, 2008).

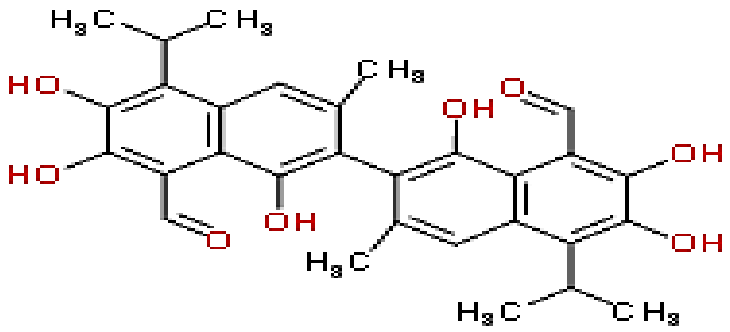

Figure 3: Chemical structure of gossypol (chemspider.com, 2013)

It melts at temperatures around 200°C depending on its polymorphic form. Many polymorphic forms and crystalline solvates of gossypol exist depending on the solvent of crystallization. As racemic gossypol forms a 1:1 crystalline complex (solvate) with acetic acid that is less soluble in some organic solvents than gossypol itself, acetic acid is commonly used when isolating gossypol (Alexander*et al.*, 2008).

The presence of a polyhydroxylated aromatic aldehyde moieties make gossypol exhibits complex tautomerism, which influences its chemical reactions. In addition to the symmetrical forms, the two halves of the molecule may adopt different tautomeric forms, giving rise to numerous molecular species (Alexander*et al.*, 2008). Moreover, water loss from the hemiacetal form results in reversible formation of anhydrogossypol and oxidation of gossypol produces gossypolone. Because rotation around the bond connecting the two halves of the gossypol molecule is hindered, the molecule is non-planar and has a two-fold symmetry axis. The gossypol molecule is therefore chiral due to axial dissymmetry. Hence, two enantiomers [(+) and (–)] of gossypol exist; (+)-

Gossypol has the (*S*) configuration, and (–)-gossypol the (*R*)- configuration (Huang *et al*., 1988; Freedman *et al*., 2003).

The aldehyde groups present in the main tautomer of gossypol (the dialdehyde form) react readily with amines, including free amino groups in proteins and form imines (Schiff bases), which can be further transformed to other products (Strom-Hansen *etal*., 1989). Thus, reaction of gossypol with amino groups present in side-chains of amino acid residues and with other amino groups present in proteins (N-terminals of peptide chains) leads to formation of so-called bound gossypol, i.e., gossypol-protein conjugates. Such covalently protein-bound gossypol is not extractable with organic solvents, but can be liberated as free gossypol by treatment with acids. Protein-bound gossypol is formed upon storage of cottonseed products and its formation is accelerated by heat and moisture, the conditions encountered during processing of cottonseed. Although gossypol bound to proteins is considered less toxic or non-toxic, it may diminish the quality of proteins. Because the compound forms tight complexes with some metal ions, bound gossypol presumably also includes a fraction of gossypol that is bound as insoluble metal complexes, particularly with iron. Part of the bound gossypol can, in principle, be liberated in the digestive tract of animals, but the evidence for this is lacking (Alexander*et al.,* 2008). Gossypol is a dimeric or bis-naphthalene natural compound. The naphthalene rings, however, are derived from sesquiterpenes of the cadinane family. The cadinanes are formed in the biogenetic cascade from the bisabolane intermediate by a series of putative 1,2-shifts and cyclization (Alexander*et al.*, 2008).

2.8.2 Effects of processing of whole cottonseed on gossypol content

Currently, there are three methods of processing cottonseed meal or cake from whole cottonseeds, namely mechanical extraction (screw processing), pre-pressed solvent extraction and direct solvent extraction. These methods use different temperatures, pressures and cooking times. All the three forms of cottonseed meal produced have similar nutritional values (Jones, 1981).

Jones (1981) and Rogers *et al.* (2002), stated that when cubed or pelleted, cottonseed meal is also known as cottonseed cake. The cake is the residue of the mechanical extraction and retains higher oil compared to the meal. During processing, the rupture of the pigment glands containing the gossypol and other complex polyphenols allow the binding of these highly reactive compounds with the components of the meal. Thus the portion of gossypol which has combined with the sulphur containing amino acids in protein during condensation of gossypol is known as bond *gossypol* while the remaining uncombined gossypol is called free *gossypol* (Pons and Hoffpauir, 1957; Alexander *et al.,* 2008).

The concentration of free gossypol in cottonseed meal is influenced by the method of oil extraction. Jones (1981) reported the following representative values of free and total gossypol content for screw pressed (0.04 and 1.02%), pre-pressed solvent extracted (0.05 and 1.13%) and direct solvent extracted (0.30 and 1.04%) cottonseed meals. This is an indication that although total gossypol content does not differ greatly, the physiologically active free gossypol content is much greater in direct solvent-extraction method than in the other two processes. Rogers *et al*. (2002) stated that

higher temperatures and pressures favour the formation of stable bonds between gossypol and other molecules.

2.8.3 Biosynthesis of gossypol

The metabolic fate of gossypol has been the subject of several investigations. Plasma gossypol (PG) concentrations directly reflect gossypol absorption in the gut and metabolism by the liver. When cottonseed is fed, gossypol in the free form that escapes detoxification within the fore-stomachs is available for absorption in the small intestine. It is assumed that binding of gossypol to amino acids of dietary and bacterial origin reduces gossypol availability. When cottonseed escapes rumen digestion, plasma gossypol concentrations increase dramatically (Santos *et al.,* 2002 and Prieto *et al*., 2003).

Mena *et al*. (2001) fed lactating Holstein cows varying amounts of gossypol from whole linted Upland cottonseed or cottonseed meal. Gossypol in cottonseed is mostly in the free form and gossypol in cottonseed meal is mostly in the bound form. Plasma gossypol concentrations were directly related to intake of free gossypol. The major site for gossypol detoxification is the reticulum-rumen (Calhoun *et al*., 1995). When lint on whole cottonseed is removed, passage rate of cottonseed through the digestive tract was increased (Coppock *et al*., 1985), which suggests that rumen retention time was reduced. When whole linted cottonseed was compared with whole Pima and ground Pima in the diet of finishing steers, feeding whole linted cottonseed increased ruminal digestion of organic matter and acid detergent fiber (Zinn, 1995), supporting the hypothesis that rumen retention time of the cottonseed might be decreased by lack of

lint and by seed processing through cracking or grinding. It was observed that cracked Pima cottonseed had a smaller mean particle size and a greater bulk density than whole linted Upland cottonseed (Santos *et al*., 2002). Such changes in the physical characteristics of feedstuffs are usually associated with an increased ruminal passage rate.

Mena *et al*. (2001) proposed that gossypol detoxification within the rumen might be reduced when rumen retention time of gossypol containing feeds is reduced. Processing of cottonseed by pelleting and addition of iron sulfate to the diet seem to decrease gossypol availability and plasma gossypol concentrations in growing steers and lactating dairy cows (Santos *et al.*, 2002). However, when iron sulfate was added to diets of lactating cows containing cracked Pima cottonseed, milk production decreased (DePeters*et al.*, 2000).

The greatest amount of free gossypol accumulation was also observed in the liver followed by kidney and lastly the heart when lambs were fed whole cottonseed or cottonseed products for a long time (Nikokyris *et al.*, 1991). The preferential accumulation of gossypol in the liver has also been studied in several livestock animals, such as in sheep (Morgan 1990), swine (Sharma *et al.*, 1966), broiler chickens (Gamboa *et al.*, 2001) and rainbow trout (Roehm *et al.*, 1967).

The concentration of gossypol increased linearly in plasma, liver, kidney, and muscle with dietary levels of free gossypol. Liver had the highest concentration of total gossypol (71.4-313.6 mg/kg dry matter) followed by kidney (9.2 to 36.3 mg/kg dry matter), plasma (3.0-14.6 μg/ml), and muscle (2.1-9.8 mg/kg dry matter). The

proportion of (-)-gossypol was higher than that of (+)-gossypol by 16-27 % in all tissues (Gamboa *et al.*, 2001).

2.8.4 Gossypol toxicity

Gossypol exhibits a variety of biological actions, which range from apparently highly specific pharmacological activities on certain macromolecular targets such as enzymes involved in maturation of mammalian sperm to more unspecific binding to proteins (Abou-Donia, 1976).

2.8.4.1 Gossypol toxicity in monogastrics

Acute toxicity has been shown in the heart, lung, liver, and blood cells, resulting in increased erythrocyte fragility. Post mortem findings include generalised oedema and congestion of lungs and liver, hydrothorax, hydroperitoneum, and degeneration of heart fibres (Tanksley, 1990).

Acute gossypol intoxication of monogastric animals causes circulatory failure. Single-dose oral LD50 of racemic gossypol is 2400-3340 mg/kg for rats, 500-950 mg/kg for mice, 350-600 mg/kg for rabbit, 550 mg/kg for pigs and 280-300 mg/kg for guinea pigs. Sub-acute toxicity causes pulmonary oedema and symptoms of malnutrition. Dogs appear to be quite sensitive (repeated oral dose of 10-200 mg/kg for a month was fatal).

The two enantiomers of gossypol have markedly different biological effects. In comparison with (+)-gossypol, the (–)-enantiomer generally exhibits more pronounced effects. For example, (–)-gossypol is more cytotoxic (Band *et al.*, 1989; Benz *et al.*,

1990; Blackstaffe *etal.*, 1997; Shelley *et al.*, 1999), binds more strongly to proteins (Wang *et al.*, 1992; Oliver *etal.*, 2005), is the active anti-spermatogenic agent, and is considered more toxic than the (+)- gossypol (Matlin *et al.*, 1985; Lindberg *et al.*, 1987; Bailey *et al.*, 2000; Lordelo *et al.*, 2005). Heywood *et al.* (1985) and Heywood (1988) reported that racemic gossypol acetic acid was administered in cynomolgus monkeys at 25 mg/kg BW per day for 13 weeks, and most of the animals died.

Morphological changes in general included necrotic myocardial fibres and fibrosis in the heart, the presence of vacuolar hepatocytes, extensive changes in the kidney including dilation, epithelial vacuolation and degeneration with calcification and dystrophic mineralisation and unilateral testicular atrophy (except in the monkey that survived the experiment whose testicular morphology was normal). At lower doses, reproductive toxicity was observed (Heywood *et al.*, 1985, Heywood, 1988).

2.8.4.2 Gossypol toxicity in ruminants

For many years, it was assumed that only non-ruminant animals were susceptible to gossypol toxicity. Reports earlier this century of injury to cattle being fed cottonseed products were thought to be gossypol related, but were eventually discovered to be a vitamin A deficiency (Adams *et al.*, 1960). Reiser and Fu (1962) identified the mechanism by which functional ruminants avoid gossypol toxicity. These researchers found that the detoxification was a result of free gossypol becoming bound to soluble proteins in the rumen. The gossypol most likely binds to the epsilon-amino group of lysine. This bond is not broken by the proteolytic enzymes secreted in the lower gut.

It is known, however, that ruminants are susceptible to gossypol toxicity if the rumen detoxification process is somehow bypassed. Intravenous administration of gossypol in sheep resulted in similar symptoms to those seen in non-ruminants (Danke *et al.*, 1965). Commercial beef cattle that are consuming cottonseed meal as a protein source in mixed feed or as a supplement are very unlikely to be in danger of a toxicity situation. There are at least two reasons for this: the rumen's detoxification abilities and the fact that a mature beef animal in a production situation usually would not receive enough cottonseed cake to tax the rumen's gossypol detoxification process (Martin, 1990).

Over the decades, the largest proportion of cottonseed meal has been fed to beef cattle and this is still the case. This extensive practical use did much over the years to build confidence in cottonseed meal and tempted dairy and sheep producers to use large amounts of meal almost all of it without incident (Martin, 1990). Cottonseed meal was included in rations of many classes of livestock like dairy calves, high-producing dairy cows and lambs. However, these additional uses for meal were not as well supported by scientific data, and the unsupported use of cottonseed cake in several types of rations has resulted in some apparent toxicity conditions reported in the farm press (Martin, 1990).

The concern that this high level of meal feeding could tax the rumen's gossypol detoxification ability prompted research to answer this question. Lindsey *et al.* (1980) found gossypol in the plasma and livers of dairy cows fed a diet containing 45% direct solvent and screw-press process cottonseed meal. This level of meal was much higher than what is usually used commercially. However, the purpose of the study was to determine if the rumen could be overloaded with free gossypol. The fact that free

gossypol was found in the plasma and livers, indicates that there is a limit to how much free gossypol can he detoxified in the rumen of lactating dairy cows. The study, however, did not reveal how much gossypol could be safely handled by the high producing milk cow. Observations in the study did lead the researchers to believe that a high free gossypol intake caused the animals to be more susceptible to ill effects caused by heat stress and other stresses incurred in today's production system (Martin, 1990).

The subject of gossypol with regard to young ruminants is an area of more uncertainty. In order for gossypol to be detoxified, the animal must have a functioning rumen. Gossypol toxicity in young ruminants has received much attention, even to the point of making producers and feed manufacturers wary of including the safe levels of cottonseed meal (CSM) supported by available research data. The symptoms of gossypol toxicosis reported in young ruminants are similar to those listed earlier for non-ruminants. These symptoms appear to be most prevalent after long-term feeding of unsafe levels (greater than 70days, more than 100 ppm free gossypol). Death may result and may be preceded by the typical symptoms or it may be sudden. Fatalities have been reported to occur up to 30 days after the removal of CSM from the diet (Holmberg *et al.*, 1988). Tool Vera (1997) reported that the maximum amount of gossypol in ruminant diets should be 10-20 mg/kg.

2.8.5 Mechanisms of gossypol toxicity

In several studies the mechanisms of toxicity of gossypol have been explored (Tso and Lee, 1982; Ali and El-Sewedy, 1984; Gawai *et al.*, 1995; Kovacic, 2003; Fiorini *et al.*,

2004; Zhou *et al.*, 2008). Toxicity is shown against the reproductive system, heart, liver, and membranes (Kovacic, 2003).

Gossypol and its metabolites exert pro- and anti-oxidant potential (Kovacic, 2003 and Zhou *et al.*, 2008). Considerable evidence points to oxidative stress, formation of reactive oxygen species, and DNA scission, characteristics of redox-cycling by electron transfer in biosystems (Kovacic, 2003). The chemistry of gossypol is very relevant to its unique actions. The two aldehyde groups can easily bind to proteins via aldehyde-amino group linkage. Gossypolone, the in vivo oxidation product of gossypol, may form a redox system with its corresponding hemiquinone, leading to free radical generation (Yu *et al.*, 1996).

In rats treated intraperitoneally for 5 days with 5 mg/kg body weight of racemic gossypol per day biochemical changes in liver enzymes were observed with a decrease in cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase. However, the treatment did not affect levels of cytosolic glutathione S transferase (GST) and the serum enzymes sorbitol dehydrogenase and alanine aminotransferase, which are indicators of liver damage (Ali and El-Sewedy, 1984). With respect to GST isozymes, gossypol is a reversible inhibitor (Lee *et al.*, 1982). Furthermore, gossypol binds to microsomal membranes, inhibits DNA synthesis, and causes depletion of iron and glutathione in mammalian cells (Gawai *et al.*, 1995).

Gossypol also specifically inhibits the 11β-hydroxy-steroid dehydrogenase (11β-HSD), which oxidises cortisol to inactive cortisone in the kidney and other tissues of importance in electrolyte potassium and sodium regulation. Episodes of hypokalemia

observed in clinical trials for anti-spermatogenic effect are the clinical consequence of inhibition of 11β-HSD (Song *et al.,* 1992; Waites *et al.,* 1998; Reidenberg, 2000).

Another mechanism relevant to the anti-fertility effects of gossypol is the ability to block gap junction intercellular communication (GJIC) in Sertoli cells, required for spermatogenesis, which has been demonstrated in cultured human and rat cells (Herve *et al*., 1996). *In vitro* experiments in Sertoli cells showed a rapid cytoplasmic delocalization of connexin 43 (a gap junctional protein important for Sertoli cells to regulate spermatogenesis). In addition N cadherin and connexin 43 protein expressions were decreased (Fiorini *et al.,* 2004; Zhou *etal.,* 2008).

2.8.6 Gossypol and reproduction

Liu (1957) reported that not a single childbirth was reported during a period of 10 years in one area of China during the 1930s and 1940s. This was attributed to the fact that for economic reasons villagers had switched from cooking with soybean oil to crude cottonseed oil. During the late 1970s, Chinese researchers reported on clinical trials of gossypol as a male contraceptive that were undertaken after observing that consumption of large quantities of crude cottonseed oil causes reduced fertility. Those first clinical trials of gossypol as an oral contraceptive for men reported high efficacy, reversibility, and few side effects (Wang *et al.,* 2009).

2.8.7 Effect of gossypol on male reproduction

The recent interest in gossypol's effect on male reproduction is a result of reports in the 1970s of the contraceptive effect of gossypol in men in China. These observations were the basis of research to determine the value of gossypol as a male anti-fertility agent in laboratory animals and humans. In the Chinese studies, a dose level of 20 mg of free gossypol per day for two months resulted in infertility in 99.98% of the 4,000 men in the study (Guan-Zhen *et al.* 1986).

Infertility resulting from gossypol has also been found to be reversible as early as six weeks after the removal of gossypol (Sotelo *et al.,* 1982). The research area has been relatively active (Xue, 1985) and several researchers have attempted to determine if the results seen with men in China are applicable to other species.

Species variability in the susceptibility of males to antifertility effects of gossypol is evident in the literature. Male rabbits were reported to be insensitive to antifertility effects yet demonstrated other signs of gossypol intoxication (Chang *et al*., 1980).

Hahn *et al*. (1981) reported that,pregnancy rates in mice bred to gossypol-treated males indicated that the mouse maintains fertility for at least 5 to 8 week after gossypol exposure. Results for rats (Shepu *et al*., 1980; Hahn *et al*., 1981; Kalla *et al.,* 1982; Weinbauer *et al*., 1982; Wong *et al*., 1984) and hamsters (Hahn *et al*., 1981; Waller *et al*., 1981; Sakesena and Salmonsen, 1982) indicate that antifertility responses are both dose- and time-dependent.

The effects of gossypol on hormone levels in male humans, rats, hamsters, rabbits, and monkeys have been reviewed comprehensively by Qian and Wang (1984)and Hoffer

(1985)**.** Inconsistent findings between and within species in concentrations of serum FSH, LH and testosterone in response to gossypol render any conclusions speculative.

In several species of monkeys, gossypol acetic acid has also been shown to inhibit spermatogenesis at oral doses of 0.5 mg/kg per day 5 days a week in male bonnet monkeys (*Macaca radiata*) after 3 months of treatment (Kalla *et al.*, 1984), and 5 mg/kg body weight per day in cynomolgus monkeys (*Macaca fascicularis*) after 6 months of treatment (Shandilya *et al.*, 1982). At high doses (25 mg/kg per day), racemic gossypol acetic was lethal and also induced unilateral testicular atrophy in cynomolgus monkeys after 11–48 days of dosing (Heywood *et al.*, 1985, Heywood 1988).

The effects of gossypol on male reproduction include immature spermatozoa, detached sperm heads and a decrease in number and mobility of spermatozoa (Ruttle, 1989). Some of the causes for these problems have been attributed to a degeneration of testicular tissue (Chase *et al*., l990), a decrease in the number of sperm reaching maturation and an increase in the percent abnormal sperm. It was observed that it takes approximately six weeks after gossypol is removed from the diet for the semen to return to normal (Ruttle, 1989).

In a study, the testicular micrometry of Teddy male goats fed dietary gossypol indicated a degeneration of spermatogenic cell layers of the seminiferous tubules (Zahid *et al.*, 2002). In the bovine (Stahnke, 1986; Chase *et al*., 1989; Jimenez *et al.,*1989) and ovine (Arshami andRuttle, 1989) single ejaculates collected from postpubertalmales receiving gossypol-containing feedstuffsshowed no consistent anomalies in spermconcentration or motility or abnormalities evaluatedusing the light microscope.

Kramer *et al*. (1991) observed a decrease in testicular steriodogenesis and spermatogenesis in pubescent bulls and rams. In peripubertal bulls, gossypol increased spermatozoa abnormalities. The number of sperm with proximal droplets was significantly higher in gossypol-treated bulls, suggesting testicular degeneration. But these effects were reversible (Hassan *et al*., 2004). Chenoweth *et al.* (1993) also observed increased mid-piece abnormalities in young bulls fed cottonseed meal. Randel *et al.* (1992) observed extensive damage to the germinal epithelium in both rams and bulls fed diets containing gossypol. Guedes and Soto-Blanco (2010) studied the effect of cottonseed cake on sperm quality of rams. They fed the rams a diet containing 0.5 kg/head/day of cottonseed cake for 120 consecutive days. They concluded that cottonseed cake had no effect on semen quality.

However, El-Mokadem *et al.* (2012) observed that feeding cottonseed cake at 48 % of the diet to rams causes a reduction in ejaculate volume, sperm output, serum testosterone concentration and forward motility of the sperm. They also observed an increased total sperm abnormality. The effects were counteracted by administration of selenium to rams consuming dietary gossypol.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out at the Department of Theriogenology and Production, Ahmadu Bello University Zaria, situated in the Northern Guinea Savannah, and lying between latitudes 11º 1573' N and between longitude 7º 64989' E at an elevation of 646 m above sea level. The mean annual rainfall in the area is 1100 mm lasting from May to October (816 mm/month). Mean daily temperatures during the wet season are 25^{o}C and mean relative humidity of 72%. The dry season lasts from November to April, the mean daily temperature ranges from 14 to 36^{o}C and the relative humidity of 20-30% (www.world66.com).

3.2 Experimental Animals and Management

Fifteen (15) apparently healthy Yankasa rams with clinically normal genitalia, aged 18-24 months (aged using dentition) and weighing 20 ± 3 kg were purchased from a livestock market in Makarfi Local Government Area and randomly divided into 3 groups of 5 rams each. Each ram was housed in separate sheltered pens with a floor space of 1.0 m^2 /ram. Feeding and watering troughs were provided for each pen. They were acclimatized for two weeks during which they were screened for haemoparasites and helminthes and treated appropriately.

3.3 Experimental Design

The 15 rams were randomly divided into 3 groups of 5 animals each. Treatments were allocated at random using the table of random numbers (Steel and Torrie, 1980). The first group, the animals were given a diet containing 48 % of whole cottonseed. In the second group,animals were given a diet containing 48 % of cottonseed cake and the third group served as a control (and were given a diet that contained no free gossypol, with groundnut cake as a source of protein). Diet composition is presented in Table 3.1. Treatment period extended for 100 days to represent the duration of 2 spermatogenic cycles in rams (49 days) as reported by Pineda (2003). The chemical composition of the diet determined using proximate analysis is presented in Table 3.2.

Table 3.1: Composition of Experimental Diets

Item	Group A (WCS)	Group B (CSC)	Group C (Control)
Groundnur cake (%)	-	-	40
Whole cottonseed (%)	48	-	-
Cottonseed cake (%)	-	48	-
Wheat bran (%)	50	50	58
Limestone + Salt (%)	2	2	2

WCS- Whole Cottonseed; CSC- Cottonseed Cake

Table 3.2: Chemical Composition of Experimental Diets

Item	**A (WCS)**	**B(CSC)**	**C(Control)**
DM, %	94.15	94.94	93.92
CP, %	21.08	22.75	15.78
EE, %	27.97	16.13	21.89
CF, %	9.55	12.20	5.80
NFE, %	39.10	49.89	50.43
ASH, %	6.00	6.20	5.82

DM= Dry matter, CP= Crude Protein, EE= Ether extract, CF= Crude fibre, NFE= Nitrogen free extract; WCS-Whole Cottonseed, CSC-Cottonseed cake

The rams were fed with the concentrate supplement at the rate of 3 % of their body weight per head per day. The diets were offered to the rams at 0800h daily. During each feeding time, the rams were given the concentrate ration and were allowed to finish it. Subsequently, the roughage ration was offered but in a separate feeding trough in the pens. Fresh water was provided to the rams *ad libitum* and changed every morning.

3.4 Scrotal Circumference and Live weight Measurements

Scrotal circumference in centimeters (cm) of each ram was measured and recorded weekly using a flexible measuring tape at the widest scrotal diameter by applying pressure with a hand above the head of the epididymis, thereby gently forcing the testes into the scrotum. Then the flexible measuring tape was placed at the widest scrotal diameter and the reading was taken.

Similarly, the body weight in kilogrammes (Kg) of ram was measured and recorded weekly using a clinical weigh balance (Tesco® Basic weigh scale, USA). This was both before and during the experiment period.

3.5 Testicular Ultrasonography

Ultrasonography was used to evaluate the testicles of the rams to determine changes from the normal homogeneous testicular parenchyma. Ultrasound imaging was carried out on each ram monthly and as described by Ahmad *et al*. (1999) with slight modifications. A B-mode, portable scanner (Medison SA600V from Krusse Denmark) was used, fitted with a 3.5 MHz linear-array transcutaneous transducer. The area

examined was shaved and ultrasound coupling gel was applied. Both the testes and the epididymides were imaged in the transverse and the longitudinal planes.

3.6 Blood Sample Collection and Evaluation

Five (5) ml of blood were collected via jugular veno-puncture using a 21 G, 1 1/2 inch hypodermic needle for haematology. The blood was collected into ethylenediamine tetra acetic acid (EDTA) container for hematology. This was done on a weekly basis. Whole blood samples were analyzed for packed cell volume (PCV), haemoglobin concentration, total protein, lymphocytes, eosinophils, basophils, monocytes and neutrophils counts according to the methods described by Coles (1986).

3.7 Semen Collection

Prior to the commencement of the experiment, semen samples were collected and evaluated weekly for two weeks to establish a baseline data. During the experimental period, semen samples were collected using electro-ejaculator as described by Jibril *et al.* (2011), from rams in each group and evaluated. Semen samples were collected weekly for a period of 14 weeks from each ram in the morning between 09.00 and 11.00 am using a portable battery powered electro-ejaculator (Lane Manufacturing Inc. No. 72707C). The rams were adequately restrained and the prepuce washed and dried. The bipolar rectal probe of the electro-ejaculator was lubricated using petroleum jelly and inserted into the rectum and switched on. This resulted in erection and subsequently ejaculation. The first ejaculate was collected in a calibrated tube. The time

taken from onset of stimulation to first ejaculation, volume, consistency and colour of the ejaculate was noted. The ejaculated semen was placed in a flask with warm water at 37ºC. This procedure was repeated for rams in each group.

3.8 Semen Evaluation

Semen samples collected were evaluated as described by Zemjanis (1970). This included the visual or gross evaluation of the ejaculate soon after collection for volume and colour as well as microscopically for motility, concentration, live-dead ratio and morphology.

Three different colour categories of creamy, milky and watery were used for scoring semen as described by Zemjanis (1970).

Gross motility was determined by examining a drop of the semen on a pre-warmed glass slide under a light microscope at x 100 magnification.

The percentage dead sperm was determined by using the eosin-nigrosin vital staining technique. This technique was based on the principle that eosin-nigrosin penetrates and stains dead sperm cells, while live sperm cells repel the stain. A thin smear of the semen sample was made on clean grease free glass slide and stained with eosin-nigrosin stain. One hundred stained and unstained sperm cells were counted using a light microscope and percentage for each group was estimated.

Semen concentration was determined using the haemocytometer. With a micropipette, 25 µl of the semen sample was aspirated and diluted with 5 ml of 3 % NaCl inside a test tube. The outside of the pipette was wiped out to remove any adhering semen. A cover slip was placed on the haemocytometer and 10 µl of the diluted semen was

placed under the cover slip on each side of the haemocytometer. The haemocytometer was carefully placed in a pre-wetted chamber and the lid was closed and left for 5 minutes. It was the removed and placed on a microscope (× 40) and the sperm cells counted in five squares of onechamber. The semen concentration was calculated as follows:

Concentration (sperm cells/ml) = Average count per square × DF × 10^4

DF= Dilution Factor

Spermatozoa abnormalities were determined by making a thin smear of the semen sample on clean grease free glass slide and fixed with buffered formol saline. One hundred sperm cells were counted per slide using light microscopy at × 40 magnification.

3.9 Histological Examination of the Testes

At the termination of the experiment, two rams per group were slaughtered and the testes collected. Histological examination of the testis was done as described by Zahid *et al.* (2002). The removed testicles were dissected and tissue samples measuring approximately 10×10×5 mm in size were taken from the proximal, middle and distal portion of the half of the testis and placed in 10 % formalin for four days (for fixation). After fixation, the tissues were dehydrated, infiltrated with liquid paraffin and embedded in paraffin blocks and sectioned at 5 microns thickness. Each section was stained with hematoxylin and eosin (H&E) using standard staining procedures according to Luna (1968). Each slide was examined under light microscopy.

3.10 Determination of Gonadal Sperm/spermatid and Epididymal Sperm Reserves

This was done using the method of Rekwot *et al.* (1994)with slight modifications.

The weight and volume of each testis was determined and recorded. The tunica albuginea was carefully removed. Each testis was homogenized in 35 ml of saline with antibiotic and blended for about two minutes. The homogenate volume, after rinsing the blender container with 35 ml of saline and adding the effluent, was measured. About 5 ml of the homogenate was transferred to a conical flask and further dilution was made with 20 ml of saline and the homogenate was stored at 5^{0} C for 24 hours to allow sperm cells to ooze out of the tissues. Finally, the testicular sperm/spermatid concentration was determined with a haemocytometer. The concentration of spermatozoa was determined using the erythrocyte counting chamber of a haemocytometer that was crossed with microscopic grids containing small squares.

For epididymal sperm reserve, the epididymis was carefully separated from the testis using a scalpel blade. The lengths and weights of the caput, corpus and cauda portions were determined. These parts were minced separately in 20 ml of saline using a sharp scissors. The homogenate was stored at 5^{0}C for 24 hours. It was then filtered through gauze and the volume of the filtrate measured. One (1) ml of the filtrate was diluted with 2 ml of saline and the concentration of spermatozoa was determined with a haemocytometer. Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares according to the method of Rekwot *et al.* (1994).

3.11 Data Analysis

Data collected were expressed as means (and standard errors of mean, SEM) and were analyzed using the software GraphPad Prism 5.0 (GraphPad Software Inc. U.S.A., 2007). Significance of differences between treatments means were determined at $P \leq 0.05$ with Tukey-Kramer multiple comparison test of repeated measure analysis of variance (ANOVA).

CHAPTER FOUR

4.0 RESULTS

4.1 Live weight Changes and Scrotal Circumferences

The mean (± SEM) of body weights (Kg) and scrotal circumferences (cm) of the Yankasa rams in experimental groups A (fed whole cottonseed diet) and B (fed cottonseed cake) for 14 weeks are presented on Tables 4.1 and 4.2 respectively.

The mean body weights of the rams increased through out the feeding period and did not differ significantly (P = 0.4820) between the experimental groups A and B and the control group as shown in Table 4.1.

Table 4.3 shows the mean scrotal circumference of Yankasa rams in experimental groups A and B and the control. The mean scrotal circumference increased for the first 6 weeks and started decreasing at 8 weeks. The mean scrotal circumference of rams in group A and B were significantly (P= 0.0001) lower than that of rams in the control group from 9 to 14 weeks of the experiment (21.2 ± 0.50 cm at week 9 to 18.8 ± 0.97 cm at week 14 for group A and B vs 25.6 ± 0.51 cm at week 9 to 26.8 ± 0.58 cm at week 14 for control).

Table 4.1: Mean (± SEM) values of live weight (Kg) of Yankasa rams fed whole cottonseed, cottonseed cakeand control diets

	Body Weight (kg)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	19.60 ± 0.24	19.40 ± 0.51	18.80 ± 0.37
2	20.40 ± 0.51	20.20 ± 0.58	19.40 ± 0.60
3	20.80 ± 0.49	20.40 ± 0.51	19.40 ± 0.60
4	21.10 ± 0.19	20.50 ± 0.22	19.70 ± 0.44
5	21.20 ± 0.20	21.00 ± 0.32	20.20 ± 0.37
6	21.50 ± 0.22	21.40 ± 0.24	21.20 ± 0.37
7	22.00 ± 0.16	22.10 ± 0.24	21.60 ± 0.62
8	22.60 ± 0.24	22.60 ± 0.24	22.20 ± 0.58
9	23.00 ± 0.32	23.40 ± 0.40	22.40 ± 0.51
10	23.60 ± 0.51	24.60 ± 0.51	22.60 ± 0.24
11	25.40 ± 0.68	25.60 ± 0.51	23.80 ± 0.20
12	27.00 ± 0.63	26.40 ± 0.40	24.80 ± 0.20
13	29.00 ± 0.45	27.60 ± 0.51	26.20 ± 0.37
14	29.60 ± 0.24	27.80 ± 0.37	26.80 ± 0.37

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P-value = 0.4820

Table 4.2: Mean (± SEM) values of scrotal circumference (cm) of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

	Scrotal Circumference (cm)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	22 ± 0.51	21.6 ± 0.81	21.2 ± 0.73
2	22.2 ± 0.58	21.6 ± 0.81	21.2 ± 0.73
3	22.4 ± 0.51	21.8 0.73	21.8 ± 0.58
4	23.4 ± 0.51	23.00 ± 0.63	21.8 ± 0.58
5	25.6 ± 0.24	24.2 ± 0.58	23.8 ± 0.20
6	26.6 ± 0.51	25.4 ± 0.24	25.8 ± 0.37
7	25.8 ± 0.58	25.4 ± 0.24	26.0 ± 0.32
8	24.2 ± 0.66	24.8 ± 0.20	26.2 ± 0.37
9	22.6 ± 0.60^{a}	22.2 ± 0.37^{a}	25.6 ± 0.51^{b}
10	21.2 ± 0.58^{a}	21.4 ± 0.40^{a}	25.6 ± 0.51^{b}
11	19.6 ± 0.75^{a}	20.4 ± 0.40^{a}	25.6 ± 0.51^{b}
12	19.2 ± 0.80^{a}	19.6 ± 0.51^{a}	25.6 ± 0.51^{b}
13	18.8 ± 0.97^{a}	18.6 ± 0.75^{a}	26.0 ± 0.45^{b}
14	18.8 ± 0.97^{a}	18.6 ± 0.75^{a}	26.8 ± 0.58^{b}

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P-value = 0.0001, a, b: Means with different superscripts on the same row are significantly different.

4.3 Haematological Parameters

The mean (± standard errors of means, SEM) of packed cell volume (PCV), haemoglobin concentration (Hb), total protiens (TP), white blood cells count (WBC), neutrophils and lymphocytes are presented in Tables 4.4 to 4.9.

Generally, the PCV increased from the onset of the experiment to week 7 (in group A) and week 5 (in group B). The PCV of rams in the experimental group A were significantly lower (P = 0.0001) than those of the control group until week 8 (24.6 ± 0.86 vs 30.75 ± 1.44 %) to week 14 (16.8 ± 1.72 vs 32.75 ± 0.95 %) as shown on Table 4.3. Similarly, the PCV of the rams in the experimental group B were significantly lower (p = 0.0001) than those of the control group from week 8 (23.6 ± 1.72 vs 30.75 ± 0.95 %)) to week 14 (14.8 ± 3.08 vs 32.75 ± 0.95 %). The lowest mean PCV was observed at week 14 and were 16.80 ± 1.72 % and 14.80 ± 3.08 % for groups A and B, respectively. There was no significantly difference between the mean PCV values of groups A and B.

Mean (± SEM) haemoglobin (Hb) concentrations (g/dL) followed a similar trend with that of the mean PCV. TheHb concentrations of group A and B were significantly lower (P = 0.00001) than those of the control group from week 8 to 14 (Table 4.4). The lowest mean haemoglobin concentrations were observed at week 14 and were 5.60 ± 0.69 g/dL and 4.93 ± 0.27 g/dL for groups A and B, respectively.

The mean values for total protein, WBC count, neutrophils and lymphocytes for the experimental group rams did not differ significantly from the control group rams (see Table 4.5-4.8).

Table 4.3: Mean (± SEM) values of packed cell volume (PCV) of Yankasa rams fed whole cottonseed and cottonseed cakeand control diets.

	PCV (%)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	27.60 ± 1.29	26.20 ± 0.73	29.25 ± 0.48
2	29.00 ± 1.95	27.80 ± 1.39	28.75 ± 0.75
3	28.40 ± 1.63	27.60 ± 0.75	29.75 ± 1.11
4	29.00 ± 1.70	26.80 ± 0.80	28.25 ± 1.25
5	27.60 ± 1.21	27.00 ± 0.55	29.50 ± 1.19
6	28.00 ± 0.84	25.80 ± 0.37	30.75 ± 0.75
7	27.00 ± 0.55	25.20 ± 1.59	30.50 ± 0.96
8	24.60 ± 0.81^{a}	23.60 ± 1.72^{a}	30.75 ± 1.44^{b}
9	22.00 ± 0.84^{a}	22.40 ± 2.77^{a}	29.00 ± 0.58^{b}
10	22.60 ± 1.47^{a}	21.20 ± 2.99^{a}	30.25 ± 0.95^{b}
11	19.60 ± 0.75^{a}	19.80 ± 2.69^{a}	30.25 ± 1.65^{b}
12	18.80 ± 1.02^{a}	19.20 ± 2.92^{a}	32.50 ± 2.25^{b}
13	17.60 ± 1.36^{a}	15.20 ± 2.67^{a}	35.25 ± 1.65^{b}
14	16.80 ± 1.72^{a}	14.80 ± 3.08^{a}	32.75 ± 0.95^{b}

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P = 0.0001; a,b Means with different superscript on the same row are statistically significant.

Table 4.4: Mean (± SEM) values of haemoglobin concentration (g/dL) of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

	Haemoglobin (g/dL)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	9.20 ± 0.43	8.73 ± 0.33	9.75 ± 0.29
2	9.67 ± 0.24	9.27 ± 0.23	9.58 ± 0.18
3	9.47 ± 0.47	9.20 ± 0.41	9..92 ± 0.17
4	9.67 ± 0.32	9.32±0.27	9.42 ± 0.29
5	9.20 ± 0.19	8.93 ± 0.30	9.83 ± 0.24
6	9.33 ± 0.17	9.00 ± 0.42	10.25 ± 0.18
7	9.00 ± 0.27	8.60 ± 0.21	10.17 ± 0.19
8	8.20 ± 0.24[a]	8.40 ± 0.22[a]	10.25 ± 0.36[b]
9	7.33 ± 0.29[a]	7.87 ± 0.40[a]	9.67 ± 0.29[b]
10	7.53 ± 0.39[a]	7.47 ± 0.42[a]	10.08 ± 0.27[b]
11	6.53 ± 0.37[a]	7.07 ± 0.28[a]	10.08 ± 0.33[b]
12	6.20 ± 0.46[a]	6.60 ± 0.20[a]	10.83 ± 2.20[b]
13	5.87 ± 0.40[a]	6.40 ± 0.34[a]	11.75 ± 0.39[b]
14	5.60 ± 0.69[a]	4.93 ± 0.27[a]	10.92 ± 0.36[b]

WCS-Whole cottonseed, CSC- Cottonseed Cake, P = 0.0001; [a,b] Means with different superscripts on the same row are significantly different.

Table 4.5: Mean (± SEM) values of total protein concentration (g/dL) of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

	Total Protein (g/dL)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	6.50±0.21	6.54±0.04	6.62±0.12
2	6.52±0.19	6.62±0.09	6.64±0.15
3	6.46±0.20	6.68±0.14	6.72±0.13
4	6.48±0.21	6.78±0.15	6.76±0.12
5	6.64±0.11	6.76±0.16	6.90±0.11
6	6.72±0.10	6.84±0.14	6.86±0.18
7	6.62±0.16	7.00±0.14	6.84±0.12
8	6.58±0.20	6.92±0.10	6.82±0.14
9	6.66±0.22	7.04±0.07	6.98±0.12
10	6.68±0.22	7.08±0.10	6.92±0.12
11	6.80±0.17	7.06±0.09	6.98±0.07
12	6.86±0.14	7.04±0.04	6.92±0.12
13	6.74±0.17	7.12±0.05	6.86±0.07
14	6.72±0.12	7.18±0.10	6.88±0.10

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P = 0.06

Table 4.6: Mean (± SEM) values of white blood cell count ($\times 10^9$/L) of Yankasa rams fed whole cottonseed, cottonseed cake and control diets**.**

	WBC ($\times 10^9$/L)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	10.50 ± 0.50	9.86 ± 0.10	9.96 ± 0.04
2	10.24 ± 0.32	9.56 ± 0.23	10.16 ± 0.35
3	10.20 ± 0.36	9.68 ± 0.24	9.80 ± 0.37
4	9.92 ± 0.17	9.76 ± 0.32	9.96 ± 0.32
5	9.88 ± 0.23	9.44 ± 0.27	9.86 ± 0.37
6	9.82 ± 0.37	9.84 ± 0.38	10.16 ± 0.47
7	10.06 ± 0.70	10.00 ± 0.06	9.70 ± 0.30
8	9.86 ± 0.24	9.44 ± 0.23	10.00 ± 0.00
9	9.76 ± 0.25	10.26 ± 0.19	9.60 ± 0.24
10	10.62±0.40	10.00±0.16	9.32±0.21
11	10.60±0.24	9.80±0.37	9.76±0.19
12	10.04±0.16	9.96±0.32	9.80±0.37
13	9.86±0.56	9.94±0.32	10.00±0.45
14	9.66±0.19	10.20±0.37	10.20±0.37

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P = 0.06

Table 4.7: Mean (± SEM) values of neutrophils (%) of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

	Neutrophils (%)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	48.80±0.80	41.80±2.35	45.08±0.28
2	39.20±0.58	40.20±1.56	52.10±0.22
3	40.80±0.49	41.00±1.84	42.34±0.68
4	41.00±1.05	41.80±1.39	49.01±1.02
5	41.20±1.20	41.80±1.59	44.08±0.93
6	42.40±0.93	44.00±1.51	50.05±0.43
7	40.80±0.86	41.40±1.72	48.24±0.15
8	41.20±1.16	42.40±1.72	42.00±1.74
9	41.20±1.39	44.00±1.05	48.40±1.75
10	41.60±0.68	43.00±1.34	43.21±1.21
11	42.00±1.14	43.60±0.51	48.23±1.02
12	41.80±1.36	43.60±0.51	44.50±1.46
13	43.00±1.26	41.80±1.36	42.22±0.22
14	42.40±1.60	45.20±0.86	44.33±0.01

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P = 0.07

Table 4.8: Mean (± SEM) values of lymphocytes (%) of Yankasa rams fedwhole cottonseed, cottonseed cake and control diets.

	Lymphocytes (%)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	51.20 ± 1.17	58.20 ± 1.20	54.92 ± 1.12
2	60.08 ± 1.21	59.80 ± 1.11	47.90 ± 1.83
3	58.40 ± 1.17	59.00 ± 1.30	57.60 ± 1.77
4	59.20 ± 1.89	58.20 ± 0.92	57.00 ± 1.88
5	58.60 ± 1.70	58.20 ± 1.03	50.00 ± 0.60
6	57.20 ± 1.96	56.00 ± 1.07	54.00 ± 1.53
7	59.20 ± 1.36	58.60 ± 0.75	54.99 ± 1.25
8	58.80 ± 2.48	57.60 ± 1.02	51.00 ± 2.52
9	58.80 ± 2.21	56.00 ± 1.69	58.00 ± 0.97
10	58.00 ± 2.63	57.00 ± 1.60	51.60 ± 1.59
11	58.00 ± 2.06	56.00 ± 0.20	56.70 ± 0.49
12	57.00 ± 1.71	56.40 ± 1.80	51.70 ± 1.07
13	57.20 ± 1.29	58.20 ± 1.56	55.50 ± 1.69
14	57.40 ± 1.77	54.80 ± 1.07	55.70 ± 2.19

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P= 0.6112

4.4 Semen Characteristics

The mean values (± Standard Error of Mean, SEM) of ejaculate volume, gross motility, concentration, dead sperm percentage and sperm morphology are presented in Tables 4.9-4.14. The lowest and highest mean ejaculate volume of the experimental rams was 0.26 ± 0.02 and 1.0± 0.24 ml, respectively. There was no statistically significant difference ($p > 0.05$) in the mean ejaculate volume between the group C (fed control diet) and groups A and B fed whole cottonseed (WCS) and cottonseed cake (CSC), respectively, as shown in table 4.9.

Table 4.9: Means (± SEM) values of ejaculate volume of Yankasa rams fedwhole cottonseed, cottonseed cake and control diets.

Week	Ejaculate Volume(ml)		
	A(WCS) n = 5	B(CSC) n = 5	C(Control) n = 5
1	0.40 ± 0.10	0.64 ± 0.24	0.72 ± 0.24
2	0.50 ± 0.14	0.66 ± 0.19	0.48 ± 0.04
3	0.48 ± 0.09	0.62 ± 0.10	0.72 ± 0.22
4	0.88 ± 0.12	0.86 ± 0.25	0.68 ± 0.13
5	0.66 ± 0.09	0.58 ± 0.18	0.86 ± 0.12
6	0.52 ± 0.13	0.52 ± 0.12	0.62 ± 0.10
7	0.44 ± 0.04	0.42 ± 0.07	0.40 ± 0.08
8	0.44 ± 0.04	0.48 ± 0.05	0.96 ± 0.27
9	0.34 ± 0.04	0.32 ± 0.02	1.12 ± 0.24
10	0.26 ± 0.02	0.50 ± 0.03	0.86 ± 0.06
11	0.30 ± 0.03	0.38 ± 0.06	0.78 ± 0.18
12	0.38 ± 0.06	0.94 ± 0.52	0.72 ±0.10
13	0.32 ± 0.05	0.36 ± 0.05	0.82 ± 0.13
14	0.30 ± 0.03	0.34 ± 0.06	0.80 ± 0.13

WCS- Whole cottonseed, CSC- Cottonseed Cake, P = 0.3572

The mean semen gross motility increased until weeks 6 and 5 in groups A and B, respectively. The mean semen gross motility decreased significantly in groups A and B ($p < 0.05$) compared to group C from week 8 to 14. Similarly, there was a significant ($p < 0.05$) decrease in semen gross motility of the rams in group A compared to those of group B. The lowest gross motility for groups A and B were observed at week 14 and were 16.50 ± 4.00 % and 37.50 ± 4.30 %, respectively. The highest mean percentage gross motility was observed in the control group at week 14 which was 90.9 ± 6.37 % (Table 4.10).

Table 4.11 shows the mean semen concentration in millions/ml of the three experimental groups. There was no statistically significant difference ($p > 0.05$) in the semen concentration between the control group (group C) and groups A and B. However, the lowest mean semen concentration was observed in group B (161.2 ± 30.02) at week 14 of the experiment while the highest was observed in the control group at week 14 (475.40 ± 28.05 × 10^6/ml).

Table 4.12 shows the mean values of percentage live of the spermatozoa of Yankasa rams fed WCS and CSC for 14 weeks. The mean values of percentage live spermatozoa decreased in groups A and B from weeks 8 and 6, respectively. There was a significant decrease in percentage live spermatozoa ingroups A and B compared to the control ($p < 0.05$) at weeks 10 and 11 of the experiment while that of group B differed from control ($p < 0.05$) at weeks 10 to 14 of the experiment. There was no statistically significant difference between groups A and B ($p > 0.05$). The lowest mean viability was observed in group B at week 13 and 14 with mean percentage live spermatozoa of 66.4 ± 4.99 % and 67.0 ± 5.76 %, respectively.

The mean total sperm abnormalities increased in the treatment groups A and B and were significantly higher ($p < 0.05$) than that of the control group from week 8 to week 14 of the experiment as shown in Table 4.13. The mean total sperm abnormality of group B was significantly higher ($p < 0.05$) than that of group A at week 12 to 14 of the experiment. The highest mean sperm abnormality in groups A and B was 37.6 ± 1.50 % observed at week 14 and 46.8 ± 1.62 % observed at week 13, respectively. The mean values of detached head (DH) for group Bincreased significantly from the those of the control group at week 8 ($p < 0.05$) and week 9 ($p < 0.05$) while there was no statistically significant difference between the mean DH values of group A and that of the B as well as the control group. The mean mid-piece defects (MPD) for group B increased significantly compared to the control from week 9 to week 14 ($p < 0.05$) while that of group A was not significantly different from the control group. The mean CT for groups A and B was increased significantly compared that of the control group ($p < 0.05$). However, there was no statistically significant difference in the mean values of BT and FT both between treatment groups and between treat groups and the control group.

Table 4.10: Mean (± SEM) values of semen gross motility of Yankasa rams fedwhole cottonseed, cottonseed cake and control diets.

	Gross motility (%)		
Week	A (WCS) n = 5	B(CSC) n = 5	C(CONTROL) n = 5
2	67.00 ± 8.30	84.80 ± 6.40	82.00 ± 6.37
3	73.00 ± 7.00	78.80 ± 7.34	80.00 ± 6.52
4	74.80 ± 9.08	90.60 ± 4.39	86.00 ± 3.32
5	92.40 ± 4.69	86.00 ± 4.30	77.00 ± 3.39
6	89.80 ± 2.55	72.00 ± 6.44	85.00 ± 4.64
7	57.00 ± 7.00^{a}	50.00 ± 6.12^{a}	80.80 ± 2.40^{b}
8	34.00 ± 9.27^{a}	41.00 ± 5.34^{a}	75.00 ± 3.39^{b}
9	43.00 ± 3.74^{a}	46.00 ± 5.34^{a}	90.60 ± 4.39^{b}
10	46.00 ± 11.77^{a}	35.00 ± 7.75^{a}	83.80 ± 6.08^{b}
11	41.00 ± 6.96^{a}	65.00 ± 8.51^{a}	88.60 ± 4.62^{b}
12	36.00 ± 8.57^{a}	54.00 ± 9.14^{a}	85.80 ± 4.14^{b}
13	17.00 ± 5.83^{a}	38.00 ± 5.61^{b}	91.80 ± 3.28^{c}
14	16.00 ± 4.00^{a}	37.00 ± 4.90^{b}	90.00 ± 2.73^{c}

WCS- Whole Cottonseed, CSC- Cottonseed Cake; $P \leq 0.0001$; $^{a, b, c}$: Means on the same row with different superscripts are significantly different.

Table 4.11: Mean (± SEM) values of semen concentration (× 10^6/ml) of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

	Semen concentration (×10^6/ml)		
Week	A(WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	146.60 ± 47.15	195.20 ± 72.79	170.60 ± 21.50
2	335.80 ± 42.94	334.80 ± 30.21	287.00 ± 67.55
3	297.20 ± 40.72	288.40 ± 20.85	268.40 ± 62.48
4	311.40 ± 44.46	179.80 ± 29.56	423.60 ± 39.33
5	222.20 ± 45.82	331.60 ± 42.58	324.40 ± 41.10
6	310.00 ± 48.88	301.60 ± 61.37	282.60 ± 22.21
7	223.60 ± 54.80	273.20 ± 75.22	313.60 ± 27.27
8	350.80 ± 43.89	455.40 ± 33.44	266.00 ± 18.63
9	179.20 ± 14.32	230.20 ± 59.31	335.40 ± 54.51
10	272.00 ± 10.33	304.20 ± 69.20	332.45 ± 30.25
11	335.60 ± 48.90	286.00 ± 87.83	386.80 ± 23.40
12	235.60 ± 70.10	118.00 ± 30.02	351.40 ± 20.02
13	335.20 ± 13.07	153.40 ± 34.48	365.20 ± 37.14
14	160.80 ± 32.65	169.00 ± 45.85	475.40 ± 28.05

WCS- Whole Cottonseed, CSC- Cottonseed Cake; P > 0.05

Table 4.12: Mean (± SEM) values of percentage live spermatozoa of Yankasa rams fedwhole cottonseed, cottonseed cake and control diets.

	Live spermatozoa (%)		
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	86.0 ± 1.76	87.2 ± 2.52	89.0 ± 1.87
2	85.0 ± 2.24	83.6 ± 2.23	86.6 ± 2.93
3	89.6 ± 1.63	84.8 ± 2.75	84.8 ± 1.96
4	84.2 ± 2.94	82.0 ± 1.22	82.6 ± 1.66
5	83.0 ± 1.22	81.4 ± 2.23	83.8 ± 2.27
6	84.0 ± 2.92	80.8 ± 4.56	82.4 ± 3.43
7	84.2 ± 3.56	77.4 ± 0.67	82.0 ± 2.35
8	82.2 ± 2.06	72.2 ± 4.22	85.0 ± 2.10
9	76.6 ± 0.92	73.6 ± 4.76	82.8 ± 2.08
10	71.4 ± 2.56^{a}	70.2 ± 5.66^{a}	84.6 ± 2.04^{b}
11	70.4 ± 2.94^{a}	74.4 ± 6.04^{a}	84.4 ± 2.23^{b}
12	73.4 ± 1.44^{a}	70.8 ± 4.80^{a}	82.4 ± 2.48
13	72.6 ± 2.18^{a}	66.4 ± 4.99^{a}	84.2 ± 1.93^{b}
14	73.6 ± 2.16^{a}	67.0 ± 5.76^{a}	83.2 ± 2.06^{b}

WCS- Whole Cottonseed, CSC- Cottonseed Cake; $^{a, b, c}$: Means with different superscripts are significantly different ($P<0.05$).

Table 4.13: Mean (± SEM) valuesof percent sperm morphological abnormality of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

		Abnormal spermatozoa (%)	
Week	A (WCS) n = 5	B (CSC) n = 5	C (Control) n = 5
1	8.2 ± 0.52	10.0 ± 0.66	15.8 ± 0.22
2	12.8 ±0.57	13.0 ± 0.86	12.4 ± 0.33
3	14.4 ±0.92	13.4 ± 0.24	9.6 ± 2.22
4	14.8 ±1.06	15.6 ± 0.92	14.9 ± 1.88
5	18.8 ±1.32	16.4 ± 0.80	12.4 ± 2.45
6	22.8 ±1.18	19.8 ± 0.97	15.0 ± 0.32
7	27.8 ±1.31	26.0 ± 0.89	19.8 ± 1.55
8	29.4 ±2.61^{a}	37.4 ± 1.02^{a}	20.7 ± 2.65^{b}
9	29.1 ±1.73^{a}	31.4 ± 1.85^{a}	17.2 ± 0.25^{b}
10	33.4 ±2.15^{a}	32.0 ± 2.24^{a}	22.6 ± 1.50^{b}
11	31.4 ±1.89^{a}	39.3 ± 2.76^{a}	16.1 ± 0.98^{b}
12	35.8 ±1.45^{a}	40.2 ± 2.33^{b}	22.3 ± 0.26^{c}
13	37.3 ±1.31^{a}	46.8 ± 1.62^{b}	19.2 ± 1.32^{c}
14	37.6 ±1.50^{a}	43.7 ± 0.98^{b}	22.3 ± 2.88^{c}

WCS- whole cottonseed, CSC- Cottonseed cake; ^{a,b,c}: Means on the same row with different superscripts are significantly different. ($P < 0.0001$)

4.5 Gonadal and Epididymal Weight, Length Spermatid/Spermatozoa Reserves

There were no gross pathological changes observed on the testes of all the rams. Tables 4.14 and 4.15 show the gonadal and epididymal sperm/spermatids reserves of Yankasa rams (n = 3) in the experimental groups A and B as well as the control group. There was no statistically significant difference ($p > 0.05$) in the testicular weight, length and sperm reserves between the experimental groups A and B and the control group. The gonadal sperm reserves were not significantly different between the treatment groups A and B. Similarly, mean values for epididymal weight, length and sperm reserves were found to be lower in the experimental groups A and B compared to the control group, although not statistically significant ($P = 0.0857$).

Table 4.14: Mean (± SEM) values of testicular weight, length and spermatozoa reserves of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

	Groups					
	A (WCS) n =3		**B (CSC) n = 3**		**C (Control)n = 3**	
Para-meter	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**
Weight (g)	11.83± 0.88	11.90 ± 0.22	7.92± 0.39	7.98 ± 0.32	15.05 ± 0.23	15.18 ± 0.20
Length (cm)	7.25 ± 0.14	7.29 ± 0.17	5.26± 0.37	5.29 ± 0.38	7.40 ± 0.23	7.45 ± 0.23
GSR(× 10^6/g)	59.76±4.18^a	16.33 ±1.85^a	23.67±6.17^a	16.00± 2.64^a	110.33 ± 1.20^c	87.33 ± 3.84^c

WCS- Whole Cottonseed, CSC- Cottonseed Cake, GSR- Gonadal sperm reserves; p= 0.0433 a,b: Means on the same row with different superscripts are significantly different.

Table 4.15: Mean (± SEM) values of epididymal weight, length and spermatozoa reserves of Yankasa rams fed whole cottonseed, cottonseed cake and control diets.

Parameter	Portion	**Groups**		
		A (WCS) n = 3	B (CSC) n = 3	C (Control) n = 3
Weight (g)				
	Right Caput	3.05 ± 0.57	2.03 ± 0.99	8.4 ± 0.10
	Left Caput	4.16 ± 0.47	3.54 ± 0.66	6.34 ± 0.29
	Right Corpus	1.30 ± 0.44	1.11 ± 0.53	2.04 ± 0.25
	Left Corpus	1.27 ± 0.40	1.31 ± 0.52	1.16 ± 0.23
	Right Cauda	5.34 ± 0.56	4.07 ± 0.64	6.42 ± 0.22
	Left Cauda	5.14 ± 0.40	3.18 ± 0.91	6.90 ± 0.21
Length (cm)				
	Right Caput	4.17 ± 0.38	2.94 ± 0.81	5.73 ± 0.27
	Left Caput	4.43 ± 0.20	3.33 ± 0.60	5.82 ± 0.13
	Right Corpus	4.83 ± 0.20	4.45 ± 0.65	4.88 ± 0.66
	Left Corpus	4.83 ± 0.20	3.50 ± 0.80	4.00 ± 0.12
	Right Cauda	4.37 ± 0.32	3.37 ± 0.84	6.00 ± 0.58
	Left Cauda	4.37 ± 0.32	3.37 ± 0.84	6.00 ± 0.58
ESR ($\times 10^6$)				
	Right Caput	41.67 ± 4.41	14.33 ± 5.46	218.67 ± 13.86
	Left Caput	23.00 ± 4.73	23.33 ± 3.53	145.33 ± 5.46
	Right Corpus	23.33 ± 6.01	21.00 ± 0.58	185.00 ± 20.21
	Left Corpus	24.33 ± 8.09	22.67 ± 11.17	133.00 ± 4.36
	Right Cauda	248.33 ± 75.96	103.33 ± 6.77	733.67 ± 8.76
	Left Cauda	119.33 ± 9.24	95.33 ± 3.71	455.67 ± 43.53

WCS- Whole Cottonseed, CSC- Cottonseed Cake; ESR- Epididymal sperm reserve;p = 0.05

4.6 Testicular Histopathological Changes

The testicular histopathological changes in the experimental group A and B are shown in Plates I and II, respectively.Microscopic lesions observed in testicular photomicrographs of the rams' testis in group A were depletion of spermatogenic cell layers and arrest of spermatogenesis at the spermatocyte and spermatids levels and an exfoliation of the spermatogonia. There was vacuolation of the germ layers in the seminiferous tubules (Plate I). These changes are associated with testicular degeneration.

Similarly, microscopic lesions in testicular photomicrographs for rams in group B include depletion of spermatogenic cells layers in the seminiferous tubules which was more severe than those in group A (Plate II).

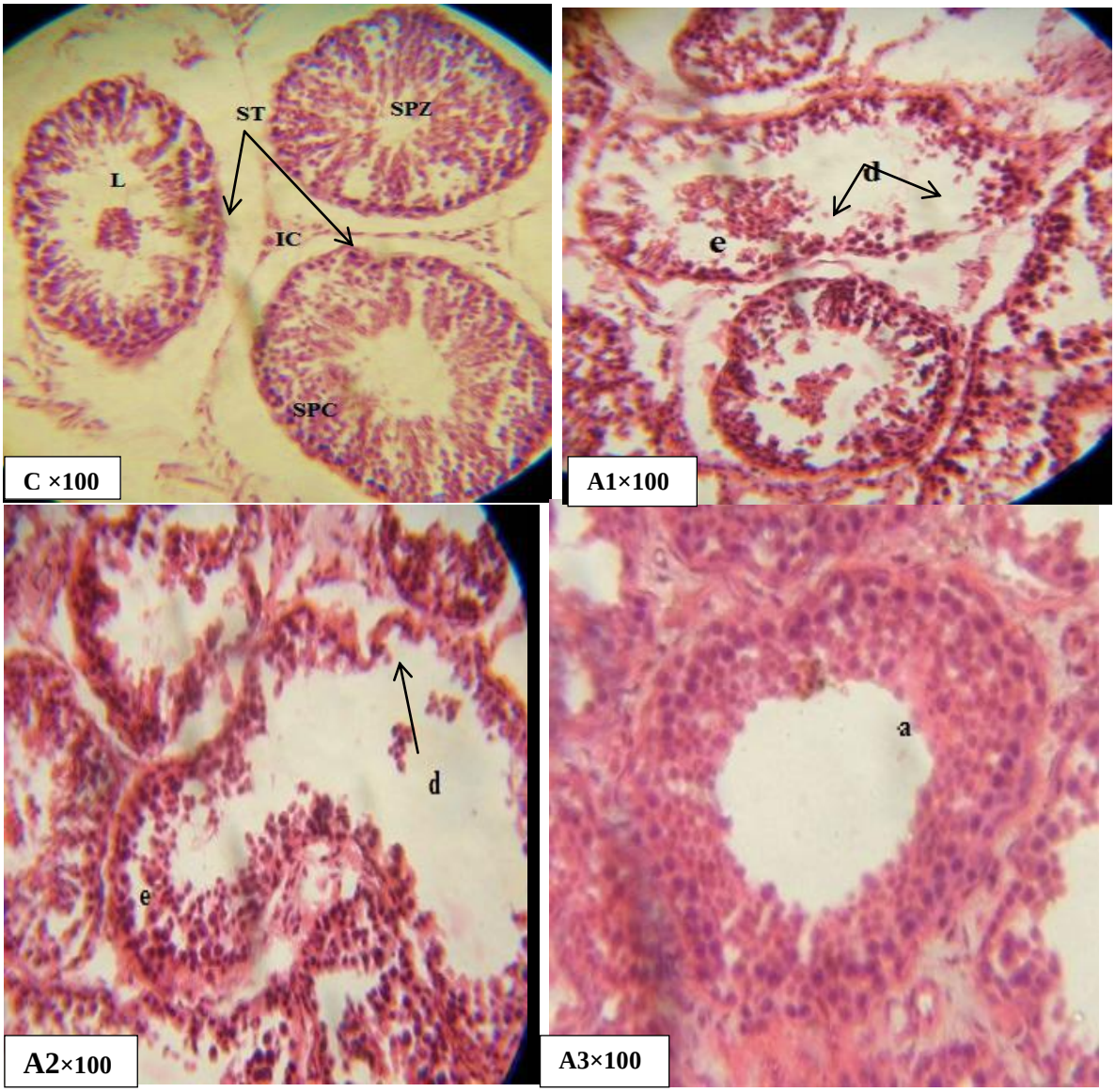

Plate I: Photomicrographs of the testis of the Yankasa rams. **C**. Normal histology of the testis of rams in the control (Group C). Note the seminiferous tubules (ST) with thick layer of spermatogenic cells (**SPC**), and the lumen (**L**) with spermatozoa (**SPZ**) and interstitial cells (**IC**). **A1 and A2.** Histopathologic changes in testis of rams in group A.(**d)** severe depletion of spermatogenic cell layers and (**e**) exfoliation of germ cell layers. **A3 (a)** shows arrest of spermatogenesis at spermatocytes (arrows in D) (H & E).

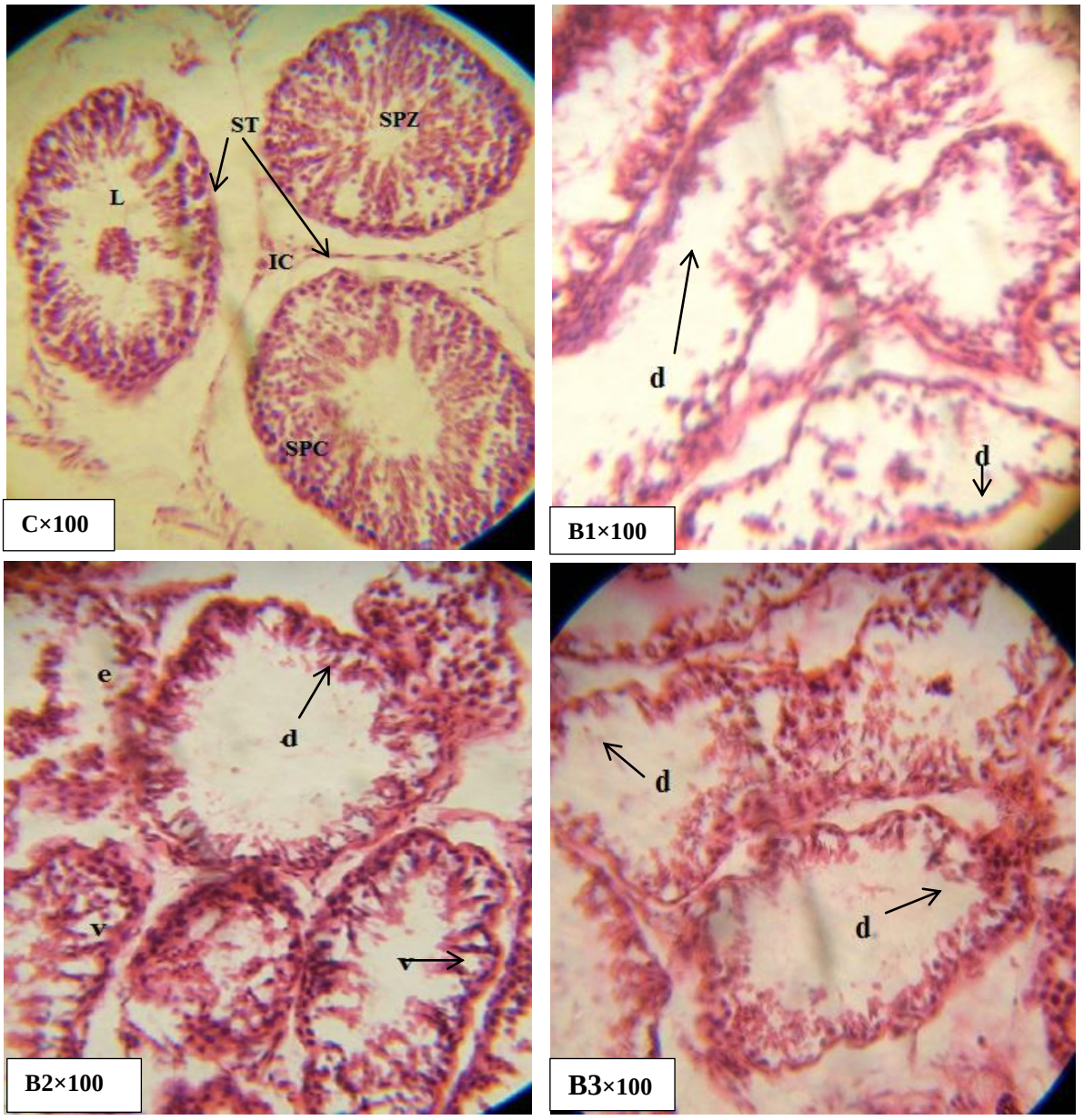

Plate I1: Photomicrographs of the testis of the Yankasa rams. **C**. Normal histology of the testis of rams in the control group (Group C). Note the seminiferous tubules (**ST**) with thick layer of spermatogenic cells (**SPC),** and the lumen (L) with spermatozoa (SPZ) and interstitial cells (IC). **B1 and B2**. Histopathologic changes in testis of rams in group B. (**d**) severe depletion of spermatogenic cell layers. **B3:** Severe depletion of spermatogenic cell layers (**d**), exfoliation of germ cell layers (**e**), vacuolation of germ cell layers (v) (H & E).

4.7 Testicular Ultrasonographic Changes

Testicular ultrasonograms for the rams in both experimental and control groups are presented in Plates III and IV. Testicular ultrasonograms in Plate III show the normal testicular architecture of the rams in group C (Control) with a hyperechoic mediastinum and echogenic testicular parenchyma. Plate IV shows the ultrasonograms of the rams in experimental groups A and B respectively. In both groups, there is a hyperechoic appearance of the testicular parenchyma indicating some level of testicular degeneration.

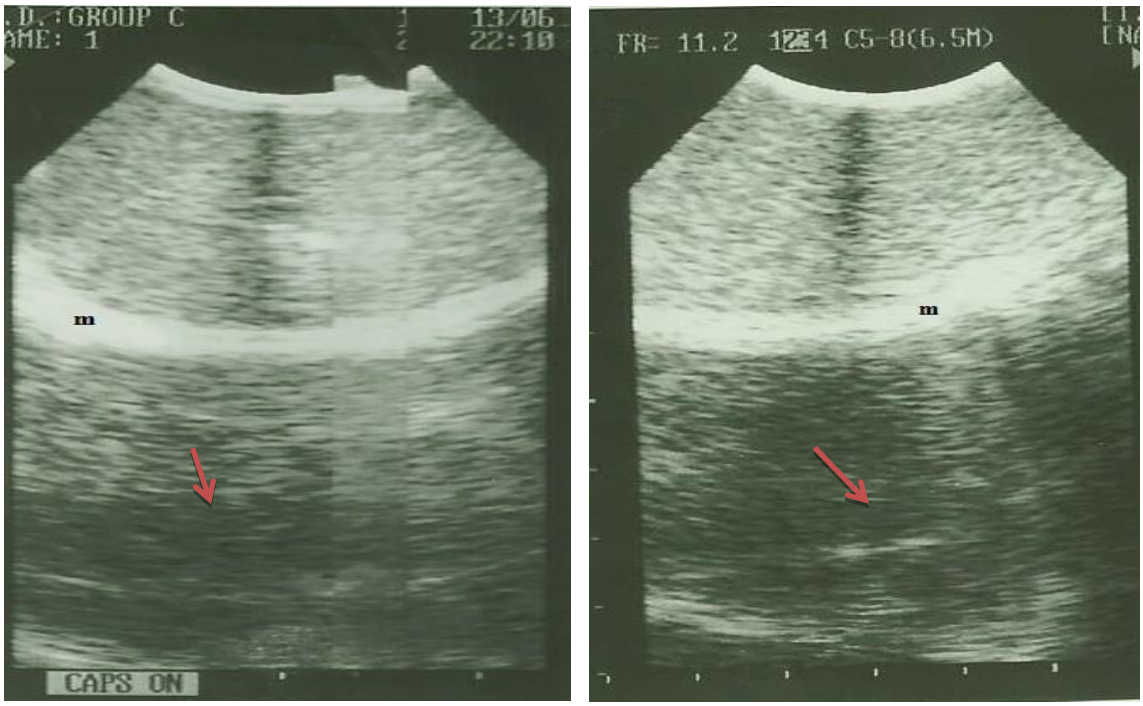

Plate III: Transverse ultrasonogramsof the testis of Yankasa rams in the control group. Note the hyperechoic mediastinum (m) and the echogenic appearance of the testicular parenchyma (arrows)

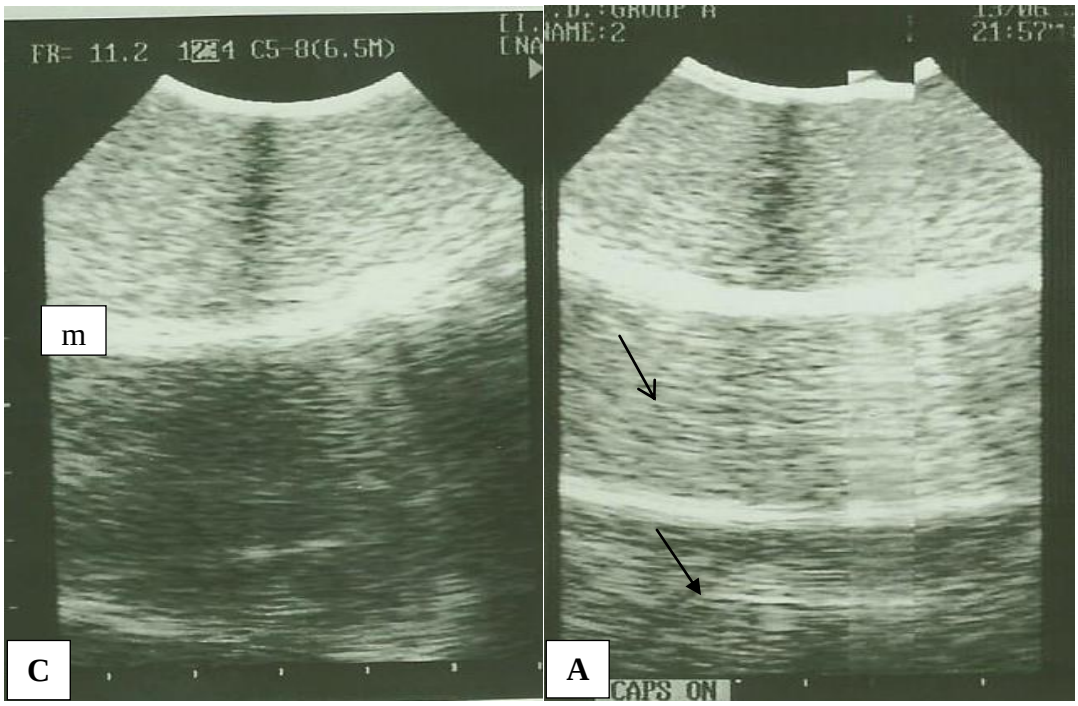

Plate IV: Transverse ultrasonogramsof the testis of Yankasa rams. **C**. Normal testis for rams in group C. Note the hyperechoic mediastinum (m) and the echogenic appearance of the testicular parenchyma. **A:** Testis of rams in group A showing loss of echogenicity and hyperechoic testicular parenchyma (see arrow in **A**).

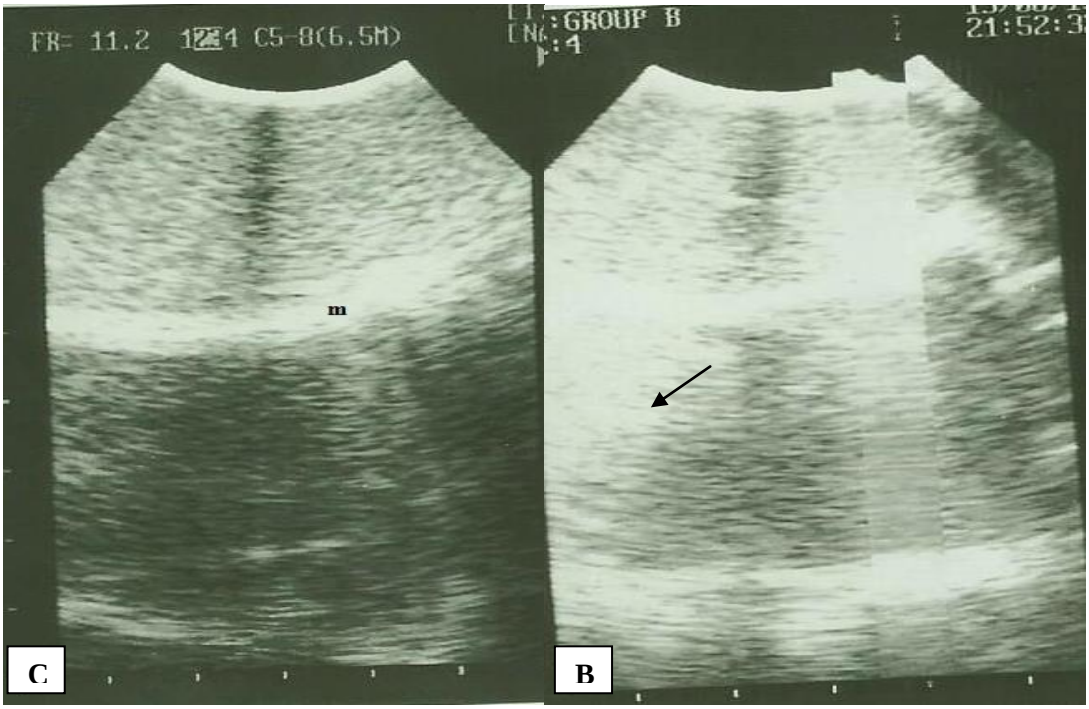

Plate V: Transverse ultrasonogramsof the testis of Yankasa rams. **C**. Normal testis for rams in group C. Note the hyperechoic mediastinum (m) and the echogenic appearance of the testicular parenchyma. **B:** Testis of rams in group B showing loss of echogenicity and hyperechoic testicular parenchyma (see arrow in **B**).

CHAPTER FIVE

5.0 DISCUSSION

The mean values of the body weight of Yankasa rams in the control group obtained were within reference values for this breed reported byOsuhor *et al.* 2004, Afolayan *et al.* (2006) and Adama *et al.* (2011). These values were lower than those reported by Adu and Ngere (1979) and Mohammed *et al.* (2012). The mean values of the body weight of Yankasa rams in the experimental groups did not differ significantly from those of the control group. This could be due to the fact that whole cottonseeds are high in energy (due to their fat content), crude protein and fibre and also because ruminants have the ability to detoxify (to a large extent) the free gossypol found in the whole cottonseeds by binding it to amino acids in the rumen as demonstrated by Santos *et al*. (2002). Thus, the rams in group A gained weight through out the feeding period. Similarly, cottonseed cake is a good protein source for ruminants and it is palatable and hence, supplementation with a source of degradable energy increases the efficiency of cottonseed meal. This explains why the rams in group B also had increased body weights through out the experiment.

This finding is in agreement with the findings of Kalla *et al*. (1981); Stahnke (1986); Jimenez *et al*. (1989); Singh and Rath (1990); Akhtar (1997); Zahid *et al.* (2003a) and Cunha *et al*. (2012) who reported non-significant effect of gossypol feeding on live weight of rats, Brangus bulls, Holstein bulls, mice, Nili-Ravi buffalo bulls, Teddy male goats and Santa Ines rams, respectively. Gossypol in cottonseed cake and whole cottonseed is considered an anti-nutritional factor when used in high percentage (> 40

% and total gossypol of > 20 mg/kg.) in dairy cows (Barraza *et al.*, 1991; Lindsey *et al.*, 1980), calves (Rogers *et al.*, 1975), sheep (Danke *et al.*, 1965;Morgan *et al.*, 1988) and goats (East *et al.*, 1994). Similarly, Velasquez-Pereira *et al.* (1998) found that using undecorticated cottonseed meal in Holstein bulls diet recorded lower daily gain compared with soyabean meal diet. The present study is not in agreement with these findings. This could be due to high concentration of dietary protein (in both WCS and CSC) which could have affected the absorption andmetabolism of gossypol by supplying a greater numberof free *epsilon*-amino groups for gossypol to combine with inthe digestive tract and then be excreted and byfacilitating the catabolism and detoxification of theabsorbed gossypol.

Scrotal circumference (SC) has high correlation with sperm production, service capacity and sexual development (Yarney *et al*., 1990). In this study, gossypol significantly decreased the scrotal circumference of Yankasa rams in the experimental groups A and B. This finding may be due testicular degeneration observed in the rams of groups A and B. This result agrees with the finding of by Andreazzi *et al*. (1995), who observed smaller scrotal circumference in goats receiving 30 % of whole cottonseed in relation to the control diet.

This finding diverged from that reported by Kramer *et al.* (1991) who observed no difference in the scrotal circumference of pubescent rams receiving gossypol-containing diet and those receiving control diet. In the same way, Jimenez *et al*. (1989), working with bulls, observed that gossypol intake had no influence on the scrotal circumference. This could be because these researchers used lower inclusion rates of gossypol-containing diets (30 % cottonseed meal and 40 % whole cottonseed, respectively). In

this study, the 48 % inclusion rate of both WCS and CSC might have provided adequate exposure to gossypol that caused testicular degeneration which led to a decrease in scrotal circumference in the treatment

The mean packed cell volume (PCV) values obtained for rams in the control group in this study were within the normal range for Yankasa sheep reported by Addass *et al.* (2010) (30.25 ± 0.99 % 32.55 ± 0.43 %) and higher than that reported by Bello and Tsado (2013) (22.4 ± 0.23 % to 29.4 ± 0.55 %). These values were higher than those reported for West African Dwarf sheep (Olayemi *et al.*, 2000, Addass *et al.*, 2010, Adelokun *et al.*, 2012; Aye, 2013), Uda and Balami sheep (Addass *et al.*, 2010). In this study, the mean values of PCV and haemoglobin concentration (Hb) of Yankasa rams in experimental groups A and B (fed whole cottonseed and cottonseed cake respectively) were significantly lower than the rams in the control group (fed gossypol-free diet). Decreased PCV and Hb are symptomatic of gossypol toxicity as reported by several researchers. Warren *et al.* (1988) reported decreased PCV in wethers fed 45 % and 42 % raw cottonseed meal and whole cottonseeds, respectively. Lindsey *et al.* (1980) reported a decreased PCV in mature dairy cows fed excessive amounts of cottonseed meal (6.6 and 42.7mg of free gossypol/kg body weight/day). Similar findings were reported in pigs (Jarquin *et al.* 1966, Braham *et al.*, 1967). Decreased PCV and Hb was also reported by Matondi *et al.* (2007) in goats; Wyse *et al.* (1991) in cattle, Apata (2010) in chicks; Mitruka and Rawnsely (1977) and Adeyemo and Longe (2007) in broiler chickens; Amao *et al.* (2012) in rabbits and Rinchard *et al.* (2003) in fish. Conversely, Nagalaskshi *et al.* (2001) reported that there was no significant

difference in the PCV and Hb concentration of lambs fed control diet and those fed cottonseed meal. Similar findings were reported by Nwoha (1995) in rats and Kailas and Thirumalesh (2011) in dairy cows.

The detrimental effect of gossypol-containing diets on erythrocytes is attributable to the increased erythrocytes fragility induced by gossypol coupling with iron (Gray *et al.*, 1993; Willard *et al.,* 1995; Matondi *et al.*, 2007; Nicholson, 2012). The binding of gossypol with iron forms a gosyypol-iron complex which inhibits the absorption of iron, consequently affecting erythropoiesis which leads to anaemia (Lindsey *et al.*, 1980). The effect of anaemia on testicular function has been described by Bomhard and Gelbke (2013) as being secondary to systemic oxygen deficit (hypoxaemia).

The mean values of total proteins, white blood cell (WBC) counts, lymphocytes and neutrophils did not differ significantly between the control and the experimental groups. These findings were similar to those observed by Nagalaskshi *et al.* (2001) in lambs, Solaiman (2007) in male goats and Amao *et al.* (2012) in rabbits. However, Soto-Blanco *et al*. (2012) reported reductions in counts of leukocytes, specifically of lymphocytes and neutrophils at a lesser extent, in cottonseed supplemented adult rams receiving 35% and 45% cottonseed meal. They concluded that cottonseed was not completely detoxified by the extrusion process and that residual gossypol may be involved in lymphocyte deficiency. This was attributed to gossypol's cytotoxic effects and apoptosis of lymphocytes (Quintana *et al.*, 2000, Yurtcu *et al.*, 2003 and Xu *et al.*, 2009). This was not observed in the present study. Similarly, gossypol was found to induce lymphocytopenia in male wistar rats (Akingbemi and Aire, 1994). Adeyemo and

Longe (2007) observed elevated values of total serum proteins in broiler chickens fed cottonseed cake.

There was no statistically significant difference between the mean ejaculate volume of the rams in the experimental and the control groups. Nevertheless, there was a decrease in mean ejaculate volume in groups A and B compared to the control group. This finding agrees with that of Guedes and Soto-Blanco (2010) in which they observed no statistically significant difference in the mean ejaculate volume of rams fed 0.5 /animal/day of cottonseed meal for 120 consecutive days. However, this finding contrasts that of El-Mokadem *et al.* (2012) who observed a significant decrease in semen volume for rams fed two levels of dietary gossypol (9 and 14 mg/kg/day or 30 and 48 % cottonseed meal) for 8 weeks.

The non-significant the effect of gossypol on ejaculate volume has also been reported by other workers such as Zahid *et al.* (2003b) in Teddy male goats (fed 1750 mg/kg and 2370 mg/kg of gossypol, respectively), Smith *et al.* (1991) in Holstein bulls (fed 64 and 75 mg/kg body weight of free gossypol) and Akhtar (1997) in Nili-Ravi buffalo bulls (fed cottonseed meal with 1.68 g/kg of gossypol).

The study showed that the mean percentage gross motility of the semen for Yankasa rams obtained in the pre-experimental period was within the normal range reported by Garner and Hafez (2000), and those reported for West African Dwarf rams by Oyeyemi *et al.* (2009). These values are higher than those reported for Yankasa rams by Jibril *et al.* (2011). However, rams in groups A and B (fed whole cottonseed and cottonseed cake, respectively), had significantly lower mean percentage gross motility compared to

those in the control group. This concur with the findings of Zhujun and Yila (1995), Cunha *et al.* (2012) and El-Mukadem *et al.* (2012) in rams; Zahid *et al.* (2003b) in male goats, Chase *et al.* (1994) and Chenoweth *et al.* (1994) in bulls, Hong *et al.* (1989) in man, Swan *et al*. (1990) in rats. The values obtained for mean gross motility in the experimental rams are lower than the values reported by Kimberling and Parsons (2009) as satisfactory for classification of reproductive potential in rams (> 40 %).

The detrimental effect of gossypol on spermatozoa motility is attributable to induction of mitochondrial specific injury to the sperm tail through inhibition of redox-cycling by electron transfer (Kovacic, 2003).

The mean semen concentration obtained for the control rams is within the normal range reported for Yankasa rams by Jibril *et al.* (2011). These values also concur with those reported by Oyeyemi *et al.* (2009) for West African Dwarf rams. On the contrary, these values are lower than those reported by Osinowo *et al.* (1988) in Yankasa rams and Garner and Hafez (2000); Pineda (2003) in temperate breeds.

In this study, the mean values of semen concentration in the experimental groups A and B did not differ significantly from those in the control group. A similar finding was reported by Arshami and Ruttle (1989), Guedes and Soto-Blanco (2010), Cunha *et al.* (2012) in rams; Zahid *et al.* (2003b) in male goats by Stahnke (1986); Arshami and Ruttle (1988), Kramer *et al.* (1991) and Chase *et al.* (1994) in bulls.

Conversely, Velasquez-Pareira *et al.* (1998) reported a reduction in semen concentration in gossypol-treated bulls while El-Mokadem *et al.* (2012) reported an increase in semen concentration in gossypol-treated rams. In the present study, the semen concentration in both experimental groups did not differ significantly from the

control group and this could be due to the fact that there is an increased amount of non-degradable protein intake from whole cottonseed and cottonseed cake which increases daily sperm production. Similar findings were reported by Rocha *et al.*(1996).

The mean percentage live spermatozoa values observed in the control group agree with reference values reported for sheep by Oyeyemi *et al.* (2009), Ptaszynska (2009) and Jibril *et al.* (2011). Similar values were also reported for bulls by Rekwot *et al.* (1987) and Zahid *et al.* (2003a) for goats. El-Mokadem *et al.* (2012) who reported that gossypol increased the percentage of dead spermatozoa. This agrees with the finding of our study in which the percentage of dead spermatozoa significantly increased at weeks 10 and 12 of the experiment for rams fed whole cottonseed and week 10 to 14 for rams fed cottonseed cake. This finding also concur with that of Velasquez-Periera *et al.* (1998) who reported an increase in percentage dead spermatozoa in bulls treated with gossypol. Zahid *et al.* (2003b) reported that there was no significant difference in the percentage dead spermatozoa between the control and gossypol treated goats.

The mean values for percentage total sperm abnormalities obtained in the rams of the control group are similar to normal values reported for Yankasa rams by Osinowo *et al.* (1988) and Jibril *et al.* (2011) and Oyeyemi *et al.* (2009) for West African Dwarf rams. Gossypol has been reported to cause morphological changes in the germ cells due to gossypol-induced inhibition of the synthesis of sperm-cell histones and other nuclear proteins that stabilize the structure of DNA (Ye *et al.,* 1989). The finding of our study supports this assertion. There was an increase in percentage total abnormal sperm from week 8 to the end of the experiment in the gossypol-treated groups. This agrees with the findings of Amann and Schanbacher (1983) who reported that the interval between

damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 60 days, depending upon the site of damage. The mean values observed for total sperm abnormalities for the experimental rams were higher than that recommended by Kimberling and Parsons (2009) as satisfactory for classification of reproductive potential in rams (≤10 %).

There was also a higher percentage total sperm abnormality in the rams in group B compared to those of group A due to the fact that cottonseed cake contains more bound gossypol than free gossypol which is more readily detoxified in the rumen. Conversely, whole cottonseed contains more of free gossypol than bound gossypol. This finding agrees with those of Chenoweth *et al.* (1994),Velasquez-Periera *et al.* (1998), Santos *et al.* (2008) in bulls; Arshami and Ruttle (1989), El-Mokadem *et al.* (2012) in rams; Zahid *et al.* (2003a) in goats; Hikum and Hoffer (1987), Swan *et al.* (1990) in rats and Mohan *et al.* (1989) in cocks. Guedes and Soto-Blanco (2010) reported a contrary finding that gossypol had no effect on total spermatozoa abnormality in rams fed cottonseed cake. Specifically, the mean values for head, mid-piece and tail defects for the rams in groups A and B differed significantly from those of the rams in the control group. These findings clearly indicate the association of spermatozoa defects and the reduction in percentage forward motility in the gossypol treated rams.

The effects of gossypol in this study were manifested in the semen characteristics at or above 49 days (7 weeks) in the rams of the experimental groups. This period (i.e. 49 days) corresponds with the period of spermatogenic cycle in the ovine species (Pineda, 2003). This also confirms that the effect of gossypol on spermatogenesis is time-

dependent as reported by Randel *et al.* (1992). They stated that the effects of gossypol in the male are both dose- and time-dependent.

Testicular and epididymal weight, length and sperm reserves were not significantly affected by gossypol. Gossypol-treated rams in groups A and B however, had numerically lower mean values of testicular and epididymal weight, length and sperm reserves than those of the control group. Similar findings were reported by Kramer *et al.* (1991) in rams fed diets containing gossypol.

Chenoweth *et al.* (1994) found a similar trend where gossypol-fed bulls had the lowest testicular volumes and weights, but they failed to find statistical differences. These results are consistent with studies that did not find any effect of gossypol on testicular weights (Jimenez *et al.,* 1989; Chase *et al.,* 1994; Chenoweth *et al.,* 1994).

Amao *et al.* (2012) reported a contrary finding in rabbit bucks fed with cottonseed cake. They reported marked severe reductions in testicular sperm reserve, daily sperm production and daily sperm production per gram testis. This observation is consistent with similar work in rodents (Coulson *et al.,* 1980; Sakesena and Salmonsen, 1982), bulls (Velasquez Pereira *et al*., 1998) and rabbit (Taha *et al.,* 2006). This anti-spermatogenic effect has been attributed mainly to gossypol present in the cottonseed cake which causes damage to the germinal epithelium of the seminiferous tubules (Arshami and Ruttle, 1988).

In the present study, histopathological examination of the testicular tissue indicated depletion of spermatogenic cell layers of the seminiferous tubules in the testis of the ramsin groups A and B, which led to larger lumens of the seminiferous tubules and fewer germ cells. There was also failure of maturation of spermatocytes and

degeneration of spermatids and an exfoliation of the spermatogonia. These changes are consistent with testicular degeneration (Foster and Ladds, 2007). These changes were more severe in the rams fed cottonseed cake (group B). This would not normally be expected because whole cottonseed (WCS) contains more of free gossypol than cottonseed cake (CSC). However, the availability of free gossypol in WCS for detoxification in the rumen affects its absorption and bioavailability. Furthermore, the tolerance of free gossypol in ruminants has been attributed to binding between soluble proteins and free gossypol in the rumen (Reiser and Fu, 1962).

These findings are in agreement with those of Arshami and Ruttle (1989) who reported similar microscopic changes in testicular tissue of rams fed cottonseed rations as a source of gossypol. Similar findings were reported by Chongthammakun *et al.* (1986); Arshami and Ruttle (1988); Singh and Rath (1990); Chase *et al.* (1994) Zahid *et al.* (2002) in monkeys, bulls, mouse and goats respectively.

Changes from the normal ultrasonographic homogeneous testicular parenchyma such as hyperechoic appearance are associated with testicular degeneration. In testicular degeneration, the testicular parenchyma loses its tissue architecture and becomes hyperechoic over time (Goyal and Hemon, 2007).

In the present study, ultrasonograms of the testis of gossypol-treated rams indicated hyperechoic appearance which implies testicular degeneration. The testis of the rams in the control group had echogenic appearance. Similar findings were reported by Ahmad and Noakes (1995) in two male goats with testicular degeneration. This finding implies that ultrasonographic methods could be a useful means of diagnosing intrascrotal lesions in domestic animals.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The semen characteristics and testicular morphology as well as the live weight changes and some haematological parameters of Yankasa rams fed whole cottonseed and cottonseed cake were observed for 14 weeks (98 days). On termination of the experiment, the histological changes and gonadal and epididymal sperm reserves of the testes of the rams were also evaluated.

From the findings of this work, the following conclusions were made:

1. Dietary gossypol from whole cottonseed and cottonseed cake fed at 48 % of the diet to Yankasa rams significantly altered their semen percentage gross motility, percentage live-dead ratio and morphology. The semen volume and concentration were not significantly affected by gossypol.
2. Of the sperm abnormalities evaluated, detached head (DH), mid-piece defects (MPD), coiled tail (CT) were significantly higher in Yankasa rams fed whole cottonseed and cottonseed cake. Thus, gossypol caused increased abnormality of the head, mid-piece and tails which could be responsible for decreased gross motility in the semen of gossypol-treated rams.
3. Of the haematological parameters evaluated, gossypol reduced the packed cell volume (PCV) and haemoglobin concentrations of the rams while other parameters were unaffected.

4. The scrotal circumference was significantly decreased by gossypol while the body weights of rams in the experimental groups were not significantly different from those of the control group.
5. Gossypol-induced testicular degeneration in testes of rams of the experimental groups by inducing depletion and vacuolation of spermatogenic cell layers of the seminiferous tubules, arrest of spermatogenesis at the spermatocytes levels and degeneration of spermatids as well as exfoliation of the spermatogonia from the basal membrane of the seminiferous tubules. These changes were more severe in rams fed cottonseed cake (group B).
6. Gossypol caused gross morphologic changes in the testes of rams of the experimental group as evaluated by ultrasonography. There was testicular degeneration evident by loss of homogeneity and hyperechoic appearance of the testes in the ultrasonograms. These changes were more severe in group B rams.

6.2 Recommendations

1. High levels of gossypol containing feeds such as whole cottonseed and cottonseed cake should not be fed for prolonged period of time to rams intended for breeding purposes.

2. High levels of gossypol containing feeds such as whole cottonseed and cottonseed should not be fed for prolonged periods (more than 8 weeks) to rams with anaemia.

3. Further studies should be carried out to determine the molecular effects of gossypol on the spermatozoa and the effects of dietary gossypol on the reproduction of female Yankasa sheep.

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APPENDICES

APPENDIX I: Mean (± SEM) values of semen characteristics, body weight and scrotal circumference of the experimental rams in groups A, B and C during the acclimatization period.

Parameter	**A (WCS)**	**B (CSC)**	**C (Control)**
	n = 5	**n = 5**	**n = 5**
Body Weight (Kg)	20.60 ± 0.24	21.40 ± 0.51	19.80 ± 0.37
Scrotal Circumference (cm)	22.0 ± 0.71	22.2 ± 0.92	21.6 ± 0.74
pH	6.6 ± 0.24	7.4 ± 0.24	7.0 ± 0.00
Colour	Creamy	Milky	Creamy
Volume (ml)	0.44 ± 0.05	0.78 ± 0.25	0.72 ± 0.13
Motility (%)	81.8 ± 6.69	84 ± 3.67	83.8 ± 5.19
Concentration (x 10^6/ml)	274.6 ± 7.52	206.0 ± 3.34	267.2 ± 5.55
Live Spermatozoa (%)	89.0 ± 1.76	88.2 ± 2.52	90.0 ± 1.87
Abnormal sperm cells (%)	8.2 ± 0.52	10.0 ± 0.66	9.8 ± 0.22

WCS- Whole Cottonseed, CSC- Cottonseed Cake

APPENDIX II: Mean (± SEM) values of sperm abnormalities for rams in Group A

Week	DH	MPD	BT	CT	FT	Total(%)	Normal Sperm (%)
1	1.8 ±0.58	2.2±0.49	1.6±0.55	1.6 ±0.51	1.0 ±0.31	8.2 ±0.52	91.8 ± 0.52
2	2.2 ±0.49	2.2±0.49	2.0 ±0.54	2.2 ±0.49	2.2 ±0.49	12.8 ±0.57	87.2 ± 0.57
3	2.2 ±0.49	2.4±0.86	2.6 ±0.51	4.8 ±0.86	2.4 ±0.86	14.4 ±0.92	85.6 ± 0.92
4	2.4 ±0.86	3.2±0.92	4.8 ±0.86	4.4 ±0.88	4.4 ± 0.88	14.8 ±1.06	85.2 ± 1.06
5	4.0 ±0.89	2.2 ±0.49	3.8 ±1.71	4.0 ±0.89	4.8 ± 0.97	18.8 ±1.32	81.2 ± 1.32
6	4.0 ±0.89	4.2 ±0.8	6.6 ±1.53	4.6 ±1.32	3.4 ±0.76	22.8 ±1.18	77.2 ± 1.18
7	7.6 ±1.74	4.0 ±0.49	5.4 ±1.20	5.2 ±1.11	5.6 ±2.16	27.8 ±1.31	72.2 ± 1.31
8	5.2 ±1.47	4.8 ±0.97	9.2 ±0.80	6.2 ±2.11	4.0 ±0.89	29.4 ±2.61	70.6 ± 2.61
9	4.3 ±1.24	5.2 ±1.47	7.6 ±2.63	7.6 ±2.15	4.4 ±1.24	29.1 ±1.73	70.9 ± 1.73
10	7.8 ±1.56	6.2 ±1.01	9.2 ±1.01	6.2 ±2.11	4.0 ±0.89	33.4 ±2.15	66.6 ± 2.15
11	6.8 ±0.40	4.8 ±0.97	7.2 ±2.38	5.6 ±2.06	7.0 ±2.10	31.4 ±1.89	68.6 ± 1.89
12	7.8 ±1.41	7.8 ±1.41	8.6 ±1.99	5.8 ±1.48	5.8 ±1.86	35.8 ±1.45	64.2 ± 1.45
13	10.2 ±0.41	6.9 ±1.52	6.8 ±0.87	7.0 ±2.10	6.4 ±2.20	37.3 ±1.31	62.7 ± 1.31
14	10.4 ±0.43	8.2 ±1.62	7.6 ±1.32	6.0 ±1.98	5.4 ±1.86	37.6 ±1.50	62.4 ± 1.50

DH-Detached head, MPD- Mid-piece defect, BT- Bent tail, FT- Free tail

APPENDIX III: Mean (± SEM) values of sperm abnormalities for rams in Group B

Weeks	DH	MPD	BT	CT	FT	ABNORMAL	NORMAL
1	2.4 ± 0.51	3.0 ± 0.71	3.0 ± 0.71	0.0 ± 0.00	1.6 ± 0.51	10.0 ± 0.66	90.0 ± 1.20
2	4.0 ± 1.04	2.4 ± 0.81	3.2 ± 1.16	1.2 ± 3.38	2.2 ± 0.66	13.0 ± 0.86	87.0 ± 0.88
3	4.0 ± 0.63	1.6 ± 0.40	3.8 ± 1.16	1.4 ± 0.40	2.6 ± 0.68	13.4 ± 0.24	86.6 ± 0.84
4	3.8 ± 0.12	2.6 ± 0.40	4.0 ± 1.38	2.0 ± 1.05	3.2 ± 1.16	15.6 ± 0.92	84.4 ± 1.56
5	3.2 ± 1.68	1.8 ± 0.58	5.4 ± 1.25	2.2 ± 0.20	3.8 ± 1.43	16.4 ± 0.80	83.6 ± 1.15
6	9.0 ± 1.18	3.4 ± 0.40	0.0 ± 0.00	4.6 ± 0.93	2.8 ± 1.39	19.8 ± 0.97	80.2 ± 0.98
7	9.4 ± 2.18	2.0 ± 0.55	4.6 ± 1.88	6.2 ± 1.85	3.8 ± 1.66	26.0 ± 0.89	74.0 ± 1.25
8	8.4 ± 1.76	8.2 ± 2.77	6.8 ± 1.48	6.8 ± 0.98	7.2 ± 1.83	37.4 ± 1.02	62.6 ± 2.12
9	9.0 ± 1.52	3.4 ± 1.82	8.0 ± 1.41	8.4 ± 1.66	2.6 ± 1.78	31.4 ± 1.85	68.6 ± 2.90
10	9.8 ± 1.17	8.2 ± 3.40	8.4 ± 2.76	5.6 ± 0.80	5.4 ± 1.94	32.0 ± 2.24	68.0 ± 2.34
11	8.7 ± 1.74	6.2 ± 2.48	10.4 ± 0.74	8.8 ± 1.36	5.2 ± 1.39	39.3 ± 2.76	60.7 ± 2.10
12	13.0 ± 1.48	7.2 ± 1.70	6.2 ± 1.40	9.2 ± 1.89	4.6 ± 1.47	40.2 ± 2.33	59.8 ± 3.55
13	9.4 ± 2.10	9.6 ± 2.99	10.2 ± 0.75	10.2 ± 1.28	7.4 ± 2.04	46.8 ± 1.62	53.2 ± 3.25
14	8.8 ± 1.72	10.1 ± 1.80	10.2 ± 0.75	6.6 ± 2.24	8.0 ± 1.10	43.7 ± 0.98	56.3 ± 3.11

DH-Detached head, MPD- Mid-piece defect, BT- Bent tail, FT- Free tail

APPENDIX IV: Mean sperm abnormalities for rams in Group C

Weeks	DH	MPD	BT	CT	FT	TOTAL	NORMAL
1	2.8 ± 0.37	3.4 ± 0.58	4.3 ± 0.73	4.5 ± 0.58	0.8 ± 0.37	15.8 ± 0.22	84.2 ± 0.22
2	3.2 ± 0.20	2.2 ± 0.37	2.2 ± 0.66	2.4 ± 1.60	2.4 ± 0.24	12.4 ± 0.33	87.6 ± 0.33
3	1.4 ± 0.36	0.6 ± 0.40	2.0 ± 0.70	2.2 ± 0.49	3.4 ± 0.50	9.6 ± 2.22	90.4 ± 2.22
4	2.4 ± 0.51	5.6 ± 0.51	3.0 ± 1.00	1.5 ± 0.87	2.4 ± 0.51	14.9 ± 1.88	85.1 ± 1.88
5	2.6 ± 0.37	3.8 ± 1.24	4.8 ± 0.73	0.0 ± 0.00	1.2 ± 0.81	12.4 ± 2.45	87.6 ± 2.45
6	2.8 ± 0.55	2.6 ± 0.50	5.2 ± 1.65	1.2 ± 1.45	3.2 ± 1.47	15.0 ± 0.32	85.0 ± 0.32
7	3.0 ± 0.73	3.4 ± 0.51	4.4 ± 1.91	3.0 ± 1.40	6.0 ± 1.36	19.8 ± 1.55	80.2 ± 1.55
8	2.8 ± 0.87	4.4 ± 1.63	4.6 ± 1.94	4.1 ± 1.26	4.8 ± 2.07	20.7 ± 2.65	79.3 ± 2.38
9	4.4 ± 1.28	3.0 ± 0.71	3.2 ± 1.74	5.2 ± 1.61	1.4 ± 1.35	17.2 ± 0.25	82.8 ± 3.21
10	4.4 ± 1.69	6.2 ± 2.09	4.2 ± 2.19	4.0 ± 0.63	3.8 ± 1.50	22.6 ± 1.50	77.4 ± 0.98
11	3.6 ± 0.80	5.3 ± 1.59	4.8 ± 1.47	0.0 ± 0.00	2.4 ± 1.32	16.1 ± 0.98	83.9 ± 4.32
12	3.2 ± 0.92	6.2 ± 1.18	3.2 ± 1.32	4.3 ± 0.75	5.4 ± 2.22	22.3 ± 0.26	77.7 ± 0.88
13	2.8 ± 0.87	2.4 ±0.45	3.2 ± 1.77	6.2 ± 1.75	4.6 ± 1.29	19.2 ± 1.32	80.8 ± 2.12
14	5.4 ± 1.50	5.6 ± 2.75	4.8 ± 1.93	3.2 ± 1.50	3.3 ± 0.98	22.3 ± 2.88	77.7 ± 3.22

DH-Detached head, MPD- Mid-piece defect, BT- Bent tail, FT- Free tail